UNIVERSITE DES SCIENCES ET TECHNOLOGIES DE LILLE

U.F.R. DE BIOLOGIE

HDR

Mémoire présenté par

Florence PICQUET

Pour obtenir

L'HABILITATION A DIRIGER DES RECHERCHES
Section : Sciences Naturelles

PLASTICITE MUSCULAIRE ET MESSAGES AFFERENTS

Soutenue le 03 JUILLET 2008

JURY

Dr G. Butler-Browne, Directeur de Recherche INSERM, Paris	**Rapporteur**
Dr M. Jamon, Directeur de Recherche INSERM, Marseille	**Rapporteur**
Pr G. Lensel-Corbeil, Professeur des Universités, Lille 2	**Rapporteur**
Pr C.A. Maurage, Professeur des Universités, Praticien Hospitalier, Lille 2,	**Examinateur**
Pr L. Stevens, Professeur des Universités, Lille 1	**Examinateur**
Pr M. Falempin, Professeur des Universités, Lille 1	**Directeur**

Travail réalisé au Laboratoire de Plasticité Neuromusculaire
Université des Sciences et Technologies de Lille

Sommaire

CURRICULUM VITAE

I – ETAT CIVIL

Nom	:	PICQUET
Prénoms	:	Florence, Marguerite, Laure
Date et lieu de naissance	:	09/05/1968 à Saint-Omer (Pas-de-Calais)
Nationalité	:	Française
Situation professionnelle	:	Maître de Conférences, classe normale
Adresse administrative	:	Laboratoire de Plasticité Neuromusculaire
		EA 4052, Bâtiment SN4
		Université des Sciences et Technologies de Lille,
		59655 Villeneuve d'Ascq cedex
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II – TITRES ET DIPLOMES UNIVERSITAIRES

1991-1992 → Diplôme d'Etudes Approfondies (DEA) des Sciences de la Vie et de la Santé cohabilité par l'Université des Sciences et Technologies de Lille (Lille 1) et l'Université du Droit et de la Santé de Lille (Lille 2), option Neurosciences. Stage du DEA réalisé au Laboratoire de Plasticité Neuromusculaire (Université des Sciences et Technologies de Lille) sous la direction du Pr Y. Mounier.

Titre du mémoire : Etude de la relation structure/fonction des protéines contractiles du muscle soleus de singe. 37 pages
Mention Assez Bien.

1992-1996 → Doctorat d'Université. Allocataire de recherche du Ministère de la Recherche et de l'Enseignement Supérieur.

Directeur de thèse : Pr Y. Mounier ; Mention Très Honorable, Félicitations du jury

Titre de la thèse : Etude au cours du développement post-natal des propriétés structurales et fonctionnelles du soleus de rat en conditions normale et

d'immobilisation. 184 pages, soutenue le 03 Décembre 1996 devant un jury composé de :

- M. FALEMPIN, **Président** : Professeur des Universités, Université des Sciences et Technologies de Lille,
- G.S. BUTLER-BROWNE, **Rapporteur** : Directeur de Recherches INSERM, Paris V,
- G. RAYMOND, **Rapporteur** : Directeur de Recherches, Université de Poitiers,
- J.P. VILAIN, **Examinateur** : Professeur des Universités, Université des Sciences et Technologies de Lille,
- A. GUELL, **Examinateur** : Responsable des Programmes Sciences de la Vie, CNES, Paris,
- Y. MOUNIER, **Directeur** : Professeur des Universités, Université des Sciences et Technologies de Lille

Depuis 1998 → Inscription à l'**Habilitation à Diriger des Recherches** (HDR) au Laboratoire de Plasticité Neuromusculaire, Université des Sciences et Technologies de Lille (USTL)
Directeur d'HDR : Pr M. Falempin

III – FONCTIONS EXERCEES EN ENSEIGNEMENT

	1992-1993	**1995-1996**	**1996-1997**	**1998-2006**
Fonction exercée	Vacataire	Attaché Temporaire d'Enseignement et de Recherche (ATER), section 66	ATER, section 66	MCU, section 69
Enseignement dispensé	Travaux pratiques de physiologie cellulaire	Travaux pratiques et travaux dirigés de physiologie cellulaire	Travaux pratiques et travaux dirigés de physiologie cellulaire et physiologie de la nutrition	Cours, travaux dirigés et travaux pratiques en physiologie et neurophysiologie
Nombre d'heures (éq.TD)	60	96	96	210
Lieu d'exercice	USTL	USTL	Université du Littoral-Côte d'Opale	USTL

En tant que maître de conférences, mon activité d'enseignement est constituée annuellement d'environ 192 heures équivalent TD répartis de la façon suivante dans différentes unités d'enseignement (UE), depuis la mise en place du parcours LMD :

- **Parcours Licence (L) (environ 100 h éq TD):**

Semestre 2 : Cours, Travaux dirigés et travaux pratiques de l'UE Physiologie Cellulaire

Semestre 3 : Cours et travaux dirigés de l'UE Grandes Fonctions Physiologiques

Semestre 5 : Cours et travaux dirigés de l'UE Neurophysiologie Intégrée

Semestre 6 : Cours et travaux dirigés de l'UE Physiologie du Mouvement, cours de l'UE Adaptations Physiologiques, cours de l'UE Modèles Animaux

- **Parcours Master (M) (environ 80 h éq TD):**

Master 1 Semestre 1 : Travaux dirigés de l'UE MO1 : Génie Génétique, Biologie et Physiologie Cellulaires

Master 1 Semestre 1 : Travaux dirigés de l'UE MP2 : Motricité et Sensibilité

Master 1 Semestre 1 : Cours de l'UE MP3 : Pathologies Actuelles

Master 1 Semestre 1 : Travaux pratiques de l'UE M03 : Pratiques en Biologie et Biotechnologies – Physiologie Intégrée

Master 1 Semestre 2 : Cours et travaux dirigés de l'UE MP5 : Physiologie Neuromusculaire

Master 1 Semestre 2 : Cours de l'UE MP8 : Adaptations Physiologiques aux Conditions Extrêmes

Master 1 Semestre 2 : Cours de l'UE MB9 : Motricité Digestive

Master 1 Semestre 2 : UE MP4 : Encadrement d'étudiants lors des Travaux d'Etudes et de Recherche

- **Hors LMD : CAPES et Agrégation (environ 12 h éq TD)**

Mise au point de Travaux Pratiques (Physiologie Respiratoire)

- **<u>Responsabilités au titre de l'enseignement</u>**

A ces heures d'enseignement dispensées s'ajoute la responsabilité de 2UE, l'une en licence, l'autre en master :

- Depuis 2004, responsable de l'UE "Adaptations Physiologiques" en S6 dans le cadre de la LMD
- Depuis 2006, responsable de l'UE "Adaptations Physiologiques en situations extrêmes" en M1 dans le cadre de la LMD

IV – ACTIVITES ADMINISTRATIVES

- Depuis 1999, membre élue du comité de gestion du Centre Commun de Microscopie et d'Imagerie Cellulaire de l'USTL
- Depuis 2000, membre élue du comité de gestion de l'Animalerie de l'USTL
- Depuis 2003, membre élue du CEVU
- Depuis 2003, membre suppléant élue de la commission de spécialistes 66-69 de l'USTL

V – RECONNAISSANCE SCIENTIFIQUE

- Bénéficiaire de la PEDR depuis 2003
- Référé externe pour l'Agence Spatiale Canadienne
- Référé externe pour plusieurs journaux scientifiques : Am J Physiol, J Appl Physiol, Exp Neurol, J Histochem Cytochem, Muscle Nerve, Pflugers Arch
- Responsabilité de contrat AFM, en 2003, thème retenu : « Deafferentation and muscular consequences »

VI – SOCIETES SAVANTES

- Membre de la Société des Neurosciences Française
- Membre de l'Association des Physiologistes

VII – COLLABORATIONS SCIENTIFIQUES

- **Dr V. BOUET** UMR 6185 - CNRS, Neurodegenerescence: models and therapeutic strategies, University of Caen Basse-Normandie, CYCERON, Bd H Becquerel - BP 5229, F-14074 Caen Cedex, France
- **Dr G. S. BUTLER-BROWNE**, UMR CNRS 7000, Faculté de Médecine, Paris VI, 105 Boulevard de l'Hôpital, 75634 Paris Cedex 13, France.
- **Pr V.R. EDGERTON**, Brain Research Institute, University of Californie, Los Angeles CA 90095-1527, Etats-Unis.
- **Pr M. LACOUR,** Laboratoire de Neurobiologie Intégrative et Adaptative, CNRS UMR 6149, Université de Provence, 52, Faculté de St Jérôme, Case 361, 13397 Marseille Cedex 20, France.
- **Pr J. PETIT**, Faculté des Sciences du Sport et de l'Education Physique, Université de Bordeaux 2, Domaine Universitaire, 12 Avenue Camille Jullian, 33607 Pessac Cedex, France.
- **Dr P. POULAIN**, INSERM U 422, Place de Verdun, 59045 Lille Cedex, France.

VIII – PARTICIPATION A L'ENCADREMENT DE JEUNES CHERCHEURS

En accord avec le Pr M. Falempin, directeur du Laboratoire de Plasticité Neuromusculaire, j'ai participé à l'encadrement (apprentissage des techniques expérimentales, participation à la mise au point de protocole expérimentaux et aux expérimentations, analyse des résultats) de jeunes chercheurs, en DEA puis en thèse de 3ème cycle à hauteur de 50%.

A. DEA

1998-1999 → DE-DONCKER L.: *Effets de la stimulation des récepteurs cutanés sur l'atrophie musculaire développée en situation d'hypodynamie-hypokinésie*, DEA Sciences de la vie et de la santé, option Neurosciences, cohabilité Lille 1-Lille 2

2001-2002 → ROZE D.: *Etude préliminaire de la régénération du nerf alvéolaire inférieur après section et suture conventionnelle*, DEA Sciences de la vie et de la santé, option Neurosciences, cohabilité Lille 1-Lille 2

2001-2002 → KASRI M. : *Conséquences chez le rat d'une labyrinthectomie unilatérale sur les propriétés morphologiques, contractiles, histochimiques et électromyographiques du muscle soleus*, DEA Sciences de la vie et de la santé, option Neurosciences, cohabilité Lille 1-Lille 2

B. Thèses d'Université

1999-2002 → DE-DONCKER L. : *Influence de la microgravité simulée sur les afférences cutanées plantaires et sur les afférences proprioceptives du muscle soleus de rat*, 185 pages, USTL

2002-2005 → TREFFORT N. : *Effets de l'hypodynamie-hypokinésie sur les caractéristiques morphologiques et électrophysiologiques des Organes Tendineux de Golgi. Conséquences de la microgravité simulée au niveau sur les neurotransmetteurs médullaires,* 163 pages, USTL

IX – PUBLICATIONS SCIENTIFIQUES

A. Mémoires personnels

PICQUET, F. (1992)
Etude de la relation structure/fonction des protéines contractiles du muscle soleus de singe.
Mémoire de DEA, Sciences de la Vie et de la Santé, option Neurosciences, Lille I., 37 p.

PICQUET, F. (1996)
Etude au cours du développement post-natal des propriétés structurales et fonctionnelles du soleus de rat en conditions normales et d'immobilisation.
Mémoire de Thèse d'Université, Sciences de la Vie et de la Santé, option Neurosciences, Lille I., 184 p.

B. Publications dans des revues internationales à comité de lecture

P1. CORDONNIER, C., STEVENS, L., **PICQUET, F.** & MOUNIER, Y. (1995). Structure-function relationship of soleus muscle fibres from the rhesus monkey. *Pflügers Arch. Eur. J. Physiol., 430, 19-25.*

P2. **PICQUET, F.**, STEVENS, L., BUTLER-BROWNE, G. S. & MOUNIER, Y. (1997). Contractile properties and myosin heavy chain composition of newborn rat soleus muscles at different stages of postnatal development. *J. Muscle Res. Cell Motility, 18, 71-79.*

P3. MOUNIER, Y., **PICQUET, F.** & STEVENS, L. (1997). Postnatal muscle development in unloading conditions. *Int. J. Sports Medicine, 18, 1-2.*

P4. **PICQUET, F.**, STEVENS, L., BUTLER-BROWNE, G.S. & MOUNIER, Y. (1998). Differential effects of a six-day immobilization on newborn rat soleus muscles at two developmental stages. *J. Muscle Res. Cell Motility, 19, 743–755.*

P5. DE-DONCKER, L., **PICQUET, F.** & FALEMPIN, M. (2000). Effects of cutaneous receptor stimulation on the muscular atrophy developed in hindlimb unloading condition. *J. Appl. Physiol., 89, 44-51.*

P6. **PICQUET, F.,** CANU, M.H. & FALEMPIN, M. (2000). Phenotypic changes in the composition of muscular fibres in rat soleus motor units after 14 days of hindlimb unloading. *Pflügers Arch. Eur. J. Physiol., 440, 229-235.*

P7. KISCHEL, P., STEVENS, L, MONTEL, V., **PICQUET, F.** & MOUNIER, Y. (2001)**.** Plasticity of monkey triceps muscle fibers in microgravity conditions. *J. Appl. Physiol., 90, 1825-1832.*

P8. CANU, M.H., STEVENS, L.; RICART-FIRINGA, C., **PICQUET, F**. & FALEMPIN, M. (2001). Effect of the β_2-agonist clenbuterol of the locomotor activity of rat submitted to a 14-day period of hypodynamia-hypokinesia. *Behav. Brain Res., 122, 103-112.*

P9. **PICQUET, F.,** BOUET, V., CANU, M.H., STEVENS, L., MOUNIER, Y., LACOUR, M. & FALEMPIN, M. (2002). Contractile properties and myosin expression in rats born and reared in hypergravity. *Am. J. Physiol., 282, R1687-R1695.*

P10. DE-DONCKER, L., **PICQUET, F.,** BUTLER-BROWNE, G. & FALEMPIN, M. (2002). Expression of myosin heavy chain isoforms along intrafusal fibers of soleus muscle spindles in normal and after 14 day hindlimb unloaded rats. *J. Histoch. & Cytoch., 50, 1543-1553.*

P11. DE-DONCKER, L., **PICQUET, F**., PETIT, J. & FALEMPIN, M. (2003). Characterization of spindle afferents in rat soleus muscle using ramp-and-hold and sinusoidal stretches. *J. Neurophysiol., 89, 442-449.*

P12. DE-DONCKER, L., **PICQUET, F**., PETIT, J. & FALEMPIN, M. (2003). Effects of hypodynamia-hypokinesia on the muscle spindle discharges of rat soleus muscle. *J. Neurophysiol., 89, 3000-3007.*

P13. **PICQUET, F**. & FALEMPIN, M. (2003). Compared effects of hindlimb unloading versus terrestrial deafferentation on muscular properties of the rat soleus. *Exp. Neurol., 182, 186-194.*

P14. CAMPANA, A., RONDI-REIG, L., TOBIN, C., LOHOF, A., **PICQUET, F**., FALEMPIN, M., WEITZMAN, J.B. & MARIANI, J. (2003). P53 inactivation leads to impaired motor synchronization in mice. *Eur. J. Neurosci., 17, 2135-2146.*

P15. **PICQUET, F**., DE-DONCKER, L. & FALEMPIN, M. (2003). Expression of myosin heavy chain isoforms in rat soleus muscle spindles after 19 days in hypergravity. *J. Histochem. Cytochem., 51, 1479-1489.*

P16. KASRI, M., **PICQUET, F.** & FALEMPIN, M. (2004). Effects of unilateral and bilateral labyrinthectomy on rat postural muscle properties : the soleus. *Exp. Neurol., 185, 143-153*.

P17. BOZZO, C., STEVENS, L., BOUET, V., MONTEL, V., **PICQUET, F**., FALEMPIN, M., LACOUR, M. & MOUNIER, Y. (2004) Hypergravity from conception to adult stage : effects on contractile properties and skeletal muscle phenotype.*J. Exp. Biol., 207, 2793-2802*.

P18. **PICQUET, F**., DE-DONCKER, L. & FALEMPIN, M. (2004). Enhancement of hybrid fiber types in rat soleus muscle after clenbuterol administration during hindlimb unloading. *Can. J. Physiol. & Pharmacol., 82, 311-318.*

P19. MOUNIER, Y., MONTEL, V., **PICQUET, F**., STEVENS, L., BASTIDE, B. & FALEMPIN, M. (2005) Dual effect of deafferentation on contractile characteristics and sarcoplasmic reticulum properties in rat soleus muscle fibers. *J. Appl. Physiol., 99, 542-548.*

P20. **PICQUET, F**., BOUET, V., COCHON, L., LACOUR, M. & FALEMPIN, M. (2005). Changes in rat soleus muscle phenotype consecutive to a growth in hypergravity and in normogravity. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 289, R217-R224.*

P21. TREFFORT, N., **PICQUET, F**., PETIT, J. & FALEMPIN, M. (2005) The structure and response properties of Golgi tendon organs in control and hypodynamia-hypokinesia rats. *Exp. Neurol., 195, 313-321.*

P22. DE-DONCKER, L., KASRI, M., **PICQUET, F**. & FALEMPIN, M. (2005). Impact of a reduction or a lack of afferent feedback on the soleus EMG activity, L_5 efferent and afferent neurograms in the rat. *J. Exp. Biol., 208, 4585-4592.*

P23. CANU, M.H., TREFFORT, N., **PICQUET, F**., DUBREUCQ, G., GUERARDEL, Y., FALEMPIN, M. (2006). Concentration of amino acid neurotransmitters in the somatosensory cortex of the rat after surgical or functional deafferentation. *Exp. Brain Res., 173, 623-628.*

P24. TREFFORT, N., DUBREUCQ, G., CANU, M.H., GUERARDEL, Y., FALEMPIN, M. & **PICQUET, F.** (2006). Variations in amino acid neurotransmitters in the rat ventral spinal cord after hypodynamia-hypokinesia. *Neurosci. Lett., 403, 147-150.*

<u>Synthèse des travaux</u> :

Premier auteur	Deuxième auteur	Dernier auteur
8 fois	7 fois	1 fois

C. Autres publications

A1. STEVENS, L., **PICQUET, F**., CATINOT, M.P. & MOUNIER, Y. (1996). Differential adaptation to weightlessness of functional and structural characteristics of rat hindlimb muscles. *J. Gravitational Physiol., 3, 54-57.*

A2. LANGLET, C., CANU, M.H., **PICQUET, F**. & FALEMPIN, M. (1999). Short-term plasticity in primary somatosensory cortex of the rat after hindlimb suspension. *J. Gravit. Physiol., 6, 59-60.*

A3. MOUNIER, Y., STEVENS, L., SHENKMAN, B., KISCHEL, P., LENFANT, A.M., MONTEL,V., CATINOT, M.P., TOURSEL, T. & **PICQUET, F**. (2000). Effect of space-flight on single fiber function of triceps and biceps muscles in Rhesus monkeys. *J. Gravit. Physiol., 7, 51-52.*

A4. LIBERSA, P., ROZE, D., LIBERSA, J.C., COURTAND, G. & **PICQUET, F**. (2003). Preliminary results and evidence of early regeneration in inferior alveolar nerve fibers. *Surg. Radiol. Anat. 24(6), 354-357.*

D. Communications avec résumés

C1. MOUNIER, Y., STEVENS, L., CORDONNIER, C. & **PICQUET, F**. (1993). Differential adaptation of rat skeletal muscles to weightlessness. *Symposium "Animals in Space", Bordeaux, 15-17 mars.*

C2. **PICQUET, F**., CORDONNIER, C., STEVENS, L. & MOUNIER, Y. (1993). Structure-function relationship of different muscle fiber types. *International Union of Physiological Sciences, Glasgow, Août. Proceedings du congrès, 3, p 67.*

C3. MOUNIER, Y., CORDONNIER, C., **PICQUET, F**. & STEVENS, L. (1993). Relationship between contractile and structural properties of monkey soleus fibers. *Congrès de l'Association des Physiologistes de langue française, Bordeaux. Arch. Int. Physiol. Bioch. Bioph., 104, A 101.*

C4. MOUNIER, Y., CORDONNIER, C., HELLEC, S., MONTEL, V., **PICQUET, F**. & STEVENS, L. (1993). Evolution of the structure-function relationship of the contractile proteins of monkey triceps muscle in weightlessness conditions (Biocosmos 2229). *International Biocosmos Symposium, Moscou, 15-18 décembre.*

C5. CORDONNIER, C., STEVENS, L., **PICQUET, F**. & MOUNIER, Y. (1993). Analysis of structure-function relationship on soleus atrophied fibers. *European Conference on Muscle Contraction and Cell Motility, Bern. J. Muscle Res. Cell Motility, 15, p. 177, 1994.*

C6. STEVENS, L., **PICQUET F**. & MOUNIER Y. (1994). Effect of six day immobilization on the structure-function relationship in the soleus of new-born rats. *European Muscle Congress, Bochum, 11-14 septembre.*

C7. STEVENS, L., **PICQUET, F**. & MOUNIER, Y. (1995). Evolution de la relation structure-fonction des protéines contractiles de soleus de rat au cours du développement postnatal. *Réunion d'Azay-le-Ferron, (Physiologie Musculaire), 18-19 mai.*

C8. **PICQUET, F**., STEVENS, L. & MOUNIER, Y. (1995). Evolution des propriétés contractiles du soleus de rat à différents stades du développement postnatal. *Congrès de la Société de Physiologie, Strasbourg, 20-23 décembre. Arch. Physiol. & Biochem., 103, D9.*

C9. **PICQUET, F**., STEVENS L. & MOUNIER, Y. (1996). Effects of a six day immobilisation on functional characteristics and MHC composition in new bom rat soleus muscles. *European Muscle Congress, Montpellier, 14-17 septembre. J. Muscle Res. Cell Motility, 18, p. 204, 1997.*

C10. **PICQUET, F.**, STEVENS, L. & MOUNIER, Y. (1996). Contractile properties and MHC composition on rat skinned fibre bundles during postnatal development in relation with the innervation state. *European Muscle Congress, Montpellier, 14-17 septembre. J. Muscle Res.Cell Motility, 18, p. 205, 1997.*

C11. **PICQUET, F**., STEVENS, L. & MOUNIER, Y. (1997). MHC composition and contractile properties in normal and immobilized soleus muscles during postnatal development, in relation with the innervation maturation. *Société Française de Biologie du Développement. Colloque : Développement & Evolution, Dourdan, 29-30 mai.*

C12. MOUNIER, Y., STEVENS, L., SHENKMAN, B., KISCHEL, P., LENFANT, A.M., MONTEL, V., CATINOT, M.P., TOURSEL, T. & **PICQUET, F**. (1998). Effect of space flight on single fiber function of triceps and biceps muscles from Rhesus monkeys. *International symposium on Bion 11 space flight results, Moscou, 24-25 juin.*

C13. CANU, M.H., LANGLET, C., **PICQUET, F**. & FALEMPIN, M. (1999). Plasticité du cortex somatosensoriel du rat après une période d'hypodynamie-hypokinésie : étude électrophysiologique. *4ème Colloque de la Société des Neurosciences, Marseille, 26-28 mai.*

C14. LANGLET, C., CANU, M.H., **PICQUET, F**. & FALEMPIN, M. (1999). Short term plasticity in primary somatosensory cortex of the rat after hindlimb suspension. *20th Annual International Gravitational Physiology Meeting, Orlando (USA), 6-11 juin.*

C15. **PICQUET, F**., LANGLET, C., CANU, M.H. & FALEMPIN, M. (1999). Alteration of motor unit composition in the rat soleus muscle after 14 days of hindlimb unloading. *20th Annual International Gravitational Physiology Meeting, Orlando (USA), 6-11 juin.*

C16. **PICQUET, F**., CANU, M.H., DE-DONCKER, L. & FALEMPIN, M. (2000). Réorganisation phénotypique des unités motrices du muscle soleus de rat après 14 jours d'hypodynamie-hypokinésie. *Congrès International de Myologie, Nice, 27-31 mars.*

C17. DE-DONCKER, L., **PICQUET, F**., CANU, M.H. & FALEMPIN, M. (2000). Effets de la stimulation des récepteurs cutanés sur l'atrophie du soleus de rat pendant 14 jours d'hypodynamie-hypokinésie. *Congrès International de Myologie, Nice, 27-31 mars.*

C18. DE-DONCKER, L., **PICQUET, F**. & FALEMPIN, M. (2000). Functional effects of plantar stimulation on the atrophied soleus muscle of rat during unloading. *13th I.A.A. "Humans in Space Symposium", Santorini (Grèce), 20-26 mai.*

C19. DE-DONCKER, L., **PICQUET, F.** & FALEMPIN, M. (2000). Sole plantar stimulation : a countermeasure for rat soleus atrophy observed during unloading. *BAM' 2000 - Basics & Applications of Muscle plasticity, Abano Terme (Padova - Italy), 11-13 juin.*

C20. BOUET, V., **PICQUET, F**., STEVENS, L., CANU, M.H., FALEMPIN, M. & LACOUR, M. (2000). Modifications des propriétés des muscles extenseurs de la cheville chez le rat conçu, né et élevé en hypergravité. *17ème réunion du Club Locomotion, Villeneuve d'Ascq, 5-6 octobre.*

C21. DE-DONCKER, L., **PICQUET, F**. & FALEMPIN, M. (2000). Stimulation de la sole plantaire : une contre-mesure pour l'atrophie du muscle soleus de rat placé 14 jours en hypodynamie-hypokinésie. *17ème réunion du Club Locomotion, Villeneuve d'Ascq, 5-6 octobre.*

C22. DE-DONCKER, L., **PICQUET, F**. & FALEMPIN, M. (2000). Effets fonctionnels de la stimulation plantaire sur l'atrophie du muscle soleus de rat placé 14 jours en hypodynamie-hypokinésie. *Congrès LARC, Lille, 20 octobre.*

C23. **PICQUET, F**., DE-DONCKER, L. & FALEMPIN, M. (2001). Are muscular modifications induced by hindlimb unloading counteracted by clenbuterol administration ? *22nd Annual International Gravit. Physiol. Meeting, Budapest (Hongrie), 22-27 avril.*

C24. DE-DONCKER, L., **PICQUET, F**., PETIT, J. & FALEMPIN, M. (2001). Etude, après l'application d'un étirement en rampe, de la décharge des terminaisons primaires et secondaires issues du fuseau neuromusculaire du muscle soleus de rats contrôles et de rats placés 14 jours en hypodynamie-hypokinésie. *5ème Colloque de la Société des Neurosciences, Toulouse, 28-31 mai.*

C25. DE-DONCKER, L., **PICQUET, F**., PETIT, J. & FALEMPIN, M. (2001). Caractéristiques de décharge des terminaisons primaires et secondaires issues des fuseaux neuromusculaires du muscle soleus de rats contrôles et de rats après une exposition de 14 jours en hypodynamie-hypokinésie. *4ème Colloque du Club du Motoneurone, Batz sur Mer, 19-22 septembre.*

C26. CANU, M.H., STEVENS, L., **PICQUET, F**. & FALEMPIN, M. (2001). Effet du clenbutérol sur l'activité locomotrice de rats soumis à 14 jours d'hypodynamie-hypokinésie. *18ème réunion du Club Locomotion, Marseille, 4-5 octobre.*

C27. FALEMPIN, M., DE-DONCKER, L. & **PICQUET, F**. (2002). Hindlimb unloading induces changes in the muscular afferent discharges in rat soleus muscle spindles. *12th Conference on Space Biology and Aerospace Medicine, Moscou, 10-14 juin.*

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PRESENTATION SYNTHETIQUE

DE MES TRAVAUX DE RECHERCHE

L'ensemble de mes travaux de recherche porte sur la plasticité neuromusculaire lors de situations de non-utilisation (immobilisation, hypodynamie-hypokinésie….).

En septembre 1991, j'intègre le Laboratoire de Plasticité Neuromusculaire dirigé par le Pr Y. Mounier dans le cadre de la préparation d'un Diplôme d'Etudes Approfondies (DEA). La thématique générale du Laboratoire consiste à déterminer au cours d'une atrophie fonctionnelle, la part des facteurs d'origine myogène ou neurogène dans la transformation d'un muscle lent des membres postérieurs (tel que le soleus) vers un muscle exprimant un phénotype plus rapide. Les travaux relatifs à ce thème de recherche sont menés simultanément par les 2 équipes présentes dans le Laboratoire de Plasticité Neuromusculaire. L'une de ces équipes dirigée par le Pr Y. Mounier s'intéresse à la "plasticité des structures cellulaires impliquées dans la contraction musculaire lors d'atrophies fonctionnelles". L'autre équipe dirigée par le Pr M. Falempin étudie plus particulièrement la "contraction musculaire et son contrôle sensori-moteur lors de dysfonctionnements".

Au cours du stage de DEA, les travaux entrepris portaient sur l'étude des fibres du muscle soleus de singe. Ils ont été réalisés sur des fibres musculaires isolées et pelées chimiquement à l'EGTA. **J'ai ainsi établi que les propriétés fonctionnelles (sensibilité et affinité relatives aux ions calcium et strontium) varient suivant les types de fibres musculaires et qu'elles sont associées à des caractéristiques structurales bien définies et notamment à des combinaisons spécifiques d'isoformes de protéines contractiles, motrices et régulatrices. Ces travaux ont abouti d'une part à la présentation de mon mémoire de DEA en Juin 1992 (mention AB) et d'autre part, à une publication (P1) et 3 communications.**

En 1992, grâce à l'obtention d'une allocation de recherche MRT, j'ai pu entreprendre une thèse d'Université dans la même équipe. Il s'agissait d'étudier la plasticité des fibres musculaires de rat nouveau né en fonction de l'état d'innervation (poly- ou mono-synaptique). Ma thématique de recherche s'inscrit alors plus précisément sur l'étude des protéines contractiles et leur capacité d'adaptation au cours du développement post-natal en conditions normales ou perturbées par une immobilisation. Il s'agissait de voir si l'induction d'une période d'immobilisation des membres postérieurs à différents stades du développement post-natal (caractérisés par des états d'innervation différents) peut moduler ou modifier l'installation des différentes formes de protéines contractiles musculaires. Celles-ci seraient ensuite à l'origine de propriétés fonctionnelles nouvelles. L'immobilisation d'animaux jeunes

constitue un modèle expérimental idéal qui permet d'apprécier les capacités d'adaptabilité ou de transformation du muscle face à une situation nouvelle.

Pour juger des effets de l'installation de l'innervation monosynaptique définitive, nous avons comparé les caractéristiques du muscle soleus de rats nouveau-nés normaux à celles de muscles soleus de rats nouveau-nés immobilisés. Deux périodes déterminantes du développement post-natal ont été choisies : avant (6 jours post-natals) et après (17 jours post-natals) l'installation de l'innervation définitive monosynaptique. Les résultats obtenus montrent que l'immobilisation en flexion plantaire du soleus induit, quel que soit le stade développemental, une atrophie musculaire significative d'environ 50 %. Cependant, en fonction de l'état d'innervation, les résultats diffèrent. En effet, l'immobilisation du soleus avant l'apparition de l'innervation définitive (6ème jour post-natal), ralentit son évolution vers un type lent, et le maintient en un état indifférencié. Au contraire, l'immobilisation du muscle soleus à un stade où l'innervation est monosynaptique entraine la transformation de celui-ci vers un muscle de type rapide. **La part de l'innervation dans la plasticité musculaire est prépondérante puisqu'elle est à même d'influer sur le comportement du muscle face à une situation nouvelle.**

Ces travaux ont fait l'objet de ma Thèse d'Université soutenue en décembre 1996 (Mention Très Honorable et Félicitations du jury) et ont donné matière à 3 publications (P2, P3, P4) et à 6 communications.

Après la soutenance de ma thèse, mes activités de recherche se sont orientées vers l'étude de la plasticité neuromusculaire en relation avec la nature du message nerveux sensori-moteur lors de conditions expérimentales particulières (microgravité, déafférentation, hypergravité…).

En septembre 1998, j'ai été nommée Maître de Conférences à l'USTL au Laboratoire de Plasticité Neuromusculaire dans l'équipe du Pr M. Falempin. La problématique de recherche qui m'a été confiée était la suivante : étudier les capacités d'adaptation du système neuromusculaire face à une situation de non-utilisation des membres postérieurs. L'examen des données de la littérature montre que dans ces conditions se développe rapidement une atrophie musculaire significative ainsi qu'une transition phénotypique des fibres musculaires. Les altérations les plus importantes ont été mises en évidence dans les muscles lents à vocation posturale, fonction que ces muscles perdent lors d'une situation de non-utilisation (microgravité, immobilisation, ténotomie….). Parmi ceux-ci, le muscle soleus, muscle

extenseur de la cheville, présente les transformations les plus significatives. Ainsi il a été démontré qu'il développait une remarquable plasticité lors d'une variation environnementale. En plus de la mise en place précoce d'une atrophie, ce muscle normalement lent montre une évolution vers un type rapide (transition lent → rapide des protéines contractiles, acquisition d'un comportement contractile rapide).

Cependant, de nombreux points restaient à éclaircir :

- ***lors d'un épisode d'hypodynamie-hypokinésie***

1. Que devenait l'unité motrice, véritable entité contractile musculaire ?
2. Les propriocepteurs (fuseau neuromusculaire et organe tendineux de Golgi) étaient-ils capables de plasticité ou au contraire étaient-ils des structures figées ?
3. Quelle était la part exacte du message afférent dans la transformation du muscle soleus ?

- ***à l'inverse en situation d'hypergravité***

4. Que devenaient le muscle soleus et ses propriocepteurs lors d'une sur-utilisation ?

Pour répondre à ces différents points, nous avons eu recours à plusieurs modèles expérimentaux mis au point chez l'animal :

1. Un modèle d'hypodynamie-hypokinésie (HH) dit de microgravité simulée qui permet de reproduire les effets de la microgravité réelle. Ce modèle mis point en raison de la rareté et du coût des missions spatiales permet d'induire une absence de charge corporelle et une réduction des mouvements musculaires, conséquences qui sont observées lors d'un séjour en micropesanteur ou lors d'immobilisation fonctionnelles. L'avantage majeur de ce modèle est de préserver l'intégrité du système neuromusculaire.

2. Un modèle de déafférentation périphérique qui permet une section sélective des racines dorsales en provenance du muscle soleus. Il devient possible de déterminer sur le plan musculaire l'impact exact d'une suppression totale des

messages afférents par comparaison avec les données obtenues chez les animaux HH.

3. Un modèle d'hypergravité obtenu par centrifugation chronique qui permet d'augmenter l'effet de charge corporelle et qui constitue une situation en miroir de la microgravité. La comparaison des données obtenues avec ce modèle et celles consécutives à l'HH permet de vérifier s'il existe une continuité des transformations musculaires en fonction du facteur gravité.

Ces travaux ont jusqu'à présent donné lieu à 28 publications de niveau international et 50 communications présentées dans des congrès nationaux (n = 25) et internationaux (n = 25). Une partie de ces travaux sera présentée plus en détails dans la suite de ce mémoire.

POSITION DU PROBLEME

RAPPELS BIBLIOGRAPHIQUES

Depuis le premier vol spatial du cosmonaute russe Youri Gagarine en 1961, les hommes n'ont cessé d'imaginer des moyens de plus en plus innovants et perfectionnés pour accéder et évoluer durablement dans l'espace. Ainsi, la station spatiale internationale (ISS) dont la durée de vie est estimée au minimum à une dizaine d'années représente l'une de ces étapes majeures. Le but final est de permettre à l'homme de vivre et travailler dans l'espace (cf site internet du CNES). De plus, le succès des dernières missions de la NASA vers la planète Mars va probablement relancer la conquête spatiale. En effet, le gouvernement des Etats Unis compte consacrer plus de 12 milliards de dollars à ce projet au cours des cinq prochaines années. Ces perspectives sous-entendent la reprise des vols habités de longue durée. Il faut cependant souligner que l'une des caractéristiques les plus marquantes d'un séjour dans l'espace est sans conteste l'absence quasi-totale de gravité (que l'on appelle encore impesanteur). Or au fil de l'évolution, notre organisme s'est adapté à la présence de la pesanteur terrestre. Les effets de la pesanteur conditionnent toute notre existence, depuis notre développement embryonnaire jusqu'au fonctionnement harmonieux de nos grands systèmes physiologiques : perception de l'espace et du temps, sens de l'orientation, circulation des liquides corporels etc… La mise en impesanteur conduit nécessairement à une altération progressive de certaines fonctions physiologiques. Parmi celles-ci, la fonction neuromusculaire est l'une des plus perturbées. Cependant, le tissu musculaire est aussi l'un des plus plastiques puisqu'il s'adapte de façon remarquable aux changements environnementaux. Les perturbations les plus marquantes sont la mise en place d'une atrophie musculaire associée à des pertes de force et à des changements d'expression des protéines contractiles. Ces manifestations sont préjudiciables au bon déroulement d'une mission spatiale. C'est pourquoi la compréhension des mécanismes de plasticité du système neuromusculaire est plus que jamais d'actualité. En outre, il est également nécessaire de mettre en place des stratégies permettant de contrer ces phénomènes.

Dans ce cadre, la thématique du Laboratoire de Plasticité Neuromusculaire s'inscrit dans l'étude des altérations musculaires lors de situations de non-utilisation des membres. La situation de non-utilisation peut être la conséquence d'une diminution de la gravité comme cela est observé lors d'un vol spatial ou lors d'un alitement prolongé volontaire (expériences de bed rest) ou non (immobilisation par plâtrage, vieillissement, pathologie). Compte tenu de

la rareté et du coût des vols spatiaux ainsi que de la sélection drastique des équipes scientifiques admises à y participer, différents modèles terrestres ont été mis au point pour étudier le développement des altérations musculaires. La plupart des travaux scientifiques que j'ai réalisés ont été obtenu par utilisation du modèle d'hypodynamie-hypokinésie (HH) mis au point sur le rat. L'hypodynamie consiste en une absence de charge corporelle et l'hypokinésie en une réduction des mouvements. Dans ce modèle, la fonction de support antigravitaire de la musculature est réduite par non-utilisation des membres postérieurs. Ce modèle, encore appelé modèle du rat suspendu est largement utilisé internationalement (Morey-Holton et Globus, 2002) et reproduit les effets de la microgravité réelle (Morey et coll., 1979) sur les systèmes osseux et neuromusculaire. Il permet donc d'étudier au sol les conséquences physiologiques des vols spatiaux. Ce modèle peut aussi servir à tester des moyens de contre-mesure de l'atrophie musculaire (vibration tendineuse, électrostimulation, etc…). En faisant varier la durée de la suspension, il est également possible de graduer le degré d'atrophie musculaire et de contrôler dans une certaine mesure l'ampleur de la transformation phénotypique musculaire. En effet, le modèle d'HH est à l'origine de transition des isoformes de myosine dans le sens lent → rapide avec apparition d'isoformes habituellement non exprimées dans les muscles lents normaux et dont il est ainsi possible d'étudier la régulation génique.

La thématique de recherche de l'équipe « plasticité sensori-motrice » du Laboratoire est d'étudier l'impact des messages nerveux moteur et sensitif sur les propriétés contractiles musculaires. En effet, le message nerveux est essentiel au déterminisme des propriétés contractiles musculaires et à la mise en place d'un phénotype musculaire stable (Kugelberg, 1976). Le modèle d'HH utilisé au Laboratoire offre l'avantage de préserver l'intégrité du système neuromusculaire. Il permet également l'étude des caractéristiques des messages nerveux afférent et efférent, avant, pendant et après l'HH. Dans la littérature, il a été émis l'hypothèse que certaines des altérations musculaires résulteraient d'une modification ou d'une absence du message nerveux sensitif (Riley et coll., 1990). Dans le cadre de mes travaux et pour répondre à cette problématique, j'ai donc été amenée à mettre au point un modèle de déafférentation périphérique (section des racines dorsales de la moelle épinière) afin de déterminer la part de l'innervation afférente dans le développement de l'atrophie musculaire et des transformations phénotypiques qui y sont associées.

Notre dernier objectif est de déterminer si l'hypergravité pouvait induire des modifications « en miroir » vis-à-vis de la microgravité et s'il existait ou non une continuité de modifications selon le degré de gravité : hypogravité ⇔ normogravité ⇔ hypergravité. Le

modèle utilisé est celui de l'hypergravité obtenu par centrifugation chronique. Le niveau d'hypergravité a été fixé à 2G qui est perçu comme 2 fois la charge corporelle. Ce modèle permet la réalisation d'études de longue durée à la fois sur des animaux adultes et sur des animaux extrêmement jeunes.

I – DONNEES OBTENUES EN HYPODYNAMIE-HYPOKINESIE

Notre but n'est pas de faire ici une revue exhaustive des résultats obtenus (ce qui a déjà donné matière à de nombreuses revues de questions) mais de rappeler brièvement l'essentiel des données expérimentales établies à partir du modèle d'HH.

A. Atrophie corporelle et musculaire, Pertes de forces

Une des adaptations les plus marquantes à l'HH est le développement d'une atrophie musculaire (Edgerton et Roy, 1996). Cette atrophie est consécutive à une diminution de 46% de la synthèse protéique couplée à une augmentation de la dégradation des protéines (Thomason et Booth, 1990 ; Booth et Kirby, 1992). De plus, l'HH s'accompagne d'une suractivation de facteurs tels que la myostatine qui régulent négativement la masse musculaire (Carlson et coll., 1999). Cette suractivation serait un des évènements déclencheurs de la plasticité musculaire : atrophie et transitions phénotypiques (Wittwer et coll., 2002). L'atrophie musculaire apparaît comme un marqueur précoce de la transformation musculaire puisqu'elle peut être détectée chez le muscle soleus dès 4 jours d'HH (Stevens et coll., 1999 b). Elle atteint 50 % à 14 jours d'HH (Falempin et coll., 1990) et 63 % après 5 semaines d'HH (Morey et coll., 1979). Des études de cinétique ont démontré que la majeure partie des adaptations physiologiques du muscle soleus observées à 28 jours comme le degré d'atrophie musculaire sont similaires à celles constatées après 206 jours d'HH (Elder et McComas, 1987). Selon ces auteurs, ces données suggèrent que 28 jours sont suffisants pour que le muscle soleus présente un nouvel état physiologique stable. En HH, la mise en place de l'atrophie musculaire s'accompagne d'une diminution de la taille de tous les types de fibres

musculaires (Ohira, 2000). Cependant, il n'a pas été décrit de perte de fibres (Templeton et coll., 1988). Cependant, une hypothèse récente suggère qu'une absence de gravité en conditions réelle ou simulée de longue durée pourrait compromettre la survie motoneuronale et par là même induire une dégénérescence musculaire sévère (Whang, 1999). Enfin, il a également été proposé que la microgravité pourrait être assimilée à un modèle de vieillissement puisque dans ces 2 situations, des changements similaires tels que des pertes de force et de masse musculaires ont été décrits (Biolo et coll., 2003).

En association avec le développement de l'atrophie musculaire, l'HH induit l'apparition d'une perte de force musculaire plus importante pour les muscles de type lent. Ces pertes de force ont été mises en évidence sur muscle entier (Winiarski et coll., 1987 ; Falempin et coll., 1990 ; Edgerton et Roy, 1996 ; Falempin et In-Albon, 1999 ; De-Doncker et coll., 2000) mais aussi sur fibres isolées et pelées (Gardetto et coll., 1989 ; Stevens et coll., 1990 ; Toursel et coll., 1999). Si la perte de force reste significative lorsqu'elle est normalisée à la section transversale du muscle ou des fibres, celle-ci s'accompagne d'une perte de protéines contractiles corrélée à une augmentation du tissu non contractile et du volume interstitiel (Ohira, 2000).

B. Evolution des cinétiques de contraction

Les conditions d'HH ont également des effets sur les propriétés mécaniques musculaires et plus particulièrement sur les paramètres cinétiques. Ces paramètres (Winiarski et coll., 1987) sont classiquement mesurés sur une secousse simple obtenue après une stimulation isolée d'intensité maximale. Ce sont le temps de contraction (TC, exprimé en msec) et le temps de demi-relaxation (HRT pour Half Relaxation Time, exprimé en msec). Le moyen d'obtention de ces paramètres est illustré par la figure 1. Un troisième indicateur de cinétique (P_x/P_0 : rapport de la tension tétanique développée lors d'une stimulation à X Hz rapportée à la tension tétanique maximale développée pour une stimulation à 100 Hz) est utilisé pour différencier les muscles de type lent des muscles de type rapide. Pour les muscles lents il s'agit du rapport P_{20}/P_0 et pour les muscles rapides, du rapport P_{40}/P_0. En fait, d'un point de vue physiologique, les rapports P_{20}/P_0 et P_{40}/P_0 correspondent pour chaque type musculaire au rapport des tétanos non fusionnés sur la réponse tétanique maximale fusionnée. Classiquement, il a été reporté une diminution des valeurs des critères cinétiques du muscle soleus après 2 semaines d'HH. Ceci reflète l'existence d'une transition d'un phénotype lent

vers un phénotype rapide (Winiarski et coll., 1987 ; Falempin et coll., 1990 ; Thomason et Booth, 1990). Ainsi, Falempin et In-Albon (1999) et De-Doncker et coll. (2000) décrivent une diminution de ces paramètres de l'ordre de 30%. Les valeurs des paramètres cinétiques sont également modifiées en ce qui concerne les unités motrices du muscle soleus. Les unités motrices lentes montrent une valeur de HRT augmentée de 20% après HH alors que les unités rapides présentent une diminution de TC de l'ordre de 20% et de HRT de 15% (Leterme et Falempin, 1996). Ces auteurs ont également mis en évidence, sur la base des critères cinétiques, une nouvelle population d'unités motrices intermédiaires dans le muscle soleus des rats HH.

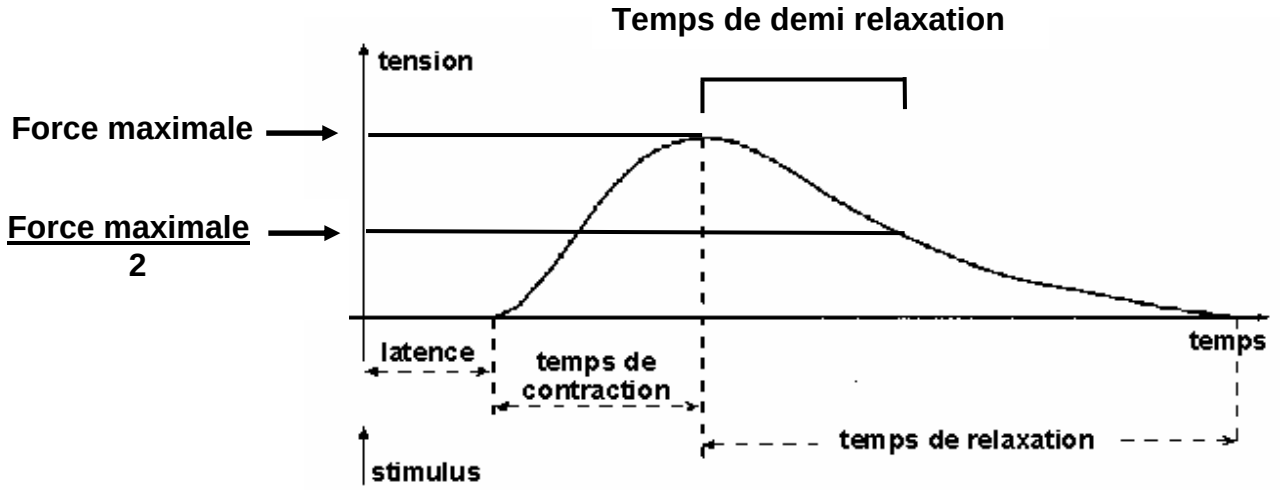


Figure 1 : Méthode de mesure des paramètres force et de cinétique (temps de contraction et temps de demi-relaxation) à partir de l'enregistrement d'une secousse musculaire simple ou twitch

C. Modifications phénotypiques

Les muscles squelettiques sont composés de fibres musculaires, éléments polynucléés qui comportent essentiellement des protéines contractiles. Le reste du volume fibrillaire est occupé par le réticulum sarcoplasmique qui est en relation avec la membrane plasmique ou sarcolemme. Les protéines contractiles sont organisées en deux filaments : un filament fin composé d'actine à laquelle s'ajoutent la tropomyosine et le complexe des troponines et un filament épais composé de myosine. La myosine comporte deux chaînes lourdes (ou MHC pour myosin heavy chain) qui portent l'activité ATPasique et quatre chaînes légères ou MLC (pour myosin light chain). L'organisation musculaire est illustrée par la figure 2. Les fibres musculaires ont été classées en fonction de leur contenu en isoformes de MHC. Ainsi les fibres lentes appelées fibres de type I expriment l'isoforme lente de la MHC, les fibres rapides de type II, les isoformes IIA, IIB, ou IIX (Pette et Staron 1990, 1997). Cependant, il existe également des fibres hybrides coexprimant plusieurs isoformes de MHC. La transition des isoformes de MHC se fait selon un schéma privilégié dit de « next-neighbor rule » qui est le suivant : MHC I ⇔ MHC IIA ⇔ MHC IIX ⇔ MHC IIB. La MHC I (ou MHC Iβ) est l'isoforme lente et la MHC IIB, à l'inverse, est l'isoforme la plus rapide (Pette et Staron, 1997). De façon classique, la proportion de fibres hybrides est considérée comme un bon indicateur de l'état de transformation d'un muscle (Fauteck et Kandarian, 1995 ; Allen et coll., 1996 ; Talmadge et coll., 1996). L'HH induit une diminution progressive de la proportion en fibres lentes de type I dans le muscle soleus (Templeton et coll., 1984 ; Martin et coll., 1988 ; Ohira et coll., 1992 ; Stevens et coll., 1999 b ; Ohira, 2000). Par contre les muscles rapides tels que la partie médiane du gastrocnémius et le tibialis anterior ne sont pas modifiés (Roy et coll., 1987 ; Graham et coll., 1989 ; Jiang et coll., 1992 ; Ohira, 2000). La perte de fibres de type I dans le muscle soleus est concomittante avec une émergence des fibres de type rapide exprimant la MHC IIA (Stevens et coll., 1999 b; Ohira, 2000). De plus, des isoformes habituellement non exprimées telles que la MHC IIX et la MHC IIB apparaissent après 2 semaines d'HH. Leur proportion augmente ensuite graduellement jusqu'à 28 jours d'HH (Fauteck et Kandarian, 1995 ; Caiozzo et coll., 1998 ; Stevens et coll., 1999 b). Enfin, il faut également citer que l'expression de l'isoforme MHC Iα, habituellement décrite dans les fuseaux neuromusculaires (Pedrosa-Domellof et coll., 1991 ; Soukup et al., 1995), est renforcée aussi bien dans le muscle soleus que dans le muscle gastrocnémius (Stevens et coll., 2000 b).

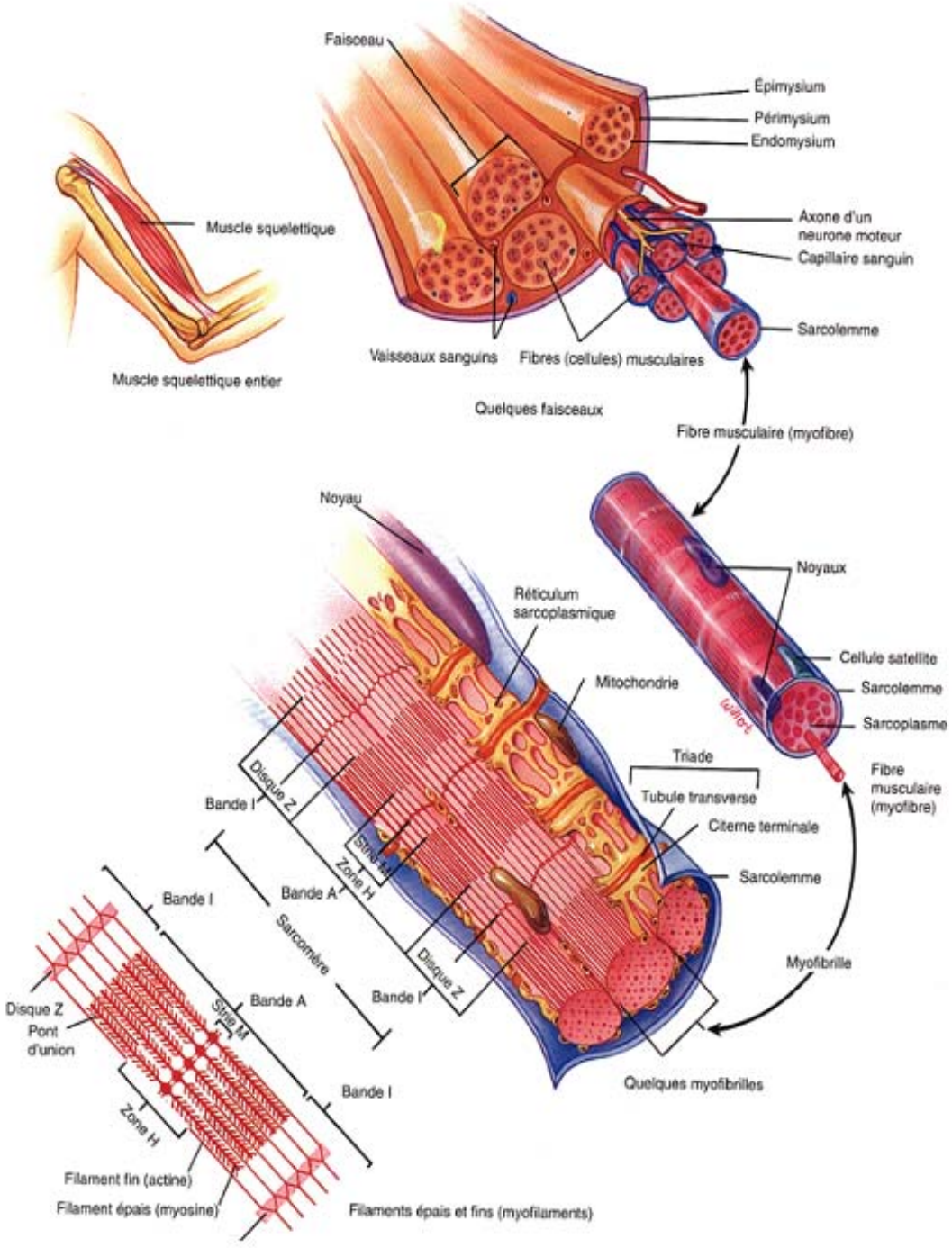


Figure 2 : Organisation générale du muscle strié squelettique, d'après Tortora et Grabowski, 2002.

Cette surexpression de la MHC Iα correspondrait à une étape intermédiaire dans la transition MHC Iβ → MHC IIA.

Un séjour en HH entraîne également l'apparition de nombreuses populations de fibres hybrides, c'est-à-dire exprimant plus de 2 isoformes de myosine (Fauteck et Kandarian, 1995, Talmadge et coll., 1996 ; Stevens et coll., 1999 a ; Ohira, 2000). Ainsi sur fibres isolées, Stevens et coll. (1999 a) ont démontré une transition des fibres hybrides : diminution des fibres hybrides MHC I + MHC IIA au profit de l'apparition de fibres hybrides MHC I + MHC IIA + MHC IIX et MHC I + MHC IIA + MHC IIX + MHC IIB. Ces deux dernières populations représentent chacune respectivement 23% et 13% des fibres du muscle soleus après 7 jours d'HH. Ces transitions se retrouvent également au niveau des ARNm puisqu'une augmentation à la fois des trancripts et des isoformes de MHC rapides a été décrite sur le muscle soleus après 28 jours d'HH (Stevens et coll., 1999 a,b). Cependant en ce qui concerne la MHC I, la diminution de la protéine n'est pas corrélée à une diminution du transcript ce qui suggère d'après les auteurs l'existence d'une dégradation protéique accrue de cette isoforme (Stevens et coll., 1999 b).

Les transitions des isoformes de MHC dans le sens lent → rapide s'accompagnent également d'une transition lent → rapide pour les isoformes de chaînes légères de myosine ou MLC, (Stevens et coll., 2000 a, 2004). Ainsi une fibre isolée hybride en termes de MHC peut également l'être en termes de MLC. La transformation phénotypique vers un type plus rapide a été aussi mise en évidence sur les protéines régulatrices du muscle soleus : les troponines C, T et I (Kischel et coll., 2001 ; Bastide et coll., 2002 ; Stevens et coll., 2002 ; Kischel et coll., 2005).

En conclusion, les protéines contractiles sont toutes sensibles, mais à des degrés divers, aux effets de l'HH.

D. Activité électromyographique (EMG) au cours de la suspension

L'EMG est la mesure de l'activité électrique globale du muscle. L'examen de la littérature révèle des discordances en ce qui concerne le décours et le niveau d'activité électromyographique (EMG) au cours d'un épisode d'HH. Ainsi, pour les muscle soleus, plantaris et gastrocnémius, l'activité EMG diminue dès la mise en suspension des rats (Alford et coll., 1987 ; Ohira M et coll., 2002 ; Ohira Y et coll., 2002 ; Kawano et coll., 2004 ; De-Doncker et coll., 2005). Durant la première journée d'HH, l'activité EMG reste inférieure à

celle des animaux contrôles. Pour Blewett et Elder (1993), elle est diminuée de 57% en ce qui concerne le soleus et de 66% en ce qui concerne le plantaris. Pour Ohira M et coll. (2002) et Ohira Y et coll. (2002), la réduction d'activité atteint 75% pour les muscles soleus et plantaris, par contre l'activité EMG du muscle tibialis anterior est augmentée. L'activité EMG reste inférieure d'environ 60% au niveau contrôle pendant la première semaine d'HH. Ensuite, elle augmente graduellement et à partir du 9ème jour, présente des valeurs identiques au niveau pré-HH (Ohira M et coll., 2002 ; De-Doncker et coll., 2005) puis devient supérieure à celle d'un muscle contrôle (De-Doncker et coll., 2005). Par contre, pour Kawano et coll. (2004), le niveau d'activité pré-HH (donc contrôle) n'est atteint qu'au bout de 14 jours de suspension. Enfin, pour Blewett et Elder (1993), jusqu'à 28 jours d'HH, les soleus des rats HH ne retrouvent pas l'activité EMG identique à celle des animaux contrôles. La nature de l'EMG est également modifiée par l'HH puisque pour le muscle soleus, celui-ci passe d'une activité tonique chez l'animal contrôle à une activité phasique. Cette activité phasique fait place à nouveau à une activité tonique dès la fin de la mise en HH (De-Doncker et coll., 2005). Trois jours plus tard, l'activité tonique est redevenue strictement identique à celle des animaux contrôles. Lorsque la suspension est prolongée jusqu'à neuf semaines, l'activité EMG des muscles soleus, plantaris et gastrocnemius est réduite par rapport à celle des animaux contrôles et ce, à partir de la 7ème semaine (Ohira Y. et coll., 2002). Au cours de la suspension, l'activité EMG du tibialis anterior est augmentée par rapport au niveau contrôle pendant toute la durée de l'étude, soit 9 semaines de suspension (Ohira Y. et coll., 2002).

Quoi qu'il en soit, malgré une disparité quantitative de certains résultats, il apparaît que l'EMG est modifié suite à l'HH.

E. Activité EMG après la suspension

L'évolution de l'activité EMG a également été étudiée ***en situation de repos*** dès la remise en condition terrestre des rats après 9 semaines de suspension (Ohira Y. et coll., 2002). Ainsi, par comparaison avec les muscles contrôles, l'activité EMG des muscles soleus et plantaris est significativement diminuée alors que celle du muscle gastrocnemius est normale. Par contre, l'activité du muscle tibialis anterior est augmentée lorsque les rats retrouvent une condition terrestre. De plus, les rats sont incapables de soutenir une position normale et présentent des variations fréquentes de leur posture. Cette incapacité à soutenir une position de type pronateur a été attribuée à l'hyperextension, au cours de la suspension, des

articulations du genou et de la cheville des pattes postérieures. Dès que les animaux présentent un changement de position, des variations rapides de l'activité EMG peuvent être détectées : le muscle soleus devient actif sur de courtes périodes (5 - 10 secondes) alors que parallèlement, les autres muscles ne présentent pas de variation significative de leur activité EMG. Dans un deuxième temps, le muscle tibialis anterior devient actif à son tour tandis que l'activité EMG du soleus décroît. Ensuite, les animaux changent à nouveau de position. Au bout d'une semaine de retour en conditions terrestres, l'activité EMG devient qualitativement et quantitativement équivalente à celle d'un animal contrôle.

Des mesures de l'activité EMG ont été faites ***après la suspension lors d'épisodes locomoteurs*** (marche sur tapis roulant). Il a été démontré une réduction de l'EMG des muscles fléchisseurs plantaires (soleus, plantaris et gastrocnémius latéral) dès le retour en conditions terrestres, leur activité restant synchrone (Ohira Y et coll., 2002). Seul le tibialis anterior présente une activité renforcée. Chez certains animaux, des signes de co-activation entre fléchisseurs plantaires et dorsaux peuvent être détectés, ceci n'ayant jamais été rapporté chez le rat contrôle. Pour Langlet et coll., (2000), si l'activité EMG du muscle soleus et de la partie rouge du muscle gastrocnémius est bien diminuée après 14 jours d'HH, par contre celle de la partie blanche du muscle gastrocnémius est augmentée. Pour ces auteurs, ces résultats indiquent que la partie blanche du gastrocnémius serait davantage activée lors d'un effort musculaire après suspension.

Pendant la locomotion sur tapis roulant, à faible vitesse les cycles locomoteurs du muscle soleus de rats ayant été 7 et 14 jours en HH sont augmentés. De plus, ces animaux montrent de nombreuses hyperextensions de la cheville (Canu et Falempin, 1996, 1998). Une étude de la locomotion fictive de rats HH montre également une hyperactivité du neurogramme des nerfs innervant les muscles extenseurs des membres postérieurs (Canu et coll., 2001). Ces résultats seraient la conséquence de modifications des propriétés intrinsèques des motoneurones.

Une synthèse des principaux résultats relatifs aux mesures d'EMG pendant et après la suspension est illustrée par la Figure 3.

EMG du muscle soleus de rat en condition TERRESTRE

EMG Tonique →

EMG du muscle soleus de rat en conditions d'HYPODYNAMIE-HYPOKINESIE

J 0 → Silence électrique

J 1 → J 7 → EMG Phasique < EMG contrôle

Au delà de J 7 →

- **J 28** : EMG < EMG CONTROLE (Blewett et Elder, 1993)
- **J 9** : EMG > EMG CONTROLE (De-Doncker et coll., 2005)
- **J 14** : EMG > EMG CONTROLE (Kawano et coll., 2004)

Figure 3 : Synthèse des principaux résultats obtenus lors de l'étude de l'EMG en conditions terrestre et d'HH

F. Messages nerveux afférent/efférent

L'évolution des neurogrammes moteur et sensitif a également été étudiée au cours de l'HH. Kawano et coll. (2004) ont étudié le niveau d'activité du neurogramme efférent et afférent sur les racines L5 ventrale et dorsale. Les résultats montrent une diminution de ceux-ci suite à la mise en HH respectivement de 37 % et 20 %.

La diminution du neurogramme efférent persiste jusqu'au 8ème jour. Il augmente ensuite pour atteindre 130 % de la valeur pré-HH. L'activité EMG du muscle soleus ne montre pas une telle augmentation ce qui indique que le neurogramme efférent ne reflète pas que les variations d'EMG du muscle soleus. En effet par utilisation d'un marqueur fluorescent injecté au niveau de la plaque motrice et transporté de façon rétrograde, il a été démontré que le niveau spinal L5 contenait également les corps cellulaires des motoneurones innervant les muscles gastrocnémius, plantaris et EDL (Kawano et coll., 2004). Ainsi, l'EMG du gastrocnémius ne change pas lors de l'HH alors que celui du tibialis anterior est légèrement augmenté (Kawano et coll., 2004). Le neurogramme efférent est donc le reflet de la décharge de tous les motoneurones dont les corps cellulaires sont localisés en L5 et qui innervent différents muscles des membres postérieurs. Des études récentes ont également démontré après HH une diminution de taille des somas des motoneurones innervant le muscle soleus, ce qui pourrait consister un indicateur de changement de l'excitabilité des motoneurones du muscle soleus (De-Doncker et coll., 2006).

La diminution du message nerveux afférent enregistré sur la racine dorsale L5 persiste pendant les 8 premiers jours d'HH. A partir du 9ème jour, l'activité afférente réaugmente et atteint un niveau supérieur à celui des animaux contrôles (Kawano et coll., 2004 ; De-Doncker et coll., 2005). De plus cette activité montre une transition tonique vers un état plus phasique (De-Doncker et coll., 2005).

II – DONNEES SUR L'HYPERGRAVITE

Les effets de l'hypergravité (HG) sur le système neuromusculaire ont fait l'objet, à ce jour, d'études moins nombreuses et moins développées que celles relatives à l'HH ou à la microgravité réelle. Cependant, elles offrent l'avantage de permettre la réalisation d'études de longue durée. Les données obtenues peuvent différer suivant 3 facteurs : la durée de l'HG, l'espèce ou l'âge des animaux. Les travaux les plus nombreux ont été réalisés sur des périodes de 2-3 semaines d'HG pour une comparaison avec les durées les plus fréquentes des missions spatiales dont la caractéristique majeure est la microgravité.

Des travaux récents ont établi que des rats adultes placés à 2G pendant 2 semaines réalisent plus rapidement des tâches d'apprentissage que ne le font les animaux contrôles (Mitani et coll., 2004). De plus, chez le rat adulte soumis à 14 jours d'HG (2G), il a été démontré des changements de poids de l'animal (diminution) et des masses musculaires (augmentation relative), (Vasques et coll., 1998). Ces données morphologiques sont complétées par des mesures biochimiques démontrant des variations des taux des enzymes du métabolisme (Chi et coll., 1998) et un niveau d'expression des MHC peu modifié sur le rat adulte (Martin et Romond, 1975 ; Roy et coll., 1996).

Lorsque l'HG est imposée à un stade plus jeune, des changements dans les proportions relatives des MHC peuvent être mis en évidence. Ainsi, Martrette et coll. (1998) décrivent sur des rats soumis à l'HG entre le 11ème jour de gestation et le 7ème jour post-natal, une diminution de la myosine embryonnaire associée à une augmentation de la myosine périnatale. Des rats jeunes (30 jours post-natals) soumis à plusieurs semaines d'HG montrent des transitions phénotypiques musculaires (augmentation de la proportion de myosine lente : Martin, 1980). L'importance des modifications observées semble être en relation avec la durée de l'HG.

Enfin, les données les plus pertinentes ont été obtenues lorsque les animaux sont conçus, nés et élevés en HG. Un séjour en HG allonge la durée de la gestation qui passe de 21 à 23 jours et les rats nouveaux-nés sont plus petits (Sajdel-Sulkowska et coll., 2001). Le poids du cerveau ainsi que l'expression des hormones thyroïdiennes sont également réduits (Sajdel-Sulkowska et coll., 2001). En ce qui concerne des jeunes rats élevés en HG, leur comportement moteur est retardé et plus particulièrement les réflexes directement dépendants des entrées vestibulaires (Wubbels et De Jong, 2000 ; Bouet et coll., 2004). Les durées des potentiels d'action des motoneurones, évoqués par stimulation des racines ventrales de la moelle épinière lombaire sont augmentées après HG (Brocard et coll., 2003). Le

développement de l'innervation monoaminergique est retardé à la naissance des rats HG. Au cours du développement postnatal et jusqu'à l'âge adulte (8 mois), l'innervation reste anarchique (Gimenez y Ribotta et coll., 1998), les perturbations les plus importantes ayant été détectées dans la partie ventrale de la moelle épinière. Ainsi à 8 mois le nombre de synapses de la moelle épinière ventrale des rats HG reste inférieur à celui des rats contrôles. Il a également été mis en évidence une diminution du nombre de connections synaptiques dans l'oreille interne et les noyaux cérebelleux des rats HG (Krasnov, 1991). Plus récemment, des données obtenues sur fibres musculaires isolées montrent que chez les rats HG, les protéines régulatrices lentes sont surexprimées dans le muscle soleus et l'affinité des fibres musculaires et leur force développée sont augmentées (Bozzo et coll., 2004).

En conclusion, l'HG se révèle être un inducteur de plasticité nerveuse et musculaire.

PRINCIPAUX RESULTATS

Dans la suite de ce mémoire, nous avons choisi de présenter les résultats les plus marquants des travaux que j'ai réalisés pour cette habilitation à diriger les recherches. Les articles scientifiques relatifs aux différents points développés sont mis en annexe du mémoire.

Le premier point abordé concernera l'étude des modifications phénotypiques et contractiles des unités motrices du muscle soleus suite à une période d'HH. En effet, les données de la littérature montrent une altération du message moteur lors d'une période d'HH et des transitions phénotypiques musculaires qui pourraient laisser prévoir un changement des unités motrices. Cependant, ce point n'avait jamais été étudié.

Les résultats obtenus nous ont ensuite amenés à nous intéresser aux entrées proprioceptives du muscle soleus et à son message nerveux sensitif ce qui constituera le deuxième point de cette présentation. En effet, le message nerveux moteur est directement lié au message nerveux sensitif et on peut se demander ce que deviennent les caractéristiques électrophysiologiques des propriocepteurs (fuseaux neuromusculaires et organes tendineux de Golgi) lors d'épisodes d'HH et quel est l'impact du message nerveux afférent dans le développement de la plasticité neuromusculaire.

Enfin, compte-tenu de nos résultats obtenus en HH, nous avons voulu déterminer si une augmentation de la gravité obtenue par hypergravité (HG) pouvait induire certaines transformations musculaires et proprioceptives inverses de celles constatées en HH. Autrement dit, l'HG est-elle le miroir exact de l'HH ? Ce point sera discuté dans la troisième partie.

La dernière partie sera consacrée aux perspectives de nos travaux de recherche.

DEVENIR DES UNITES MOTRICES DU MUSCLE SOLEUS APRES DEUX SEMAINES D'HH

Après une période d'HH, le muscle soleus, muscle lent postural présente des modifications importantes sur les plans morphologique, histochimique, et biochimique (Edgerton et Roy, 1996). L'HH est responsable de l'induction d'une atrophie musculaire et de l'émergence des fibres de type rapide (Pette et Staron, 1997). Le type d'une fibre étant en étroite corrélation avec son contenu en MHC, les isoformes rapides de cette protéine contractile seront donc surexprimées après HH. En parallèle des transitions d'isoformes de MHC, le muscle soleus acquiert des caractéristiques contractiles de type rapide (Falempin et coll., 1990). Si les travaux bibliographiques se rapportant aux modifications musculaires après HH sont nombreux et homogènes en ce qui concerne l'évolution du muscle entier, les études rapportant le comportement contractile de l'unité motrice sont rares. Parmi celles-ci, un travail préliminaire entrepris au Laboratoire a examiné les propriétés mécaniques des unités motrices du soleus chez le rat contrôle et après HH (Leterme et Falempin, 1996). Ces auteurs ont déterminé que le muscle soleus contrôle contenait 85% d'unités motrices de type lent et 15% d'unités motrices de type rapide, identifiées sur la base de leurs comportements contractiles. Après HH, ces 2 populations d'unités motrices sont encore présentes mais la proportion d'unités lentes a fortement diminuée (50%) et celle d'unités motrices rapides, augmentée (24%). De plus, une nouvelle population d'unités motrices, totalement inexistante chez les rats contrôles apparaît (26%), avec des caractéristiques cinétiques de type intermédiaires.

Cependant, la composition des unités motrices en termes de types de fibres musculaires restait à déterminer. Ce point a constitué le but de notre étude. Après une déplétion glycogénique, la proportion de chaque type de fibres au sein d'une unité motrice a été déterminée par typage ATPasique couplée à une coloration de la teneur en glycogène des fibres musculaires. Les fibres d'une unité motrice sont déplétées de leur contenu en glycogène par une stimulation nerveuse répétée en conditions d'ischémie ; elles seront donc blanches lors de la coloration.

Nos résultats attestent de l'existence de 3 populations d'unités motrices après HH dans le muscle soleus, lente, rapide et intermédiaire contre 2 (lente et rapide) pour le muscle contrôle. Ils montrent également la réalité de l'atrophie musculaire qui concerne tous les types de fibres et la conversion lente → rapide. En ce qui concerne les muscles contrôles, il apparaît que les unités identifiées comme lentes d'après leur comportement contractile sont homogènes et ne contiennent que des fibres de type lent. Par contre les unités motrices contrôles caractérisées comme FR (rapides et résistantes à la fatigue) sont hétérogènes (fibres de type I lent, IIC intermédiaire, et IIA rapide). Pour le groupe HH, toutes les unités motrices sont hétérogènes : les unités identifiées comme lentes contiennent en fait une majorité de fibres de type I et quelques fibres de type IIC. Dans les unités motrices intermédiaires et rapides, les 3 types I , IIC et IIA sont présents. Pour les unités motrices intermédiaires, le type I est prédominant alors que pour les unités motrices rapides (FR), le type IIA est majoritaire.

La présence d'unités hétérogènes dans le muscle contrôle peut être le reflet d'un vieillissement musculaire puisque durant celui-ci, les unités motrices de type FR acquièrent progressivement un typage plus lent du à un renforcement de la proportion en fibres de type I (Ansved et Larsson, 1989). Pour le muscle HH, notre hypothèse est que le type de fibres prédominant (type I pour les unités lentes, type IIA pour les unités rapides) dicte le comportement contractile de l'unité motrice. Par contre, les unités motrices intermédiaires comportent une grande quantité de fibres de type I en association avec des fibres de type IIC et IIA. Cependant, ces unités motrices présentent des caractéristiques contractiles qui diffèrent des unités lentes et rapides, ce qui indique qu'elles constituent bien une population à part entière. Le fait que la totalité des unités motrices du muscle soleus des animaux HH soit hétérogène atteste de la transformation musculaire lent → rapide. Plusieurs facteurs pourraient expliquer cette hétérogénéité : le patrimoine génétique et la nature du message nerveux moteur. En effet, il est bien connu que dans les muscles adultes, certaines fibres proviennent de myotubes de deuxième génération qui sont aptes à exprimer les isoformes rapides de la myosine (DeNardi et coll., 1993). Ensuite au cours du développement post-natal ces fibres présenteront progressivement un phénotype lent par suite de la répression des gènes des myosines rapides (DeNardi et coll., 1993). Or il a été démontré que la présence de l'innervation était indispensable au maintien de la myosine lente (Butler-Browne et Whalen,1984). En conséquence, on peut supposer qu'au sein d'une même unité motrice cohabitent des fibres qui en fonction de leur origine développementale sont capables de réexprimer des isoformes rapide de myosine alors que d'autres en sont incapables : ces

éléments peuvent ainsi expliquer l'hétérogénéité au sein des unités motrices. La nature du message nerveux moteur pourrait également expliquer l'hétérogénéité des fibres musculaires d'une même unité motrice. En effet, de nombreuses études ont démontré que le message nerveux moteur était modifié au cours de l'HH puisqu'il passe d'un type tonique à un type phasique (Blewett et Elder, 1993 ; Ohira Y et coll., 2002 ; Kawano et coll., 2004 ; De-Doncker et coll., 2005). Cette transition serait ainsi perçue différemment suivant l'origine génétique des fibres musculaires.

En conclusion, les résultats que nous avons obtenus peuvent être pris en compte pour expliquer certaines modifications qui apparaissent sur le plan fonctionnel lors de la locomotion après un épisode de microgravité simulée : apparition de nombreuses hyperextensions de la cheville et instabilité posturale (Canu et coll., 1998).

Ces travaux sont présentés en annexe dans l'article paru dans Pflügers Archives sous le titre : « Phenotypic changes in the composition of muscular fibres in rat soleus motor units after 14 days of hindlimb unloading, (Picquet F., Canu M.H. and Falempin M.) ».

L'analyse des données bibliographiques montre l'existence de modifications des messages nerveux moteur suite à une période d'HH. Puisque le message nerveux efférent est fortement altéré au cours de l'HH et que ces altérations ont des répercussions importantes sur le fonctionnement et la structure du muscle, nous avons choisi de nous intéresser à l'évolution du message afférent musculaire suite à une période d'HH puisque ceci n'avait jamais été réalisé auparavant. Nous avons étudié plus particulièrement les propriocepteurs : fuseau neuromusculaire et organes tendineux de Golgi dont le fonctionnement est probablement modifié pendant la mise en HH.

ETUDES DES PROPRIETES ELECTROPHYSIOLOGIQUES ET DU CONTENU EN ISOFORMES DE MYOSINE DES FUSEAUX NEUROMUSCULAIRES DU MUSCLE SOLEUS DE RAT APRES DEUX SEMAINES D'HH

Les fuseaux neuromusculaires (FNM) sont des propriocepteurs très impliqués dans le contrôle des activités posturales et locomotrices. Ils sont insérés en parallèle des fibres musculaires classiques également appelées fibres extrafusales. Ces récepteurs encapsulés d'une longueur moyenne de 3 à 5 mm comportent des fibres dites intrafusales de 3 types : Bag1, Bag2 (également appelées fibres à sac) et chaîne dont le contenu en myosine est hautement spécialisé. Le contenu en myosine des fibres intrafusales, d'une part et leur éloignement vis-à-vis de la capsule, d'autre part permettent de définir trois zones A, B et C. Les FNM comportent une innervation sensitive (fibres Ia et II) et motrice (motoneurones β-squelettofusimoteur et γ-fusimoteur). Une illustration de la structure du fuseau neuromusculaire est donnée à la figure 4. Leur stimulus naturel est à la fois le degré et la vitesse de l'étirement musculaire. Les données de la littérature montrent que lorsque les conditions environnementales changent, la décharge des fibres afférentes des FNM est modifiée. En effet, une ténotomie entraîne une augmentation de la fréquence de décharge des fuseaux neuromusculaires (Hnik et Lessler, 1973 a, b). L'immobilisation musculaire chez le chat induit une légère augmentation de la décharge des fibres Ia (Gioux et Petit, 1993). Dans ces deux conditions, les muscles sont en position raccourcie et par conséquent le stimulus naturel des FNM est inexistant. Puisque pendant l'HH, le muscle soleus se trouve en position raccourcie (Riley et coll., 1990),on peut légitimement supposer qu'il existe dans ces conditions, une modification de la décharge des fibres Ia et II des FNM, pendant et après un épisode d'HH. Les données bibliographiques relatives à l'étude in vivo de la décharge des fibres afférentes Ia et II des FNM sont très abondantes sur le chat. Par contre, ce point concernant le rat est quasi inexistant dans la littérature (Andrew et coll., 1973 ; Miwa et coll., 1995) aussi bien en conditions contrôle qu'expérimentale.

Notre premier objectif a donc été d'identifier électrophysiologiquement les fibres afférentes issues des FNM par des protocoles de stimulation pertinents (étirements en rampe et sinusoïdaux) qui permettent une classification rapide de ces fibres. Dans un second temps, nous avons étudié le devenir de la décharge de ces fibres afférentes après 14 jours d'HH. Enfin, nous nous sommes intéressés au contenu en isoformes de myosine des fibres

intrafusales afin de vérifier s'il existait une plasticité fusoriale comparable à la plasticité musculaire.

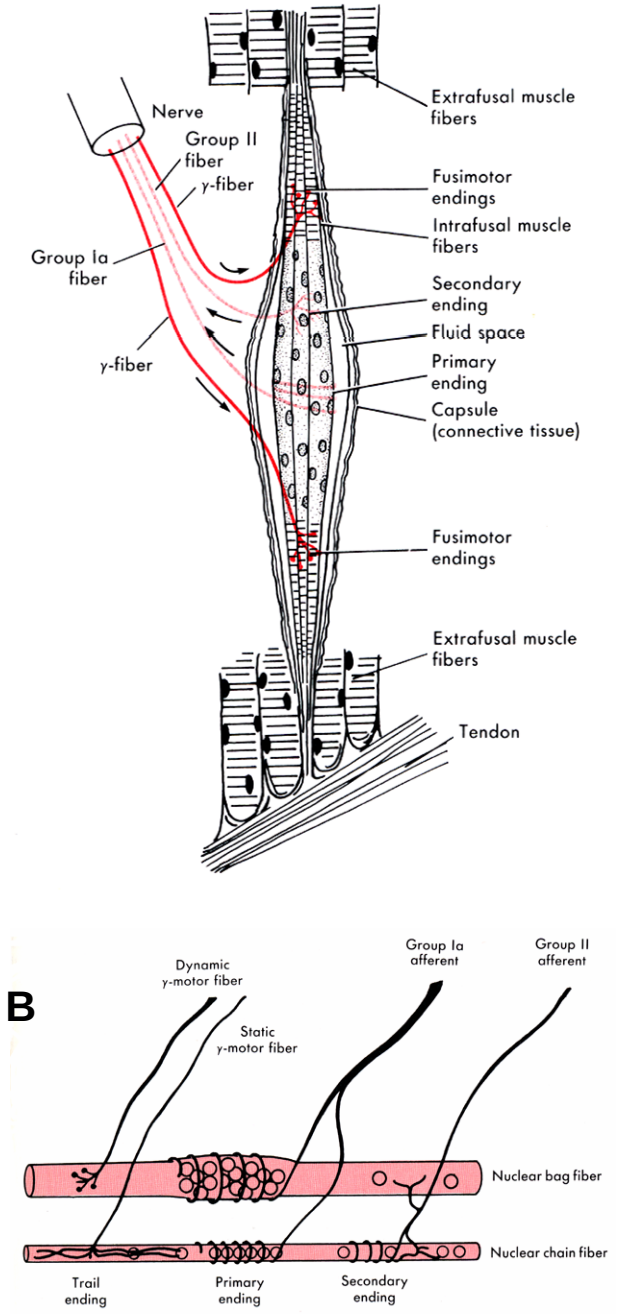


Figure 4 : A : Représentation schématique d'un fuseau neuromusculaire

B : Détail de l'innervation sensorielle et motrice des fibres à sac et à chaîne, d'après Berne et Levy, 1993.

Nos résultats montrent qu'il est possible de distinguer rapidement les fibres afférentes Ia des fibres II en retenant certains paramètres mesurés lors de d'étirements en rampe ou sinusoïdaux. Ainsi il est aisé de distinguer les fibres Ia des fibres II par l'allure de leur décharge lors d'un étirement en rampe : ces deux types de fibres se caractérisent par une décharge de base correspondant à une longueur musculaire de repos, un pic de décharge (ou pic dynamique, plus important pour les fibres Ia) qui traduit l'étirement musculaire puis une stabilisation de la décharge (décharge statique) à la nouvelle longueur musculaire. Lors du retour à la longueur musculaire de départ, les fibres Ia cessent de décharger tandis que les fibres II maintiennent leur décharge (diminution de la décharge et retour à des valeurs équivalentes à celle précédant l'étirement). Ces fibres peuvent également être différenciées lors de stimulations sinusoïdales ou de vibrations (simulations sinusoïdales à haute fréquence). Les fibres II présentent une décharge qui suit parfaitement une stimulation sinusoïdale jusqu'à une amplitude de 1 mm, alors que les fibres Ia cessent de décharger dès que l'amplitude de la sinusoïde atteint 0.12 mm. Les fibres Ia sont également plus sensibles aux vibrations de haute fréquence (jusqu'à 150 Hz) et de petite amplitude (0.12 mm) que les fibres II. Dans ces conditions, la réponse des fibres Ia se caractérise par le « driving », c'est-à-dire que leur fréquence de décharge suit la fréquence de stimulation sinusoïdale.

D'après l'ensemble de ces paramètres, nous avons pu caractériser les deux populations de fibres afférentes (Ia et II) responsables de l'innervation sensitive du FNM. Ces résultats nous ont servi de base pour étudier l'évolution des décharges afférentes de ces fibres après 14 jours d'HH.

Après HH, l'allure globale de la décharge des fibres Ia et II n'est pas changée. Cependant, certains des paramètres caractérisant les réponses des fibres afférentes intrafusales sont significativement augmentés. A la fois pour les fibres Ia et II, la décharge de repos des fibres, leur index dynamique (différence entre la valeur du pic dynamique et la valeur de la décharge statique) et la décharge statique. Concrètement, la réponse d'une fibre afférente est plus importante pour un même étirement après deux semaines d'HH. La sensibilité du fuseau est donc changée.

Nous avons également étudié le contenu en isoformes de myosine des fuseaux neuromusculaires du muscle soleus, à l'aide d'une batterie d'anticorps spécifiques, chez les rats contrôles et HH. Nous avons pu ainsi identifier les 3 types de fibres intrafusales (Bag1, Bag2 et chaîne). Nos résultats montrent que les FNM sont globalement plus résistants à l'HH puisqu'ils ne présentent pas d'atrophie significative. Le nombre de FNM ainsi que le nombre de fibres intrafusales du muscle soleus ne sont pas non plus changés. Par contre, les

répartitions des isoformes de myosine sont modifiées. Chez les fibres Bag1, nos résultats mettent en évidence une diminution de la myosine lente et une augmentation de la MHC slow tonic. Le taux d'expression de la MHC α-cardiaque est également réduit le long des fibres Bag1 et Bag2. Nous avons également mis en évidence une néoexpression des MHC α-cardiaque et lente dans les fibres à chaîne.

L'ensemble de nos résultats montre que l'étirement musculaire est mieux détecté par les FNM après HH, puisque pour un même étirement la réponse sensitive est augmentée. Cette augmentation de décharge ne peut pas être expliquée par des modifications de la structure des FNM (nombre et taille des fibres intrafusales, contenu en myosine) puisque comme le montrent nos données obtenues en immunohistochimie, ceux-ci semblent très résistants à l'HH. Par contre, il existerait une augmentation de l'élasticité musculaire (donc une diminution de la raideur) due en partie à l'émergence des fibres de type rapide qui sont moins raides que ne le sont les fibres de type lent (Petit et coll., 1990) et en partie à une adaptation des structures tendineuses (Canon et Goubel, 1995 ; Almeida-Silveira et coll., 2000). Des transitions des isoformes de collagène (type I → type III, soit dans le sens raide → moins raide) ont également été mises en évidence lors de l'HH (Miller et coll., 2001). Plus récemment, Rosant et coll. (2006) ont mis en évidence une légère augmentation de la sensibilité des FNM en condition dynamique et une forte augmentation en condition statique après 21 jours d'HH. Des études ont montré une corrélation positive entre la raideur passive et l'augmentation de la sensibilité des FNM dans les muscles contrôles (Rosant et Pérot, 2006). En ce qui concerne les muscles soleus HH, une telle corrélation n'a pu être mise en évidence que lors de tests en condition expérimentale dynamique : ainsi dans ces conditions, une augmentation de raideur musculaire pourrait expliquer une augmentation de sensibilité fusoriale (Rosant et coll., 2006). Lors de conditions expérimentales statiques, une telle relation n'a pu être mise en évidence ce qui indiquerait l'existence d'autres facteurs responsables de l'augmentation de la sensibilité des FNM. Dans ces conditions, Rosant et coll. (2006) ont attribué l'augmentation de sensibilité fusoriale à l'apparition de fibres intrafusales surnuméraires à chaîne qui participent très largement à l'élaboration de la sensibilité statique des FNM.

L'augmentation de décharge des FNM pourrait intervenir dans les changements posturaux et locomoteurs mis en évidence chez le rat après une période d'HH. Des travaux réalisés au Laboratoire (Canu et Falempin, 1996, 1998) ont montré qu'après HH, la marche

sur tapis roulant était perturbée (hyper-extensions de la cheville, instabilité posturale). De plus, la durée des cycles locomoteurs et la durée d'activation du muscle soleus sont augmentées. Pour expliquer ce résultat, l'hypothèse formulée par ces auteurs était l'existence d'une hyper-excitabilité des motoneurones α innervant le muscle soleus ce qui aurait en retour pour effet d'augmenter le niveau de contraction du muscle. Par la suite, il a été mis en évidence lors d'expériences de locomotion fictive une hyperactivité du neurogramme des nerfs innervant les muscles extenseurs des membres postérieurs du rat HH (Canu et coll., 2001). Ceci traduit une hyperactivité des corps cellulaires des motoneurones. Il a été démontré qu'une période d'inactivité renforçait l'efficacité de la connexion synaptique Ia-motoneurone α (Webb et Cope, 1992) et augmentait la taille des potentiels post-synaptiques excitateurs. Ainsi l'excitabilité des motoneurones serait renforcée. Des données de la littérature ont également montré qu'une déafférentation périphérique avait pour conséquence une hyper-extension de la cheville (Hnik et coll., 1981 ; Walro et coll., 1997 ; Wang et coll., 1997). Dans notre modèle, durant l'HH, la décharge des fibres afférentes des FNM est probablement très réduite voire absente puisqu'il n'existe plus d'effet « poids corporel » ce qui aurait pour conséquence d'induire une hyper-excitabilité des motoneurones α du muscle soleus. Cependant, nous mesurons la décharge afférente après l'HH, ce qui pourrait correspondre à une réactivation des FNM. Le fait que la décharge afférente soit augmentée induirait une activation accrue des motoneurones α ce qui en retour participerait au développement de l'hyper-extension de la cheville qui peut être constaté lors de la reprise des activités posturales et locomotrices.

Ces travaux sont respectivement présentés en annexe dans deux articles parus dans Journal of Neurophysiology sous les titres : « Characterization of spindle afferents in rat soleus muscle using ramp-and-hold and sinusoidal stretches, (De-Doncker L., Picquet F., Petit J. and Falempin M.) » et « Effects of hypodynamia-hypokinesia on the muscle spindle discharges of rat soleus muscle, (De-Doncker L., Picquet F., Petit J. and Falempin M.) », et dans un article paru dans Journal of Histochemistry and Cytochemistry sous le titre : « Expression of myosin heavy chain isoforms along intrafusal fibers of rat soleus muscle spindles after 14 days of hindlimb unloading, (De-Doncker L., Picquet F., Butler-Browne G. and Falempin M.) ».

ETUDE ELECTROPHYSIOLOGIQUE ET MORPHOLOGIQUE DES ORGANES TENDINEUX DE GOLGI SUITE A DEUX SEMAINES D'HH

Nous nous sommes intéressés aux organes tendineux de Golgi (OTG) qui représentent la deuxième catégorie de propriocepteurs musculaires. Les OTG, situés à la jonction myotendineuse ou myo-aponévrotique, sont constitués de faisceaux de collagène enfermés dans une capsule à partir de laquelle émerge une fibre afférente Ib (Zelena et Soukup, 1983). A une extrémité, ils sont donc connectés au tendon et à l'autre extrémité, ils sont disposés en série avec des fibres musculaires (5-10) provenant de différentes unités motrices. Les OTG sont donc disposés en série avec ces fibres musculaires qui sont appelées fibres « en série » ; les autres fibres musculaires non reliées à l'OTG sont appelées fibres « en parallèle ». Au sein d'un même muscle, chaque OTG est relié à plusieurs unités motrices mais chaque unité motrice est également en relation avec plusieurs OTG. Ces propriocepteurs sont donc à même de coder directement les variations de la force développée lors de contraction musculaire.

L'HH est à l'origine de nombreuses altérations musculaires telles que des transitions phénotypiques lent → rapide. Ces transitions conduisent à l'émergence des myosines de type rapide dans le muscle soleus et à la modification des unités motrices (Picquet et coll., 2000). D'une part, l'HH induit l'apparition d'une nouvelle catégorie d'unités motrices à comportement contractile intermédiaire entre les unités motrices lentes et rapides, classiquement décrites. D'autre part, elle conduit à la formation d'unités motrices hétérogènes exprimant des types différents de fibres musculaires. L'élasticité musculaire est également modifiée puisque les isoformes de collagène de type I sont graduellement remplacées par les isoformes de collagène de type III qui est plus compliant (Miller et coll., 2001). De plus, selon Canon et Goubel (1995), il suffirait de trois semaines d'HH pour que le muscle devienne plus élastique ce qui correspond en à la transformation des fibres lentes en fibres rapides, ces dernières étant plus élastiques. Puisque chaque unité motrice possède au moins une fibre musculaire en liaison avec un OTG et que la nature même de ces unités motrices est modifiée, il semblait logique de penser que le comportement électrophysiologique des OTG soit altéré suite à une période d'HH. Nous avons donc étudié les caractéristiques de décharge des OTG ainsi que leur morphologie après 14 jours HH.

Afin d'étudier la morphologie des OTG, nous avons réalisé des coupes sériées le long de la jonction myotendineuse du muscle soleus. Nos résultats montrent que la surface et la longueur de l'OTG sont inchangées après HH. Celui-ci ne subit aucune atrophie contrairement au muscle. Dans ces conditions, le rapport de la surface de l'OTG sur la surface musculaire est multiplié par 16. Ces résultats indiquent que les OTG sont beaucoup plus résistants lors d'une situation de non utilisation telle que l'HH.

Les paramètres électrophysiologiques caractérisant la décharge des OTG ont été mesurés sur des muscles soleus contrôles et sur des muscles soleus HH présentant les signes d'une transformation musculaire lent → rapide (atrophie et perte de force significatives, acquisition d'un comportement contractile plus rapide). La longueur musculaire a été réglée à la longueur permettant d'obtenir une réponse contractile maximale (secousse simple ou twitch). La décharge caractéristique des OTG a été mesurée lors de contractions tétaniques fusionnées des unités motrices en relation avec l'OTG considéré. Cette décharge des OTG est caractérisée par un pic de fréquence (ou pic dynamique) qui se produit lors de l'établissement du tétanos suivi d'une stabilisation de la décharge (décharge statique) qui peut être mesurée lors du plateau tétanique. Les OTG identifiés ne présentaient pas de décharge de base. Les valeurs de pic dynamique et de décharge statique sont classiquement très dispersées. Nous nous sommes donc intéressés aux mesures de sensibilités (sensibilité dynamique et sensibilité statique) qui sont normalisées à la force musculaire développée par l'unité motrice en série avec l'OTG étudié. Ces sensibilités sont respectivement les rapport des fréquences de décharge instantanée de l'OTG lors de contractions tétaniques fusionnées (temps de montée du tétanos, valeur du plateau tétanique). Ces paramètres offrent l'avantage de normaliser les réponses des OTG et de comparer celles-ci d'un groupe à l'autre (Davies et coll., 1995). Ils rendent compte respectivement de la sensibilité à la vitesse de contraction et au niveau de force musculaire. Après HH, la sensibilité dynamique n'est pas modifiée. Par contre, la sensibilité statique est augmentée quelle que soit la valeur de la fréquence tétanique de stimulation. Ceci peut être expliqué par une diminution de la force des unités motrices tandis que la décharge statique reste constante. La diminution de force des unités motrices est la conséquence de l'atrophie de tous les types de fibre musculaire (Picquet et coll., 2000).

Plusieurs hypothèses peuvent être formulées pour expliquer l'augmentation de la sensibilité statique des OTG. Si on écarte pour l'instant les hypothèses de modification des propriétés intrinsèques des OTG après HH, nous pouvons supposer que l'augmentation de la sensibilité statique est due à des paramètres musculaires et/ou tendineux. Les UM en série avec un OTG donné ont des fibres en série et des fibres en parallèle. Chez le rat contrôle, les

unités motrices sont homogènes : les fibres musculaires qu'elles soient en série ou en parallèle avec l'OTG présentent un typage semblable et des valeurs de raideur comparable. Après HH, il a été décrit (Picquet et coll., 2000) une hétérogénéisation de ces UM. En plus d'une perte de force, les fibres musculaires d'une même UM présentent des typages et des valeurs de raideur différents. On peut penser qu'il existe une diminution de la raideur des composantes élastique (composante élastique série) et contractile (ponts actine/myosine) qui résulte de la transition phénotypique lent → rapide (Petit et coll., 1990 ; Canon et Goubel, 1995). Ceci induirait une augmentation globale de l'élasticité musculaire qui serait associée à une augmentation de l'élasticité des tendons (Miller et coll., 2001). Concrètement, en dépit d'une baisse de la force musculaire, celle-ci serait mieux transmise à l'OTG qui continuerait ainsi après HH de percevoir les variations de force musculaire et remplirait toujours son rôle de détecteur de contraction musculaire.

Ces travaux sont présentés en annexe dans l'article paru dans Experimental Neurology sous le titre : « The structure and response properties of Golgi tendon organs in control and hypodynamia-hypokinesia rats, (Treffort N., Picquet F., Petit J. and Falempin M.) ».

EFFETS D'UNE DEAFFERENTATION PERIPHERIQUE SUR LE MUSCLE SOLEUS

Puisque les données bibliographiques ont montré que le message nerveux moteur était modifié après HH (Kawano et coll., 2004) *et qu'il existait des altérations du message afférent* (Kawano et coll., 2004 ; De-Doncker et coll., 2005)*, nous avons voulu essayer de déterminer quelle pouvait être la part de ce message nerveux sensitif dans le développement des transformations neuromusculaires après HH. Pour ce faire, nous avons choisi de supprimer le message afférent en utilisant une technique de déafférentation périphérique du muscle soleus par section spécifique des racines dorsales L4 et L5. L'évolution des propriétés contractiles et phénotypiques du muscle soleus déafférenté a été étudiée et comparée à celle d'un rat HH.*

Les principales conséquences de l'HH sont l'apparition d'une atrophie musculaire conjuguée à des pertes de force significatives et chez le muscle lent, des transitions phénotypiques lent → rapide. Ces transformations musculaires ont été attribuées en partie à une modification du message nerveux moteur qui, dans un premier temps, disparaît, puis évolue d'un type tonique vers un type phasique. En HH, les pattes postérieures ne sont plus en contact avec le sol ce qui nécessairement réduit ou altère le message nerveux sensitif provenant des récepteurs somesthésiques localisésdans la sole plantaire. Cette situation peut donc être assimilée à une période de déafférentation fonctionnelle. Les effets sur le muscle d'une réduction ou d'une absence de message nerveux afférent n'avaient jamais été étudiés. Le but de notre étude était de déterminer si une absence de message nerveux afférent pouvait avoir un impact sur les propriétés contractiles et morphologiques du muscle soleus et sur son contenu en isoformes de myosine. Les résultats obtenus ont été comparés avec ceux provenant d'animaux soumis à l'HH. Ainsi, il devenait possible de déterminer dans les altérations musculaires celles qui découlaient directement d'une absence de message afférent et celles qui étaient dues à une modification du message nerveux moteur. Pour ce faire, nous avons réalisé une déafférentation sélective en sectionnant les racines dorsales L4 et L5 qui contiennent les corps cellulaires des motoneurones innervant le muscle soleus (Kawano et coll., 2004). Les propriétés contractiles, la section transversale des fibres ainsi que le contenu en myosine du muscle soleus ont ensuite été mesurées.

Nos résultats montrent que la déafférentation par ablation des racines dorsales induit l'apparition d'une atrophie musculaire moins marquée que lors de l'HH. Lors de la déafférentation, l'atrophie musculaire est principalement due à une atrophie sélective des fibres hybrides et des fibres de type rapide. Les fibres I lentes ne sont pas atrophiées. Le niveau de force musculaire est réduit après déafférentation mais de façon moins drastique que lors de l'HH. Parmi les paramètres contractiles seul le temps de demi-relaxation de la secousse simple musculaire est modifié après déafférentation, ce qui pourrait être la conséquence d'un changement d'isoforme (lent → rapide) des Ca^{2+}-ATPases du réticulum sarcoplasmique (Loukianov et coll., 1998). Ceci est donc en accord avec l'augmentation de la vitesse de reprise du Ca^{2+} par le réticulum sarcoplasmique observée après déafférentation (Mounier et coll., 2005). Les autres paramètres cinétiques tels que le temps de contraction et le rapport P_{20}/P_0 ainsi que le contenu en isoformes de myosine ne sont pas significativement modifiés après déafférentation, contrairement à ce qui se produit suite à l'HH. Les paramètres cinétiques sont directement reliés au contenu en MHC (Leterme et Falempin, 1994), qui est lui-même dépendant de l'innervation motrice (Pette et Vrbova, 1985). Puisque dans notre étude, le contenu en MHC ainsi que les paramètres cinétiques sont inchangés, notre hypothèse était que le message nerveux moteur n'était pas modifié après 2 semaines de déafférentation. Cette hypothèse fut confirmée par la suite lors d'expériences de mesure chronique de l'EMG du muscle soleus et du neurogramme efférent enregistré au niveau de la racine ventrale L5 sur des rats préalablement déafférentés (Kasri, 2005). Les résultats ont montré que, dès la déafférentation, que le message nerveux moteur et l'EMG sont quantitativement diminués. Le troisième jour suivant la déafférentation, ces deux paramètres réaugmentent et montrent des niveaux d'activation supérieurs à ceux d'un animal contrôle. A partir du quatrième jour, l'activité du neurogramme efférent et de l'EMG se stabilise à un niveau pré-déafférentation. Ce que nous avions supposé s'est donc révélé exact.

L'étude des caractéristiques du message afférent en condition chronique lors de l'HH a démontré que ce message diminue significativement dès la mise en HH puisque dès cet instant les pattes postérieures des rats ne sont plus en contact avec le sol. La nature du message est également modifiée puisqu'il passe d'un type tonique à un type phasique. Durant la première semaine d'HH, le message afférent reste qualitativement inférieur à celui d'un animal contrôle. A partir du $9^{ème}$ jour d'HH, les bouffées afférentes augmentent en amplitude et en durée pour atteindre un niveau d'activité équivalent à celui d'un enregistrement contrôle. Dès le $12^{ème}$ jour d'HH, le message sensitif est quantitativement supérieur à celui d'un rat en condition terrestre (De-Doncker et coll., 2005). Une première hypothèse attribue la

réapparition du message afférent à une réduction du nombre et de la longueur des sarcomères ce qui permet une diminution de la longueur musculaire et induit un ré-étirement passif du muscle (Kawano et coll., 2004). Celui-ci provoque en retour le développement d'une tension passive musculaire qui contribue à la réactivation de la décharge électrophysiologique des fuseaux neuromusculaires. Une autre interprétation de la réapparition du message nerveux afférent est en relation avec les influx supra-spinaux. L'augmentation progressive de l'influx afférent pendant l'HH serait la conséquence d'un renforcement des influx excitateurs supraspinaux descendants. Une telle hypothèse avait déjà été avancée pour expliquer une augmentation de l'activité EMG lors de déafférentation (Hnik et coll., 1981 ;1982). A ce phénomène s'ajouterait une diminution de la teneur en neurotransmetteurs inhibiteurs (GABA) qui a été décrite après HH au niveau du cortex somesthésique (D'Amelio et coll., 1996).

La synthèse de nos résultats montre que la déafférentation est essentiellement caractérisée par une atrophie significative et une perte de force sans que le contenu en myosine soit modifié. L'HH a des effets plus marqués : atrophie et pertes de force importantes, émergence des myosines rapides aux dépens de la myosine lente. La diminution du message afférent au cours de la première semaine d'HH montre que cette condition expérimentale peut bien être considérée comme une déafférentation fonctionnelle mais uniquement durant cette période. Cependant, l'HH ne se limite pas à une réduction des messages afférents puisque certaines de ses conséquences (transition lent → rapide) ne sont pas dues à une disparition des entrées afférentes mais plutôt à une altération du message nerveux moteur.

Ces travaux sont présentés dans un article paru dans Experimental Neurology sous le titre : « Compared effects of hindlimb unloading versus terrestrial deafferentation on muscular properties of the rat soleus muscle, (Picquet F. and Falempin M.) ».

SYNTHESE DES TRAVAUX
SUR L'HH ET LES MESSAGES AFFERENTS

L'ensemble de nos travaux a permis de mieux déterminer les conséquences de l'hypodynamie-hypokinésie sur le système musculaire. Nous avons pu démontrer que celui-ci était complètement remodelé jusque dans la composition de l'entité musculaire, à savoir l'unité motrice. Celles-ci deviennent hétérogènes quant à leur composition en types de fibres. Cette transformation remet ainsi en cause la notion initiale d'unité motrice qui veut qu'au sein d'une même unité motrice, les fibres musculaires présentent un type semblable. L'hétérogénéité des unités motrices peut avoir pour origine une modification du message nerveux efférent puisque l'expression des protéines contractiles est directement dépendante de ce paramètre. Cette altération du message moteur serait ensuite différemment perçue par les fibres musculaires en fonction de leur origine génétique. Puisque nos travaux nous permettaient de supposer un changement des influx nerveux d'origine motrice, il semblait ensuite important de déterminer quel pouvait être le facteur déclenchant de ces modifications. Notre hypothèse était que la mise en HH entraînait un silence afférent partiel ou total puisqu'à partir de cet instant, les soles plantaires ne sont plus en contact avec le sol. De plus, le muscle soleus présente immédiatement une position raccourcie. Dans un premier temps, nous avons voulu vérifier si le message sensitif était modifié après HH. Nous avons donc examiné le comportement électrophysiologique et la morphologie des principaux propriocepteurs (fuseau neuromusculaire et organe tendineux de Golgi). Les résultats nous ont montré que le message proprioceptif était bien modifié après une période d'HH. Le contenu en myosine des fuseaux neuromusculaires est également différent. L'expression des myosines intrafusales étant dépendante à la fois du message afférent et efférent, on peut légitimement supposer une modification des influx nerveux moteurs et/ou sensitifs au cours de l'HH. Dans un second temps, nous avons voulu estimer la part des messages afférents dans les transformations musculaires consécutives à l'HH. Pour ce faire, nous avons étudié les propriétés contractiles et phénotypiques du muscle soleus à partir d'animaux déafférentés. D'après nos résultats, il apparaît que les pertes de force et l'atrophie musculaires sont la conséquence d'un manque d'influx afférents. La comparaison de nos données avec les résultats obtenus après HH montre que les diminutions de force contractile et de masse musculaire sont plus importantes que celles uniquement mises en évidence lors d'une déafférentation, ce qui suggère deux choses :

1) le message afférent est très probablement modifié au cours de l'HH, 2) il existerait en plus un facteur autre que le message sensitif qui serait également à l'origine de la perte de masse et de force musculaires. La résultante de ces deux conséquences serait l'apparition marquée d'un déficit en masse et en force musculaires, déficit qui est observé lors de l'HH. Une hypothèse serait que la position musculaire raccourcie pourrait influer sur le niveau d'atrophie et de force musculaire développée.

VARIATIONS DU CONTENU EN ISOFORMES DE MYOSINE DES FUSEAUX NEUROMUSCULAIRES SUITE A 19 JOURS D'HYPERGRAVITE

Si en microgravité, le muscle soleus est en position raccourcie, ce qui réduit sans doute la quantité d'influx afférents, on peut supposer qu'à l'inverse, en hypergravité, celui-ci sera en position étirée ce qui pourrait augmenter la décharge afférente. L'augmentation supposée de cette décharge afférente pourrait en retour renforcer le message nerveux moteur, dont l'influence accrue se répercuterait sur la nature des protéines contractiles. Ainsi, entre deux conditions expérimentales extrêmes (hypo et hypergravité), il pourrait exister des transformations musculaires inverses. C'est l'hypothèse que nous avons voulu présenter dans la suite de ce mémoire : nous nous sommes donc intéressés d'une part au contenu en myosine des fibres intrafusales de rats adultes après un séjour court (19 jours) en hypergravité. D'autre part, nous avons étudié les propriétés contractiles et les proportions relatives des isoformes de myosine du muscle soleus chez des rats adultes n'ayant connu qu'un environnement hypergravitaire.

Les études établissant la nature et l'importance des modifications neuromusculaires en microgravité réelle ou simulée sont particulièrement nombreuses. Une de nos précédentes études, présentée dans ce mémoire, avait mis en évidence une modification du contenu en isoformes de MHC des fibres intrafusales suite à une période d'HH de 15 jours sur des rats adultes. Tous nos protocoles d'investigation avaient jusqu'à présent été mis au point et appliqués à la situation 0G (HH) versus 1G (gravité normale). A l'inverse, le devenir des propriétés phénotypiques des propriocepteurs (FNM) a fait l'objet de peu d'études en hypergravité. Nos motivations étaient de démontrer l'existence éventuelle d'un continuum entre les étapes 0G ⇔ 1G ⇔ 2G (hypergravité) ce qui aurait permis de mieux cerner le ou les facteurs à l'origine de la plasticité neuromusculaire détectée en microgravité réelle ou simulée.

Nos expérimentations axées sur l'hypergravité ont été menées en collaboration avec le Dr Shenkman de l'IMBP (Institut Médical des Problèmes Biologiques) de Moscou. Des rats adultes ont été placés 19 jours en centrifugeuse à 2G. L'organisme perçoit alors 2 fois son poids corporel. Nous avons reçu des biopsies musculaires de soleus sur lesquelles nous avons réalisé une étude du contenu en isoformes de MHC des fibres musculaires intrafusales. Ceci

permettait de comparer les effets de 2G par rapport à 0G et 1G sur des animaux (rats Wistar adultes) placés environ 2 semaines en gravité différente de 1G. Une symétrie d'effets 1G $\rightarrow$ 0G et 1G $\rightarrow$ 2G sur les propriocepteurs pouvait être suspectée et a donc été contrôlée.

Les données recueillies nous permettent d'affirmer qu'il n'existe aucune atrophie ni hypertrophie des fibres intrafusales. Nos résultats démontrent que le long des fibres bag 1, l'expression des MHC I et MHC α-cardiac est augmentée alors que l'expression des MHC IIA et MHC slow-tonic est diminuée. Les fibres bag 2 montrent une diminution du marquage anti MHC IIA, MHC slow-tonic et MHC rapides. Chez les fibres à chaînes, seuls les marquages immunologiques dirigés contre les MHC IIA et MHC rapides (IIA + IIX + IIB) sont significativement réduits.

Nos résultats démontrent que l'hypergravité a un réel impact sur le phénotype des fibres intrafusales. De plus, pour certaines isoformes de MHC (MHC I, MHC α-cardiac et MHC slow-tonic) elle induit des effets opposés à ceux de l'HH. En effet, contrairement à l'HH, le muscle soleus en HG est en position étirée et en conséquence l'activité afférente doit être augmentée. Par voie réflexe (Bernstein et Goldberg, 1995), l'activité motrice et fusimotrice est également plus importante, puisque les afférences IA se projettent à la fois sur les neurones moteurs et fusimoteurs. Le taux d'expression des isoformes de MHC intrafusales est en relation directe avec l'existence d'une innervation sensitive et/ou motrice comme le montrent les expériences de déefférentation/déafférentation et dénervation musculaire (Pedrosa et coll., 1990 ; Wang et coll., 1997 ; Soukup et coll., 1999). Ainsi, le taux de MHC α-cardiac (Wang et coll., 1997) et de MHC I (Harris et coll., 1989) est directement dépendant de la présence de l'innervation motrice. En conséquence, bien qu'aucune mesure de l'EMG n'ait été faite pour des raisons techniques, nous pouvons supposer qu'une augmentation de ces MHC reflète un renforcement de l'activité nerveuse motrice pendant l'HG. La diminution du taux de MHC slow-tonic peut indiquer soit une variation de la balance efférence/afférence avec une prépondérance des influx moteurs soit une diminution du message nerveux sensitif ce qui dans notre cas semble peu probable. En effet il a été démontré qu'une augmentation de l'activité afférente renforçait le taux de MHC rapides (Wang et coll., 1997) ce qui est le cas après HG.

En conséquence, l'HG semble induire des changements contraires à ceux de l'HH, c'est-à-dire un renforcement des activités nerveuses sensitives et motrices ce qui conduirait à l'émergence des myosines de type lent.

Ces travaux sont présentés dans un article paru dans Journal of Histochemistry and Cytochemistry sous le titre : « Expression of myosin heavy chain in rat soleus muscle spindles after 19 days of hypergravity, (Picquet F., De-Doncker L. and Falempin M.) ».

ETUDE DES PROPRIETES CONTRACTILES ET PHENOTYPIQUES MUSCULAIRES SUITE A UN DEVELOPPEMENT EN HYPERGRAVITE SUIVI D'UNE PERIODE DE MISE EN NORMOGRAVITE

Nous avons eu l'opportunité de participer à un projet commun financé par le CNES axé sur les relations existant entre l'hypergravité et le développement pré et post-natal. En collaboration avec l'équipe du Pr Lacour de Marseille, nous avons été amenés à apporter un volet relatif à la contraction musculaire sur des rats conçus, nés et élevés jusqu'à l'âge de 100 jours dans un environnement à 2G. Ce programme nous est apparu très intéressant puisqu'il intégrait le rôle du facteur gravité dans le développement de l'individu et dans notre cas, dans l'acquisition du phénotype musculaire. En outre, au cours de ma thèse, j'avais précédemment mené des travaux sur l'influence d'un « unloading », par plâtrage en position raccourcie du membre inférieur, appliqué à des rats nouveau-nés (Picquet et coll., 1997 ; 1998). L'opportunité de comparer nos résultats était donc très attractive.

Nous avons choisi d'étudier les propriétés contractiles et le contenu en isoformes de myosine du muscle soleus et de son agoniste rapide, le plantaris, sur des rats n'ayant connu que l'hypergravité. En fonction des résultats obtenus, nous avons poursuivi notre programme en plaçant ensuite ces rats en gravité normale pour une durée de 15, 30, 60 et 120 jours. Notre objectif était de vérifier si les modifications observées sur le muscle soleus étaient réversibles à 1G, ce qui pour des animaux élevés en HG constituait une situation « d'hypogravité ».

Nos résultats montrent que les rats conçus, nés et élevés en HG jusqu'à 100 jours ont une croissance plus faible que celle des contrôles à 1G mais la force musculaire maximale (P_0) développée par le soleus augmente (+ 43 % sur muscle entier, in situ). Les cinétiques de contraction deviennent très ralenties ce qui est corroboré par l'existence d'un seul type de fibres n'exprimant que de la MHC I lente. Rappelons qu'en normogravité, les muscles soleus comprennent des fibres « pures », n'exprimant que la MHC I ou la MHC IIA et des fibres hybrides coexprimant les deux isoformes.

Il est donc clair que le facteur 2G induit des transformations dans l'expression des MHC et dans le développement de la force musculaire opposées à celles produites à 0G sur le muscle soleus. De plus, le muscle plantaris, de type rapide, subit également une perte de

masse associée à la diminution de masse corporelle et une augmentation de la force relative tandis que ses cinétiques de contraction diminuent, le muscle devenant ainsi plus rapide. Ce résultat est associé à un renforcement de la proportion de fibres hybrides coexprimant les isoformes les plus rapides (MHC IIX, MHC IIB), au détriment des isoformes MHC I et MHC IIA. Il est donc intéressant de noter que l'hypergravité exerce des effets marqués sur un muscle rapide pour lequel aucune étude n'a rapporté d'effet aussi net à 0G.

Puisqu'il existe une réelle plasticité musculaire après hypergravité, il nous paraissait important d'approfondir les caractéristiques musculaires des animaux soumis à 2G et de déterminer si les transformations phénotypiques du muscle soleus sont définitives ou au contraire si ces animaux gardent leur capacité d'adaptation lorsque l'environnement gravitaire change. Les durées de mise en normogravité ont été choisies afin de vérifier s'il existe un âge critique permettant ou non la mise en place d'un phénotype musculaire « normal » (coexpression de myosine lente et rapide).

Cette programmation nous permettait de vérifier si le muscle soleus était une structure figée lorsque son développement pré et post-natal s'était réalisé en hypergravité ou au contraire s'il pouvait être affecté par la normogravité, sachant que celle-ci représentait une situation « d'hypogravité simulée » pour les rats conçus, nés et élevés en HG.

Nos résultats montrent qu'au cours de la maturation post-natale, les rats témoins acquièrent progressivement un phénotype lent tout en exprimant la MHC IIA en faible proportion (10% à 220 jours). Par contre, les rats nés en HG puis placés en normogravité expriment exclusivement la MHC I jusqu'au 220ème jour ; ils présentent également des propriétés contractiles plus lentes que celles des témoins appareillés. Ces animaux montrent de plus des signes de croissance musculaire et corporelle ralentie même après un séjour long (120 jours) en normogravité. Une hypothèse serait que le muscle soleus qui a un rôle antigravitaire percevrait la normogravité comme une « hypogravité » et serait donc moins sollicité qu'en HG ce qui contrecarrerait sa croissance. La surexpression de la MHC I serait la conséquence de la mise en place d'un message nerveux moteur exacerbé donc plus tonique en HG. Ceci laisserait supposer l'existence de périodes-clés dans la maturation nerveuse et musculaire. En cas de diminution de la gravité (de 1G à 0G), la transition des isoformes de myosine habituellement observée se fait dans le sens lent → rapide mais dans ce cas, la fonction locomotrice est réduite, le contact des soles plantaires avec le sol n'existe plus et le muscle présente une position raccourcie. Bien que la mise en normogravité corresponde à une forme d'hypogravité pour les rats HG, le contact des soles plantaires avec le sol existe toujours et le muscle n'est plus constamment en position raccourcie : ceci pourrait participer

au maintien de l'expression de la myosine lente. Il a été démontré qu'après HG, la posture et la locomotion étaient modifiées : les animaux maintenus en hypergravité pendant deux semaines se déplacent plus lentement que les rats maintenus en normogravité et leur stabilité posturale est augmentée (D'Amelio et coll., 1998). Ces deux facteurs pourraient également favoriser l'émergence de la myosine lente.

Le bilan de ces travaux montre que l'hypergravité peut être considérée dans certains cas, comme un miroir de la microgravité (transformation musculaire lent → plus lent), mais elle est également capable d'induire des modifications plus profondes habituellement non détectées en microgravité (transition rapide → plus rapide du muscle plantaris). C'est, de plus, un modèle expérimental de choix qui permet la réalisation aisée d'études : de longue durée sur des animaux adultes ; du développement à partir d'animaux extrêmement jeunes. Ces types d'expérimentations sont en effet difficilement réalisables en microgravité réelle ou simulée, pour des raisons techniques et des problèmes de coût important.

En conséquence, ce modèle ouvre de nouvelles perspectives d'études des phénomènes de plasticité nerveuse et musculaire.

Ces travaux sont présentés dans deux articles parus dans American Journal of Physiology sous les titres : « Contractile properties and myosin expression in rats born, and reared in hypergravity, (Picquet F., Bouet V., Canu M.H., Stevens L., Mounier Y., Lacour M. and Falempin M.) » et « Changes in rat soleus phenotype consecutive to a growth in hypergravity followed by normogravity », (Picquet F., Bouet V., Cochon L., Lacour M. and Falempin M.).

CONCLUSION GENERALE

ET

PERSPECTIVES

L'ensemble de nos travaux menés au cours de ces dernières années nous a permis de mieux cerner l'étendue de la plasticité nerveuse et musculaire. En confrontant nos résultats obtenus en hypo et en hypergravité, nous avons pu déterminer qu'un changement de gravité induisait un remaniement profond du muscle.

Un séjour en microgravité simulée (HH) sera à l'origine :

- de l'apparition d'une atrophie significative,
- d'une hétérogénéisation des unités motrices,
- d'une modification électrophysiologique des propriocepteurs,
- les proportions relatives des isoformes de myosine intra et extra fusales sont changées et montrent une transition dans le sens lent → rapide.

Les travaux que nous avons menés montrent qu'un épisode d'hypergravité aura pour conséquences :

- l'acquisition d'un phénotype complètement lent pour le muscle soleus avec une surexpression des isoformes lentes de protéines,
- une modification du contenu en isoformes des myosines intrafusales, avec acquisition de formes lentes.

Ces altérations interviennent dans l'existence des modifications comportementales telles que la présence d'une instabilité posturale et de nombreuses hyperextensions de la cheville observées après HH (Canu et Falempin, 1996, 1998). De plus, au cours de l'HH, les messages nerveux moteurs et sensitifs sont qualitativement et quantitativement diminués au moins durant la première semaine (Kawano et coll., 2004 ; De-Doncker et coll., 2005). Après cette période, la nature de ces messages tend progressivement vers la normale, ce qui suggère un mécanisme adaptatif en relation avec la longueur musculaire qui diminue pour mieux se conformer à l'angle des articulations du genou et de la cheville. Enfin au bout de deux semaines d'HH, l'activité EMG et le neurogramme afférent deviennent plus importants que ceux d'un animal contrôle ce qui, pour l'EMG, a été attribué à une influence accrue des influx supra-spinaux (Hnik et coll., 1981 ; De-Doncker et coll., 2005), et pour l'activité nerveuse afférente, à une meilleure élasticité musculaire (Kawano et coll., 2004 ; De-Doncker et coll., 2005). Si l'étude en condition terrestre des neurogrammes afférent et efférent in vivo est

techniquement contraignante, les difficultés sont encore plus importantes en hypergravité : c'est pourquoi nous ne disposons pas de données concernant l'évolution des messages nerveux moteur et sensitif en HG. Cependant, dans la mesure où l'expression des isoformes de myosine est modifiée et que celle-ci dépend du message nerveux moteur, nous pouvons supposer qu'il est également altéré après HG.

Cependant, de nombreuses questions concernant la plasticité nerveuse et musculaire restent encore sans réponse. Déterminer précisément le ou les facteurs initiateurs de cette plasticité nécessitera à la fois une meilleure connaissance d'éléments jusqu'ici peu étudiés, notamment les motoneurones et les neurotransmetteurs médullaires qui peuvent moduler l'activité de ces derniers, mais également les récepteurs de ces neurotransmetteurs qui sont eux-mêmes associés à des canaux ioniques. Les valeurs des conductances ioniques sont en effet, modulatrices du comportement électrophysiologique des motoneurones (Cormery et coll., 2005).

Les motoneurones peuvent être considérés comme des centres intégrateurs recevant de nombreux influx excitateurs et inhibiteurs dont la balance régule l'activité motoneuronale. Or après HH, il a été démontré que les enregistrements des neurogrammes globaux reflétant l'activité des motoneurones innervant les muscles des membres postérieurs étaient augmentés (Canu et coll., 2001). Plusieurs hypothèses peuvent être formulées pour expliquer cette activité accrue du neurogramme. Il pourrait s'agir d'une modification de la balance entre influx excitateurs et inhibiteurs et/ou d'une variation de l'activité intrinsèque des motoneurones. Récemment, les propriétés électrophysiologiques des motoneurones lents et rapides composant le nerf tibial ont été déterminées par enregistrement intracellulaire (Cormery et coll., 2005) à la fois sur des rats contrôles et des rats soumis à 2 semaines d'HH. Ainsi sur la base du temps de demi-diminution de l'hyperpolarisation (AHP ½ ou AHP half decay time) qui est un critère important d'identification chez les animaux contrôles, une classification des motoneurones lents et rapides a pu être établie : les motoneurones lents présentent une valeur moyenne de AHP ½ supérieure à 20 msec alors que les motoneurones rapides montrent une valeur inférieure à 20 msec. Les motoneurones lents montrent également une valeur de résistance d'entrée (2.7 MΩ) supérieure à celle des motoneurones rapides (1.7MΩ). Les autres paramètres tels que la valeur du potentiel de membrane, le seuil d'apparition du potentiel d'action, la rhéobase ne permettent pas de distinguer les différents types de motoneurones. Après HH, la distinction entre les motoneurones lents et rapides peut encore être réalisée grâce au critère AHP ½ (Cormery et coll., 2005). Pour les motoneurones

rapides du groupe HH, le seuil de déclenchement du potentiel d'action est décalé vers des valeurs plus dépolarisantes. A la fois pour les motoneurones lents et rapides, la quantité minimale de courant nécessaire pour déclencher une réponse neuronale sous forme de décharge de potentiel d'action est augmentée. La relation entre les intensités de stimulation et les valeurs des fréquences de décharge obtenues est ainsi décalée vers la droite, ce qui indique une diminution de l'excitabilité neuronale pour les deux types de motoneurones. Ainsi, tous les motoneurones deviennent moins excitables et le comportement électrophysiologique des motoneurones lents se rapproche significativement de celui des motoneurones rapides (Cormery et coll., 2005). La transition motoneuronale lent → rapide, qui se traduit par un déplacement vers la droite de la relation fréquence de décharge / courant de stimulation, implique que la quantité de courant nécessaire soit plus importante pour provoquer une décharge de fréquence donnée. Ceci pourrait avoir une incidence dans la façon dont sont recrutés les motoneurones lors d'un exercice volontaire et donc expliquer les altérations locomotrices mises en évidence après HH (Canu et Falempin, 1997 ; 1998). Ainsi, compte tenu de l'augmentation de la valeur de la rhéobase et du décalage de la relation fréquence de décharge / courant de stimulation vers la droite, le recrutement des motoneurones nécessaires pour réaliser un mouvement donné sera plus difficile (Cormery et coll., 2005).

Ces modifications électrophysiologiques des motoneurones obtenues lors d'enregistements intracellulaires après 2 semaines d'HH semblent à priori en désaccord avec les données obtenues sur l'EMG. En effet celui-ci montre une diminution significative durant les premiers jours d'HH avant un retour à des valeurs égales (Kawano et coll., 2004) ou supérieures aux valeurs contrôles (De-Doncker et coll., 2005). L'hypothèse retenue pour expliquer ces résultats serait que les motoneurones percevraient tout d'abord la diminution de l'activité EMG qui a lieu au cours des premiers jours de suspension et ensuite adapteraient leur fréquence de décharge aux conditions expérimentales qui leur sont imposées (Cormery et coll., 2005). Ainsi, ce serait les changements musculaires induits par l'HH qui seraient à l'origine des altérations des motoneurones. Les modifications musculaires après HH (atrophie, transition phénotypique de nombreuses protéines, hétérogénéisation des unités motrices, etc...) sont relativement plus conséquentes que celles des motoneurones. On peut donc supposer que les changements musculaires précédent l'apparition des changements motoneuronaux. Ceci suggère donc que les fibres musculaires seraient capables de produire certaines substances qui en agissant en retour sur le comportement des motoneurones seraient donc à l'origine des modifications des propriétés électrophysiologiques des motoneurones. L'existence d'un facteur présent au niveau musculaire mais agissant de façon rétrograde sur le

système nerveux a été précédemment mise en évidence par Gonzalez et Collins (1997). Ces auteurs ont en effet réalisés des injections dans le muscle gastrocnémius d'une neurotrophine : le BDNF (Brain Derived Neurotrophic Factor). L'examen, 5 jours après injection, des propriétés électrophysiologiques des motoneurones innervant le muscle gastrocnémius révèle une diminution des valeurs de la rhéobase et de la capacité membranaire des motoneurones. Ainsi, le BDNF peut se révéler un modulateur de l'excitabilité neuronale et par là même être inducteur d'une plasticité neuronale (Dupont et coll., 2005).

Une hypothèse séduisante pour expliquer les transitions des motoneurones serait qu'une substance habituellement inactive ou inexistante en condition terrestre serait produite sous une forme active par les muscles des animaux suspendus. Cette substance agirait ensuite de manière rétrograde sur la synthèse et l'incorporation des canaux ioniques motoneuronaux qui sont eux-mêmes modulateurs de l'excitabilité neuronale. Ainsi on peut supposer que les changements d'excitabilité des motoneurones seraient la conséquence d'une distribution différente des canaux ioniques sur la membrane des motoneurones et/ou d'une modification des conductances ioniques. Des expériences de modélisation des motoneurones mises au point par Dai et coll. (2002) permettent par simulation informatique de « compartimenter » un neurone en 5 parties caractéristiques (axone, cône d'émergence de l'axone, soma, dendrites proximale et distale) dont les paramètres (diamètre, longueur, résistance et capacité membranaires, conductances ioniques….) sont fixés par l'expérimentateur. Un tel modèle avec des valeurs de résistance membranaire et de rhéobase comparables à ce qui a été décrit dans la littérature constitue une représentation fiable d'un motoneurone médullaire de chat. Cormery et coll. (2005) ont montré qu'une réduction de la conductance sodique de 25 % et 15 % respectivement dans le segment initial de l'axone et dans le corps cellulaire, provoquait une augmentation de la valeur de la rhéobase, du seuil de déclenchement des potentiels d'action et un décalage vers la droite de la relation fréquence de décharge / courant de stimulation. Des résultats identiques sont obtenus lorsque la conductance potassique à rectification retardée est augmentée de 55 % dans le cône d'émergence de l'axone et de 42 % dans le soma. Les modifications d'autres conductances ioniques provoquent en plus d'autres altérations (variations du potentiel de repos, de la durée et de l'amplitude du potentiel d'action, de la durée de l'hyperpolarisation….). Il a également été suggéré qu'un courant entrant de calcium et de sodium serait impliqué dans le maintien du seuil de déclenchement de la décharge motoneuronale (Heckman et coll., 2005).

Le comportement des motoneurones est sous la dépendance d'influx de deux types : ionotropiques et métabotropiques (ou neuromodulateurs). Les influx métabotropiques consistent en l'activation d'un récepteur couplé à une protéine G qui est elle-même le point de départ de l'activation d'une cascade intracellulaire de seconds messagers. Ceux-ci à leur tour peuvent moduler le fonctionnement des canaux ioniques cellulaires. Les influx d'origine ionotropique consistent en l'arrivée de neurotransmetteur se fixant sur des récepteurs postsynaptiques spécifiques couplés à des canaux ioniques. En fonction du type de canaux ioniques activés, le motoneurone sera dépolarisé (neurotransmetteur excitateur : glutamate) ou hyperpolarisé (neurotransmetteurs inhibiteurs : glycine ou GABA). Les récepteurs de ces neurotransmetteurs sont couplés à des canaux ioniques sodique, potassique, calcique et chlore et il a été suggéré qu'une variation de conductance ionique pouvait être à l'origine des changements des propriétés électrophysiologiques des motoneurones (Cormery et coll., 2005).

Si l'existence et la répartition des récepteurs ionotropiques a été assez bien étudiée au niveau des motoneurones médullaires des rats jeunes (Palecek et coll., 1999 ; Abdrachmanova et coll., 2000 ; Stegenga et Kalb, 2001 ; Marvizon et coll., 2002), peu de travaux se sont intéressés à l'expression de ces récepteurs chez l'adulte. Ce sont ces points qui seront abordés dans le cadre de nos perspectives de recherche.

Nous proposons d'aborder cette thématique sous plusieurs aspects :

- Lors d'épisodes d'hypodynamie-hypokinésie
  - Etude du contenu en neurotransmetteurs médullaires
  - Etude de la distribution des récepteurs de ces neurotransmetteurs

Notre programme sera réalisé sur des rats adultes où l'on peut considérer que le système neuromusculaire est mature.

RECHERCHE DES VARIATIONS DES NEUROTRANSMETTEURS MEDULLAIRES APRES HH

Une première partie de ce travail est parue sous le titre « Variations in amino acid neurotransmitters in the rat ventral spinal cord after hindlimb unloading » dans Neuroscience Letters (403 : 147-150, 2006).

L'activité des neurones moteurs innervant les muscles des membres postérieurs est la synthèse d'influx supra-spinaux et d'informations sensorielles périphériques qui peuvent être aussi bien excitateurs qu'inhibiteurs. En conséquence, les motoneurones sont hautement sensibles à un changement environnemental et leur excitabilité pourrait être modulée par une altération du contenu en neurotransmetteurs médullaires. Ainsi des études réalisées après section de moelle épinière dans la région thoracique (T12-T13) montrent des modifications des enzymes liées au métabolisme du GABA et du glutamate (Tillakaratne et coll., 2000).

Notre hypothèse est la suivante : l'HH pourrait être à l'origine de modifications dans l'expression de certains neurotransmetteurs médullaires. Les modifications des messages nerveux afférent et efférent, et de l'activité électromyographique mises en évidence lors de l'hypodynamie-hypokinésie (De-Doncker et coll., 2005) pourraient impliquer également des variations de la quantité de neurotransmetteurs libérés au niveau médullaire. Il nous a semblé judicieux de focaliser notre attention sur les principaux acides aminés présents dans la moelle épinière. Deux sont connus comme étant des neurotransmetteurs excitateurs (acide glutamique et acide aspartique) et deux comme neurotransmetteurs inhibiteurs (glycine et GABA). *L'acide glutamique* ou glutamate est un agoniste du récepteur ionotropique N-méthyl-D-aspartate (NMDA) mais aussi des récepteurs à l'acide α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) et kaïnate (Dingledine et coll., 1990a et b, Kalb et coll., 1992). Le glutamate est le neurotransmetteur excitateur principal du SNC (Johnson, 1972). *L'acide aspartique* ou aspartate est exclusivement un agoniste du récepteur NMDA (Patneau et Mayer, 1990). *La glycine* ou glycocolle est l'agoniste du récepteur ionotropique glycine (Legendre, 2001). Elle a également un rôle de neuromodulateur puisqu'elle peut se lier au récepteur NMDA (Kleckner et Dingledine, 1988). *Le GABA* (γ-amino butyric acid) est synthétisé à partir d'une décarboxylation du glutamate via la glutamate décarboxylase (GAD).

Le GABA est l'agoniste des récepteurs inhibiteurs $GABA_A$ et $GABA_B$. De plus, le GABA et la glycine sont coexprimés dans les neurones de la moelle épinière (Jonas et coll., 1998).

Les expérimentations ont été réalisées à partir de rats contrôles et de rats soumis à 7 et 14 jours d'hypodynamie-hypokinésie (groupes HH7 et HH14). L'analyse qualitative et quantitative des neurotransmetteurs médullaires a été réalisée sur les segments spinaux ventraux L_4 et L_5. Ces niveaux correspondent à l'emplacement des corps cellulaires des motoneurones innervant les muscles des membres postérieurs : tibialis anterior et plantaris pour le niveau L_4, soleus, gastrocnemius médian et latéral et extensor digitorum longus pour le niveau L_5. Les neurotransmetteurs ont été mis en évidence grâce à une technique d'HPLC iPAD (chromatographie liquide de haute précision à détection ampérométrique pulsée intégrée). Leur profil d'élution a été obtenu par comparaison à une solution étalon contenant un mélange de 20 acides aminés. La quantification a été réalisée par intégration de la surface des pics correspondants à chaque neurotransmetteur.

Les résultats montrent que :

- au niveau segmentaire L_4, il n'y a aucune variation quantitative des acides aminés entre les groupes contrôles, HH7 et HH14. Cette absence de modification peut être liée au fait que ce niveau segmentaire contient les corps cellulaires des motoneurones de muscles très peu modifiés par les conditions d'HH ;
- au niveau segmentaire L_5, l'aspartate, le glutamate, le GABA et la glycine varient significativement. Leurs proportions sont augmentées dans le groupe HH7 par rapport aux animaux contrôles. Par contre, leurs valeurs moyennes indiquent un retour vers des données contrôles dans le groupe HH14. Ce niveau segmentaire contient les corps cellulaires des motoneurones innervant les muscles les plus transformés par l'HH ;
- il n'existe pas de variation de l'équilibre entre les neurotransmetteurs inhibiteurs (GABA et glycine) et excitateurs (glutamate et aspartate) entre les groupes contrôles, HH7 et HH14.

En conclusion, cette étude montre pour la première fois que l'HH est à l'origine d'une surexpression, transitoire, des principaux neurotransmetteurs excitateurs et inhibiteurs dans les segments médullaires où sont localisés les corps cellulaires des motoneurones innervant les muscles les plus transformés. Ces travaux préliminaires ont été réalisés en collaboration avec les Drs Y. Guérardel (Laboratoire de Glycobiologie Structurale et Fonctionnelle, UMR 8576, Université de Lille 1) et G. Dubreucq (Société Biodex, St Amand-les-Eaux).

PROJET DE RECHERCHE

Les effets de l'HH tels que l'atrophie et les pertes de force musculaires ont des répercussions sur les capacités locomotrices et posturales. Des études ont montré chez le rat une altération des performances locomotrices après 2 (Canu et Falempin, 1996 ; Canu et coll., 2005) et 9 semaines (Ohira Y et coll., 2002) d'HH. Or, il existe dans la moelle épinière un générateur central de locomotion (CPG) dont l'activité en conditions normales est sans cesse modulée par les influx afférents d'origine cutanée, proprioceptive ou articulaire. Pendant la période d'HH, ces messages sont altérés, ce qui peut avoir des conséquences sur le fonctionnement du CPG et sur la libération des neurotransmetteurs médullaires. Ainsi, Laporte et coll. (1995) ont rapporté une diminution de la quantité de sérotonine médullaire après section de racines dorsales. Croul et coll. (1998) ont montré qu'un écrasement du nerf sciatique induisait, dans la moelle épinière, une augmentation du nombre de récepteurs au glutamate de type NMDA. Il a également été démontré qu'après une lésion médullaire, il était possible de faciliter et renforcer la récupération de la locomotion par administration d'agonistes des récepteurs glycinergiques (Edgerton et coll., 1997 ; de Leon et coll., 1999) et noradrénergiques (Chau et coll., 1998 ; Giroux et coll., 2001). Des études ont démontré en outre que les motoneurones ainsi que les interneurones du groupe I de la moelle épinière étaient directement sous influence excitatrice des monoamines (Heckman et coll., 2003, Theiss et Heckman, 2005). Ces substances sont en conséquence capables de modifier l'excitabilité neuronale en modifiant l'efficacité synaptique. Nous ne pouvons donc exclure une possible variation de ces monoamines dans la moelle épinière des rats soumis à une période d'HH.

Nous comptons compléter notre étude sur les neurotransmetteurs médullaires par HPLC en nous intéressant aux catécholamines ou monoamines encore appelées amines biogènes (noradrénaline, adrénaline, dopamine) et à la sérotonine.

Cependant, cette approche nécessitera la maîtrise d'une technique HPLC différente de celle employée lors de l'étude des neurotransmetteurs de type acides aminés. Ce projet demandera donc une période de mise au point technique. Nous comptons employer la technique d'HPLC de partage en phase inverse, par détection électrochimique.

RECHERCHE DES VARIATIONS DES RECEPTEURS DES NEUROTRANSMETTEURS MEDULLAIRES APRES HH

RAPPELS SUR LES RECEPTEURS NMDA

Le récepteur NMDA est un récepteur ligand ionotropique composé d'un assemblage hétéro-oligomérique de sous-unités NR1, NR2 et parfois de sous-unités NR3 (Dingledine et coll., 1999). La figure 5 illustre un exemple de récepteur au NMDA. La sous unité NR1 est ubiquitaire. Le site de fixation du glutamate se trouve sur la sous-unité NR2. La glycine a un site de fixation sur la sous-unité NR1. Elle se révèle donc être un coagoniste du récepteur (Kleckner et Dingledine, 1988). Cependant, l'hypothèse de l'activation par la glycine seule n'a pu être confirmée ou repoussée (Dingledine et coll., 1999). Les sous-unités NR1 et NR2A sont les principales isoformes présentes dans les motoneurones de la moelle épinière lombaire de Rat adulte (Marvizon et coll., 2002 ; Stegenga et Kalb, 2001). L'expression de NR2 est un facteur limitant pour un récepteur NMDA fonctionnel (Myers et coll., 1999). En effet, il a été démontré que l'ouverture du canal est plus importante pour les récepteurs hétéromériques composés d'une sous-unité NR1 et de 4 sous-unités NR2 (Mori et Mishina, 1995). Ainsi, les changements dans la synthèse de NR2A peuvent moduler l'activité du récepteur NMDA (Wood et coll., 1996). Les mécanismes intrinsèques de régulation du récepteur NMDA sont des processus complexes impliquant la boucle membranaire M2 de la sous-unité NR1, et sont donc sous la dépendance de la glycine (Dingledine et coll., 1999). Le récepteur NMDA permet l'entrée dans la cellule des ions sodium et la sortie des ions potassium. Il permet surtout l'entrée des ions calcium dans la cellule sous la dépendance du type de sous-unité NR2 (Burnashev et coll., 1995 ; Stern et coll., 1992). L'entrée massive du calcium permet à son tour la dépolarisation du neurone qui pourrait être modifiée après HH.

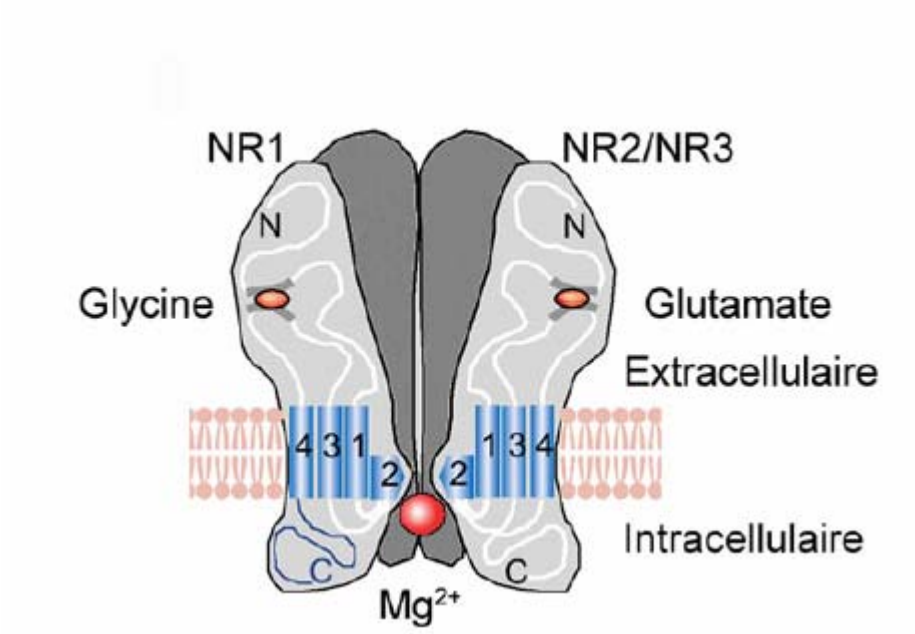


Figure 5 : Représentation schématique d'un récepteur de type NMDA composé de la sous unité NR1 possédant un site de fixation à la glycine et de sous unités NR2 (site de fixation au glutamate) et NR3. D'après Carroll et Zukin (2002) et Cull-Candy et coll. (2001).

RAPPELS SUR LES RECEPTEURS GLYCINE

Le récepteur ionotropique glycine est un récepteur pentamérique constitué de deux types de sous-unités, α et β (Langosch et coll., 1988). Le site d'action de la glycine se situe sur la sous-unité α (Leite et coll., 2000). Il a également pour agoniste la taurine et la β-alanine. La fixation de la glycine au récepteur entraîne une ouverture du canal qui permet l'entrée des ions chlore, créant une hyperpolarisation inhibitrice du neurone (Legendre, 2001).

PROJET DE RECHERCHE

Nous approfondirons nos travaux sur l'étude des neuromédiateurs médullaires en nous intéressant à leurs récepteurs. Une étude quantitative globale de ces récepteurs sera entreprise par immunoblot. Les expérimentations seront réalisées sur des fragments de moelle épinière des niveaux segmentaires L_4 et L_5 prélevés sur des rats adultes contrôles et soumis à l'HH. La surface des bandes d'intérêt sera mesurée par densitométrie. Nous nous intéresserons d'abord aux récepteurs prédominants dans la moelle épinière, c'est-à-dire les récepteurs au glutamate de type NMDA (sous unité NR1 ubiquitaire) et à la glycine. **Nous pourrons ainsi déterminer une éventuelle variation de la quantité totale de récepteurs après HH.**

Les données nécessiteront ensuite d'être affinées grâce à une seconde approche utilisant la microscopie confocale. En effet, les expérimentations menées en western blot le sont à partir de fragments spinaux qui ne contiennent pas uniquement les motoneurones innervant le muscle soleus. Pour conforter nos précédents résultats, il est donc capital de déterminer avec précision le taux exact de récepteurs présents au niveau des motoneurones soléaires. Des sections transversales de moelle épinière seront observées en microscopie confocale après un double marquage par fluorescence des motoneurones et des récepteurs. Le marquage des corps cellulaires des motoneurones d'intérêt sera effectué par injection intramusculaire d'un marqueur fluorescent (fluorogold) à transport rétrograde. La révélation du fluorogold se fera grâce à une sonde fluorescente FITC. Le marquage des récepteurs proprement dit (glycine et NMDA) sera réalisé par des anticorps primaires spécifiques eux mêmes révélés par un anticorps secondaire marqué à la rhodamine. Les résultats obtenus

nous indiqueront la localisation membranaire ou cytoplasmique des récepteurs, ce qui pourrait être un indicateur de leur fonctionnalité.

Nous nous attendons à observer dès 7 jours d'HH une augmentation de la quantité de récepteurs qu'ils soient fonctionnels (localisés dans la membrane plasmique) ou non (localisation cytoplasmique). Ceci serait en accord avec nos résultats préliminaires (Treffort et coll., 2006) sur les neurotransmetteurs qui sont surexprimés après 7 jours d'HH. Cependant la synthèse et la mise en place de nouveaux récepteurs demandent un délai plus long qu'une production accrue de neurotransmetteurs.

ETUDE DE LA PLASTICITE NEUROMUSCULAIRE DEVELOPPEE EN HYPERGRAVITE – IMPACT DES PERIODES CLES

Une connaissance approfondie de la plasticité neuromusculaire nécessite, d'une part, de pouvoir réaliser des expérimentations sur le long terme (plusieurs semaines voire plusieurs mois) et d'autre part de pouvoir étudier la mise en place de la plasticité en ayant recours à des animaux jeunes. Les animaux en développement sont en effet plus sensibles que les adultes aux variations environnementales et leur réactivité est d'autant plus forte et plus précoce.

Pour des raisons d'éthique évidentes, le modèle d'HH ne permet pas des études scientifiques selon ces deux conditions. Il est donc nécessaire, si l'on veut étudier l'impact de la gravité sur le développement neuromusculaire, de réfléchir à l'utilisation d'un autre modèle modifiant le facteur G et suffisamment non contraignant pour permettre de réaliser une étude complète des adaptations neuromusculaires du stade embryonnaire à l'âge adulte. Actuellement, le dispositif expérimental qui remplit le mieux ces exigences est la centrifugation. Ce modèle ouvre en effet des perspectives de recherche nouvelles en permettant une étude plus exhaustive des phénomènes de plasticité.

Un autre volet de nos futures perspectives de recherche sera axé sur les effets de l'hypergravité sur les systèmes nerveux et musculaire. L'hypergravité peut être considérée par bien des aspects comme le miroir de l'hypogravité. De plus, des animaux maintenus en hypergravité puis ramenés à la normogravité se trouvent ainsi paradoxalement en « hypogravité simulée », ce qui peut constituer une condition expérimentale très intéressante.

L'influence de la gravité sur l'activité locomotrice et sur les adaptations sensorimotrices a été étudiée au cours des vols spatiaux ou en utilisant des modèles animaux mimant les effets de la microgravité. Ainsi, des études utilisant le modèle d'hypodynamie-hypokinésie, ont décrit, sur des rats adultes, des mouvements locomoteurs ralentis, des hyperextensions de la cheville (Canu et Falempin, 1996), un remodelage complexe des unités motrices (Picquet et coll., 2000) et des transitions phénotypiques lent → rapide importantes (Stevens et coll., 2000 a, 2004). Des animaux plus jeunes soumis à un vol spatial du 7ème au 16ème et du 15ème au 24ème jour post-natal présentent également des altérations locomotrices telles que des hyperextensions de l'angle de la cheville et une marche sur la pointe des pattes (Walton,

1998). Lorsque les animaux sont ramenés en normogravité, la récupération est d'autant plus rapide qu'ils sont jeunes (Walton et coll., 1992).

Les adaptations du système sensorimoteur et les performances locomotrices ont également été étudiées lors de séjours en HG. Un séjour d'HG chez des rats jeunes (du 11ème jour de gestation au 15ème jour post-natal) a pour conséquence une désorganisation du système sérotoninergique : perte de synapses, innervation anarchique (Gimenez-y-Ribotta et coll., 1998). De plus une hyperactivité des neurones médullaires a été décrite du point de vue morphologique (Krasnov, 1991). Des modifications du système vestibulaire ont également été mises en évidence après HG : diminution de la membrane otolithique (Lim et coll., 1974), diminution de la taille des otoconies utriculaires (Sondag et coll., 1996) et augmentation de taille des cellules de l'épithélium sensoriel (Wubbels et coll., 2002). En gravité normale, la mise en place de la posture et de la locomotion est conditionnée par l'existence de périodes critiques. Le développement de la posture et de la locomotion débute vers le 15ème jour post-natal pour atteindre un état stable au 21ème jour post-natal (Gramsbergen, 1998 ; Gramsbergen et coll., 1999). La première semaine de vie post-natale est, de plus, déterminante pour la mise en place du contrôle postural du train arrière des rats (Brocard et coll., 1999). Il a été établi que la mise en place du contrôle postural dépend elle-même d'une part de la maturation du système vestibulaire qui a lieu entre le 8ème jour de gestation et le 15ème jour post-natal et d'autre part de l'installation de projections descendantes provenant du tronc cérébral dès les premiers jours suivant la naissance (Clarac et coll., 1998). L'existence d'une période critique péri-natale a été mise en évidence lors d'expériences de privation vestibulaire : une privation appliquée à partir du 5ème jour post-natal entraîne un retard moteur particulièrement important alors que la même privation mais appliquée à partir du 16ème jour post-natal aura peu de conséquences (Geisler et coll., 1997). Le maintien de rats en HG pour des périodes longues (depuis le stade embryonnaire jusqu'à l'âge adulte) a des répercussions significatives sur leur comportement locomoteur lorsque ces animaux sont introduits en normogravité : les rats HG adoptent une marche plus rapide que les rats contrôles. Le positionnement de ces animaux est également plus proche du sol : diminution de la hauteur du dos et de l'angle de la cheville ce qui entraîne une position de dorsiflexion plus importante. Une locomotion équivalente à celle des rats contrôles peut être mise en évidence après 28 jours de normogravité (Bouet et coll., 2004). Ceci suggère l'existence d'un mécanisme adaptatif probablement d'origine sensorimotrice. En effet la locomotion est modulée en permanence par des influx d'origine afférente. Comparativement à la gravité normale, l'HG est responsable d'une sollicitation accrue des récepteurs vestibulaires et musculo-articulaires qui sont eux-mêmes régulateurs de

l'activité locomotrice. Ainsi, d'après Bouet et coll. (2004), lorsque des animaux élevés en HG sont placés en normogravité, les influx afférents produits en 1G par ces récepteurs dont la formation a eu lieu en HG ne sont pas adaptés au schéma locomoteur qui a été lui-même acquis en HG. Le système vestibulaire est particulièrement sensible au moindre changement de gravité. Il a été démontré qu'un développement précoce en HG induisait des modifications de la structure (taille des otoconies) du système vestibulaire (Sondag et coll., 1996). On peut donc supposer qu'une diminution de la gravité (passage de 2G à 1G) va provoquer une réduction de l'activation des motoneurones des muscles extenseurs via le faisceau vestibulo-spinal. Il en résultera une modification de l'activation musculaire avec une accentuation des fléchisseurs par rapport aux extenseurs. Un tel phénomène a déjà été mis en évidence lors de séjour de rats en microgravité (Clément et coll., 1984). Ceci pourrait expliquer la posture plus fléchie et plus proche du sol des rats à la sortie de la centrifugeuse.

Les altérations de la locomotion s'accompagnent de modifications musculaires. Ainsi, il a été mis en évidence après microgravité une relation entre un allongement de la durée du pas et une activation plus durable du muscle soleus (Canu et Falempin, 1998). On peut donc supposer que la réduction de la durée du pas après l'HG (mise des rats à 1G) est la conséquence d'une activation moindre du muscle soleus. En effet, à 2G, les muscles extenseurs sont très sollicités puisque dans ces conditions, le poids perçu est le double du poids réel. Chez des rats conçus, nés et élevés en HG, ceci peut être à l'origine de l'acquisition d'un phénotype totalement lent du muscle soleus extenseur de la cheville (Picquet et coll., 2002). Lorsque ces animaux sont amenés en normogravité, le phénotype du muscle soleus reste inchangé même après 4 mois à 1G (Picquet et coll., 2005).

L'ensemble de ces travaux suggère qu'il existe au cours des développements pré- et post-natal des périodes clés. Tout processus de maturation ayant eu lieu au cours de ces périodes critiques et avec des conditions environnementales bien définies peut s'avérer être irréversible.

A présent, compte-tenu des données de la littérature, trois périodes critiques peuvent être proposées :

- la maturation du système vestibulaire, du 8ème jour de gestation au 5ème jour post-natal,
- l'installation de l'innervation, du 11ème jour de gestation au 15ème jour post-natal,
- la mise en place de la locomotion, du 15ème au 21ème jour post-natal.

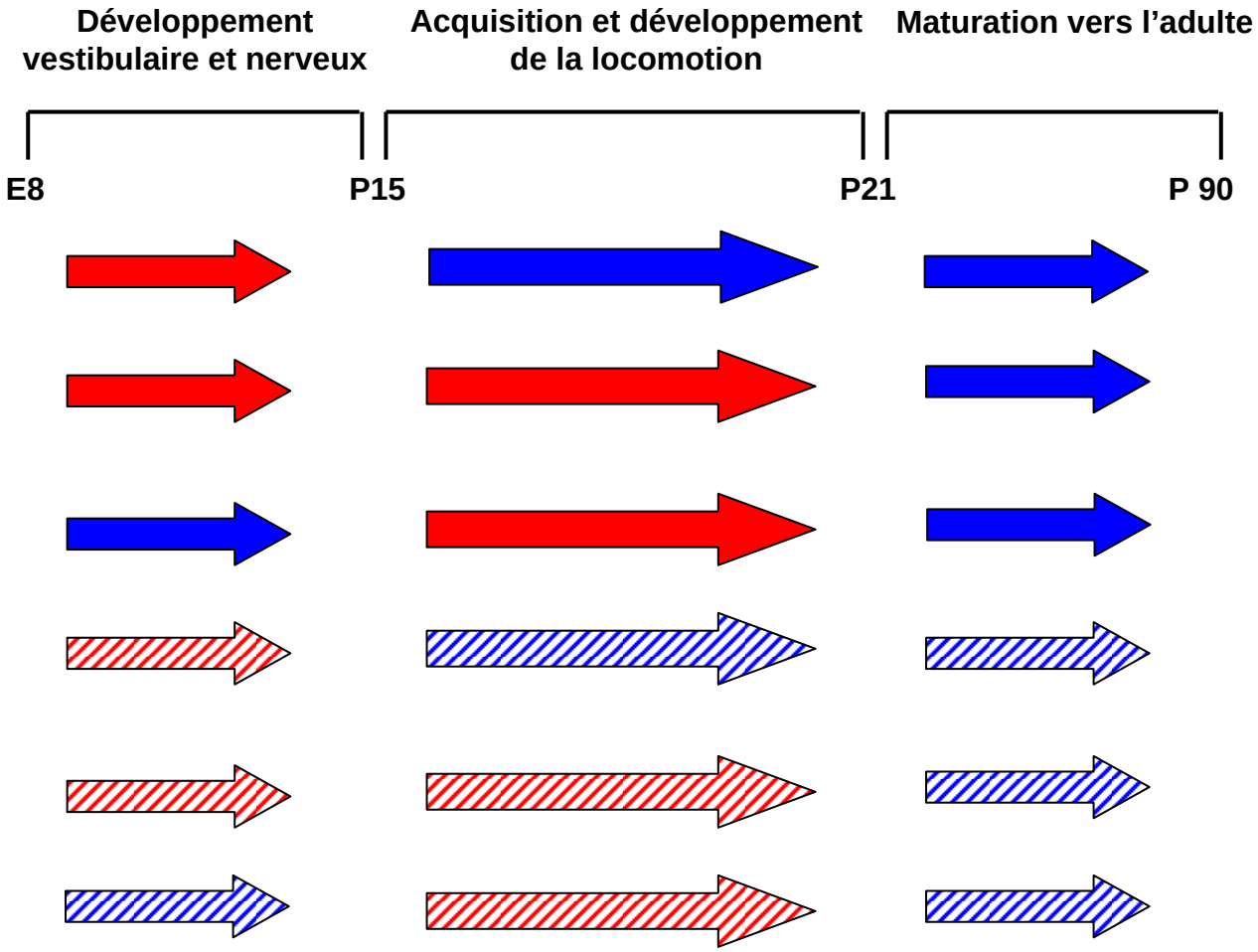
Le développement de ces systèmes est fortement influencé par le niveau de gravité.

PROJET DE RECHERCHE

Nos précédents travaux nous ont permis d'établir qu'une croissance embryonnaire et post-natale en HG affectait profondément le système musculaire (Picquet et coll., 2002).

Notre objectif sera de déterminer si un développement (pré- et post-natal) en HG au cours de l'une et/ou l'autre de ces périodes peut affecter de manière définitive ou non le système neuromusculaire.

Pour répondre à cette problématique, nous nous proposons de conjuguer hypergravité et périodes critiques du développement pré- et post-natal. Plusieurs séries expérimentales seront réalisées comme exposées dans la figure suivante.



Les flèches en bleu indiquent des séjours en normogravité, celles en rouge des séjours en hypergravité. Les animaux seront répartis différemment suivant les périodes clés du développement, depuis le 8ème jour embryonnaire (E8) jusqu'au 90ème jour post-natal (P90).

Une évaluation de l'importance des transformations musculaires (mesure de la force musculaire, identification des isoformes de protéines contractiles) et nerveuses (mesure du message efférent et afférent) sera réalisée à divers stades caractéristiques de la croissance de l'animal : après installation des systèmes nerveux et vestibulaire (P15 : 15ème jour post-natal) au sevrage, étape qui correspond également à l'acquisition de la locomotion (P21 : 21ème jour post-natal) et à l'âge adulte (P90 : 3ème mois post-natal). Nous pourrons ainsi vérifier si l'HG est en mesure de modifier durablement le système neuro-musculaire.

A plus long terme, en cas d'existence avérée d'une plasticité précoce en HG, nous pourrons compléter nos études sur les neurotransmetteurs médullaires et leurs récepteurs en

étudiant par des techniques de patch-clamp l'activité de ces récepteurs sur tranches de moelle épinière. Cette technique nécessite pour des raisons de survie cellulaire d'avoir recours à des animaux extrêmement jeunes, âgés au maximum de deux semaines. L'intérêt scientifique résidera dans le fait que leur développement n'aura pas eu lieu en normogravité. L'HH ne permet pas en effet ce type d'étude, ce qui constitue un facteur limitant pour l'avancée de notre problématique. Le modèle d'HG dans ce cas de figure sera donc très approprié, et les résultats obtenus seront sans nul doute d'un intérêt significatif pour le développement de notre thématique de recherche.

Enfin, l'utilisation de modèles murins présentant une déficience de l'appareil vestibulaire (flèches rayées) élevés dans les mêmes conditions que les animaux contrôles permettra de distinguer la part du système nerveux de celle du système vestibulaire dans les altérations neuromusculaires observées à différents stades du développement.

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ANNEXES

Pflügers Arch – Eur J Physiol (2000) 440:229–235
Digital Object Identifier (DOI) 10.1007/s004240000288

ORIGINAL ARTICLE

F. Picquet · M.H. Canu · M. Falempin

Phenotypic changes in the composition of muscular fibres in rat soleus motor units after 14 days of hindlimb unloading

Received: 10 November 1999 / Received after revision: 3 February 2000Accepted: 15 February 2000 / Published online: 14 April 2000

Abstract The main goal of this study was to identify the different fibre types of the motor units (MUs) contained in the soleus muscles from control (CONT) rats and from rats submitted to 14 days of hindlimb unloading (HU). The MU types were classified according to their contractile properties and also using glycogen depletion followed by adenosine triphosphatase (ATPase) staining. In CONT rats, the soleus muscle contained two MU types identified as slow and fast types. After HU, the MU distribution showed three populations: slow, intermediate and fast. All the MUs from HU soleus were heterogeneous in terms of fibre type composition, indicating a complex remodelling of the muscle.

Key words Motor units · Soleus · Hindlimb unloading · Rat · Glycogen depletion

Introduction

During hindlimb unloading (HU), many morphological, histochemical and biochemical properties of the postural hindlimb muscles such as the soleus are modified (for review see [10]). A period of HU induces marked atrophy, alterations in the metabolic enzyme profiles and redistribution of the histochemically defined fibre types towards faster populations (for review see [21]). These slow-to-fast transitions are related to changes in contractile proteins such as myosin heavy chain (MyHC) isoforms [23]. Moreover, recent electrophoretic techniques have demonstrated that, in addition to decreased amounts of slow MyHC and increased amounts of fast MyHC, soleus muscles from unloaded rats also express small proportions of MyHC IIX [19]. Changes also occur in the contractile characteristics. For example, in whole muscles, it is commonly recognized that the slow-twitch soleus muscles acquire faster contractile properties [11]. Only few studies have been performed on the modifications in the mechanical properties of the soleus motor units (MUs) after HU. In our laboratory, we have established previously that, in this situation, some MUs express contractile parameters intermediate between those usually described for the slow and fast type MUs [14].

Therefore, as no information existed about the phenotypic modification in the neuromuscular units, the main objective of our study was to demonstrate the effects of a HU period on the MU composition in the rat soleus muscles. The soleus MUs, both in control and in HU rats, were characterized according to their contractile parameters and their composition in muscular fibre types, using glycogen depletion and adenosine triphosphatase (ATPase) histochemistry. The motor axon conduction velocity (CV) in the MUs was also measured.

Our main results demonstrated that, after HU, all the MU types were heterogeneous with respect to their fibre type composition.

Materials and methods

Animal groups

Male Wistar rats weighing 280–300 g were divided randomly into two groups: control (CONT, n=11) and HU (n=34). HU was performed for a duration of 14 days employing the tail suspension model of by Morey [16]. A 14-day period of HU is sufficient to induce maximal muscular transformations in terms of mechanical [11] and histochemical properties [17]. All the experiments as well as the maintenance conditions of the animals were approved by both the Agricultural and Forest Ministry and National Education Ministry (Veterinary service of health and animal protection: authorization 03805).

Surgical technique and physiological measurements

The surgical technique has been described previously [14]. The muscle length was adjusted to produce the maximum twitch tension. A single MU was isolated by microdissection and splitting of ventral root L5 into fine filaments. To avoid erroneous depletion

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of non-unit muscle fibres, only one MU was studied per muscle, either in CONT or HU animals. Contraction of the MUs was induced by stimulation of the ventral root filament. The motor action potential in the muscle nerves was recorded via a monopolar platinum electrode and amplified 20,000 times (Grass P511, Quincy, Mass., USA). The recording site on the soleus nerve was 5 mm from the nerve entry in the muscle. The reference electrode was inserted into neighbouring denervated muscle mass. The electromyographic (EMG) activity was recorded using the method described [14]. The criteria for single unit activity were the all-or-nothing response for the action potential in the muscle nerve, the EMG activity and the twitch tension. The MUs were stimulated so as to record the following: (1) a single twitch, from which the maximal twitch force (P_t), the time-to-peak (TTP) and the half-relaxation time (HRT) were measured; (2) the tension/frequency relationship [14] which allowed the determination of the maximum tetanic tension (P_0) and the index P_{20}/P_0 (ratio of the tetanic tension at 20 Hz to P_0). MUs were classified as slow-fatigue resistant (S), or fast-fatigue resistant (FR), according to the TTP, the fatigue index (FI, >0.75 for S and FR MUs) and the presence or absence of a sag [3]. MUs presenting a sag at 16 and 20 Hz but not at 30 Hz were classified as intermediate type (INT).

After the fatigue test, the motor axon CV was measured. It was calculated as the quotient of the conduction distance and conduction time, measured between the stimulation artefact and the beginning of the nerve action potential. The conduction distance was measured, after post-mortem dissection, between the ventral root filament on the electrodes and the electrode position on the soleus nerve.

Glycogen depletion protocol

We glucose-loaded the animals by giving them 5% glucose in water as their drinking fluid for the 5 days before the experiment, to increase the contrast between glycogen-depleted fibres from one tetanically stimulated MU and non-depleted fibres from all other MUs. In each muscle, one MU was isolated and its contractile parameters measured. The stimulation protocol, which was started about 1 h after the end of MU characterization to avoid erroneous depletion, was that of Kugelberg [12]. Glycogen in the MUs was depleted by stimulation during ischaemia produced by clamping the popliteal artery. The MU was stimulated at 40 Hz in 500-ms trains once per second until tetanic tension fell to zero. Clamping was thereafter terminated and the unit was stimulated with the 500-ms impulse-trains once every 10 s until tetanic tension had recovered. The protocol was repeated 3–5 times. Immediately after cessation of the glycogen depletion, the soleus muscles were removed quickly, weighed, mounted in embedding medium (TEK ACT Compound, Labonord, Wavrin, France), frozen in isopentane precooled in liquid nitrogen and stored at –80 °C until analysis.

Muscle histochemistry

Serial frozen sections (10 and 20 µm thick) were cut on a cryostat at –20 °C. The 10-µm sections were stained for myofibrillar ATPase activity with acid (pH 4.3; 4.45) and alkali (pH 10.4) preincubation. The histochemical method previously described [22] allowed the characterization of types-I (slow), -IIA (fast) and -IIC (intermediate) soleus fibres. The 20-µm section was used for the periodic acid-Schiff (PAS) stain. Glycogen-depleted muscle fibres were identified as negatively stained fibres using the PAS technique [20]. The cross-sectional area (CSA) of each fibre type was measured in whole muscles (in 100 non-depleted fibres) and in the depleted MUs, using an image analyser (SAMBA 2005, Alcatel, Grenoble, France).

Ultrastructural techniques

After physiological measurements, the right soleus nerve of each studied rat was excised (~5 mm long). The nerves were immersed immediately in a phosphate-buffered saline solution (PBS 0.1 M, pH 7.0) containing 2.5% glutaraldehyde for 2 h at room temperature. After fixation, the samples were washed in 0.1 M PBS overnight at 4 °C. The soleus nerves were then post-fixed in a 1% phosphate-buffered osmium tetroxide for 2 h at room temperature, dehydrated in increasing concentrations of acetone and embedded. Ultrathin cross-sections (70 nm) were cut perpendicular to the long axis of the nerves, using an ultratome (LKB ultratome III 8800). Sections were studied after staining with uranyl acetate and lead citrate. The ultrathin cross-sections were examined under a JEOL 100 CX transmission electron microscope. For each myelinated fibre, the CSA of both the nerve fibre (maximum surface area of the fibre) and the myelin wall (obtained for each fibre by subtraction of the internal surface area from the maximum surface area) were measured using an image analyser (SAMBA 2005).

Statistical analysis

After ANOVA, the Bonferroni test was used to establish the significance of inter-group differences. Differences were considered significant at $P<0.05$. Results are expressed as mean±SD.

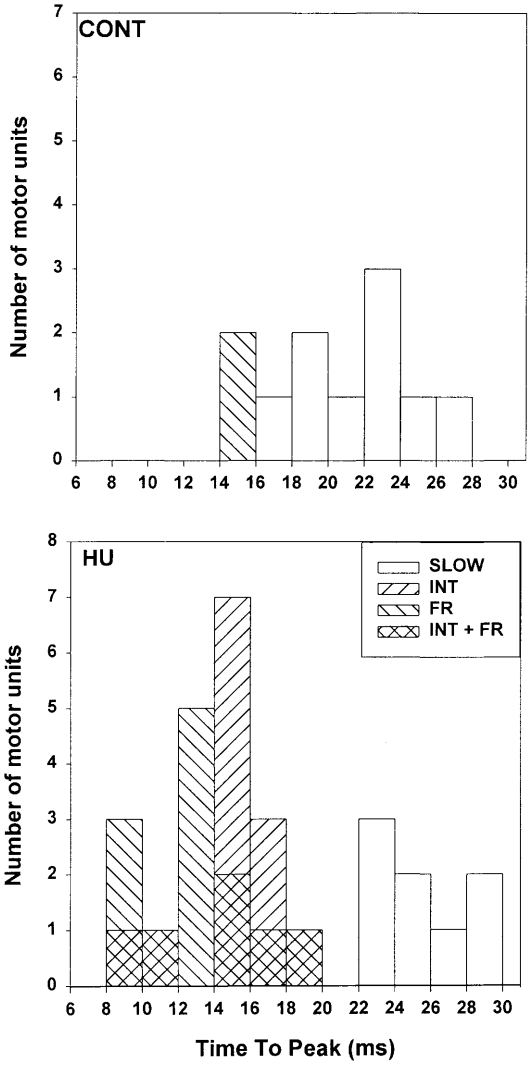

Fig. 1 Distribution histograms of the time-to-peak (TTP, in milliseconds) for each studied motor unit (MU) in control (*CONT*) and hindlimb-unloaded (*HU*) groups. The *crossed hatching* (*INT+FR*) indicates that intermediate and fast motor units had similar TTP values

Results

Body and muscle weights

After 14 days of HU, the soleus muscle mass decreased significantly from 151±4.6 (CONT, *n*=11) to 91±3.4 mg (HU, *n*=34). There was no significant difference between the body weights of the HU and CONT rats. The ratio muscle weight/body weight was therefore diminished significantly: 0.50±0.01 mg g^{-1} in CONT rats vs. 0.31±0.01 mg g^{-1} in HU rats.

Histochemical and morphometric analysis in whole muscles

The CSA of each fibre type was measured in the whole muscles (100 non-depleted fibres per type) and in all the depleted MUs. Fibre-type changes were monitored by ATPase staining. In the CONT group, the soleus muscles contained 80.7±5.3% type-I, 6.5±2.5% type-IIC and 12.8±3.6% type-IIA fibres. After HU, the composition in fibre types in the soleus muscles showed a significant decrease in type-I (67.8±1.3%) and a significant increase in both types-IIC (16.9±2.7%) and -IIA (15.3±2.4%) fibres. The HU period induced a significant decrease in the CSA of all three fibre types when compared with those expressed by the CONT rats. In the CONT soleus muscles, the mean CSAs were: 3269±241, 3370±289 and 3070±193 μm² for types-I, -IIC and -IIA fibres respectively, whereas in the HU rats CSAs were: 1798±124, 1854±152 and 1700±178 μm² respectively. No significant difference in CSAs was detected between the non-depleted and the depleted fibres in the MUs.

MU classification and contractile properties

The MU composition in the soleus muscles, expressed as a function of the twitch TTP is illustrated in Fig. 1. According to the classification of Burke et al. [3], we identified nine S-type MUs in the control soleus, characterized by the absence of a sag and by a TTP of more than 16 ms, and two FR-type MUs (sags at 16, 20, 30 and 40 Hz, TTP<16 ms). Following 14 days of HU, among the 34 studied MUs we identified 8 S-type MUs (23.50%) and 13 FR-type MUs (38.25%). One new population of MUs, never found in CONT muscles, appeared in the HU soleus muscles: 13 MUs (38.25%) of the INT-type had a sag at 16 and 20 Hz but not at 30 Hz, and a TTP closer to the fast-type MU.

Contractile properties of MUs from CONT and HU groups are reported in Table 1 . In CONT muscles, FR-type MUs were characterized by higher P_t, P_0 and P_t/P_0 values and by lower P_{20}/P_0 and TTP values compared with S-type MUs. When compared with control animals, the HU period induced, both for S-type and FR-type MUs, a decrease in P_t and P_0 values and an increase in the ratio P_t/P_0. The ratio P_{20}/P_0 increased only for the FR-type MUs. Other contractile parameters remained unchanged.

FR-type motor units were characterized by lower P_t, P_t/P_0, TTP, HRT and FI values than those expressed by slow MUs in HU soleus muscles. The INT-type MUs presented a P_t value significantly lower than the other populations of MUs expressed in the HU rats, whereas the values of P_0, TTP, HRT and FI were intermediate between those usually characterizing slow and fast-type MUs.

The P_{20}/P_0 values obtained in S-type MUs (~75%) from CONT and HU rats, and in FR-type MUs from CONT (~29%) and from HU (~32%) rats were close to those usually described in slow and fast whole muscles, whereas the INT-type MUs showed a P_{20}/P_0 ratio (~55%) intermediate between S- and FR-type MUs.

Table 1 Contractile properties and axon conduction velocity (*CV*) from control (*CONT*) and hindlimb unloading (*HU*) motor unit types. Means±SD (P_t maximum twitch force, P_0 maximum tetanic tension, P_t/P_0 ratio of P_t to P_0, P_{20}/P_0 ratio of tetanic tension at 20 Hz to P_0, *TTP* time-to-peak, *HRT* half-relaxation time, *FI* fatigue index)

Parameter	Group	Motor unit type		
		Slow	Intermediate	Fast
P_t (mN)	CONT	11.36±0.38	–	18.20±0.15*
	HU	9.36±0.07*	8.20±0.34§	8.46±0.35*§
P_0 (mN)	CONT	61.60±0.09	–	95.68±0.01*
	HU	24.23±0.01*	26.78±0.04§	27.84±0.03*§#
P_t/P_0 (%)	CONT	18.44±0.04	–	19.02±0.02*
	HU	38.62±0.02*	30.61±0.08§	30.38±0.09*§#
P_{20}/P_0 (%)	CONT	75.77±0.06	–	29.50±0.12*
	HU	75.75±0.06	55.15±0.19§	32.62±0.09*§#
TTP (ms)	CONT	22.52±2.54	–	15.40±0.40*
	HU	25.42±2.83	14.72±2.50§	12.84±2.89§
HRT (ms)	CONT	29.25±7.96	–	21.86±7.07
	HU	34.91±3.91	26.31±7.00	18.23±4.81§#
FI (%)	CONT	92.85±5.27	–	91.03±5.03
	HU	94.20±1.29	92.30±7.46	86.18±11.08
CV (m s^{-1})	CONT	28.40±3.09	–	24.58±1.41
	HU	20.95±2.29*	24.36±2.12§	25.66±3.70§

*P<0.05 vs. CONT; §P<0.05 vs. slow HU motor units; #P<0.05 vs. intermediate HU motor units

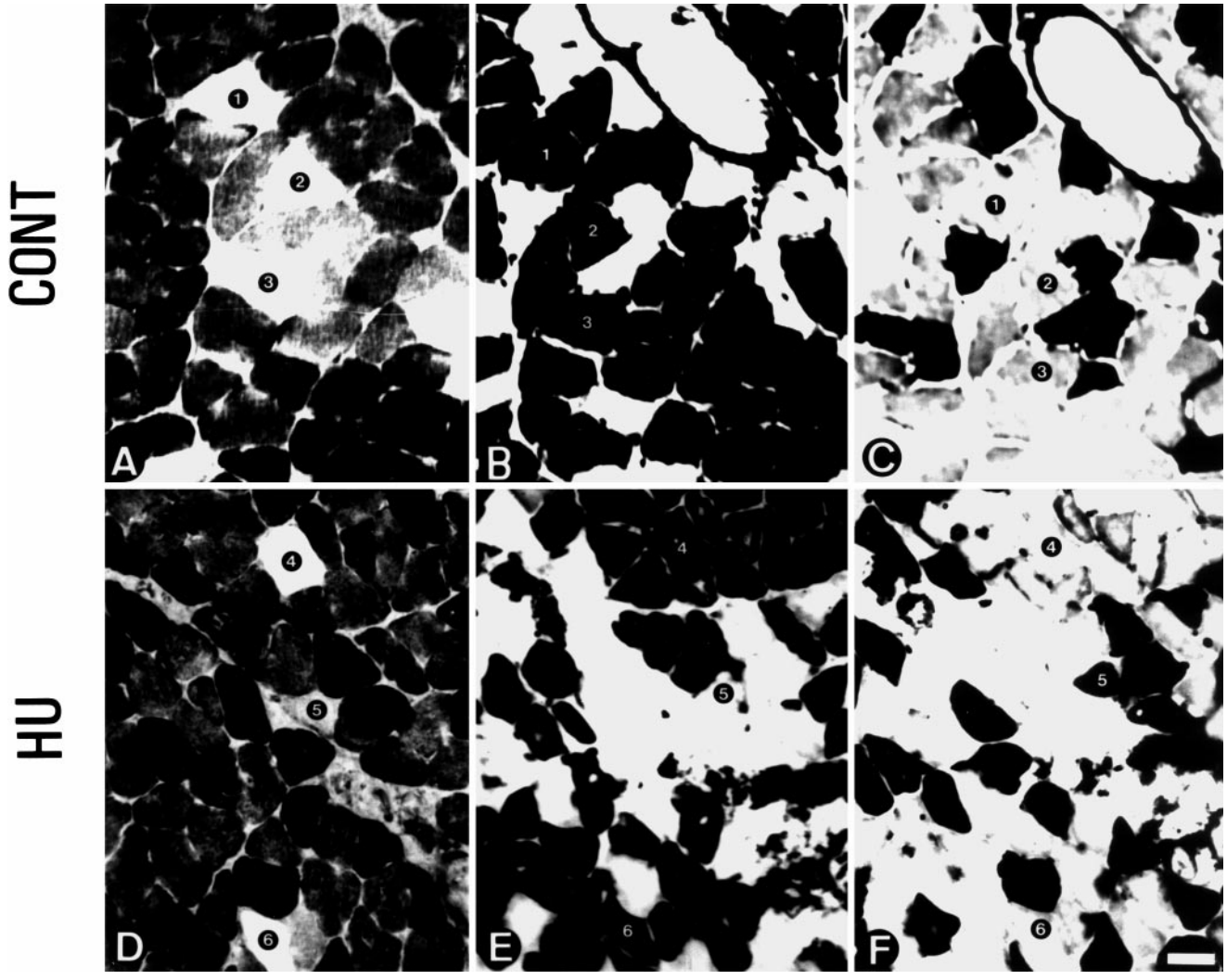

Fig. 2A–F Periodic acid-Schiff (PAS) reaction and histochemistry illustrated in slow-type MUs from CONT (**A**–**C**) and HU (**D**–**F**) rat soleus muscles. Depleted fibres were identified in PAS-stained sections (**A**, **D**) followed by preincubation at pH 4.3 (**B**, **E**) or 10.4 (**C**, **F**). Note in **B** and **C** the presence of a capillary not observed in **A**. The *numbers* indicate fibres referred to in the text. The *scale bar* represents 70 µm for **A**–**C** and 60 µm for **D**–**F**

Composition of the MUs

The fibre types contained in each MU were determined by PAS staining followed by ATPase staining. Figure 2 shows depleted S-type MU in CONT (panels A–C) and HU (panels D–F) muscles. The depleted fibres were characterized in serial sections using the PAS reaction (A, D) followed by acid (B, E) or alkaline (C, F) preincubation. In CONT muscles, the fibres labelled 1–3 were identified as slow type I; in HU muscles, the fibres labelled 4 and 6 were identified as type I, and fibre 5 as type IIC. There was no significant difference in the MU size after HU, since the average size of MUs was 78±15 fibres in CONT muscles and 83±18 in HU muscles. However, to normalise the data relative to the different fibre types contained in each MU, we expressed each different type as a percentage of the total number of fibres contained in the MUs.

The composition of MUs in fibre types is given in Fig. 3. In the CONT muscles, the MUs classified as S-type were characterized by a homogeneous composition since all identified fibres were slow type-I, whereas the FR-type MUs had 55% type-I, 8% type-IIC and 37% type-IIA fibres. In the HU muscles, all the MUs were heterogeneous in their fibre type composition. Indeed, in the HU rats, the MUs classified as S-type according to their contractile parameters (n=8) were composed of 95% type-I and 5% type-IIC fibres. No type-IIA fibres were detected in HU slow MUs. The other two MU populations were identified as FR-type (n=13) consisting of 16% type-I, 8% type-IIC and 76% type-IIA fibres, and as INT-type (n=13) made up of 87% type-I, 10% type-IIC and 3% type-IIA fibres.

Axon CV and ultrastructural data

The axon CV was measured for each MU studied in the CONT and HU groups. The values are reported in Ta-

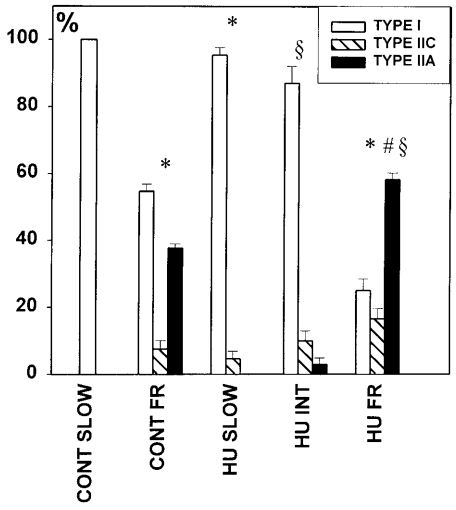

Fig. 3 Composition in fibre types of motor units in CONT and HU muscles. The MU types were determined according to their functional characteristics. The histochemical proportions in fibre types are expressed as mean±SD. **P*<0.05 vs. CONT; §*P*<0.05 vs. slow HU MUs; #*P*<0.05 vs. intermediate HU MUs

ble 1. After HU, the mean CV decreased significantly for S-type MUs compared with CONT MUs. The mean CV of INT-type MUs was significantly different from that of S-type MUs and was closer to that of FR-type MUs.

Figure 4 shows electron micrographs of ultrathin transverse sections of the soleus nerve in CONT (Fig. 4A) and HU rats (Fig. 4B). A reduction in fibre CSA was observed in the HU nerve. The CSAs of both the fibres of the soleus nerve and the myelin were measured in CONT and HU rats. Using Gauss's law, we were able to identify two populations of nerve fibres in CONT nerves according to their mean CSA: 18.8 μm^2± 8.7 in 52±13 fibres and 49.2 μm^2±9.3 in 57±15 fibres. Similarly, the histograms of myelin wall surface clearly showed a bimodal distribution for CONT rats with peaks at 12.3 μm^2±5.0 and 30.60 μm^2±7.2. After 14 days of HU, the frequency distribution of nerve fibres and myelin areas remained bimodal. The mean fibre CSA (16.2±7.6 and 45.1±8.7 μm^2) and the myelin areas were 15% and 5% respectively smaller than those determined in the CONT nerves, although the changes were not significant.

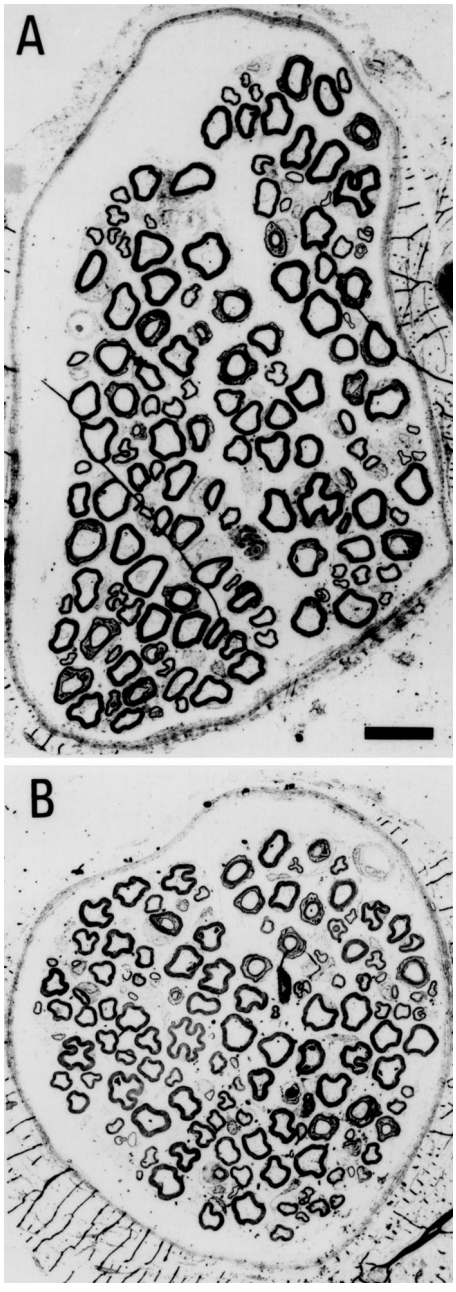

Fig. 4A, B Electron micrographs of transverse ultrathin sections of the soleus nerves from CONT (**A**) and HU (**B**) rats. The *scale bar* represents 15 µm

Discussion

Our results on morphological and functional modifications in MUs are similar to previous data [14]. As the results of the mechanical properties have already been discussed in that report, we have voluntarily chosen to focus our attention on the MyHC transitions within MUs.

Glycogen depletion followed by ATPase staining was used to show the different fibre types expressed in each MU. In the CONT rats, the MUs identified as S-type by their contractile parameters were homogeneous and contained only type-I fibres, in agreement with the literature [6, 13, 15, 18]. On the other hand, the FR-type MUs in CONT muscles were heterogeneous since they contained a mixture of types-I, -IIC and -IIA fibres, with a predominance of type I. This heterogeneity within FR-type MUs presumably represents a step towards a slower phenotype occurring during muscle maturation. Indeed, fast fibres expressing MyHC IIA in the rat soleus muscle are progressively transformed throughout muscle maturation into slow fibres expressing exclusively MyHC I [1, 4, 12]. We have proposed that, during muscle maturation,

these FR-type MUs acquire progressively a slower typing characterized by an increase in the proportion of slow fibres at the expense of fast fibres, following the sequence: type IIA → type IIC → type I [1]. Moreover, previous studies performed on single fibres have demonstrated the existence of hybrid fibres in the soleus muscle expressing both slow and fast MyHC isoforms in various proportions, their contractile behaviour being dictated by the predominant MyHC isoform. Thus, the fibres designated as hybrid slow or hybrid fast as a function of their predominant MyHC isoform, present contractile characteristics similar to those of pure slow or fast fibres [7]. In the FR-type MUs, fibres identified as type IIC contained MyHC I and MyHC IIA with MyHC IIA predominant [24], and we suggest that their contractile behaviour is identical to those of pure IIA fibres. This MU type, which was not the FR-type MU classically defined, shows nevertheless fast contractile properties probably due to the presence of types-IIC and -IIA fibres.

HU induced marked changes in the fibre composition of the MUs. Indeed, the MUs identified as S- and FR-types on the basis of their contractile parameters, expressed, respectively, types-I and -IIC fibres (predominance of type I) and types-I, -IIC and -IIA fibres (predominance of type IIA). In these two MU types, it seems that it is the predominant fibre type that dictates the functional MU type. The INT-type MUs, only detected after HU, expressed a large majority of type-I fibres and showed both types-IIC and -IIA fibres. Although the contractile properties of these MUs mostly resembled those of the FR-type MUs, they had CV and P_{20}/P_0 values intermediate between those of S-and FR-type MUs, indicating that they constituted really a new MU type. In this MU type, the predominant fibre type (type I) was not present in sufficient numbers to impose its contractile characteristics. The MU profile seemed to be dictated only by types-IIC and -IIA fibres. Moreover, the existence of other fast MyHC isoforms, like MyHC IIB and MyHC IIX, expressed in low proportions (i.e. undetectable using histochemistry) in types-IIC and -IIA fibres, is also conceivable. Indeed, fast MyHC IIB and IIX isoforms have been identified in HU soleus muscles [23].

The appearance of heterogeneous MUs with fast contractile parameters after HU probably represents a transitional state towards a fast type in the soleus muscles. Thus, the number of FR- and INT-type MUs increased concomitantly with the increase in fast type fibres detected by histochemistry in the whole soleus muscles. We suggest that the soleus fibres, identified as slow or intermediate after HU, express predominantly slow MyHC isoforms under control conditions. Indeed, several studies have demonstrated that, in adult muscles, most fibres derived from the secondary myotube generation express fast MyHC isoforms [9]. Throughout postnatal life, the fibres that express fast MyHC isoforms in the early stages of foetal and postnatal development, acquire progressively a slow profile after the elimination of fast MyHC isoforms (IIA, IIX and IIB) due to repression of the corresponding genes [9]. Previous studies [4, 5] have demonstrated that innervation is required to induce and maintain the expression of slow genes. There is, thus, evidence that, in a given soleus MU, some fibres are able to induce a re-expression of fast MyHC isoforms whereas the rest continue to express the slow MyHC isoform: the MU in question appears heterogeneous. Furthermore, the fibres called type IIA still contain a mixture of fast and slow MyHC isoforms, the relative expression of the latter being low.

Another explanation could be also suggested for the heterogeneity within a MU. After HU, there is an alteration or a modification in the soleus motor nerve message, since a change of the EMG activity from a tonic state to a phasic-like activity has already been described [2]; this transition is probably discerned differently by the muscle fibre types. The transmission of motor impulses at individual motor end-plates within MU should be also significantly altered after tail suspension since it has been demonstrated that the motor end-plates undergo structural changes after 7 days HU [8]. In the present study there were morphological and functional changes in nerve fibres following tail suspension. All or part of these modifications may contribute to the appearance of heterogeneous MUs.

Acknowledgements This work was supported by grants from the CNES (3027), from the Conseil Régional du Nord Pas-De-Calais and from the Fonds Européen de Développement Régional (F 007). The authors wish to thank Mr Loic Brunet from the Centre Commun de Microscopie Electronique et d'Imagerie Cellulaire (Université de Lille 1, 59655 Villeneuve d'Ascq, France) for his technical assistance in ultrastructural techniques.

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J Neurophysiol 89: 442–449, 2003;
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Characterization of Spindle Afferents in Rat Soleus Muscle Using Ramp-and-Hold and Sinusoidal Stretches

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Submitted 4 March 2002; accepted in final form 11 September 2002

De-Doncker, Laurent, Florence Picquet, Julien Petit, and Maurice Falempin. Characterization of spindle afferents in rat soleus muscle using ramp-and-hold and sinusoidal stretches. *J Neurophysiol* 89: 442–449, 2003; 10.1152/jn.00153.2002. The discharge properties of 51 afferents were studied in the rat soleus muscle spindles. Under deep anesthesia using a pentobarbital sodium solution (30 mg/kg), a laminectomy was performed and the right L_4 and L_5 dorsal and ventral roots were transected near their entry into the spinal cord. In situ, the minimal (L_{min}) muscle length [3 ± 0.08 (SE) cm] of the soleus was measured at full ankle extension. Unitary potentials from the L_5 dorsal root were recorded in response to ramp-and-hold stretches applied at 3 mm (S3) and 4 mm (S4) amplitudes and four stretch velocities (6, 10, 15, and 30 mm/s), sinusoidal stretches performed at four amplitudes (0.12, 0.25, 0.5, and 1 mm) and six stretch frequencies (0.5, 1, 2, 3, 6, and 10 Hz), and vibrations applied at 50-, 100-, and 150-Hz frequencies. These two kinds of stretches were performed at three different muscle lengths ($L_{min+10\%}$, $L_{min+15\%}$, and $L_{min+20\%}$), whereas vibrations were applied at $L_{min+20\%}$ muscle length. Conduction velocity of the fibers was calculated but did not allow to discriminate different fiber types. However, the mean conduction velocity of the first fiber group (43.3 ± 0.8 m/s) was significantly higher than that of the second fiber group (33.9 ± 0.9 m/s). Three parameters allowed to differentiate the responses of primary and secondary endings: the dynamic index (DI), the discharge during the stretch release from the ramp-and-hold stretches, and the linear range and the vibration sensitivity from sinusoidal stretches. The slope histogram of the linear regression based on the DI and the stretch velocity was clearly bimodal. Therefore the responses were separated into two groups. During the stretch release at a velocity of 3 mm/s, the first response group (n = 26) exhibited a pause, whereas the second (n = 25) did not. The linear range of the second ending group (0.12–1 mm) was broader than that of the first (0.12–0.25 mm). The first ending group showed a higher sensitivity to high-vibration frequencies of small amplitude than the second. In comparison with the literature, we can assert that the first and the second ending groups corresponded to the primary and secondary endings, respectively. In conclusion, our study showed that in rat soleus muscle spindles, it was possible to immediately classify the discharge of Ia and II fibers by using some parameters measured under ramp-and-hold and sinusoidal stretches.

INTRODUCTION

The postural and locomotion controls are complex and require different proprioceptive and exteroceptive receptors. In the literature, among all these receptors, the muscle spindle plays a fundamental role (McCloskey 1978; Proske et al. 2000). This stretch receptor is inserted in parallel with the extrafusal fibers (for review, see: Hunt 1990; Proske 1997). Each muscle spindle consists of a hyaluronic acid fluid-filled capsule. Inside, there is a small number of modified muscle fibers, called intrafusal fibers, each having two contractile ends, the poles, and an equatorial region almost devoid of myofibrils and containing myonuclei (Banks et al. 1982; Hulliger 1984). Two main types of intrafusal fibers are identified: the nuclear chains and the nuclear bag fibers (bag1 and bag2) differing in the way in which the nuclei are distributed, their diameters and cross sectional areas, and their immuno-histochemical properties. Two kinds of endings innervate the muscle spindle: the primary and the secondary endings are supplied in the cat by Ia and II fibers, respectively. These fibers exhibit different responses to imposed ramp-and-hold stretch (Cheney and Preston 1976a,b; Crowe and Matthews 1964; Jami and Petit 1979; Matthews 1963). The discharge of Ia fibers indicate both the muscular length changes (static sensitivity) and the velocity of length changes (dynamic sensitivity), whereas the discharge of II fibers provide mainly information about the length changes (McCloskey 1978). The sensitivity of primary and secondary endings to dynamic and static changes in muscle length are controlled by two types of motor neurons from the CNS: the fusimotor neurons (γ neurons) and the skeletofusimotor neurons (β neurons) (Banks 1994; Hunt 1990; Petit et al. 1999; Proske 1997).

The literature reporting on morphological (for review, see Arbuthnott et al. 1989; Hulliger 1984; Maier 1997), histochemical, and immunohistochemical (Pedrosa-Domellöf et al. 1991; Soukup et al. 1995) properties of rat muscle spindles is quite profuse. The data obtained in rat are in agreement with those obtained in the cat. However, although the discharge characteristics of the Ia and II fibers have been extensively studied in cat (Hunt 1990; Matthews 1972) and primate (Cheney and Preston 1976a,b), very few data are available on the discharge of rat muscle spindle afferents (Andrew et al. 1973; Hnik et al. 1977; Miwa et al. 1995). This is highly regrettable, because the rat model has been extensively used in studies based on neuromuscular disuse or training, the characteristics of rat spindle afferents being, however, less substantiated than in other spe-

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cies. Therefore the aim of our study was to determine unequivocal criteria to classify the afferent responses from passive soleus muscle spindle (i.e., without fusimotor outflow) of control rats into primary and secondary endings responses. To carry out the present work, the discharge characteristics of rat muscle spindle afferents were analyzed with ramp-and-hold stretches at different velocities and amplitudes and with sinusoidal stretches at different amplitudes and frequencies. Several parameters already defined and measured in cat (Hulliger et al. 1976; Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1978; Matthews and Stein 1969) and primate (Cheney and Preston 1976a,b) were used. However, in the literature, no criteria allow to classify unambiguously the discharges of Ia and II fibers, and several parameters are often used to perform this classification. Thus the aim of this work was to determine criteria allowing to classify easily and unequivocally the discharges of the Ia and II fibers of rat soleus muscle spindles.

METHODS

Animals

Experiments were performed on 15 male Wistar rats (IFFA CREDO, L'Arbresle, France) weighing 280–300 g. All the rats were housed individually in separate cages. They were maintained at a temperature of 25 ± 1°C with a 12/12 h circadian cycle. All the experiments as well as the maintenance conditions of the animals received authorizations from both the Agricultural and Forest Ministry and National Education Ministry (Veterinary Service of Health and Animal Protection: authorization 59-00980).

Surgical technique

Each rat was anesthetized with intraperitoneal injection of pentobarbital sodium (30 mg/kg). Supplementary injections (15 mg/kg) were provided when necessary. At the end of the experiment, the animals were killed with a lethal dose of anesthetic (100 mg/kg). Under deep anesthesia, assessed by the absence of blink reflexes, all the muscles of the thigh and lower right hindlimb were denervated except the soleus muscle. The soleus blood supply was kept intact. Under a stereomicroscope, the soleus nerve was freed and cleaned. In situ, the minimal (L_{min}) muscle length (3 ± 0.08 cm, measured at full ankle extension) and the maximal physiological (L_{max}) muscle length (4.1 ± 0.02 cm, measured at full ankle flexion) were determined.

A laminectomy was performed between L_3 and L_6. A mineral oil pool was achieved with the dorsal skin of the rat around the incision over the spine. The right L_4 and L_5 dorsal and ventral roots were transected near their entry into the spinal cord. The right dorsal L_5, containing most of the soleus afferent fibers, was severed from the other roots and was split into fine filaments until a filament contained one single fiber innervating the soleus muscle. A filament contained one single afferent fiber from the soleus muscle spindle when the stimulation of the filament triggered an all-or-none action potential in the electroneurogram recorded on the muscle nerve. Isolated afferents from muscle spindles were distinguished from Golgi tendon organs by a pause in their discharge during a twitch or a brief tetanic contraction.

The animal was then placed in a prone position on a heated steel blanket (Harvard, Les Ulis, France), and the dissected right hindlimb was placed in a bath filled with circulating thermostatically controlled (37°C) mineral oil. The tendon of the soleus muscle was severed and attached to a servo-controlled electromagnetic puller (developed in our laboratory) used to perform controlled ramp-and-hold and sinusoidal muscle stretches. The puller was coupled with a force transducer used to measure the twitch muscle.

Data recordings

The twitch tension of the soleus muscle was obtained by stimulation of the soleus nerve using a monopolar electrode located under the soleus nerve. The muscle length for maximal twitch response was measured. The soleus muscle length was then set to L_{min} (3 ± 0.08 cm). To record the electroneurogram, a monopolar platinum electrode was positioned under the soleus nerve, and a reference electrode was inserted into the neighboring denervated muscle mass. A monopolar platinum electrode placed under the dorsal root filament was used to record the afferent responses. The afferent fibers were stimulated to measure their conduction velocities. The antidromic potential was recorded on the soleus nerve neurogram. The axonal conduction velocities were calculated as the ratio of the nerve conduction distance to the antidromic spike delay. The conduction distance was measured after postmortem dissection. Muscle spindle afferent spikes were recorded using a digital tape recorder (DTR 1404, Biologic Science Instruments, Claix, France), and a CED 1401 interface with the Spike 2 processing package (Cambridge Electronic Design, Cambridge, UK), which converted the analogic discharge to an instantaneous discharge frequency.

Parameters used to identify the muscle spindle afferents

RAMP-AND-HOLD STRETCH. Ramp-and-hold stretches were applied at three different initial muscle lengths: $L_{min+10\%}$, $L_{min+15\%}$, and $L_{min+20\%}$ (110, 115, and 120% of L_{min}, respectively). These lengths were set using a micrometer and were included in the physiological range between the L_{min} and the L_{max} muscle lengths. For each length, after a prestretch of 1-mm ramp-and-hold stretches were applied with amplitude ranges of 3 mm (S3) and 4 mm (S4) at 6, 10, 15, and 30 mm/s velocities. The plateau phase was held for 5 s. Two stretches were separated by 25 s. Each series of stretches was repeated five times with the same parameters.

Several parameters were measured to characterize primary and secondary responses: the value of the resting discharge (RD) during the 0.5 s before the start of the stretch, the dynamic peak discharge (DP) that was the value of the discharge frequency at the end of the ramp phase, the final static value (FST) that was the mean value of the discharge frequency at the end of the 5-s plateau phase, the dynamic index (DI) that was the difference between the DP and the frequency at 0.5 s after completion of the stretch (Crowe and Matthews 1964; Matthews 1963), the presence or the absence of a discharge during the stretch release was also studied (Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1978), and the static sensitivity that was the difference between the static response (FST − RD) divided by the amplitude of the stretch (Boyd 1981). The significance of RD, DP, DI, and FST parameters is illustrated in Fig. 1.

SINUSOIDAL STRETCH. Sinusoidal stretches (0.5-, 1-, 2-, 3-, 6-, and 10-Hz frequencies) and vibrations (50-, 100-, and 150-Hz frequencies) were applied at 0.12, 0.25, 0.5, and 1 mm of stretch amplitudes at $L_{min+20\%}$ muscle length.

Several parameters were used: the presence of a phase lead defined as the gap between the peak of the fiber response and the peak of the sinusoidal stretch (Hunt and Wilkinson 1980), the amplitude of the afferent response equal to half the peak-to-peak response, the sensitivity to a sinusoidal stretch that was the ratio between the response amplitude and the stretch amplitude (Matthews and Stein 1969), and the modalities of the discharge of the afferent (continuous or discontinuous). For a given frequency, the range of amplitude with a continuous discharge and slight distortion was called "linear range" (Matthews and Stein 1969). The response was considered as linear when the discharge was sinusoidally modulated. The final parameter was vibration responses to 50-, 100-, and 150-Hz stretch frequencies applied at the four stretch amplitudes.

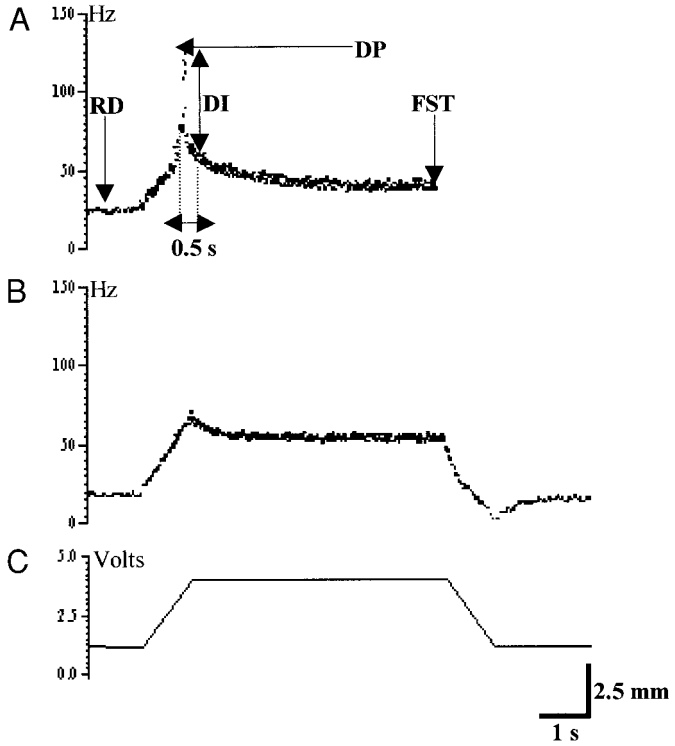

FIG. 1. Instantaneous discharge of a 1st group fiber (*A*) and a 2nd group fiber (*B*) under 3-mm ramp-and-hold stretch (*C*) applied at 3-mm/s stretch velocity. RD, resting discharge; DP, dynamic peak; DI, dynamic index; FST, final static value.

Statistical analysis

The linear regression slopes of DI as a function of the stretch velocities were determined for each muscle spindle fiber. From these slope values, a distribution histogram was achieved using GraphPad Prism 3 software to determine the presence of different fiber populations. The significant differences of results expressed as means ± SE were determined by using a nonpaired Student's *t*-test ($P < 0.05$).

RESULTS

The results are based on recordings from 51 spindle soleus afferent units of rat.

The RD, FST, static sensitivity parameters measured under a ramp-and-hold stretch, and response amplitude, sensitivity, and phase lead parameters obtained after a sinusoidal stretch did not permit to immediately distinguish two fiber populations. Therefore except for the conduction velocities, only the parameters that allowed to visually identify two kinds of fibers were retained.

Afferent discharges during ramp-and-hold stretches

Under ramp-and-hold-stretch of 3-mm amplitude applied at $L_{\min+20\%}$ and at 3-mm/s velocity, two types of responses were observed and are illustrated in Fig. 1. Both groups of responses showed a RD before the beginning of the stretch and a sustained discharge which determined the FST at the end of that phase. One type of response exhibited a high dynamic peak (125 ± 6.7 Hz) and a pause in the discharge during the stretch release. The other type of response was characterized by a lower DP value (63 ± 3.5 Hz) and a continuous discharge during the stretch release. Twenty-six responses belonged to the first group and 25 to the second group. Four responses with intermediate properties could not be classified and were not included in the sample.

Conduction velocity of afferent fibers

Figure 2 presents the histogram of the conduction velocities for 51 muscle spindle afferents. This figure does not show a bimodal distribution. However, the asymmetry of the histogram suggests the existence of two peaks, the first one ~32–36 m/s and the second ~40–44 m/s.

Dynamic index

The relations between the DI and the stretch velocity were studied at $L_{\min+10\%}$. The linear regression slopes of these relations were determined from the discharges of 51 spindle afferent fibers at $L_{\min+10\%}$ and for the two stretch amplitudes. The distribution histogram of these slopes (Fig. 3) shows a bimodal distribution. With a 3-mm amplitude stretch, the first peak was at 0.8 and the second at 4.3. With a 4-mm amplitude stretch, these peaks were shifted toward higher values, 1.3 and 5.3, respectively. Twenty-five fibers were in the part of the distribution around the first peak, and 26 fibers were in the part of the distribution around the second. Similar bimodal distributions were observed in the histograms of the DI parameter for 3- and 4-mm amplitude stretches at $L_{\min+15\%}$ and $L_{\min+20\%}$ (not illustrated). When the muscle length increased, the peak with the highest slope value was shifted toward lower slope values, whereas the first peak had the same slope value. At $L_{\min+20\%}$, the second peak had a slope of 3.3 at 3-mm amplitude and 4.8 at 4 mm.

For the three initial muscle lengths and for each stretch amplitude, we could divide the responses into two groups based on slope, and we could verify that, each time, the two groups of responses corresponded to the same two groups of afferent fibers. Our data also showed that the increase in DI values in both fiber groups linked to the increase in stretch

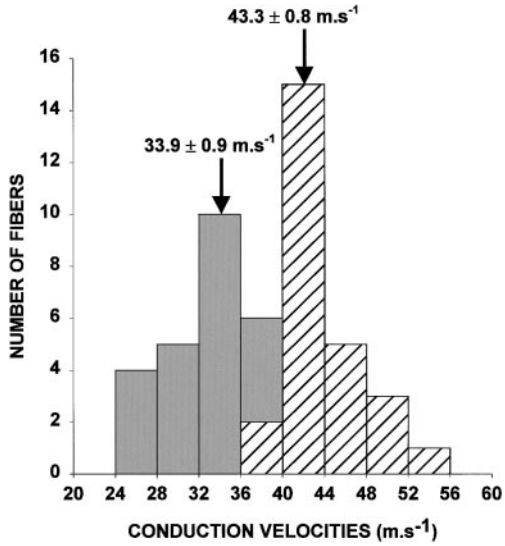

FIG. 2. Distribution histogram of conduction velocities of 51 soleus muscle spindle afferents. The values of conduction velocities (means ± SE) are indicated on the figure. ▨: 1st fiber group; □: 2nd fiber group.

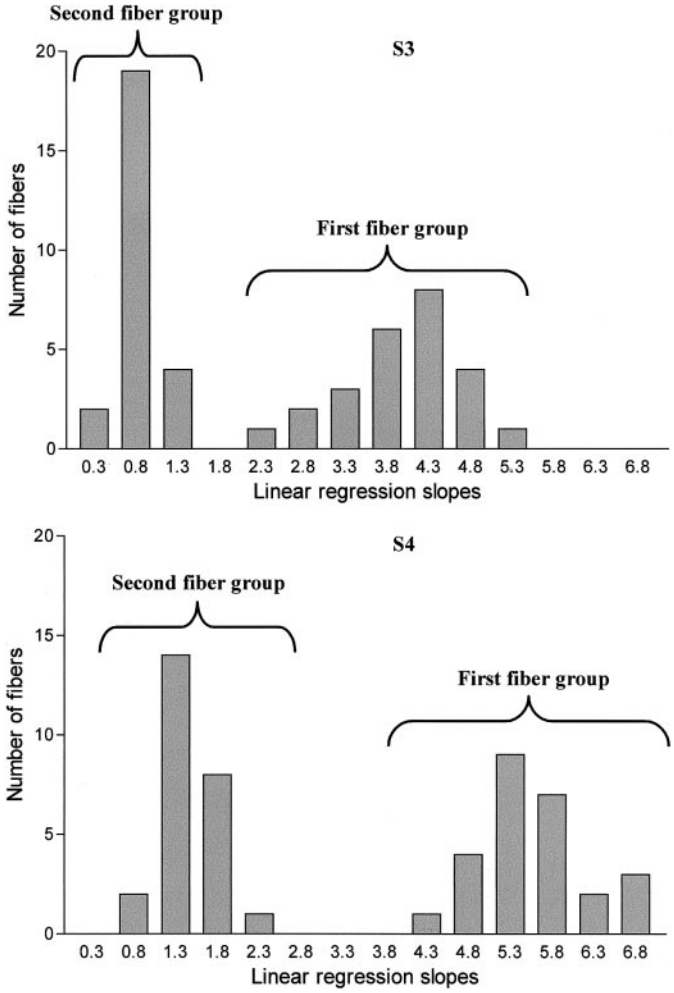

FIG. 3. Distribution histogram of the slopes of the regression lines (imp/mm) between the dynamic index and velocity of 51 fibers at $L_{min+10\%}$ muscle length and 2 stretch amplitudes (S3 and S4).

velocity was more pronounced when the initial muscle length was maintained at low length ($L_{min+10\%}$). For a constant stretch amplitude, the mean DI values of the first group of fibers increased depending on the initial muscle length (between $L_{min+10\%}$ and $L_{min+20\%}$), whereas this increase was less marked for the second group of fibers. The dynamic responsiveness of the first group fibers thus became more pronounced when the stretch amplitude was large. It is important to say that the group of fibers with higher slope values presented a high dynamic peak and a pause in the discharge during the stretch release. The group of fibers with lower slope values had a small dynamic peak and a continuous discharge during the stretch release. We assumed that the group of afferent fibers that corresponded to the highest slope values innervated primary endings and that the second group innervated secondary endings.

It should be noted that for each initial length and for each stretch amplitude and stretch velocity the mean DI values of the first group were four times as high ($P < 0.05$) as the mean DI values of the second group. The overlap in the conduction velocity values was in the 36- to 40-m/s interval. Although an overlap appears in the conduction velocity values of afferent fibers (Fig. 2), the mean conduction velocity of the first fiber group (43.3 ± 0.8 m/s) was significantly higher than that of the second fiber group (33.9 ± 0.9 m/s).

Discharge of muscle spindle afferents during sinusoidal stretches

Experiments were performed on 33 endings at $L_{min+20\%}$ under sinusoidal stretches and vibration stimuli. These endings were part of the 51 endings studied previously under ramp-and-hold stretch.

Linear range

As expected, two types of responses, (Fig. 4), were observed. Fifteen and 18 fibers belonged, respectively, to the first and second groups. The first and second fiber groups obtained under sinusoidal stretches corresponded to the first and second fiber groups previously described after ramp-and-hold stretches. Figure 4 shows the two types of responses to 0.5-Hz stretches with amplitudes ranging from 0.12 to 1 mm. The linear range of the 15 fibers of the first group extended from 0.12 mm (between 0.5 and 3 Hz) to 0.25-mm stretch amplitude (between 0.5 and 2 Hz). Beyond 0.25-mm amplitude, the discharge became discontinuous. The linear range of the 18 fibers of the second group extended from 0.12 to 1 mm of stretch amplitude between 0.5- and 3-Hz stretch frequencies.

Responses to vibrations

Vibrations were applied at $L_{min+20\%}$ to the distal tendon of the soleus muscle. Their amplitudes were within the respective linear range of the first and second fiber groups and their frequencies were of 50, 100 and 150 Hz. The results are reported in Table 1.

At 50 Hz and with a 0.12-mm stretch amplitude, the discharge of the 15 fibers of the first group were 1:1 driven (1 imp/sinusoidal cycle) by the vibration. At 100 Hz and for the same stretch amplitude, 12 fiber responses featured 1:1 driving and 3 fiber responses featured 1:2 driving. At 150 Hz and for the same stretch amplitude, 10 fiber responses featured 1:1 driving and 5 fiber responses featured a 1:2 driving. When the amplitude of vibrations was increased to 0.25 mm, the fiber responses of the first group were all driven by the vibration at the three vibration frequencies.

For the 18 fibers of the second group, driving was rarely observed with vibration amplitude ≤0.25 mm (Table 1). With a 0.5-mm vibration amplitude, 1:1 driving was the response of 14 fibers to 50-Hz vibration, 10 fibers to 100-Hz vibration, and 7 fibers to 150-Hz vibration (see Table 1 for details of non-driven afferent discharges). The discharge of every fiber of the second group was 1:1 driven by a 50-Hz vibration with a 1-mm amplitude. Almost all the fibers of this group had the same kind of response to 100- and 150-Hz vibrations (Table 1).

DISCUSSION

The aim of our study was to differentiate discharges coming from Ia and II afferent fibers. We could have distinguished Ia fibers lacking a bag1 fiber from Ia fibers innervating this intrafusal fiber by using succinylcholine. However, several authors have shown that the primary endings exclusively innervating bag2 and chain fibers displayed a similar dynamic

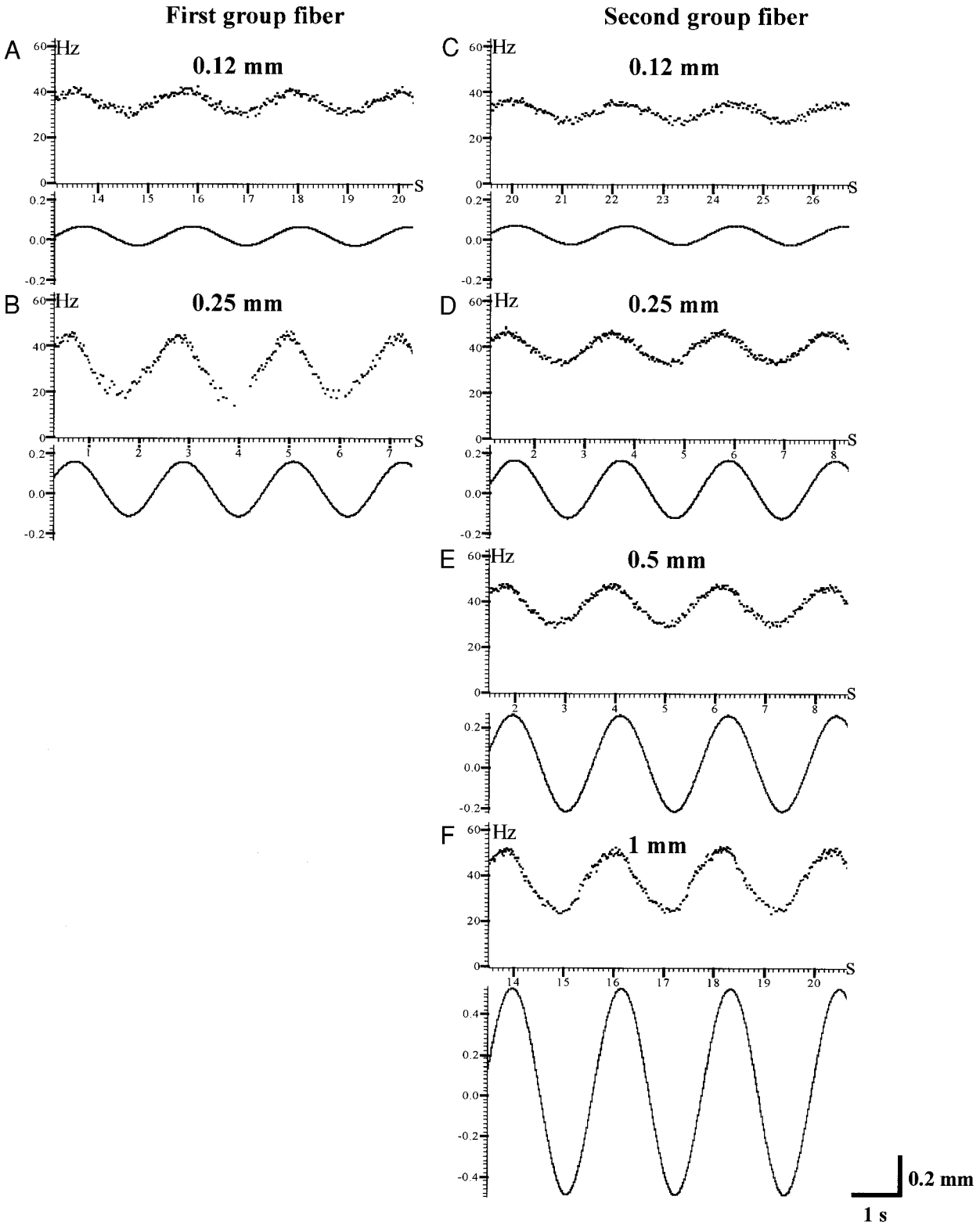

FIG. 4. Linear range of a 1st group fiber (*A* and *B*) and a 2nd group fiber (*C–F*) under 0.5-Hz sinusoidal stretch at 0.12-mm (*A* and *C*), 0.25-mm (*B* and *D*), 0.5-mm (*E*) and 1-mm (*F*) stretch amplitudes. *Top*: instantaneous discharge of fibers (Hz). *Bottom*: sinusoidal stretch.

response (under passive condition) to that of primary afferents innervating all intrafusal fiber types (Gioux et al. 1991; Scott 1991).

In the present investigation, we studied the characteristics of the discharges of rat soleus muscle spindles and we measured the discharge parameters commonly used in other species (cat, primate, human). Our results demonstrated that in rat, contrary to what had previously been reported in cat, the histogram of

TABLE 1. *Vibration sensitivity of the two fiber groups*

	First Fiber Group		Second Fiber Group			
	0.12 mm	0.25 mm	0.12 mm	0.25 mm	0.5 mm	1 mm
Amplitudes						
50 Hz	50 Hz (15)	50 Hz (15)	25 → 50 Hz (18)	25 → 50 Hz (18)	14: 50 Hz 4: 25 Hz	50 Hz (18)
Vibration frequencies						
100 Hz	12: 100 Hz 3: 50 Hz	100 Hz (15)	25 → 50 Hz (18)	3: 100 Hz 12: 50 Hz 3: 25 Hz	10: 100 Hz 8: 50 Hz	14: 100 Hz 4: 50 Hz
150 Hz	10: 150 Hz 5: 75 Hz	150 Hz (15)	25 → 50 Hz (18)	10: 75 Hz 4: 50 Hz 4: 25 Hz	7: 150 Hz 6: 75 Hz 5: 50 Hz	12: 150 Hz 4: 75 Hz 2: 50 Hz

Discharge frequency of the first group ($n = 15$; in parentheses) and second group of fibers ($n = 18$) under vibration stimuli applied at different frequencies and amplitudes. 25 → 50 Hz indicates that the discharge varied from 25 to 50 Hz.

conduction velocities was not clearly bimodal (Boyd and Davey 1968; Wei et al. 1986); it was thus hazardous to assume that all primary endings were innervated by Ia fibers and all secondary endings by II fibers. Using functional criteria to discriminate primary endings from secondary endings, we were able to show that an overlap in the conduction velocities of the afferent fibers innervating these endings exists. Such an overlap also exists in cat but is less important than in rat. Boyd (1962) and Banks et al. (1982) have shown in cat that different types of secondary endings were distinguished following their position on either side of the primary ending innervation. The S1 endings lay immediately adjacent to the primary endings and were formed by the largest-diameter II axons. The majority of these axons innervated all three fiber types. The S2 and S3 endings lay further from the primary endings, and progressively had smaller axons. The S2 ending fibers innervated mainly bag2 and/or the chain fibers, whereas the S3 endings lay predominantly on the chain fibers. Banks et al. (1982) have also shown that the diameter of the Ia axons supplying bag2 and chain fibers was generally smaller than that of the axons supplying all the intrafusal fibers. Therefore these data could explain the overlapping of fiber conduction velocities described in the rat.

Ramp-and-hold stretch

In our study, the RD and the FST parameters did not permit to immediately distinguish two fiber populations. They were therefore discarded. Our data showed that in the absence of fusimotor activity, a first fiber group stopped firing abruptly during the release of a small (3 mm) and slow (3 mm/s) stretch, whereas a second fiber group did not cease to fire. The distribution histogram of the DI linear regression slopes was clearly bimodal without overlapping, and the DI values in the first group fibers were greater than those of the second group fibers. This difference was found at all muscle lengths, velocities and stretch amplitudes. Moreover, at a given muscle length, DI values increased both with velocity and stretch amplitude in both fiber groups. However, this was less marked and variable for the second group of fibers. It has early been demonstrated during the release of a small and slow ramp-and-hold stretch that in cat, the Ia fibers ceased to fire, whereas II fibers continued (Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1978). In cat (Matthews 1963; Wei et al. 1986), primate (Cheney and Preston 1976a,b), and human (Edin and Vallbo 1990), the Ia and II fibers from hindlimb soleus muscle are characterized by well-separated ranges of DI, which permit differentiation of the fibers. The DI values of Ia fibers increased with the stretch velocity (Holm et al. 1981; Houk et al. 1981; Matthews 1963), stretch amplitude (Fisher and Schäfer 2000; Matthews 1972), and muscle length (Houk et al. 1981). On the contrary, it has been demonstrated that the DI of Ia fibers was often independent of the initial muscle length (Cheney and Preston 1976b; Matthews 1963). Furthermore, Cheney and Preston (1976b) have also observed that when the initial length was kept constant and the stretch amplitude varied, the DI of Ia fibers was often greater for low-amplitude stretches.

From our data and according to those previously described in the literature, it was tempting to suggest that our first group fibers corresponded to Ia fibers and our second group afferents to II fibers.

The differences in dynamic response between Ia and II fibers could be due to the location of Ia and II afferent fibers along the intrafusal fibers (Banks et al. 1982; Boyd 1962; Cheney and Preston 1976b) and the distinct mechanical properties of intrafusal muscle fibers innervated by Ia and II fibers (Andrew et al. 1973; Boyd et al. 1977; Hulliger 1984; Corvaja 1969; Matthews 1972; Poppele and Quick 1985; Scott 1990).

Although there was a clear difference between the DI values of the first and second group fibers, other parameters were used to confirm this classification.

Linear range of both fiber groups during a sinusoidal stretch

As the response amplitude and the phase lead did not make it possible to immediately identify two fiber populations, we only retained the parameters that allowed us to visually identify two kinds of fibers.

In our study, the amplitude of the response in both fiber groups increased linearly with the amplitude of the sinusoidal stretch over a limited range. This linear range extended from 0.12 to 0.25 mm for the first group fibers and from 0.12 to 1 mm for the second group fibers. The linear range of the second group fibers was therefore broader than that of the first group afferents at all stretch frequencies. Beyond 0.25 mm, the estimation of the response amplitude was very uncertain because the first group fibers failed to evoke action potentials during the

whole sinusoidal cycle. Similarly, in cat (Hasan and Houk 1975; Hulliger et al. 1977; Matthews and Stein 1969) and in human (Kakuda 2000), II fibers presented a broader linear range than Ia fibers. Moreover, Laporte and Emonet-Dénand (1973) have shown in cat that sinusoidal stretches <0.5 mm amplitude were sufficient to elicit bursts of impulses in Ia fibers, whereas the discharge of the II fibers was sinusoidally modulated. Therefore in accordance with these results, our data constitute another argument confirming that the first and second group fibers belong to Ia and II fibers, respectively.

Vibrations

Vibrations were applied to the distal tendon of the soleus muscle at $L_{\min+20\%}$. This length was chosen to keep the muscle and muscle spindles under tension and thus to get a better sensitivity to vibration. Indeed, the ability of spindles to get slack is known to decrease progressively at longer muscle lengths as passive tension increases (Gregory et al. 1986). Our results showed that for the three vibration frequencies (50, 100, and 150 Hz) used, the amplitude threshold of the second group fibers was higher than that of the first group fibers to produce one spike per cycle of a sinusoidal stretch. For example, at 0.12-mm stretch amplitude, almost all the first group fibers discharged at 100 Hz for vibrations applied at 100-Hz frequency, whereas the majority of the second group fibers discharged at this frequency for 1-mm stretch amplitude. Therefore to get the same discharge frequency, the thresholds of vibration amplitude were higher for the second group fibers than for the first. To confirm our data, it has been observed that high-frequency vibrations of small amplitude (0.1 mm) constituted specific stimuli for Ia fibers (Brown et al. 1967; Matthews and Watson 1981; Proske et al. 2000; Roll et al. 1989), which led these fibers to discharge to one spike per sinusoidal cycle.

To conclude, our study demonstrated that in rat soleus muscle spindles, it was possible to immediately distinguish two groups of fibers by using some significant parameters during ramp-and-hold (DI, discharge during stretch release) and sinusoidal stretches (linear range, vibration sensitivity). In comparison with data obtained by several authors, all these parameters used in combination showed that the first group fibers corresponded to Ia fibers, whereas the second group fibers were classified as II fibers.

This work was supported by grants from the Centre National d'Etudes Spatiales (3027), the Conseil Régional du Nord Pas-De-Calais, and the Fonds Européen de Développement Régional (F007).

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J Neurophysiol 89: 3000–3007, 2003;
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Effects of Hypodynamia-Hypokinesia on the Muscle Spindle Discharges of Rat Soleus Muscle

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[1]*Laboratoire de Plasticité Neuromusculaire, EA 1032, IFR 118, Bât. SN4, Université des Sciences et Technologies de Lille 1, F-59655 Villeneuve d'Ascq Cedex; and* [2]*Faculté des Sciences du Sport et de l'Education Physique, Laboratoire Commandes Motrices et Analyse du Mouvement, Université Bordeaux 2, Domaine Universitaire, F-33607 Pessac Cedex, France*

Submitted 1 October 2002; accepted in final form 2 February 2003

De-Doncker, Laurent, Florence Picquet, Julien Petit, and Maurice Falempin. Effects of hypodynamia-hypokinesia on the muscle spindle discharges of rat soleus muscle. *J Neurophysiol* 89: 3000–3007, 2003; 10.1152/jn.00875.2002. The aim of this study was to determine whether Ia and II fiber discharges of soleus muscle spindles were modified after a 14-day period of hypodynamia (absence of weight bearing) and hypokinesia (reduction of motor activity). Fifty-one and 38 afferent fibers were studied, respectively, in control and hypodynamia-hypokinesia (HH) groups. Under deep anesthesia (pentobarbital, 30 mg/kg), a L_3–L_6 laminectomy was performed. Unitary potentials from the L_5 dorsal root were recorded in response to ramp-and-hold stretches applied at two stretch amplitudes (3 and 4 mm) and four stretch velocities (6, 10, 15, and 30 mm/s) and to sinusoidal stretches applied at four stretch amplitudes (0.12, 0.25, 0.5, and 1 mm) and six stretch frequencies (0.5, 1, 2, 3, 6, and 10 Hz). In both animal groups, the Ia fibers showed higher dynamic index values, smaller linear range, and higher vibration sensitivity than the II fibers. They also exhibited a pause in their discharges during the stretch release contrary to II fibers, which displayed no pause in their responses. After HH, our results showed that for both fiber types all parameters measured under ramp-and-hold stretches (except the static sensitivity) were significantly increased and under sinusoidal stretches, the vibration sensitivity increased, and the response amplitude only increased at 0.12-mm stretch amplitude. The linear range of Ia afferents was limited to 0.12 mm, whereas it was unchanged for the II fibers. After HH, the stretches could be better transmitted to the muscle spindles, probably resulting from changes in passive mechanical properties of the soleus.

INTRODUCTION

Hypodynamia (absence of weight bearing) and hypokinesia (reduction of motor activity) conditions are present in real microgravity (space flight) or in simulated microgravity when using human or animal models. Hypodynamia-hypokinesia (HH) conditions have been reported to induce changes in many muscular properties mainly in the slow extensor muscles such as the soleus muscle (Edgerton and Roy 1996). On the contrary, no data are available concerning the effects of HH as regards the muscle spindle properties. Two kinds of endings innervate the muscle spindle: the primary and the secondary endings, which arise as the terminations of the Ia and II fibers, respectively (Hulliger 1984; Hunt 1990). The discharge of Ia fibers indicates both the muscular length changes (static sensitivity) and the velocity of length changes (dynamic sensitivity), whereas the discharge of II fibers provides mainly information about the length changes (McCloskey 1978). In a previous study, we have established a classification of rat muscle spindle afferents using ramp-and-hold and sinusoidal stretches (De-Doncker et al. 2003). Our results showed clearly that in the rat, afferent fibers could be clearly divided in two groups: the Ia and II afferents. It appears therefore that the methods that work in cats also work in rats.

In the literature, some data can be found concerning changes in the afferent fiber discharges from muscle spindles when muscles were maintained in a shortened position. In fact, in the gastrocnemius muscle, the muscle spindle structure was preserved after tenotomy, and the rate of muscle spindle discharges was increased (Hnik and Lessler 1973a). After immobilization in plantar flexion of cat peroneus longus muscle, a slight increase in both the static discharge and the dynamic index of Ia fibers in passive spindles has been observed (Gioux and Petit 1993). After a HH period, the discharge of afferent fibers from soleus muscle spindles has never been examined. In HH condition, the soleus was often in a shortened position as after tenotomy and immobilization in a plantar flexion position (Riley et al. 1990), the natural physiological stimulus of muscle spindles being thus removed. Moreover, after a HH period, modifications in elastic properties of soleus muscle and Achilles tendon have been described by several authors. A decrease in the stiffness of isolated soleus muscle (Canon and Goubel 1995) and in Achilles tendon stiffness (Almeïda-Silveira et al. 2000) was observed after 3 wk of unloading. The results obtained by Canon and Goubel (1995) were confirmed by Toursel et al. (1999) on isolated skinned fibers of rat soleus muscle. Moreover, the passive tension of in situ rat soleus muscle increased after 7 and 14 days of whole body suspension (Gillette and Fell 1996). A relative increase in the proportion of noncontractile tissue particularly in the soleus and gastrocnemius muscles has also been described (Flynn and Max 1985; Herbert et al. 1988; Templeton et al. 1984). Therefore we suggest that the stretch transmission might be affected after an episode of HH and the electrophysiological afferent discharges of endings consequently modified.

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Thus the aim of this study was to determine whether the Ia and II fiber discharges of soleus muscle spindles were modified after a 14-day period of HH.

METHODS

Animal groups

Experiments were performed on 27 male Wistar rats (IFFA CREDO) weighing 280–300 g and randomly divided into two groups: control rats (CONT: $n = 15$) and hypodynamia-hypokinesia rats (HH: $n = 12$). To maintain minimal distress, all the rats were housed individually in separate cages and were allowed food and water ad libitum. The rats were acclimatized at a 25°C room temperature with a 12:12-h light-dark cycle for 1 wk before the experiments began. Animals of the HH group were hindlimb unloaded by the tail for 14 days using Morey's model (Morey et al. 1979). Briefly, the tail of each rat was washed, cleaned, dried, and wrapped in an antiallergic orthopedic tape-adhesive plaster. This cast, covering less than half of the tail, was secured to an overhead swivel, mounted at the top of the cage and permitting free 360° rotation of the animals. The rats were unloaded at 30° head-down angle to mimic fluid shifts characteristic of weightlessness. All the experiments as well as the maintenance conditions of the animals received authorizations from both the Agricultural and Forest Ministry and National Education Ministry (Veterinary Service of Health and Animal Protection: authorization 59-00980).

Surgical technique

Fourteen days after the acclimatization period, each rat of control and HH groups was anesthetized with intraperitoneal injection of pentobarbital sodium (30 mg/kg). Supplementary injections (15 mg/kg) were provided when necessary. At the end of the experiments, the animals were killed with a lethal dose of anesthetic (100 mg/kg). Under deep anesthesia, the right soleus muscle was exposed while care was taken not to damage the main blood supply or the soleus nerve trunk. All the other muscles of the thigh and lower right hindlimb were denervated. Under a stereomicroscope, the soleus nerve was freed and cleaned. In situ, both the minimal [L_{min}: 3 ± 0.08 (SE) cm] muscle length, measured at full ankle extension, and the maximal (L_{max}: 4.1 ± 0.02 cm) physiological muscle length, measured at full ankle flexion, were determined.

A laminectomy was performed between L_3 and L_6. A mineral oil pool was achieved with the dorsal skin of the rat around the incision over the spine. The right L_4 and L_5 dorsal and ventral roots were transected near their entry into the spinal cord. The right dorsal L_5 containing the majority of the soleus afferent fibers was severed from the other roots. It was split into fine filaments until a filament that contained a single fiber innervating the soleus muscle spindles was isolated. A filament was considered to contain a single afferent fiber from the soleus muscle spindle if the stimulation (Grass Instruments S88, Quincy, MA) of the filament evoked an all-or-none action potential in the electroneurogram recorded on the muscle nerve. Isolated afferents from muscle spindles were distinguished from Golgi tendon organs by a pause in their discharge during a twitch or a brief tetanic contraction. Three or four afferent fibers were studied per animal in both CONT and HH groups.

The animal was then placed in a prone position on a heated steel blanket (Harvard), and the dissected right hindlimb was placed in a bath filled with circulating thermostatically controlled (37°C) mineral oil. The tendon of the soleus muscle was connected to a servocontrolled electromagnetic puller coupled with a force transducer (developed in our laboratory), which was used to determine the twitch muscle force and to perform controlled ramp-and-hold and sinusoidal muscle stretches.

Data recordings

The twitch tension of the soleus muscle was obtained by stimulation of the soleus nerve using a monopolar electrode located under the soleus nerve. For the CONT group, the twitch tension was 0.301 ± 0.01 (SE) N and was decreased after a HH period (0.121 ± 0.005 N) according to results obtained in the literature (Edgerton and Roy 1996; Falempin and In-Albon 1999). The muscle length for maximal twitch response was measured. The soleus muscle length was then set to L_{min} (3 ± 0.08 cm). To record the electroneurogram, a monopolar platinum electrode was positioned under the soleus nerve and a reference electrode was inserted into the neighboring denervated muscle mass. A monopolar platinum electrode placed under the dorsal root filament was used to record the afferent responses. The afferent fibers were stimulated to measure their conduction velocities. The antidromic potential was recorded on the soleus nerve neurogram. The axonal conduction velocities were calculated as the ratio of the nerve conduction distance to the antidromic spike delay. The conduction distance was measured after postmortem dissection. Muscle spindle afferent spikes were recorded using a digital tape recorder (DTR 1404, Biologic Science Instruments) and a CED 1401 interface with Spike 2 processing package (Cambridge Electronic Design) that converted the analogic discharge to an instantaneous discharge frequency.

Parameters used to identify the muscle spindle afferents

RAMP-AND-HOLD STRETCH. Ramp-and-hold stretches were applied at three different initial muscle lengths: $L_{min\ +10\%}$, $L_{min\ +15\%}$, $L_{min\ +20\%}$ (110, 115, and 120% of L_{min}, respectively). These lengths were set using a micrometer and were included in the physiological range comprised between L_{min} and L_{max} muscle lengths. For each length, after a prestretch of 1 mm maintained during all the experiments, ramp-and-hold stretches were applied with amplitude ranges of 3 mm (S_3) and 4 mm (S_4) at 6, 10, 15, 30 mm/s velocities. The plateau phase was held for 5 s. Two stretches were separated by 25 s. Each series of stretches was repeated five times with the same parameters.

Several parameters were measured to characterize primary and secondary responses: *1*) the value of the resting discharge (RD) during the 0.5 s before the start of the stretch, *2*) the dynamic peak discharge (DP), which was the value of the discharge frequency at the end of the ramp phase, *3*) the final static value (FST), which was the mean value of the discharge frequency at the end of the 5-s plateau phase, *4*) the dynamic index (DI), which was the difference between the dynamic peak (DP) and the frequency at 0.5 s after completion of the stretch (Matthews 1963), *5*) the presence or the absence of a discharge during the stretch release (Hunt et al. 1978; Hunt 1990), and *6*) the static sensitivity, which was the difference between the static response (FST – RD) divided by the amplitude of the stretch: (FST – RD/amplitude of the stretch) (Boyd 1981). The significance of RD, DP, DI, and FST parameters is illustrated in Fig. 1.

SINUSOIDAL STRETCH. Sinusoidal stretches (0.5, 1, 2, 3, 6, 10 Hz frequencies) and vibrations (50- and 100-Hz frequencies) of 0.12, 0.25, 0.5, and 1 mm amplitudes were applied at $L_{min\ +20\%}$ muscle length.

Several parameters were used: *1*) the amplitude of the afferent response equal to half the peak-to-peak response, *2*) the sensitivity to a sinusoidal stretch that was the ratio of the afferent response by the stretch amplitude (Matthews and Stein 1969), *3*) the modalities of the discharge of the afferent (continuous or discontinuous). For a given frequency, the range of amplitude in which the afferent discharge did not fall silent at any time during sinusoidal stretching and with slight distortion was called "linear range" (Matthews and Stein 1969). The response was considered as linear when the discharge was sinusoidally modulated. *4*) The vibration responses to 50- and 100-Hz stretch frequencies applied at the four stretch amplitudes were also studied.

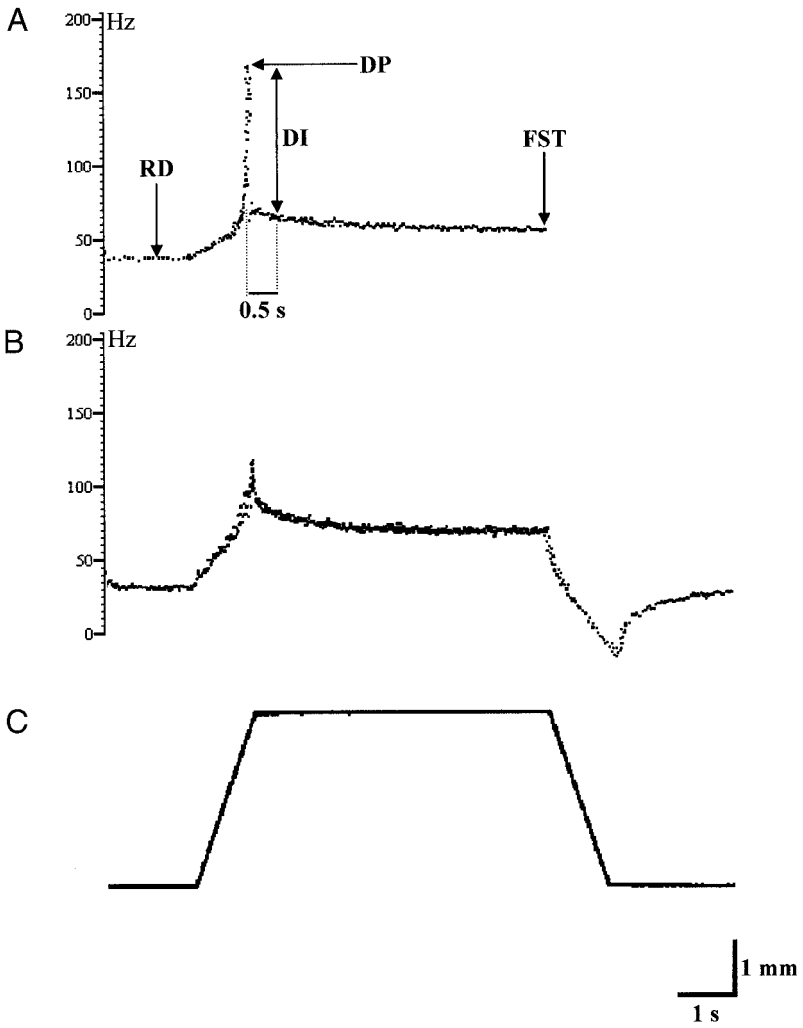

FIG. 1. Instantaneous discharge examples of Ia (*A*) and II (*B*) fibers of the hypodynamia-hypokinesia (HH) group under a 3-mm ramp-and-hold stretch (*C*) applied at 3 mm/s stretch velocity. RD, resting discharge; DP, dynamic peak; DI, dynamic index; FST, final static value.

Statistical analysis

The linear regression slopes of DI as a function of the stretch velocities were determined for each afferent fiber from muscle spindles. From these slope values, a distribution histogram was achieved using GraphPad Prism 3 software to determine the presence of two fiber populations. The significant differences of results expressed as means ± SE were established using a nonpaired Student *t*-test ($P < 0.05$). The significances between the linear regression straight lines (slopes, correlation coefficients, interception values with the *y* axis) were determined by GraphPad Prism 3 ($P < 0.0001$).

Asterisks (*) indicated a significant difference with the CONT group, and daggers (†) with the Ia fibers in the same animal group. Double daggers (‡) indicated a significant difference between linear regression straight lines of Ia and II fibers with paired fibers of the CONT group ($P < 0.0001$).

RESULTS

Fifty-one and 38 afferent fibers were respectively studied in the CONT and HH groups. The data obtained after a 14-day period of HH were compared with results of the CONT group.

Muscle length

Our results showed no significant difference between CONT (3 ± 0.08 cm, $n = 15$) and HH (2.8 ± 0.06 cm, $n = 12$) muscle lengths measured at full extension of the ankle.

Comparison of Ia and II fiber responses in CONT versus HH groups

RAMP-AND-HOLD STRETCH. Under ramp-and-hold stretch of 3 mm amplitude applied at $L_{min\ +20\%}$ and at 3 mm/s velocity, two types of responses were observed in muscle spindles of the HH group similarly to the CONT group (De-Doncker et al. 2003). These fiber responses are illustrated in Fig. 1 (*A* and *B*). However, the mean DP values of Ia ($n = 18$, 168 ± 4.9 Hz) and II ($n = 20$, 77 ± 4.2 Hz) fiber responses were significantly different from Ia ($n = 26$, 125 ± 6.7 Hz) and II ($n = 25$, 63 ± 3.5 Hz) fibers of the CONT group.

The changes in RD, DI, and FST parameters of Ia and II fibers observed after HH were illustrated in Fig. 2. This figure shows the Ia and II fiber responses of CONT (*A* and *C*) and HH (*B* and *D*) groups under 3 mm amplitude and 30 mm/s stretch velocity of a ramp-and-hold stretch.

Whatever the stretch amplitude, the muscle length, and the stretch velocities, the static sensitivities of Ia and II fibers did not change after HH.

Resting discharge. After a period of HH, the RD of both Ia and II fibers significantly increased ($P < 0.05$). This was illustrated in Fig. 3.

Dynamic index. Figure 4*A* illustrates the DI linear regression of Ia and II fibers for CONT and HH groups at $L_{min\ +10\%}$ and 3-mm amplitude. Similar figures were obtained for 3- and

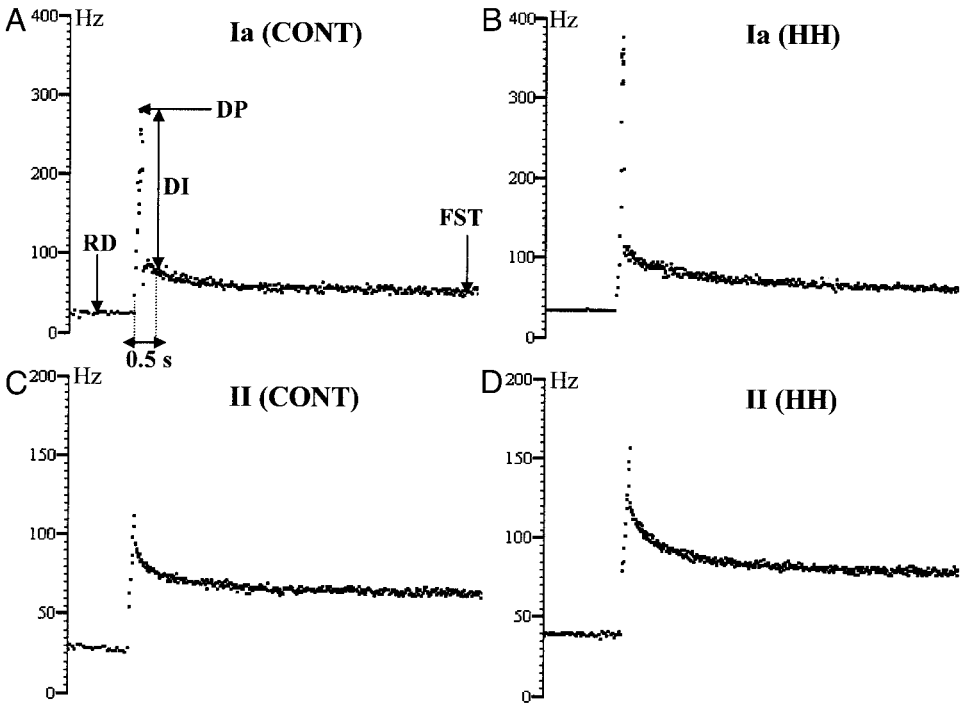

FIG. 2. Comparison of the instantaneous discharges of Ia and II fibers between the control (CONT; *A* and *C*) and HH (*B* and *D*) groups under a 3-mm ramp-and-hold stretch applied at 30 mm/s stretch velocity.

4-mm amplitudes at $L_{min\ +15\%}$ and $L_{min\ +20\%}$ (data not shown). DI increased with the stretch velocity for Ia and II fibers of CONT and HH groups. However, in both groups, whatever the stretch amplitude and the muscle length, the correlation coefficient and the linear regression slopes between DI and stretch velocities of Ia fibers were significantly higher than those of the II fibers. Therefore for Ia fibers, the increase in DI parameter was better correlated with the stretch velocity than for II fibers.

After HH, whatever the stretch amplitude and the muscle length, the DI linear regression straight lines were shifted toward higher values for both Ia and II fibers ($P < 0.0001$). However, the slopes and correlation coefficients of the linear regression straight lines were unchanged for both fiber types.

Final static value. Figure 4*B* illustrates the FST linear regression of Ia and II fibers for CONT and HH groups at 3-mm amplitude and $L_{min\ +10\%}$ muscle length. Similar variations were observed for 3- and 4-mm amplitudes at $L_{min\ +15\%}$ and $L_{min\ +20\%}$. In both animal groups, the FST values of Ia and II fibers did not increase with the stretch velocity. For both CONT and HH groups, whatever the initial muscle length and the stretch amplitude, the FST values of Ia fibers were slightly higher than those of the II afferents.

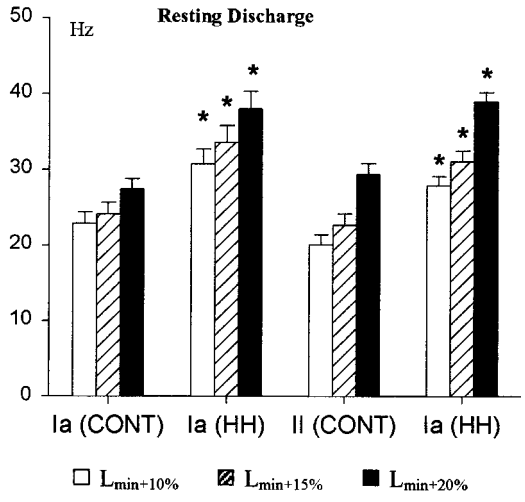

FIG. 3. Resting discharge histogram of the Ia and II fibers in the CONT and HH groups at the 3 muscle lengths. *, significant difference with the CONT group.

In HH group, whatever the stretch amplitude and the muscle length, the FST linear regression straight lines between FST and stretch velocities were shifted toward higher values both for Ia and II fibers ($P < 0.0001$).

SINUSOIDAL STRETCH. *Linear range.* Experiments were performed at $L_{min\ +20\%}$ under sinusoidal stretches and vibration stimuli on 33 and 38 endings for CONT and HH groups, respectively. Figure 5 shows the Ia and II responses of CONT and HH groups to 0.5-Hz stretches with amplitudes ranging from 0.12 to 1 mm.

For the CONT group, the linear range of Ia fibers ($n = 15$) extended from 0.12 (between 0.5 and 3 Hz) to 0.25 (between 0.5 and 2 Hz), and from 0.12 to 1 mm (between 0.5 and 3 Hz) for II fibers ($n = 18$).

After HH, the linear range of II fibers ($n = 20$) was unchanged. On the contrary, for Ia fibers ($n = 18$), the linear range was limited to 0.12 mm.

Response amplitude and sensitivity. The response amplitude linear regression straight lines of Ia and II fibers were obtained under 0.12-mm sinusoidal stretch for both animal groups (not illustrated).

For the CONT group, within the linear range of Ia afferents, II fibers had a significantly smaller response amplitude and sensitivity than Ia afferents at all stretching frequencies (Table 1). Moreover, the response amplitude of Ia and II afferents increased significantly with the stretch amplitude.

The same evolution in the response amplitude and sensitivity was observed for Ia and II fibers of the HH group except at 0.25 mm for Ia fibers when the response became nonlinear.

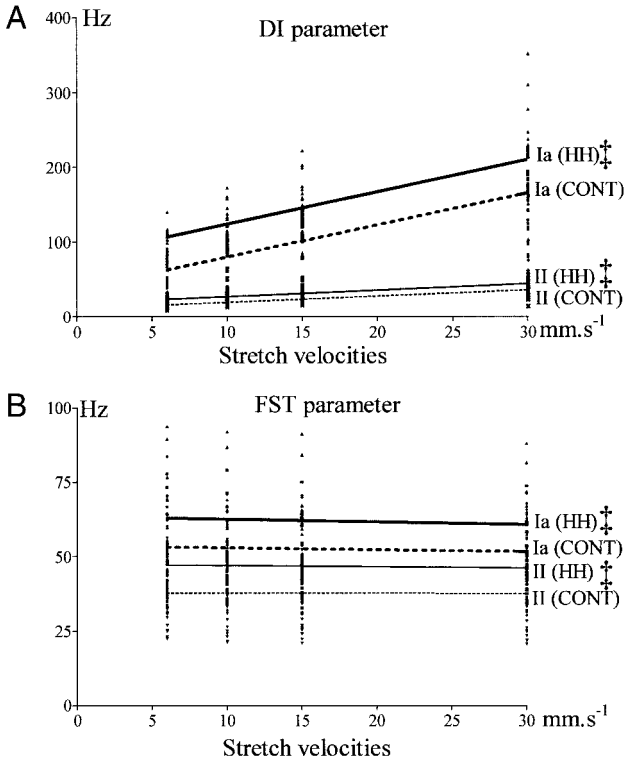

FIG. 4. Linear regression straight lines of dynamic index (DI; *A*) and final static value (FST; *B*) parameters of Ia and II fibers in CONT (51 fibers) and HH (38 fibers) groups. Thick full line, Ia fibers of HH group ($n = 18$); thick dotted line, Ia fibers of CONT group ($n = 26$); thin full line, II fibers of HH group ($n = 20$); thin dotted line, II fibers of CONT group ($n = 25$). The significance between the linear regression straight line were determined by GraphPad Prism 3 ($P < 0.0001$). ‡, a significant difference between linear regression straight lines of Ia and II fibers with the same fiber of the CONT group.

After HH, the response amplitude and sensitivity of both fiber groups were significantly ($P < 0.0001$) increased at 0.12-mm stretch amplitude (Table 1). Indeed, the linear regression straight lines of Ia and II fibers were shifted toward a higher level and the slopes increased. The slopes of linear regression straight line of Ia fibers were, respectively, 0.44 and 0.85 for CONT and HH groups and, respectively, 0.52 and 0.67 for II fibers of CONT and HH groups. More than 0.12-mm stretch amplitude, no significant difference was seen in the amplitude and sensitivity of Ia and II fibers between the two animal groups.

Vibration sensitivity. Vibrations were only applied at $L_{\text{min }+20\%}$ to the distal tendon of the soleus muscle.

For the CONT group, at 50 Hz and with an 0.12-mm stretch amplitude, the discharges of Ia fibers were 1:1 driven (1 imp/sinusoidal cycle) by vibration. At 100 Hz and for the same stretch amplitude, 12 Ia fiber responses have a 1:1 driving and 3 Ia afferent responses show a 1:2 driving. When the amplitude of the vibrations was increased to 0.25 mm, the responses of Ia fibers were all driven by the 100-Hz vibrations. For the 18 II afferents, driving was rarely observed with vibrations whose amplitude exceeded 0.25 mm. The discharge of every secondary ending was 1:1 driven by a 50-Hz vibration with a 1-mm amplitude. At 1-mm amplitude, almost all the secondary endings were 1:1 driven by a 100-Hz vibration.

After HH, all the Ia fibers were 1:1 driven by 50- and 100-Hz vibrations with 0.12-mm stretch amplitude. The vibration sensitivity of II fibers was increased. Indeed, the II afferents chiefly discharged to 1 imp/sinusoidal cycle with 0.5-mm vibrations applied at 50 and 100 Hz.

CONDUCTION VELOCITIES. The mean conduction velocities of Ia fibers were significantly higher than those of II fibers for both animal groups. The mean conduction velocities of Ia fibers were 43.3 ± 0.8 and 45 ± 0.9 m/s for CONT and HH groups ($P > 0.05$), respectively. For the II fibers, the mean in CONT group was 33.9 ± 0.9 and 35.8 ± 1.3 m/s in HH group ($P > 0.05$).

DISCUSSION

The aim of this study was to determine whether the discharges of the Ia and II fibers were modified after HH. Our results showed that under a ramp-and-hold stretch, RD, DI, and FST parameters of Ia and II fibers were significantly increased. On the contrary, the static sensitivity was unchanged. Under a sinusoidal stretch, the response amplitude and sensitivity of both fiber types were only increased at 0.12-mm stretch amplitude, the smallest value of the range used. Moreover, the vibration thresholds of II fibers were decreased. From our experiments, we can suggest that the stretch was better detected by muscle spindles after HH.

This effect could be due to a change in muscle spindle structure. Indeed, Maier et al. (1972) showed an intrafusal fiber atrophy after an immobilization of cat's medial gastrocnemius muscle in a shortened position. However, in studies of Soukup et al. (1990) and De-Doncker et al. (2002), it has been demonstrated that the muscle spindle and intrafusal fiber numbers and the cross sectional area (CSA) of intrafusal fibers were not modified after a 14-day period of HH. Therefore the previous hypothesis can be discounted. Modifications observed in Ia and II fiber discharges after a HH period could be also due to a shorter soleus muscle. However, our results showed no significant difference between CONT (3 ± 0.08 cm) and HH (2.8 ± 0.06 cm) muscle lengths measured at full extension of the ankle. Our results were in accordance with the data obtained by Gillette and Fell (1996), Edgerton and Roy (1996), and Brown et al. (1999) after 2 wk in HH. In our experiments, the effects of a possible shortening of the soleus muscle on the spindle discharges could therefore be discarded. Moreover, the muscle lengths on which stretches were applied were always expressed as a percentage of the minimum physiological muscle length (i.e., $L_{\text{min }+10\%}$, $L_{\text{min }+15\%}$, $L_{\text{min }+20\%}$) to prevent the possible effect of muscle shortening.

The effects observed on afferent responses from soleus muscle spindles after HH, could be explained by changes in muscle elasticity described in the literature. According to the model of Shorten (1987), the elastic properties of a muscle depend on several structures: the series elastic component (SEC) divided into an active part (cross bridges of fiber types) and a passive part (tendons) and the parallel elastic component (PEC: sarcolemma, titin, connective tissue). The SEC was only involved when a muscle was activated to transmit the force developed by the contractile component to the joints. On the contrary, in passive conditions, only the PEC was implied. Titin, sarcolemma and connective tissue (endomysium, perimysium, epimysium) participated in the passive resistance to stretch (Gajdosik 2001).

After HH, modifications in stiffness of soleus muscle and

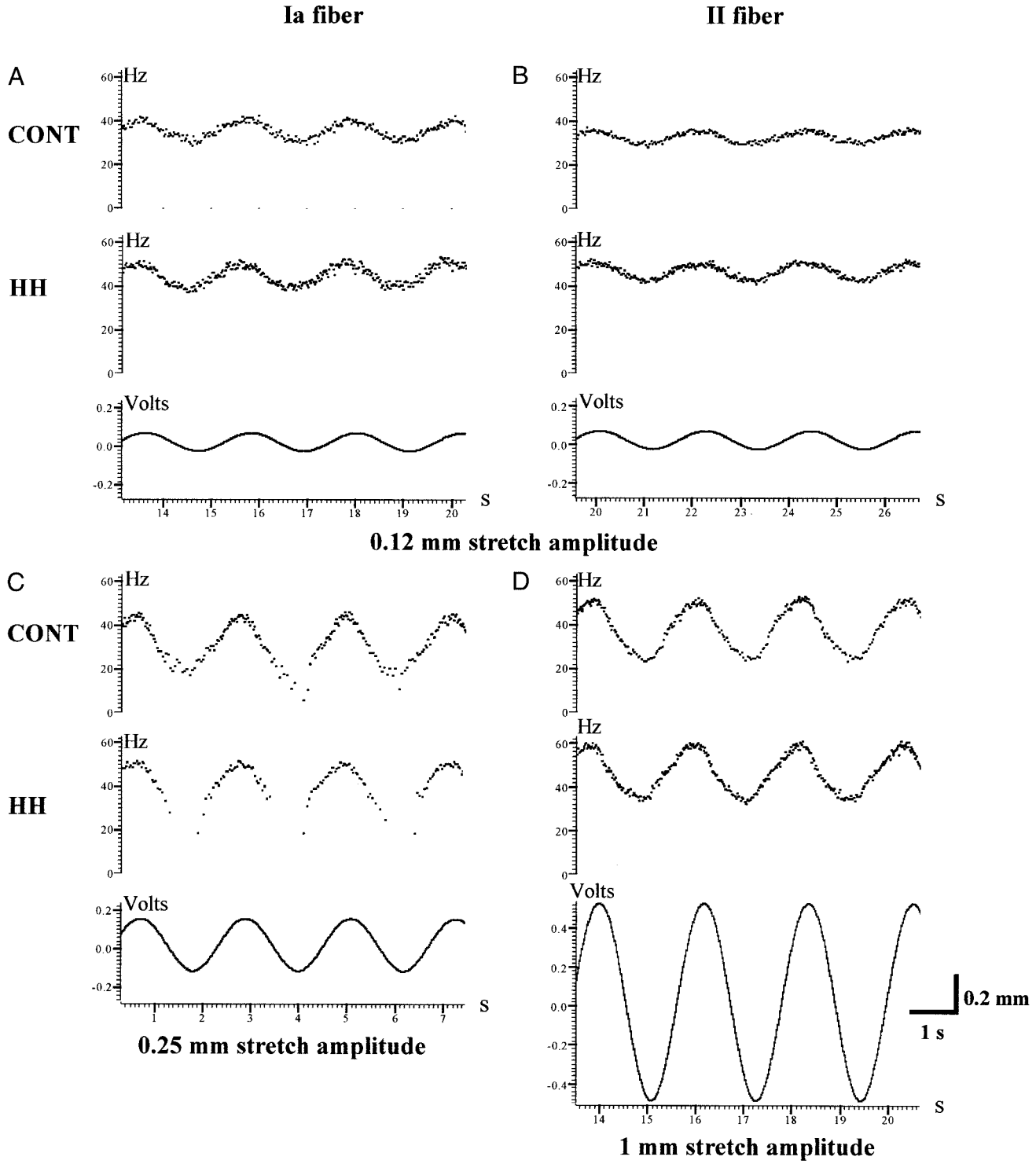

FIG. 5. Instantaneous discharges of Ia (*A* and *C*) and II fibers (*B* and *D*) under 0.5-Hz sinusoidal stretch at 0.12 mm (*A* and *B*), 0.25-mm (*C*), and 1-mm (*D*) stretch amplitudes for the CONT and HH groups. The sinusoidal stretches were expressed in volts.

Achilles tendon have been observed. However, the results were contradictory. A decrease in the stiffness of isolated soleus muscle (Canon and Goubel 1995) and in Achilles tendon stiffness (Almeïda-Silveira et al. 2000) was observed after 3 wk of unloading. These changes in elastic properties were interpreted in terms of modifications occurring in the active part and the passive part of the SEC (Canon and Goubel 1995). According to these authors, an increase in SEC compliance as a consequence of HH had probably two origins: a relative increase in fast-twitch fibers (adaptation of the active part of the SEC) and alterations in tendinous structures (adaptation of the passive part of the SEC). Indeed, hindlimb unweighting has been found to result in a higher proportion of type II fibers in the soleus muscle (Edgerton and Roy 1996). These fibers are less stiff than the slow type I fibers (Petit et al. 1990). After HH, in rat soleus muscle, Toursel et al. (1999) also reported an

TABLE 1. *Stretch frequencies at 0.12-mm amplitude*

Fiber types (groups)	0.5 Hz	1 Hz	2 Hz	3 Hz
Ia (CONT)	4.9 ± 0.2	5.5 ± 0.2	6.2 ± 0.2	6.9 ± 0.3
Ia (HH)	6.3 ± 0.3*	7.1 ± 0.4*	7.8 ± 0.4*	8.5 ± 0.4*
II (CONT)	3.3 ± 0.1†	3.9 ± 0.2†	4.3 ± 0.2†	4.7 ± 0.2†
II (HH)	4.8 ± 0.2*†	5.2 ± 0.2*†	5.8 ± 0.2*†	6.5 ± 0.2*†

Response amplitude values (means ± SE) of Ia and II fibers in control (CONT; Ia: $n = 15$; II: $n = 18$) and hypodynamia-hypokinesia (HH; Ia: $n = 18$; II: $n = 20$) groups under a 0.12-mm sinusoidal stretch applied at $L_{min+20\%}$ and at different stretch frequencies. * Significant difference with the CONT group; † significant difference with the Ia fibers in the same animal group.

increase in the compliance on a population of hybrid fast fibers, which coexpressed the MHC IIA and MHC I isoforms (MHC IIA > MHC I). Moreover, Miller et al. (2001) have demonstrated that HH induced a shift in the relative proportion of collagen isoform (type I to III) in the soleus muscle in relation to the fiber type transition (slow to fast). Thus because HH induces fiber type transitions, it could be a factor for stiffness modifications (Canon and Goubel 1995). These changes could affect the muscle passive tension as previously suggested by McHugh et al. (1999). However, it has been demonstrated recently by Kubo et al. (2001) that the passive stiffness was independent of the elasticity of tendon structures (SEC), which had no effect on the muscle performance during stretch-shortening cycle exercise. Although an immobilization in a shortened position led to an increase in the tendinous structure extensibility, it has been observed that prolonged immobilization in this position produced an increase in stiffness of the whole muscle tendon complex (Woo 1986).

Moreover Gillette and Fell (1996) have demonstrated that passive tension of the soleus muscle significantly increased in rat hindlimbs after 7 and 14 days of whole body suspension. The increased passive tension in hindlimb muscle was not attributed to a shorter muscle but was supposed to be due to changes in muscle architecture, visco-elastic properties of the muscle or its connective tissue elements, or cytoskeletal protein alterations (Gillette and Fell 1996). The major factor contributing to the passive tension in a muscle was the extensibility of the connective tissue in parallel with the muscle fibers. Indeed, Gajdosik (2001) showed that passive stiffness was influenced by lengthening deformation of the connective tissues of the endomysium, perimysium, and epimysium (PEC) of the muscle belly with a large contribution of the perimysium due to its large amount in a muscle. After HH, a decrease in the concentration of myofibrillar proteins and a relative increase in the proportion of noncontractile tissue (Flynn and Max 1985; Herbert et al. 1988; Templeton et al. 1984) in the rat soleus muscles have been observed. More recently, Brown and Hasser (1996) have also described an increase in connective tissue. Miller et al. (2001) reported an increase in collagen concentration when expressed as a function of soleus cross-sectional area. Brown et al. (1999) have shown that after 2 wk in HH, the soleus muscle stiffness remained unchanged, whereas the muscle mass decreased. In these conditions, the stiffness normalized to the muscle mass appeared increased after HH. These authors concluded that after HH, the remaining muscle tissue became stiffer. Moreover, when a muscle is stretched, the viscosity and/or stiffness of the muscle-tendon unit is reduced, which could be a factor to increase the joint range of motion (Kubo et al. 2001). However, after a period of hindlimb unloading, the range of motion of the rat soleus muscle was reduced and could thus suggest an increase in soleus muscle stiffness (Brown et al. 1999).

To conclude, the muscle spindle discharges were increased after HH. This could be in relation with an increase in the connective tissue that could contribute to a better transmission of the passive muscle stretch as previously advanced by authors in tenotomized (Hnik and Lessler 1973a) and shortened immobilized muscles (Gioux and Petit 1993; Jozsa et al. 1990; Maier et al. 1972; Tardieu et al. 1982). Maier et al. (1972) suggested that the increased of muscle spindle discharge, observed after immobilization in a plantar flexion, was due to great atrophy of extrafusal fibers. In this condition, the proportion of total tension borne by intrafusal fibers should be greater. However, modifications in mechanical properties of intrafusal fibers could not be discounted after HH. In fact, it has been demonstrated that after a chronic de-efferentation of the rat gastrocnemius, there was an increase in afferent responsiveness of muscle spindles to stretch (Hnik and Lessler 1973b). Arutiunian (1976) attributed these increases to changes in the visco-elastic properties of the intrafusal fibers. From the functional point of view, it has been demonstrated that the threshold of the tendon stretch reflex was reduced after short- and long-term space flights. This reflex occurred in response to a lower tendon tap (for review, see Edgerton and Roy 1996). Our results could explain this previous data because for a given stretch amplitude, the discharges of Ia and II fibers were increased after a HH period comparatively to those of the CONT group.

This work was supported by grants from the Centre National d'Etudes Spatiales (CNES: 3027) and the Conseil Régional du Nord Pas-de-Calais.

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Volume 50(11): 1543–1553, 2002
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

ARTICLE

Expression of Myosin Heavy Chain Isoforms Along Intrafusal Fibers of Rat Soleus Muscle Spindles After 14 Days of Hindlimb Unloading

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SUMMARY Morphological, contractile, histochemical, and electrophoretical characteristics of slow postural muscles are altered after hindlimb unloading (HU). However, very few data on intrafusal fibers (IFs) are available. Our aim was to determine the effects of 14 days of hindlimb unloading on the morphological and immunohistochemical characteristics of IF in rat soleus muscle. Thirty-three control and 32 unloaded spindles were analyzed. The number and distribution of muscle spindles did not appear to be affected after unloading. There was no significant difference in number, cross-sectional area, and histochemical properties of IF between the two groups. However, after unloading, a significant decrease in slow type 1 MHC isoform and a slight increase in slow-tonic MHC expression were observed in the B and C regions of the bag1 fibers. The α-cardiac MHC expression was significantly decreased along the entire length of the bag2 fibers and in the B and C regions of the bag1 fibers. In 12 muscle spindles, the chain fibers expressed the slow type 1 and α-cardiac MHC isoforms over a short distance of the A region, although these isoforms are not normally expressed. The most striking finding of the study was the relative resistance of muscle spindles to perturbation induced by HU. **(J Histochem Cytochem 50:1543–1553, 2002)**

KEY WORDS
rat
soleus
hindlimb unloading
intrafusal fibers
histochemistry
myosin heavy chain isoforms

THE MUSCLE SPINDLES are stretch receptors that inform the central nervous system about changes in rate and muscle length (Matthews 1981; Hulliger 1984; Hunt 1990). In the rat hindlimbs, the muscle spindles are 3–5 mm long and lie parallel to the extrafusal muscular fibers. They consist of small intrafusal fibers (IFs) surrounded by a capsule (Kucera et al. 1978). Each IF has two contractile ends, an equatorial region devoid of myofibrils but rich in myonuclei (Banks et al. 1982), and receives both sensory and motor innervation (Hunt 1990; Banks 1994). Three arbitrary regions (A, B, C) have been defined in muscle spindles: (a) the A region is composed of the equatorial and juxtaequatorial regions containing the periaxial space; (b) the B region extends from the end of the periaxial space to the end of the capsule; and (c) the C region corresponds to the extracapsular portion. Three different IF types have been identified in mammalian muscle spindles: the nuclear bag1 fibers, the nuclear bag2 fibers, and the nuclear chain fibers (Banks et al. 1982). This distinction is based on morphological criteria, i.e., myonuclei accumulation, diameter, and length (Banks et al. 1982; Hunt 1990), myofibrillar ATPase activity (Ovalle and Smith 1972; Soukup 1976; Kucera et al. 1978), and immunohistochemical characteristics (Soukup et al. 1995; Wang et al. 1997; Walro and Kucera 1999). Each IF type expresses a specific combination of myosin heavy chain (MHC) isoforms with regional variations along their length (Pedrosa–Domellof et al. 1991; Kucera et al. 1992; Soukup et al. 1995). Bag2 fibers express at least six MHC isoforms (embryonic, neonatal, slow-twitch, α-cardiac, slow-tonic, and fast-twitch). Nuclear bag1 fibers express four MHC isoforms (embryonic, slow-twitch, slow-tonic, and α-cardiac) and nuclear chain fibers express

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Received for publication January 18, 2002; accepted May 29, 2002 (2A5730).

0022-1554/02/$3.30

two MHC isoforms (neonatal and fast-twitch). The importance of the primary endings to normal spindle development has been repeatedly demonstrated by selectively eliminating the sensory or motor supply in fetal or neonatal rats (for review see Soukup et al. 1995; Soukup and Novotova 1996; Maier 1997; Wang et al. 1997). These studies showed that sensory innervation is required for the development and maintenance of muscle spindle integrity and for the expression of spindle-specific MHC isoforms. Motor innervation contributes to the diversity in the distribution of the different MHC isoforms along the length of the nuclear bag fibers. Therefore, the regulation of MHC expression within the different IF types is very complex and reflects a complex interaction of inductive and suppressive effects of motor and sensory innervation as well as the intrinsic properties of specific myoblast lineages (Kucera and Walro 1990; Pedrosa–Domellof et al. 1991; Soukup et al. 1995; Wang et al. 1997; Walro and Kucera 1999). After a period of hindlimb unloading (HU) slow postural muscles, such as the soleus (ankle extensor), become atrophied. This atrophy is characterized by a decrease in muscle weight, in cross-sectional area of muscle fibers, and in strength. It is also accompanied by modifications in contraction kinetics and in histochemical and electrophoretical properties (for review see Edgerton and Roy 1996; Ohira 2000). Very few data exist concerning the effects of HU on the IF properties (Soukup et al. 1990b). During unloading, the soleus muscle is often in a shortened position (Riley et al. 1990). Consequently, because the natural mechanical stimulus of the muscle spindles is the muscular stretch (Hulliger 1984), we hypothesized that the muscle spindles were probably little or not at all stimulated. The afferent activity of Ia and II fibers originating from these stretch receptors was probably reduced. This has never been demonstrated but was suggested by Anderson et al. (1999) to explain the inhibited tendinous reflex after HU. Ohira et al. (1992) have also suggested that the afferent input may be reduced if the muscle is shortened during HU. Moreover, during HU the reactivation of Ia fibers by tendinous vibrations is an effective countermeasure to prevent muscle atrophy developed during HU (Falempin and Fodili In-Albon 1999). These authors suggested that, in this condition, the proprioceptive information was decreased. They attributed the atrophy prevention to the reactivation of muscle spindle afferents which, in turn, augmented the excitation of the α-motor neurons. It is known that the Ia afferents not only innervate IFs but also project onto α-skeletomotor and β-skeletofusimotor neurons, and onto interneurons which, in turn, project on to γ-fusimotor neurons in the spinal cord (Bernstein and Goldberg 1995). We suggest that this reduction in afferent neural activity could modify MHC isoform expression by altering the activity of β- and γ-fusimotor neurons that innervate the contractile portion of the IF. Therefore, the aim of this study was to identify the MHC isoform distribution along IFs and to determine if this distribution was modified by unloading. A panel of eight different antibodies, in combination with ATPase labeling, was used.

Materials and Methods

Animals

Eight male Wistar rats (Iffa Credo; l'Arbresle, France) weighing 280–300 g were randomly divided into two groups of four rats: control rats and HU rats. Initially, all of the rats were housed in the same room at a constant temperature (25C) with a 12-hr:12-hr light–dark cycle and were fed and allowed water ad libitum. Animals in the HU group were unloaded for 14 days using Morey's model (Morey et al. 1979). Briefly, an orthopedic tape-adhesive plaster, covering less than half of the cleaned and dried tail, was connected to the top of the cage, where a swivel allowed 360° rotation. The rats were elevated in a head-down position (30°) so that the hindlimbs could not touch the cage floor or walls, while they were able to ambulate freely on their forelimbs. The experiments and the animal housing conditions received authorization from both the Agricultural and Forest in Ministry and the National Education Ministry (Veterinary Service of Health and Animal Protection, authorization 59-00980).

Tissue Processing

Rats were anesthetized with sodium pentobarbital (35 $mg \cdot kg^{-1}$). In both groups, the right and left soleus muscles were excised, stretched to their resting length, and immediately frozen in an isopentane solution precooled to its freezing point by liquid nitrogen. The muscles were stored at −80C until histochemical and immunohistochemical analyses were performed. The soleus muscles of the control and HU groups were cut into serial frozen transverse sections (10 μm thick) using a cryostat microtome (Leica CM 1800; Heidelberg, Germany) set at −20C. Along the muscle, every 280 μm, 11 sections perpendicular to the muscle longitudinal axis were performed. The first section was used as a negative control in the immunohistochemical analysis. The next two sections were processed for myofibrillar adenosine 5′-triphosphatase (ATPase) with acid (pH 4.3) and alkaline (pH 10.4) preincubations according to the method of Guth and Samaha (1969). The remaining sections were labeled with antibodies against the different MHC isoforms.

Antibodies and Labeling

Eight monoclonal antibodies (MAbs) were used in this study (Table 1). Binding of the primary antibodies was localized by an immunoperoxidase reaction utilizing the Novostain Universal Quick Kit (Tebu-Novocastra; Le Perray–en–Yvelines, France). Serial cross-sections were incubated in prediluted blocking serum (normal horse serum) for about 10 min. The excess serum was blotted and sections were incubated with primary antibodies diluted in PBS for 2 hr. Serial cross-sections were washed for 5 min in PBS. Then the sec-

tions were incubated for 30 min in prediluted biotinylated universal secondary antibody. At the end of 30 min, sections were washed with PBS for 5 min and incubated in ready-to-use streptavidin–peroxidase complex reagent for 30 min. To label the serial cross-sections, the peroxidase substrate solution (diaminobenzidine, DAB) was added after the sections had been washed for 5 min with PBS. Positive fibers were characterized by a brown color and negative fibers remained unlabeled. Finally, after dehydration with alcohol and toluene, the slides were mounted in Eukitt resin. The cross-sectional area (CSA) and the densitometry of the antibody labeling along the different IF types were measured using an image analyzer (SAMBA 2005; Villeneuve d'Ascq, France). To avoid a disparity in the labeling, for each antibody, all the sections were identically processed with the same diluted solution. The slides were also incubated simultaneously for 5 min in a common DAB solution. The zero of the densitometer for each preparation was adjusted on the negative fibers in the section; the slight variations of labeling between each slide were therefore discarded. The immunohistochemically labeled slides were analyzed with the image analyzer. This system was made up of a digital camera fixed to a standard optical light microscope. The camera was coupled to a computer with video monitor and image acquisition and storage modules. The measures were quantified to 256 gray levels (level 1 corresponding to black color and to 0% of light transmission, and level 256 to white color and 100% of light transmission), which were then converted automatically into optical density, taking into account the studied area. The densitometric values were expressed in percentage ($OD = \log_{10}$ (1/light transmission). According to Nibbering et al. (1986), the formation of the reaction product after DAB/H_2O_2 staining for immunoperoxidase-labeled cells indicated a linear relationship between the amount of enzyme-coupled antibodies bound to cells and the amount of enzyme reaction product. With the densitometric approach, the more elevated OD is, the more important the protein amount is. The densitometric analysis has already been used as a semiquantitative approach in rat muscular fibers (Sant'Ana Pereira et al. 1995). In muscle spindle immunohistochemistry, the immunoreactivity is usually scored as absent (−), weak to moderate (+), and strong (++). This way of expressing the results does not allow detection of fine modifications in the expression level of myosin isoforms under a given experimental condition. Consequently, we intentionally chose to express our results as values obtained using densitometric measurements. However, as for the ATPase activity (Kucera 1981), in the most nucleated equatorial region of the IFs, the MAb labeling was present only around the IFs because the central part of the IFs was not labeled. In this case, the densitometric measurement was made only on the colored part of IFs without taking into account the IF center.

Identification of IF Types

The muscle spindles were identified in both control and unloaded soleus muscles groups as encapsulations of small-diameter fibers. As stated previously, each muscle spindle can be divided into two encapsular regions (A and B regions) and one extracapsular portion (C region). Intrafusal fibers were classified as nuclear bag1, nuclear bag2, and nuclear chain fibers according to their morphology and their histochemical reaction after preincubation in acid and alkaline solutions (Soukup 1976; Kucera et al. 1978) and their MHC isoform contents, which were not uniform along their length (Pedrosa–Domellof et al. 1991; Kucera et al. 1992; Soukup et al. 1995). Rat muscle spindles usually contain four IFs: one bag1, one bag2, and two chain fibers (Kucera and Walro, 1988b). The diameter of these IFs decreases in the following order: nuclear bag2 > bag1 > chain fibers (Soukup 1976). Nuclear bag1 fibers exhibit low acid and alkaline ATPase activity along their intracapsular region but have high acid ATPase activity at their poles. Nuclear bag2 fibers have an acid-stable ATPase activity along their length and an intracapsular alkaline-stable ATPase activity that is lost beyond the capsular sleeve. Nuclear chain fibers have low acid and high alkaline ATPase activities along their entire length (Kucera et al. 1978; Khan and Soukup 1988). The nuclear bag1 and bag2 fibers were labeled with both ALD58 MAb, specific for the slow-tonic MHC isoform, and F88 MAb, specific for the α-cardiac MHC isoform. Nuclear chain fibers did not express these two MHC isoforms, except in a very short equatorial length where the slow-tonic isoform was detected (Pedrosa et al. 1989; Pedrosa–Domellof et al. 1991).

Statistical Analysis

All results were expressed as means ± SD. A Student's *t*-test was used to establish the intergroup comparisons and statistical significance was accepted at $p<0.05$.

Results

A total of 33 control muscle spindles (33 bag2, 33 bag1, 69 chain fibers) and 32 HU muscle spindles

Table 1 Antibodies used in this study and their specificity in the rat

Antibodies	Type	Dilution	MHC source	MHC isoforms	References
NCL MHCs (Tebu Novocastra)	IgG	1:20	Rabbit	1	Hoh and Hughes 1989
ALD58 (DSHB)	IgG	1:5	Chicken	Slow-tonic	Shafiq et al. 1984
SC71 (DSMZ)	IgG	1:20	Bovine	2A	Schiaffino et al. 1989
BF-F3 (DSMZ)	IgM	1:10	Bovine	2B	Schiaffino et al. 1989
MY32 (Sigma; St Louis, MO)	IgG	1:2000	Rabbit	2A, 2B, 2X	Harris et al. 1989
F88 (Coger; Paris, France)	IgG	Undiluted	Human	α-cardiac	Leger et al. 1985
NCL MHCd (Tebu Novocastra)	IgG	1:50	Newborn rat	Embryonic and neonatal	Davis et al. 1991
2B6	IgG	1:2000	Rat	Embryonic	Gambke and Rubinstein 1984

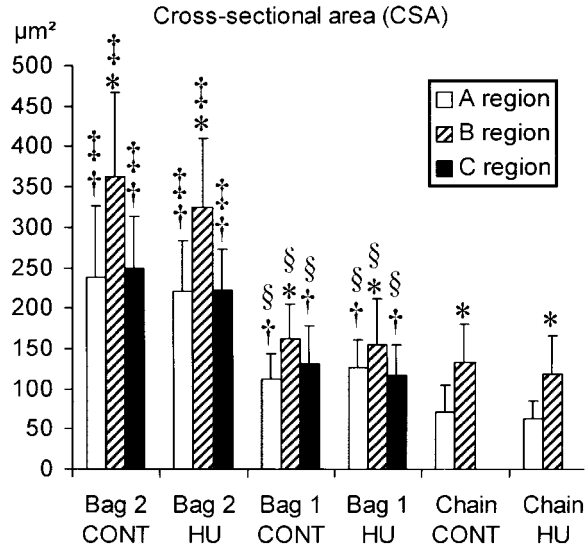


Figure 1 Histogram of cross-sectional areas (CSAs) of the three intrafusal fiber types in A, B, and C regions of the control (CONT) and hindlimb unloaded (HU) groups. Empty bar, A region; hatched bar, B region; full bar, C region. *Significant differences of CSA with the A region, †with the B region, ‡with bag1 and chain fibers, and §with chain fibers.

(32 bag2, 32 bag1, 67 chain fibers) were analyzed throughout their entire length. Most of the muscle spindles in the control and unloaded groups contained one bag1, one bag2, and two chain fibers. However, in three control and four HU muscle spindles, there were three nuclear chain fibers. There was no significant different between the number of muscle spindles in control (14.3 ± 1.5) and HU groups (13.5 ± 1.3).

Cross-sectional Areas

The CSAs of IFs are shown in Figure 1. For both control and HU groups, the CSAs of bag2 fibers were significantly larger than those of bag1 and chain fibers, and those of bag1 fibers were higher than those of chain fibers, independent of the muscle spindle region. For all IF types in both animal groups, the CSAs in the B region were significantly higher than those in the A and C regions (Figure 1). After 14 days of unloading, no significant difference in the CSAs of all IF types between control and HU groups was observed.

Histochemical Analysis

In Figure 2 (control group), the Figures 2.1–2.3 and Figures 2.4–2.6 respectively illustrative the acid (pH 4.3) and alkaline (pH 10.4) ATPase activity of IFs in the A, B, and C regions of a muscle spindle. In the muscle spindles of the control group, the nuclear bag1 fibers had low to moderate acid ATPase activity in A and B regions, but the activity was high at the end of the B region and towards the poles. After alkaline preincubation, they showed a low ATPase activity along their entire length. Nuclear bag2 fibers exhibited high acid ATPase activity and high to moderate alkali ATPase labeling along their length. However, in the more extracapsular region, the nuclear bag2 fibers lost their alkaline ATPase activity. In general, after acid preincubation, nuclear chain fibers exhibited low ATPase activity and high to moderate alkali ATPase labeling along their entire length. However, the nuclear chain fibers in the muscle spindles of Figure 2 exhibited moderate acid ATPase activity in the A region. In the most equatorial region, all IF types had a rim or no ATPase activity. Histochemical labeling did not show any difference between control and HU groups. However, it is obvious that a slight increase or decrease in one of the MHC isoforms would not necessarily be made visible by changes in ATPase activity. Moreover, ATPase labeling does not allow demonstration of the co-expression of several MHC isoforms within a fiber.

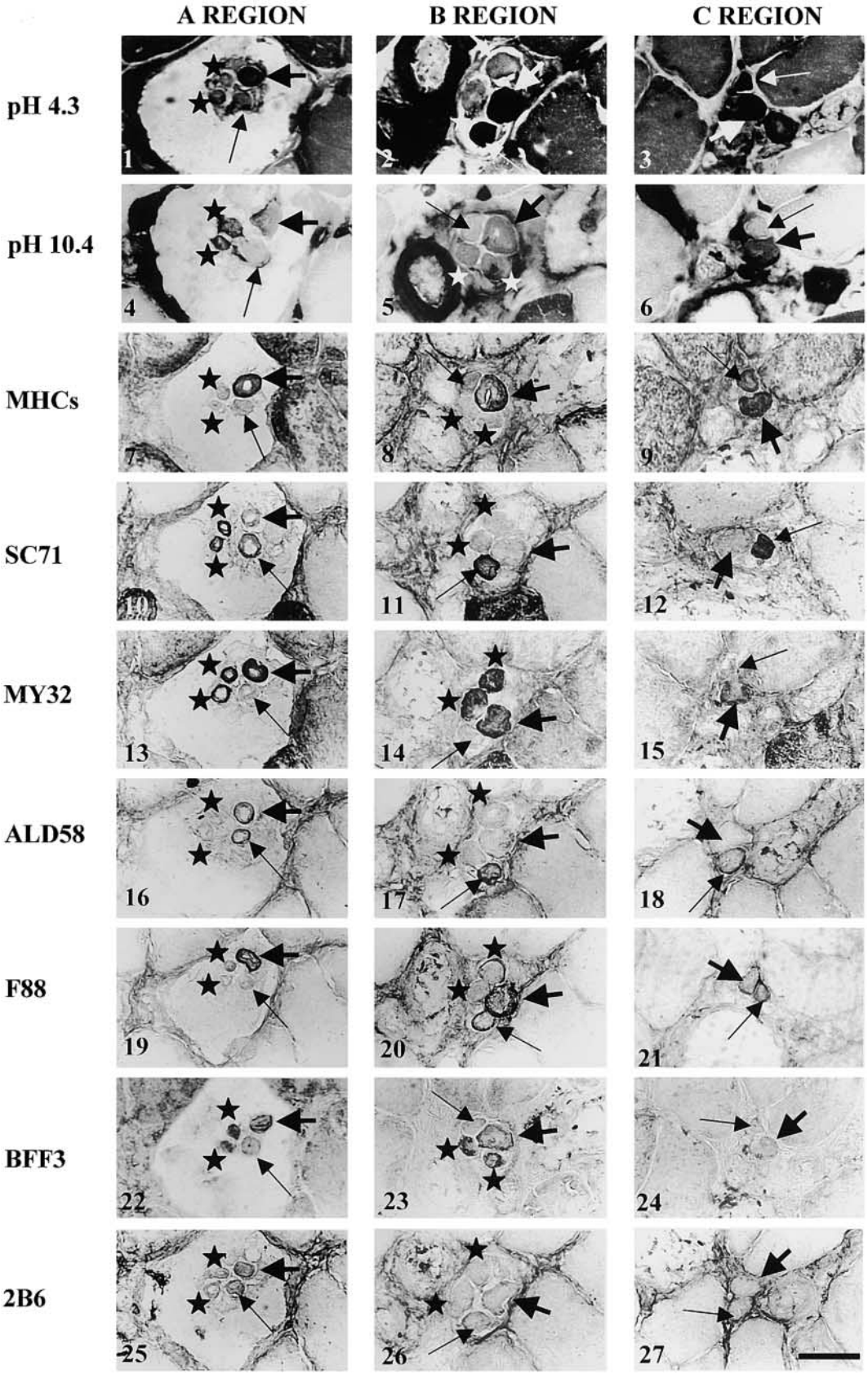
Immunohistochemistry

The regional variation of labeling with all antibodies is illustrated in Figure 2 for the control group. Figure 3 illustrates only the antibody labelings that were changed by the HU condition. The results have been refined by densitometric measurements (Table 2, control group and Table 3, HU group).

Control Group

Nuclear Bag1 Fibers. NCL MHCs (Figures 2.7–2.9) labeled only the fibers towards the poles at the end of the B region and in the C region. SC71 (Figures 2.10–2.12) and ALD 58 MAbs (Figures 2.16–2.18) strongly labeled the fibers in A and B regions and the labeling slightly decreased towards the C region (Table 2). The nuclear bag1 fibers were labeled with F88 MAb (Figures 2.19–2.21) over a short distance in the B and C regions. The binding with F88 MAb was

Figure 2 Transverse sections (10 µm thick) through the encapsulated A and B regions and extracapsular C region of a rat soleus spindle in the control group. The spindle contains one bag1 (thin arrow), one bag2 (thick arrow), and two chain fibers (star). Sections were processed for ATPase labeling with acid (pH 4.3, **2.1–2.3**) and alkaline (pH 10.4, **2.4–2.6**) preincubations and were labeled with NCL MHCs (MHCs) (**2.7–2.9**), SC71 (**2.10–2.12**), MY32 (**2.13–2.15**), ALD58 (**2.16–2.18**), F88 (**2.19–2.21**), BFF3 (**2.22–2.24**), 2B6 (**2.25–2.27**) antibodies. NCL MHCd is not shown. Bar = 40 µm.


A REGION
B REGION
C REGION
pH 4.3
pH 10.4
MHCs
SC71
MY32
ALD58
F88
BFF3
2B6
1
2
3
4
5
6
7
8
9
10
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12
13
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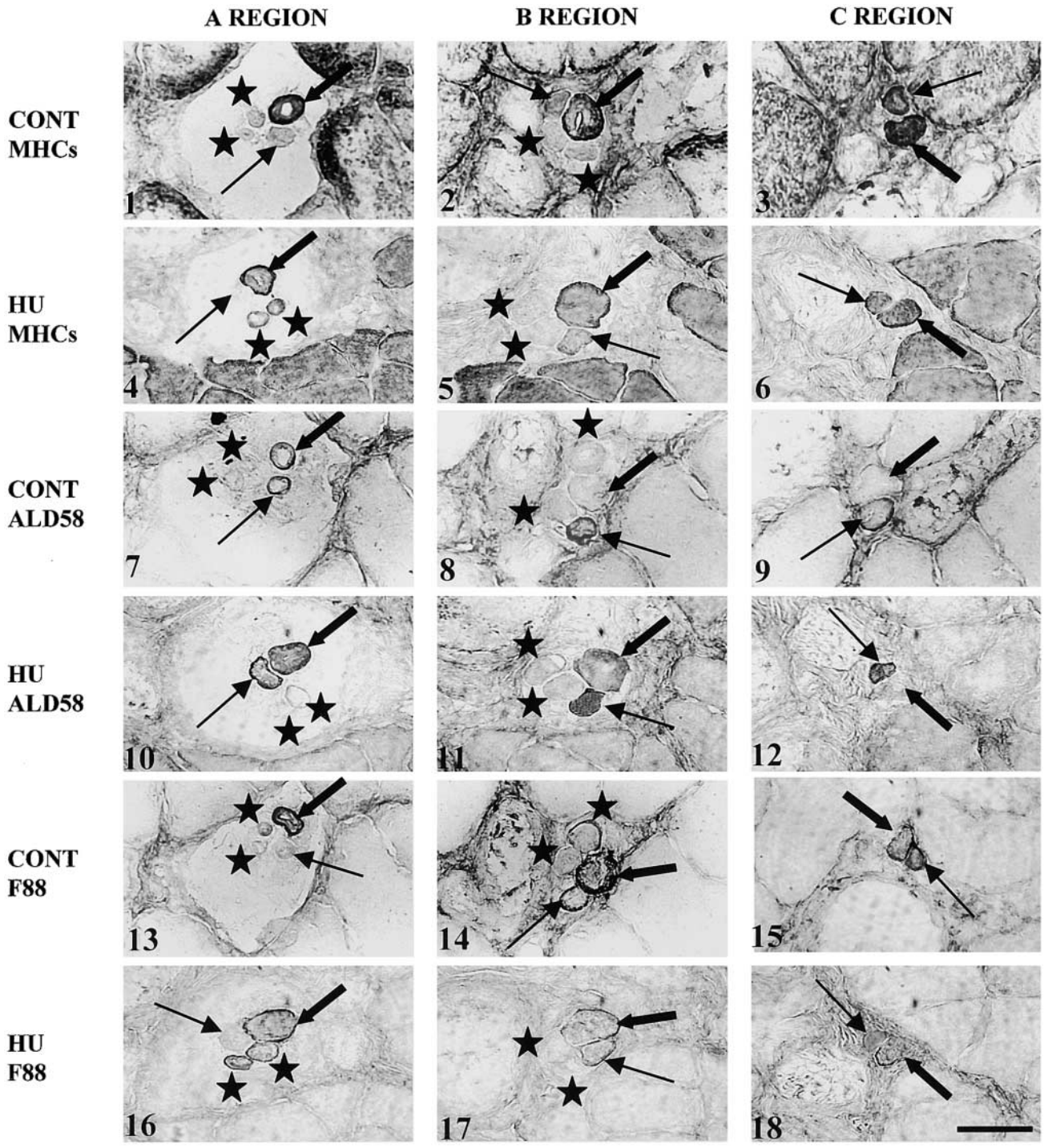

Figure 3 Transverse sections (10 μm thick) through the encapsulated A and B regions and extracapsular C region of a rat soleus spindle in the control (CONT) and in the hindlimb unloaded (HU) groups. The spindle of the HU group contains one bag1 (thin arrow), one bag2 (thick arrow), and two chain fibers (star). Sections were labeled with NCL MHCs named MHCs in the figure (CONT: **3.1–3.3**; HU: **3.4–3.6**), ALD58 (CONT: **3.7–3.9**; HU: **3.10–3.12**), F88 (CONT: **3.13–3.15**; HU: **3.16–3.18**). Bar = 40 μm.

strong to moderate in the B region and low to moderate towards the C region. The labeling with 2B6 MAb (Figures. 2.25–2.27), specific for embryonic MHC, was low in the A region. No binding was seen in the B and C regions of bag1 fibers. Whatever the IF MY32 (Figures 2.13–2.15), BFF3 (Figures 2.22–2.24), NCL MHCd MAbs did not label the nuclear bag1 fibers (not shown).

Table 2 Densitometric analysis of the immunohistochemical labeling in intrafusal fibers of the control group (CONT)

Fibers	Region	NCL MHCs	SC71	MY32	ALD58	F88	BF-F3	2B6
Bag1 (CONT) $n=33$	A	NL	27.4 ± 6.6	NL	22.1 ± 5.3	NL	NL	6.2 ± 1.1
	B	14.6 ± 5.9	27.8 ± 3.6	NL	21.6 ± 5.1	30.4 ± 7.4	NL	NL
	C	23 ± 4.9†	23.7 ± 6.2*†	NL	16.6 ± 4*†	26.6 ±6.9†	NL	NL
Bag2 (CONT) $n=33$	A	24.4 ± 4.1	15.8 ± 4.8	24.4 ± 6.7	19.4 ± 5.4	35.7 ± 10.7	10.6 ± 2.3	4.7 ± 1.6
	B	23.9 ± 4.4	NL	23.3 ± 6.6	NL	32.5 ± 10.8	8.7 ± 2.1*	NL
	C	23.6 ± 5.4	NL	19 ± 5.6*†	NL	27.6 ± 8.6*†	NL	NL
Chain (CONT) $n=69$	A	NL	23.2 ± 8.1	26.2 ± 8.1	NL	NL	9.1 ± 3.3	NL
	B	NL	NL	24.9 ± 7.2*	NL	NL	13.4 ± 2.9*	NL

*Significant difference of labeling with the A region.
†Significant difference of labeling with the B region. With the NCL MHCd antibody, no labeling (NL) was seen in any of the intrafusal fibers.

Nuclear Bag2 Fibers. NCL MHCs MAb reacted strongly with the nuclear bag2 fibers along their entire length. The nuclear bag2 fibers bound MY32 and F88 MAbs along their entire length but the binding intensity decreased from the A to the C region (Table 2). With SC71, ALD58, and 2B6 MAbs, the nuclear bag2 fibers were labeled only in the A region. BF-F3 MAb reacted in both A and B regions of bag2 fibers; the labeling was lower in the B region. NCL MHCd MAb did not label bag2 fibers.

Nuclear Chain Fibers. Nuclear chain fibers reacted with MY32 MAb in the A and B regions. BF-F3 labeled the nuclear chain fibers in the A and B regions, with higher labeling in the B region (Table 2). SC71 MAb bound only the A region of nuclear chain fibers. Our results showed no labeling with NCL MHCs, ALD58, F88, 2B6, and NCL MHCd MAbs in any region of the nuclear chain fibers.

HU Group. There was no difference with the control group in the regional variation and in the labeling intensity with SC71, MY32, BF-F3, 2B6, and NCL MHCd MAbs along the nuclear bag1 and bag2 fibers.

Nuclear Bag1 Fibers. NCL MHCs, ALD58, and F88 MAbs (Figures 3.4–3.6, 3.10–3.12, and 3.16–3.18, respectively) showed modified labeling compared to the control group (Figures 3.1–3.3, 3.7–3.9, and 3.13–3.15, respectively). Densitometric analysis, presented in Table 3, showed that type 1 MHC expression in nuclear bag1 fibers decreased significantly in both B and C regions after unloading. Moreover, the labeling intensity with ALD58 MAb, specific for slow-tonic MHC isoform, was significantly increased in the B and C regions of bag1 fibers. With F88 MAb, our results showed that the expression of α-cardiac MHC isoforms in bag1 fibers was significantly decreased in the B and C regions after unloading.

Nuclear Bag2 Fibers. After unloading, the labeling intensity with F88 MAb significantly decreased in all regions of the nuclear bag2 fibers compared with the control group. There was no difference with the control group in the regional variation and the labeling intensity with ALD58 MAb along bag2 fibers. However, although the difference was not significant, type 1 MHC isoform expression presented a slight decrease in the B and C regions of the nuclear bag2 fibers after a period of unloading.

Nuclear Chain Fibers. There was no difference with the control group in the regional variation and labeling intensity along the nuclear chain fibers with

Table 3 Densitometric analysis of the immunohistochemical labeling in intrafusal fibers of the HU group[a]

Fibers	Region	NCL MHCs	SC71	MY32	ALD58	F88	BF-F3	2B6
Bag1 (HU) $n=32$	A	NL	25.8 ± 5.8	NL	24.5 ± 4.9	NL	NL	6.4 ± 2
	B	11.1 ± 4.3§	25.5 ± 5.7	NL	24.8 ± 5.1§	22.1 ± 5.5§	NL	NL
	C	20.4 ± 4.7§†	21.6 ± 7.6*†	NL	20.8 ± 5.4§*†	17.3 ± 7.1§†	NL	NL
Bag2 (HU) $n=32$	A	22.3 ± 4.7	15.7 ± 5.2	22.9 ± 5	18.1 ± 3.9	24.2 ± 6.2§	10.2 ± 2.7	5 ± 1.3
	B	22 ± 3.7	NL	20.6 ± 4.9	NL	22.8 ± 5.6§	9.1 ± 2.3*	NL
	C	21.5 ± 4.5	NL	18.3 ± 5.9*	NL	20.2 ± 5.7§*†	NL	NL
Chain (HU) $n=41$	A	NL	21.1 ± 6.3	26.6 ± 6	NL	NL	8.3 ± 3	NL
	B	NL	NL	22.6 ± 5.5*	NL	NL	12.6 ± 3.8*	NL
Special Chain $n=26$	A	20.2 ± 3.5	Idem Chain	Idem Chain	NL	21.9 ± 3.7	Idem Chain	NL

[a]Densitometric analysis presented in percentage of bag1, bag2, and chain fibers in the HU group. Values are given for each antibody in the three different muscle spindle regions (A, B, C).
§Significant difference of labeling with control group.
*Significant difference of labeling with the A region.
†Significant difference of labeling with the B region. With the NCL MHCd antibody, no labeling (NL) was seen in any of the intrafusal fibers. The chain fibers labeled with NCL MHCs and F88 in 12 muscle spindles are called special chain fibers.

all antibodies, except for NCL MHCs and F88 MAbs. Indeed, among the 32 muscle spindles that were studied, only 12 presented nuclear chain fibers that bound the NCL MHCs and F88 MAbs (Figures 3.4 and 3.16, respectively) over a short distance of the A region (special chain in Table 3), whereas chain fibers in the control group never bound these antibodies.

Discussion

Morphological Characteristics

The number and distribution of muscle spindles in the rat soleus were not significantly different in the two experimental groups. These data are in agreement with those described previously by Soukup et al. (1990b). Our results showed that, in the control group, the bag2 fiber CSAs were greater than the bag1 fiber CSAs, which were also larger than the chain fiber CSAs. These data are in agreement with the results of other authors (Botterman and Edgerton 1975; Soukup 1976; Soukup et al. 1993). The same results were obtained after unloading. It therefore appears that IFs are more resistant to myogenic atrophy than the extrafusal fibers, as suggested earlier by other authors (Yellin and Eldred 1970; Maier et al. 1972).

Histochemical Analysis

In the control group, our data are similar to those obtained by other authors for the same species (Botterman and Edgerton 1975; Soukup 1976; Kucera et al. 1978; Kucera and Walro 1987). In some control muscle spindles, the nuclear chain fibers have moderate acid-stable ATPase activity. Soukup (1976) has also reported some differences in ATPase labeling. In the rat soleus muscle spindles, the nuclear chain fiber ATPase activity was low, medium, or high after acid preincubation.

After a 14-day period of HU, no significant difference was observed in the ATPase labeling in the IFs compared to the control group. The regional variation in ATPase labeling along the IFs is due to the non-uniform expression of MHC isoforms in the different regions of those fibers (Kucera and Walro 1989; Pedrosa et al. 1989; Kucera et al. 1992; Soukup et al. 1995).

MHC Isoform Expression of IFs in Control and HU Groups

Control Group. Our results are in agreement with other studies concerning the regional variation of labeling with NCL MHCs (Pedrosa–Domellof et al. 1991; Kucera et al. 1992; Soukup et al. 1995; Walro et al. 1997; Wang et al. 1997), MY32 (Pedrosa et al. 1989; Kucera et al. 1992), ALD58 (Kucera and Walro 1989; Kucera et al. 1992; Soukup et al. 1995; Soukup and Thornell 1997), and F88 (Pedrosa et al. 1990; McWhorter et al. 1995).

However, some differences were observed with SC71, BF-F3, 2B6, and NCL MHCd antibodies. Our results showed that SC71 bound to the encapsulated polar region of bag1 fibers and labeling was also seen in the juxtaequatorial region in bag1 and chain fibers. Our results were in accordance with those described by Kucera et al. (1992). However, in the A region of our nuclear bag2 fibers, only a low level of labeling was seen with the SC71 MAb. This difference in the labeling with SC71 between our results and those of Kucera et al. (1992) could be due to the antibody dilution. Indeed, Kucera et al. (1992) used the SC71 with a 1:600 dilution, whereas we used a 1:20 dilution.

A difference in labeling was observed in the nuclear bag1 fibers with MY32 and SC71 MAbs. Nuclear bag1 fibers were labeled by SC71 along their entire length, whereas MY32 MAb never labeled these fibers. This was surprising because the MY32 MAb is supposed to react with all fast-twitch MHC isoforms (Schiaffino et al. 1989). SC71 MAb is specific for the MHC 2A isoform and never crossreacts with other MHC isoforms. Therefore, the fast-twitch MHC isoform, recognized in nuclear bag1 fibers by the SC71 but not by MY32, could be a specific muscle spindle MHC isoform whose epitope resembles that recognized by SC71 on the MHC 2A isoform. The possible existence of other specific muscle spindle MHC isoforms not expressed in extrafusal fibers has already been suggested by other authors (Kucera et al. 1992; Pedrosa–Domellof et al. 1993). Kucera et al. (1992) suggested that the labeling intensity and the regional variation with MY32 were higher and broader than those with SC71 (MHC 2A) and BF-F3 (MHC 2B) MAbs, indicating the possible existence of a specific fast-twitch MHC isoform expressed by the IFs but not by the extrafusal fibers.

Our results show that the BF-F3 MAb bound only the nuclear bag2 and chain fibers in the A and B regions, whereas Kucera et al. (1992) showed that these fibers were labeled only in the B region. This difference with our data could therefore be explained by the dilution (1:200) that they used, since we used a lower dilution (1:10).

Only a low level of labeling or no labeling was observed with 2B6 and NCL MHCd antibodies, respectively. These labeling differences reflected either differences in antibody affinities or the existence of more than one embryonic MHC isoform (Silberstein and Blau 1986). 2B6 gave a low level of labeling in the juxtaequatorial region of the nuclear bag fibers, and bag1 fibers were more intensely labeled than the nuclear bag2 fibers. Maier et al. (1988) observed that, with 2B6 MAb diluted at 1:50 (Gambke and Rubinstein 1984), the nuclear bag1 and bag2, but not nu-

clear chain fibers, were labeled in the juxtaequatorial and polar regions. However, we observed that, at this dilution, 2B6 MAb crossreacted with the fast type 2A myosin in extrafusal fibers (data not shown). We therefore used a 1:2000 dilution to remove this crossreaction. On the other hand, Harris et al. (1989) showed that 2B6 MAb was effective at a dilution of 1:2000–1:4000 to label the embryonic MHC isoform. Therefore, the labeling observed by Maier et al. (1988) could be due to the expression of both embryonic and MHC 2A isoforms in the nuclear bag fibers. However, our results showed that nuclear chain fibers expressed the MHC 2A isoform in the A region. If the labeling pattern observed with 2B6 MAb in the nuclear bag fibers was also due to the crossreactivity with the MHC 2A isoform, in the study of Maier et al. (1988), the nuclear chain fibers should be labeled by 2B6 MAb. This is not the case. Such a surprising result could support the hypothesis that, in the nuclear bag1 fibers, the MHC isoform recognized by SC71 MAb is not the MHC 2A isoform but a specific fast-twitch isoform not recognized by 2B6 antibody. This is perhaps the reason why, in the study of Maier et al. (1988), 2B6 did not label the nuclear chain fibers at 1:50 dilution.

Kucera et al. (1992), using three different MAbs against embryonic MHC, observed either no, medium, or high-intensity labeling in IFs. Differences in antibody affinities and/or the existence of more than one embryonic MHC isoform (Silberstein and Blau 1986) cannot be ruled out. Our data showed no labeling with NCL MHCd MAb in all the IFs, which was in agreement with the results obtained by Kucera et al. (1992) using the BF-B6, another MAb specific for neonatal MHC. However, using other antibodies, it has been observed that the neonatal MHC is expressed in the nuclear bag2 and nuclear chain fibers (Pedrosa–Domellof et al. 1991; Soukup et al. 1995). Discrepancies among all these results could be related to the different specificities of the antibodies. The fact that no labeling was observed with NCL MHCd could be due to the presence of a small amount of neonatal MHC isoform that was not detected by the immunohistochemical method.

HU Group. All the observations on regional variation and labeling intensity in muscle spindles of the control group were also valid for the HU group. However, after unloading, some differences were observed in the expression level of slow type I, slow-tonic, and α-cardiac MHC isoforms.

The regulation of the α-cardiac MHC isoform expression along the length of bag fibers is under the influence of motor innervation. The expression of α-cardiac MHC appears one day after the arrival of bag1 and bag2 γ-motor innervation (Pedrosa et al. 1990). After neonatal de-efferentation, the reactivity of nuclear bag2 fibers to the anti-α-cardiac MHC isoform was decreased and was limited to a shorter portion of the fibers, whereas the nuclear bag1 fibers were unreactive (Pedrosa et al. 1990; Pedrosa–Domellof et al. 1991). Conversely, slow-tonic MHC isoform expression along the length of bag fibers is undoubtedly related to the presence of sensory innervation. Indeed, neonatal denervation (Soukup et al. 1990a; Kucera et al. 1993) and deafferentation (Kucera and Walro 1987,1988a,b) prevented the expression of the slow-tonic MHC isoform. However, after neonatal de-efferentation, the regional variation of this MHC isoform along the intrafusal bag2 fibers is modified and the slow-tonic MHC isoform is expressed more intensely and over most of their length (Soukup et al. 1990a; Pedrosa–Domellof et al. 1991).

In adult rat muscle spindles, deafferentation or de-efferentation produced less severe alterations, but the expression pattern of some MHC isoform along the IF was modified (Wang et al. 1997). Wang et al. (1997) observed that, when adult rat muscle spindles were de-efferented in the extensor digitorum longus (EDL) muscle, the nuclear bag1 fibers, which normally express the α-cardiac MHC in the outer B region, ceased to express this isoform. Moreover, bag2 fibers continued to express this isoform but less intensely than normally. Pedrosa et al. (1990) and Soukup et al. (1990a) both showed that, after denervation or neonatal de-efferentation, the expression of the α-cardiac MHC was decreased and the slow-tonic expression was increased along the length of nuclear bag fibers. According to these studies, we suggest that the decrease in α-cardiac and the increase in slow-tonic MHC isoforms in nuclear bag fibers reflect a decrease in the activity pattern of the motor nerves during unloading. This hypothesis is reinforced by the fact that, when extrafusal fibers are deprived of motor innervation (Harris et al. 1989), the expression of slow type 1 MHC isoform decreases. Similarly, our results showed that the expression of slow MHC isoform significantly decreased in the nuclear bag1 fibers after a period of unloading. However, Walro et al. (1997), who used two kinds of deafferentation, concluded that regulation of MHC expression in adult EDL muscle of rat also depended on neurotrophic factors transported anterogradely from afferent neurons to the IFs.

It is more difficult to understand why slow-twitch and α-cardiac MHC isoforms are expressed over a very short distance of the A region in nuclear chain fibers after unloading. The expression of these two MHC isoforms is usually dependent on motor innervation, whose influence decreases from the C to the A region. Therefore, the expression of slow-twitch and α-cardiac MHC isoforms over only a short portion of the A region was unexpected. No previous studies on

fetal, neonatal, or adult muscle spindles have reported a possible afferent or motor influence on the expression of slow-twitch and α-cardiac MHC isoforms in nuclear chain fibers.

To conclude, after unloading, the muscle spindle integrity and the labeling pattern of the majority of MHC isoforms were preserved. However, some differences were observed: a decrease in α-cardiac MHC expression and an increase in slow-tonic expression along the nuclear bag fibers. In the literature, it has been demonstrated that the level of α-cardiac MHC expression (Pedrosa et al. 1990; Soukup et al. 1990a; Pedrosa–Domellof et al. 1991) and the regional variations in slow-tonic MHC expression depended on motor innervation (Soukup et al. 1990a; Pedrosa–Domellof et al. 1991). Consequently, we hypothesized that the observed changes could be due to a decreased influence of motor innervation. In fact, it has been demonstrated that Ia afferents project not only onto α-skeletomotor neurons but also onto β-skeletofusimotor and γ-fusimotor neurons (Bernstein and Goldberg 1995). Therefore, the decrease in motor innervation activity could be due to decreased proprioceptive information. During unloading, the soleus muscle is often in a shortened position (Riley et al. 1990) and electromyographic activity is considerably reduced (Blewett and Elder 1993). As early suggested by Ohira et al. (1992), we supposed that the muscle spindles, which are stretch sensors, are probably little or not at all stimulated. Consequently, the afferent activity of Ia and II fibers originating from these stretch receptors might be reduced, as shown indirectly by Falempin and Fodili In-Albon (1999). We therefore conclude that proprioceptive feedback could regulate the expression of some MHC isoforms in adult muscle spindles, although changes in neurotrophic factors anterogradely transported from afferents to intrafusal fibers during hindlimb unloading cannot be ruled out.

Acknowledgments

Supported by grants from the CNES (3027) and the Conseil Régional du Nord Pas-De-Calais.

The SC-71 and BF-F3 antibodies developed by Schiaffino et al. (1989) were obtained from the Deutsche Sammlung von Mikroorganismen and Zellkulturen Gmbh (DSMZ; Braunschweig, Germany). The ALD58 antibody developed by Shafiq et al. (1984) was obtained from the Developmental Studies Hybridoma Bank (DSHB; Iowa City, IA) maintained by The University of Iowa (Department of Biological Sciences, Iowa City, IA). We thank Dr A. Kelly and Dr N.A. Rubinstein for their gift of 2B6 to Dr G.S. Butler Browne.

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Experimental Neurology 195 (2005) 313 – 321

Experimental Neurology

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Regular Article

The structure and response properties of Golgi tendon organs in control and hypodynamia–hypokinesia rats

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Received 25 March 2005; revised 13 May 2005; accepted 13 May 2005
Available online 5 July 2005

Abstract

The Golgi tendon organs (GTOs) are encapsulated mechano-receptors that, in normal conditions, monitor via Ib afferent fibers the contractile force. A 14-day period of hypodynamia, absence of weight bearing and hypokinesia, and reduction of motor activity (HH) is known to induce changes in postural muscles such as the soleus. At present, there is no data available regarding the Ib afferent feedback in normal rats (CONT group) and in rats after a hypodynamia–hypokinesia (HH group) period. Consequently, the aim of our study was to determine the HH effects on the morphological (histochemistry on gross morphology) and electrophysiological properties of the GTOs in rat soleus muscle. In the histological study, nine CONT and nineteen HH GTOs of the soleus muscle were identified. The results demonstrated that HH GTOs were morphologically similar to the CONT GTOs. Regarding the electrophysiological study, a L_2–L_6 laminectomy was performed under deep anesthesia (sodium pentobarbital, 60 mg kg^{-1}). Responses in single Ib fibers from the L_5 dorsal root to the isometric twitch and tetanic fused contractions of "in-series" motor units (MUs) were recorded. Twenty-three and twenty-eight GTO/MU pairs were studied in the CONT and HH groups, respectively. In the HH group, the Ib afferent response exhibited a decrease in dynamic peak for the high stimulation frequencies and an increase in static sensitivity for all stimulation frequencies. Our results suggest that after an HH period, the GTOs continue to fulfil their mechano-sensory function to signal the contractile force but with a higher static sensitivity.

Keywords: Golgi tendon organ; Simulated microgravity; Muscle atrophy

Introduction

Microgravity is well-known to induce changes in postural slow muscles such as the soleus. In our experimentations, we used Morey's model (Morey et al., 1979) to simulate microgravity conditions. In this model, hypodynamia (absence of weight bearing) and hypokinesia (reduction of motor activity) (HH) can be observed as in real microgravity. Among the muscle changes, a shift of muscle characteristics towards a faster type is the result of changes in motor unit types (MU), which are normally homogenous but become heterogeneous after HH (Picquet et al., 2000). Furthermore, with the same experimental model, Miller et al. (2001) have demonstrated that during slow to fast muscular changes, the relative proportion of collagen isoforms is modified. Indeed, the amount of type I collagen was gradually replaced by type III collagen isoform, which is more compliant. Moreover, as concluded by Canon and Goubel (1995), the compliance of the whole soleus muscle could be changed after 3 weeks of HH since fast fibers have been reported to be more compliant than slow fibers (Petit et al., 1990).

Previous studies carried out in our laboratory have demonstrated that the physiological response properties and the histochemical characteristics of the muscle spindle mechanoreceptor are modified after an HH period. On the contrary, there is no data concerning the possible changes in the morphological and electrophysiological properties of the second muscular mechanoreceptor: the Golgi tendon organ

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0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.05.010

(GTO). Taking into account the results described above (changes in MU type and collagen isoform), it is tempting to suggest that the structural and functional properties are modified after an HH period. It is known that GTOs are tension-sensitive mechanoreceptors present in skeletal muscles. The encapsulated collagen bundles within the GTOs are connected at one end to the whole muscle tendon or aponeurosis and at the other end to muscle fibers, called "in-series" fibers. These proprioceptors are innervated by fast conducting Ib afferent fibers (Jami, 1992). The deformations of the nerve endings, which are interlaced between the collagen bundles, generate receptor potentials (Fukami, 1981). Because the electrophysiological studies on GTOs have only been conducted in cat leg muscles (Jami, 1992), there is no physiological data available for rat GTOs. Therefore, the first and innovative aim of the present study was to establish the electrophysiological properties of normal GTOs in a rat hindlimb muscle. The second aim of the study was to observe eventual physiological and morphological changes in GTOs after a microgravity period.

Material and methods

Animals and experimental groups

The experiments were performed on 22 Wistar rats (Harlan, Gannat, France) weighing 300–320 g and randomly divided into two groups: control (CONT: n = 13) and hypodynamia–hypokinesia rats (HH: n = 9). All the rats were housed individually in separate cages and were allowed food and water ad libitum. They were maintained at a temperature of 25 ± 1°C with a 12/12-h circadian cycle. Animals of the HH group were hindlimb unloaded for 14 days using Morey's model (Morey et al., 1979). Briefly, the tail of each rat was washed, cleaned, dried and wrapped in an antiallergic orthopedic tape-adhesive plaster. This cast, covering less than half of the tail, was secured to an overhead swivel mounted at the top of the cage and which permitted free 360° rotation of the animals. The rats were unloaded at a 30° head-down angle to mimic fluid shifts classically observed in weightlessness. All the experiments as well as the maintenance conditions of the animals received authorizations from the Agricultural and Forest Ministry, National Education Ministry (Veterinary Service of Health and Animal Protection: authorization B 59-00913).

Surgical technique

The rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg kg^{-1}). Supplementary injections (30 mg kg^{-1}) were provided when necessary. The depth of the anesthesia was assessed by the absence of blink reflexes.

The dorsal and ventral spinal roots were exposed by a lumbar laminectomy from L_2 to L_6. The right limb was carefully denervated except for the nerve to the soleus muscle. The right soleus muscle and its tendon were dissected free and the soleus nerve was isolated without compromising the blood supply. Under a stereomicroscope, the soleus nerve was totally freed and cleaned from its surrounding tissues over about 3 mm.

The right hindlimb was placed in a bath filled with circulating thermostatically controlled (37°C) mineral oil. The vertebral column and the hindlimb were rigidly fixed using wire frame. The distal tendon of the soleus muscle was attached with a silk thread (gauge 2, Crepin, Paris, France) to a force transducer (FT 10, 700 Hz, Grass Instruments, Quincy, MA, USA). A pool filled with mineral oil was built with the dorsal skin of the rat to prevent the roots and the spinal cord from drying. The animal body temperature was maintained at 37°C with a servo control heating blanket (Homeothermic Blanket Control Unit, Phymep, Paris, France). The soleus muscle length was set to L_0, the length at which the muscle twitch is maximal. The amplitude of the electrical stimulation (pulse duration 0.2 ms; Grass Instruments stimulator, S8800, Quincy, MA, USA) was the minimum voltage necessary to elicit the maximal muscle twitch P_t. A monopolar recording platinum electrode was placed under the soleus nerve to record the electroneurogram. A second monopolar platinum electrode lying on an adjacent denervated muscle was used as reference. After the opening of the dura-mater, the right L_4 and L_5 dorsal and ventral roots were transected near their entry into the spinal cord.

Identification of GTOs

Ib afferent fibers were functionally isolated in the dorsal roots using the technique of Hunt and Kufler (1951). The L_4 and L_5 dorsal roots were split into fine filaments. Each filament was placed on a bipolar platinum electrode and stimulated. A filament contained a single afferent fiber if its stimulation elicited an all-or-none response in the soleus muscle nerve electroneurogram. The delay between the stimulation and the evoked response was measured.

The dorsal root filaments were placed individually on a bipolar recording platinum electrode and the soleus muscle nerve was placed on a monopolar stimulating platinum electrode. The Ib afferent fibers were identified and functionally differentiated from the muscle spindle afferents (Ia and II fibers) on the basis of (i) their discharge during the rising phase of a whole muscle twitch contraction and (ii) an absence of response during a ramp-and-hold stretch (Houk and Henneman, 1967; Matthews, 1933; Petit et al., 1997).

Then, to isolate MUs acting on the isolated GTOs, the L_4 and L_5 ventral spinal roots were split into nine or ten filaments placed individually on a bipolar stimulating platinum electrode. Each filament was subsequently divided into fine filaments until the stimulation of some of those

filaments elicited an all-or-none response in the soleus muscle nerve electroneurogram. Additional verification of MU isolation was sometimes performed by stimulating the soleus nerve and recording the all-or-none evoked response on the ventral root filament. The all-or-none response indicated that a single MU from the soleus muscle was stimulated. The effect of the stimulation of each isolated MU on the response of each isolated GTO was tested. When the response of the GTO occurred during the rising phase of the MU twitch contraction, the MU was classified as "in-series" MU (Gregory and Proske, 1979). An example of such a record is shown in Fig. 1A.

Data recording

All recordings were made under isometric conditions. For both whole muscle and MU twitch contractions, the following mechanical parameters were determined: the time-to-peak (TTP, the time from the initiation of the force until the peak force, P_t) and the half-relaxation time (HRT, duration of the decrease in force between the maximal force and the half-maximal force). For both whole muscle and MU tetanic contractions, the maximal tetanic force (P_0) and the index P_{20}/P_0 (ratio between the tetanic force developed at 20 Hz and P_0) were measured. MUs were

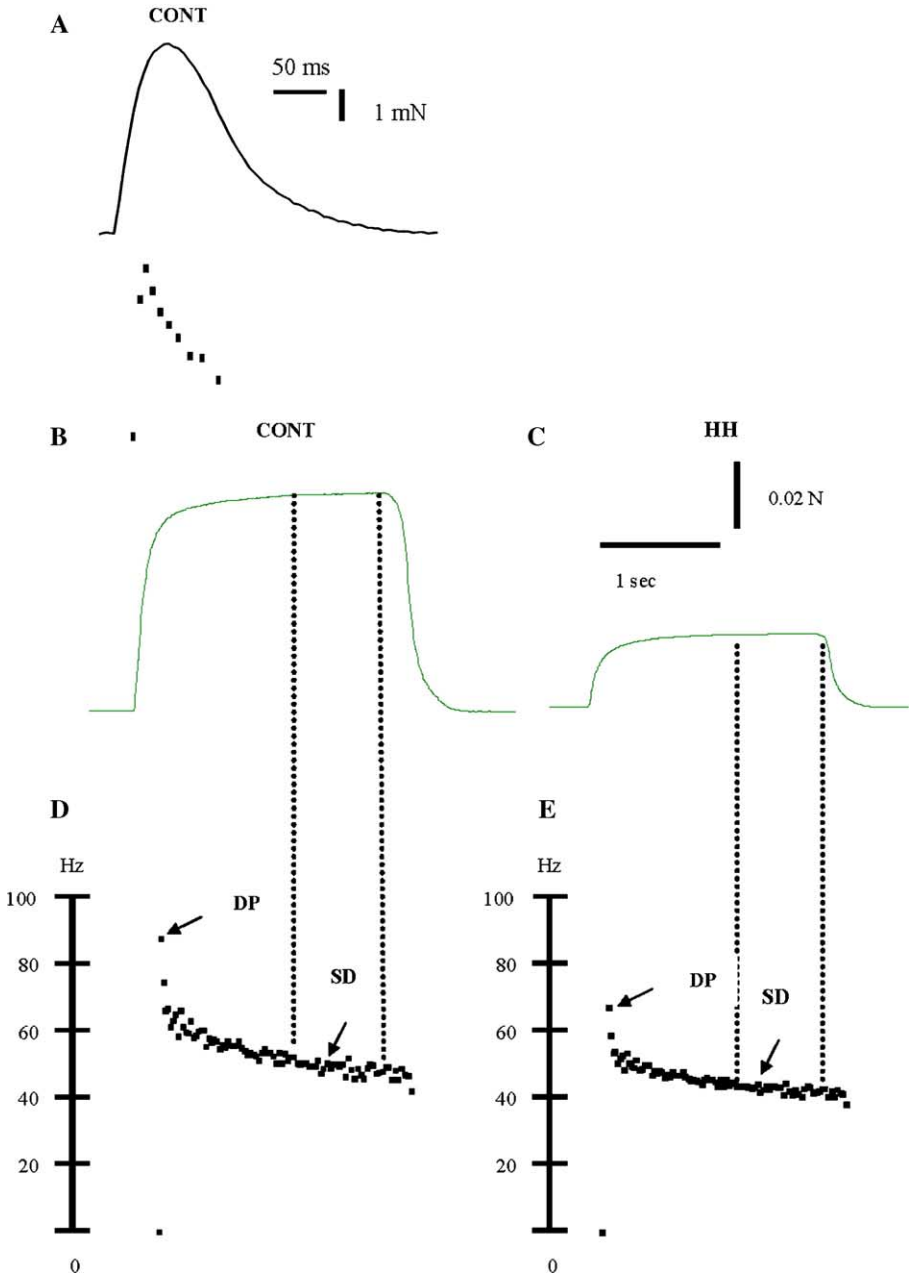

Fig. 1. (A) Discharge of a GTO during the rising phase of an MU twitch contraction. GTO spikes are represented as an instantaneous frequency display. (B–E) Response of a GTO to the representative contractions of an MU stimulated at 80 Hz in an HH muscle (right panel) and a control muscle (left panel). (B and C) MU tetanic fused force (80 Hz). (D and E) GTO responses in instantaneous frequencies. DP: dynamic peak; SD: static discharge.

classified as slow-fatigue resistant (S) or fast-fatigue resistant (FR), according to the TTP and the presence or absence of a force sag (Burke et al., 1973; Picquet et al., 2000).

For each MU/GTO couple, the motor axon was stimulated using 4 successive pulse trains (2 s duration, 2 min interval) with increasing frequencies (20, 40, 80 and 100 Hz) while the GTO afferent discharge was recorded. The dynamic peak (DP) (Davies et al., 1995; Jami and Petit, 1976), the static discharge (SD) and the MUs' tetanic force (Petit et al., 1994) were measured (Figs. 1B–E). Using these parameters, the static sensitivity (SS) was obtained by calculating the ratio between the static discharge and the tetanic force, both measured at the end of the tetanic plateau. The initial component of the dynamic sensitivity (DS) was the instantaneous frequency between the first two impulses of Ib afferent discharge divided by the rate of force development during the same time interval (Davies et al., 1995).

Histochemical analysis

At the end of each experiment, the soleus muscle was removed and the muscle wet weight (MWW) determined. Next, the muscle was cut into 3 blocks and only the distal and proximal blocks were kept. These latter blocks were then frozen in liquid isopentane precooled in liquid nitrogen and stored at −80°C for further histochemical analysis. At the end of each experiment, the animal was killed by injection of an overdose of anesthetic (100 mg kg^{-1}).

The soleus muscle blocks of the CONT and HH groups were cut into serial transverse sections (10 μm thick) using a cryostat microtome (Leica CM 1800, Heidelberg, Germany) set at −20°C. Along each extremity, fourteen 10-μm sections perpendicular to the muscle longitudinal axis were made, the 14-section series being separated by a 100-μm interval. The sections were stained using the myofibrillar ATP_{ase} method (Brooke and Kaiser, 1970; Guth and Samaha, 1969) alternatively with acid (pH 4.3) and alkaline (pH 10.4) preincubations. The histochemical method allowed the characterization of type I (slow Myosin Heavy Chain I), IIa (fast Myosin Heavy Chain IIa) and hybrid (Myosin Heavy Chain I + IIa) soleus muscle fibers. The mean cross-sectional area (CSA) of each fiber type was calculated by measuring the CSA of 200 fibers of the same type.

The GTOs were identified according to the identification criteria of Zelena and Soukup (1983) and Spielmann and Stauffer (1986). The mean CSA of each muscle fiber type, the CSA of each GTO (GTO-CSA) and the GTO intracapsular CSA were measured on ATP_{ase} sections using an image analyzer (SAMBA, Grenoble, France). GTO capsular CSA corresponded to the difference between GTO-CSA and GTO intracapsular CSA. The GTO diameter was calculated from the CSA, assuming that the transverse section of the GTO was circular.

The muscle length was always measured under the same conditions, i.e. the knee joint set at 90° and the ankle joint set at 30° (Riley et al., 1990). To normalize the data, the muscle CSA and the GTO-CSA were measured on the same slice.

Statistical analysis

The statistical analyses were made with the GraphPad software Prism 3.0. Values are expressed as means ± SEM. Significant differences were determined using a non-paired Student's t test to analyze GTO electrophysiological and muscle histochemical results and a Mann–Whitney test to analyze GTO morphological data. The number of asterisks (*) indicates the level of significance for the difference between values of a parameter measured in the HH group and in the CONT group, $*P < 0.05$; $**P < 0.01$ and $***P < 0.001$. Symbols (¶) indicate a significant difference between the values of a parameter measured for the slow MUs and the fast MUs within the HH group, $^{¶}P < 0.05$; $^{¶¶}P < 0.01$; $^{¶¶¶}P < 0.001$.

Results

Body weight, muscle weight and morphological characteristics

After 14 days of HH, the rat body weights (BW: 294.6 ± 6.7 g, $P < 0.05$) and the soleus MWW (94.9 ± 5.4 mg, $P < 0.001$) were significantly lower than those of the CONT group: 319.5 ± 3.9 g and 169.3 ± 5.7 mg, respectively. The MWW/BW ratio was significantly different ($P < 0.001$) between the CONT (0.53 ± 0.01) and HH (0.32 ± 0.01) rats.

In the CONT group, type I fiber CSA (5123 ± 131.6 μm^2) was higher than that of IIa (3097 ± 88.56 μm^2) and hybrid types (2352 ± 51.53 μm^2). The percentage differences between type I fiber CSA versus IIa or hybrid fiber CSA were 39% and 54%, respectively, while this difference was 24% between IIa and hybrid fibers. In the HH group, both IIa and hybrid fibers had smaller CSA (1185 ± 27.75 μm^2 and 1115 ± 29.31 μm^2, respectively) than type I fibers (1369 ± 34.80 μm^2), but the percent differences were only −13% and −18.5%, respectively. These values can be explained by the larger atrophy observed in type I fibers after 14 days HH. Compared to the CONT group, the percent decrease in the CSA of type I, IIa and hybrid fibers was −73%, −61% and −52.5%, respectively.

Whole muscle and MUs forces

Whole muscle and MUs mechanical properties are reported in Table 1. P_t, P_0, P_0/MWW, TTP and P_{20}/P_0 were lower in HH group muscles than in CONT group muscles. The percentage decreases were −53%, −68%, −45%, −14% and −26%, respectively. In the CONT group

Table 1
Contractile properties of both whole muscle and "in-series" MUs

	Whole muscle		Motor unit		
	CONT (n = 13)	HH (n = 9)	CONT (n = 19)	HH (n = 15)	
			Slow (n = 19)	Slow (n = 11)	Fast (n = 4)
P_t (mN)	233 ± 11.3	109.3 ± 8.2***	10.5 ± 0.5	6 ± 1***	7 ± 3
P_0 (mN)	1478 ± 110.5	478.1 ± 44.2***	5.82 ± 0.41	2.56 ± 0.37***	2.27 ± 0.77
P_t/MWW (mN.mg^{-1})	134.7 ± 5.5	116.9 ± 10.7	–	–	–
P_0/MWW (mN.mg^{-1})	9.36 ± 90	5.18 ± 60**	–	–	–
TTP (ms)	67.4 ± 1.9	58 ± 3.9*	63.47 ± 2.8	57.14 ± 2.9	32.6 ± 5.8¶¶
HRT (ms)	67.3 ± 4.4	66.8 ± 6.4	75.43 ± 6.2	87 ± 5.5	36.9 ± 6.7¶¶¶
P_{20}/P_0	0.85 ± 0.02	0.63 ± 0.03***	0.88 ± 0.03	0.75 ± 0.03**	0.52 ± 0.16¶

The contractile properties of whole soleus muscles and "in-series" MUs were measured in CONT and HH rats, the number being given in parentheses. The contractile parameters are reported as follows: **P_t**: Peak twitch tension, maximal isometric strength; **P_0**: maximum tetanic tension; **P_{20}**: tetanic tension at 20 Hz; **TTP**: time-to-peak, the time from the initiation of the force until peak force; **HRT**: half-relaxation time, measured from the peak force to the moment when force decreased to half its highest value; **MWW**: muscle wet weight.

* $P < 0.05$ significant difference with the CONT group.
** $P < 0.01$ significant difference with the CONT group.
*** $P < 0.001$ significant difference with the CONT group.
¶ $P < 0.05$ significant difference between slow MUs and fast MUs within the HH group.
¶¶ $P < 0.01$ significant difference between slow MUs and fast MUs within the HH group.
¶¶¶ $P < 0.001$ significant difference between slow MUs and fast MUs within the HH group.

muscles, only slow MUs (n = 19) were found, while in the HH group, 11 slow MUs and 4 fast MUs were identified on the basis of their contractile properties, according to Burke et al. (1973) and to Picquet et al. (2000) criteria. Fast MUs in the HH group muscles presented significant smaller values in TTP (−43%), HRT (−57%) and P_{20}/P_0 (−31%) than those observed for slow MUs in the same muscles. Slow MUs in HH group muscles had significant smaller values in P_t (−43%), P_0 (−56%) and in P_{20}/P_0 (−15%) than those observed for slow MUs in CONT group muscles.

Comparison of the GTO properties in CONT versus HH group

The electrophysiological properties were studied in the CONT (n = 13 rats) and in the HH (n = 9 rats) groups. In each experiment, between 1 and 5 Ib afferent fibers were isolated for the CONT group and between 1 and 7 Ib afferent fibers for the HH group. The identified GTOs were activated by 1 to 5 MUs in the CONT group, and by 1 to 7 MUs in the HH group. However, our study was not exhaustive and the totality of both soleus MUs and GTO were not identified. Twenty-three and twenty-eight GTO/MU pairs were studied in the CONT and HH groups, respectively.

Ib afferent fibers conduction velocity

No significant difference was observed in Ib afferent fiber conduction velocity between the CONT and HH groups (36.8 ± 3.2 m s^{-1} and 37.4 ± 1.9 m s^{-1}, respectively).

Response during a tetanic contraction

The GTO's discharge exhibited a 1:1 driving (Jami et al., 1985) only during the unfused contraction (20 Hz stimulation frequency) for the 4 HH fast "in-series" MUs, i.e. they discharged one impulse for each force oscillation. In contrast, the GTOs had a typical response during the fused contraction of the slow "in-series" MUs at the same stimulation frequency (Figs. 1B–E). Therefore, the GTO's responses to HH fast "in-series" MUs contractions at 20 Hz stimulation frequency were removed from statistical analysis. Since the DP and SD of GTO responses had similar values during the fused contractions of slow and fast HH group MUs, we pooled the values of the response parameters obtained during these contractions.

The isometric tetanic contractions of a CONT MU and an HH MU eliciting a GTO response are illustrated in Figs. 1B–E. The shapes of the GTO discharges elicited by "in-series" MUs were basically similar and characteristic of an GTO response, but, as detailed below, the discharge frequencies were somewhat different.

Absolute response frequencies

Since a GTO was activated by several MUs and since the GTO response in terms of DP and SD values are slightly different as a function of the number of in-series fiber with a given GTO, we have voluntarily selected the more characteristic response of the GTO.

Dynamic peak (DP). After a period of HH, the DP of GTO responses to single MU isometric contraction was significantly smaller ($P < 0.05$) than that observed in the CONT group. With a stimulation frequency of 80 Hz, we obtained the following values: 102 ± 18 Hz and 67 ± 8 Hz for the CONT and HH groups, respectively. For a stimulation frequency of 100 Hz, we obtained the same decrease (CONT: 110 ± 20 Hz, HH: 66 ± 6 Hz). The DP was not significantly different in GTOs responses for both groups when the MU stimulation frequency was 20 Hz or 40 Hz.

Static discharge (SD). After a period of HH, the SDs of GTO responses were similar to those observed with CONT GTOs. For the CONT group, they were 42.3 ± 6.2, 42.4 ± 7.1, 42.4 ± 7.5 and 40 ± 8.8 Hz for 20, 40, 80 and 100 Hz stimulation frequencies, respectively. For the HH group, these values were 35.9 ± 3.7, 38.7 ± 3.7, 42 ± 3.9 and 39.8 ± 3.8 Hz for 20, 40, 80 and 100 Hz stimulation frequencies, respectively.

In the CONT group, as previously described in the cat by Jami and Petit (1976), the values of these parameters were very scattered. After HH, although the scattering was still present, it was reduced compared to the CONT group. We therefore chose to use the dynamic sensitivity and the static sensitivity to objectively quantify the GTO's discharge properties since they are less influenced by MU type and myo-tendinous junction. These more reliable parameters are presented below.

Sensitivities

Dynamic sensitivity (DS). The DS of the GTO response is the ratio between the instantaneous frequency of the first spike interval in the Ib afferent discharge and the mean rate of tension development during the same interval (Davies et al., 1995). After a period of HH, the DS of GTOs responses was similar to that observed with CONT GTOs. For the CONT group, they were 0.63 ± 0.13, 0.43 ± 0.09, 0.65 ± 0.13 and 0.78 ± 0.16 Hz for 20, 40, 80 and 100 Hz stimulation frequencies, respectively. For the HH group, these values were 0.82 ± 0.17, 0.32 ± 0.06, 0.56 ± 0.11 and 0.74 ± 0.14 Hz for 20, 40, 80 and 100 Hz stimulation frequencies, respectively.

Static sensitivity (SS). After a period of HH, the SS of GTO's responses was significantly higher than that observed in the CONT GTOs responses to MUs contractions: percent increase was +111.6% at 20 Hz stimulation ($P < 0.05$), +125.9% at 40 Hz ($P < 0.01$), +118.7% at 80 Hz ($P < 0.01$) and +91.9% at 100 Hz ($P < 0.05$). This is illustrated by the SS histograms in Fig. 2 for HH and CONT groups. For the 20 Hz stimulation frequency, only the GTO responses to fused contractions were considered for analysis.

GTOs morphological analysis

Nine and nineteen GTOs were studied in the CONT and HH groups, respectively. Fig. 3 presents serial cross-sections near the midsection of an HH GTO and a CONT GTO. For both GTOs, the capsule is black and the intracapsular collagen is grey.

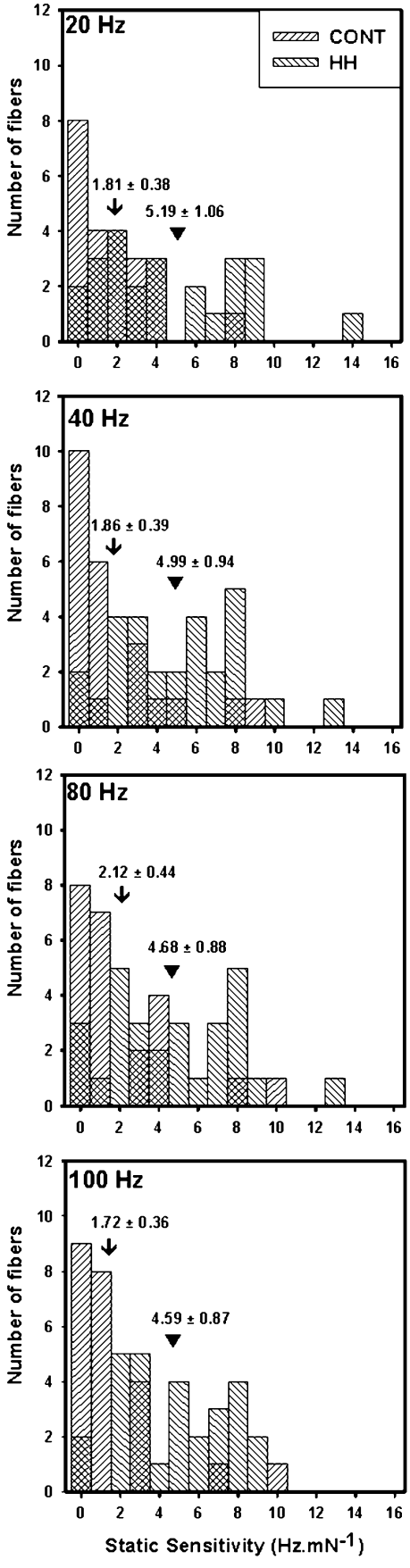

Fig. 2. Frequency distribution of the static sensitivity of GTOs in the CONT and HH groups. The number of fibers indicates the number of GTO/MU pairs. The black arrow represents the CONT group average value (±SEM) and the triangle represents the HH group average value (±SEM).

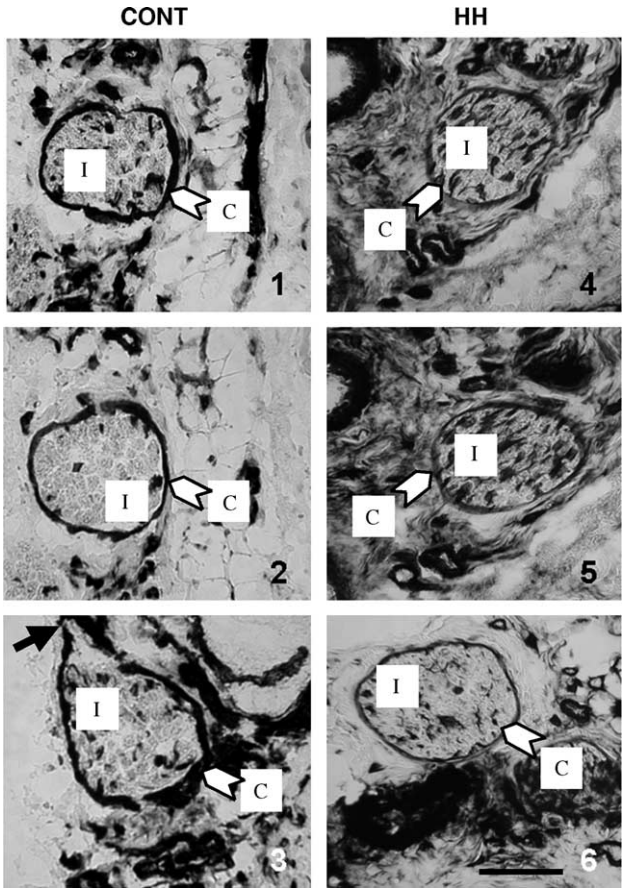

Fig. 3. Serial illustrations of GTOs. Pictures 1, 2, and 3 present a control GTO and pictures 4, 5, and 6 present an HH GTO. Pictures of the midsection are separated by 20 μm. The white arrows labelled C point the capsule. The black arrow points an excrescence that marks the exit of the Ib afferent fiber from the GTO's capsule. I: intracapsular area. Scale bar: 40 μm.

The values of the morphological parameters are shown in Table 2. After a period of HH, the ratio GTO length/muscle length and the ratio GTO diameter/GTO length were unchanged. The GTO length, the GTO capsular CSA, the GTO intracapsular CSA and the GTO-CSA remained the same in both the CONT and HH groups. The ratio GTO-CSA/muscle CSA was increased 16.8 times, by 0.058 ± 0.014 for CONT and 1.03 ± 0.3 for the HH group, respectively.

Discussion

Our results confirm that morphological and functional modifications occur in whole muscle after HH. Muscle atrophy is concomitant with the decrease in TTP and in P_{20}/P_0. The changes in kinetic parameters indicate a slow-to-fast muscle phenotype transition which has already been described in several papers (Falempin and In-Albon, 1999; Picquet and Falempin, 2003; Winiarski et al., 1987).

However, the present study examined for the first time the responses of soleus muscle GTOs to the contraction of single MUs in both CONT rats and in rats exposed to a 14-day period of HH.

GTOs identification and "in-series" MUs characteristics

In CONT rat soleus muscles, Zelena and Soukup (1983), using a histochemical method, identified an average of sixteen GTOs. In our study, by use of an electrophysiological approach, we functionally isolated, in each experiment, between one and seven Ib afferent fibers. We found one to seven MUs acting on a GTO and one to three GTOs activated by the same MU. We also observed that: (i) the rat GTO response to an "in-series" MU twitch was restricted to the rising phase of the twitch, (ii) the response to a fused MU tetanic contraction had a dynamic peak and a static discharge, and (iii) the GTO was silent during a ramp-and-hold stretch within the physiological range.

The HH effects on GTOs characteristics

After an HH period, the length, the maximal cross-sectional area (CSA), the GTO capsular CSA and the GTO intracapsular CSA were unchanged. This contrasts with the results of Józsa et al. (1988), who observed after tenotomy or muscle immobilization at a short muscle length a reduction in the intracapsular space. Consequently, the area of the capsule was increased. We found that, on the one hand, the ratio GTO diameter/GTO length, which is considered as a strain marker (Bridgman, 1970; Jami, 1992), remained unchanged, and on the other hand, that the ratio GTO-CSA/muscle CSA was increased 16.8 times after HH. The steadiness of the first ratio indicates that the morphology of GTOs is similar to CONT. The increase in the second ratio proves that the muscle fibers atrophied in HH muscles while the GTOs remained unchanged. Therefore, the stress applied to a GTO by a contracting muscle fiber should be stronger in HH than in CONT muscles. Indeed, it is commonly admitted that one GTO is activated by several MUs, which are named "in-series" MUs. However, within one "in-series" MU, only a few number of fibers really activated the GTO, these fibers are called "in-series", the rest of fibers in the MU are named "in-

Table 2
GTOs and morphological muscle parameters of the CONT and HH groups

	CONT (n = 9)	HH (n = 19)
GTO length/Muscle length	2.934 ± 0.403	3.507 ± 0.214
GTO diameter/GTO length	2.731 ± 0.384	2.559 ± 0.35
GTO length (μm)	1041 ± 143	968.6 ± 137.1
GTO capsular CSA (μm^2)	1834 ± 505.3	2330 ± 349
GTO intracapsular CSA (μm^2)	3844 ± 1258	5915 ± 1227
GTO-CSA (μm^2)	5678 ± 1677	8245 ± 1516
GTO-CSA/Muscle CSA	0.058 ± 0.014	1.03 ± 0.3**

GTOs: Golgi tendon organs; **CSA**: Cross-sectional area.
** $P < 0.01$ significant difference with the CONT group.

parallel". In case of MU stimulation, both the "in-parallel" and "in-series" fibers are activated and the "in-parallel" fibers counteracted the GTO activation by the "in-series" fibers (Spielmann and Stauffer, 1986; Zelena and Soukup, 1983). Then the GTO response was modulated as a function of the proportion of "in-series" and "in-parallel" fibers within an MU. After HH, the GTO discharge is changed. Our hypothesis is a modification of the relative proportion of "in-series" and "in-parallel" fibers. Moreover, Picquet et al. (2000) have also demonstrated that after a period of HH, the MUs became heterogeneous in their phenotypical composition. Consequently, the influence of "in-series" or "in-parallel" fiber could be related to the fiber phenotype. However, our morphological analysis only refers to the gross morphology of the GTOs, then our data did not allow to verify eventual changes both in the Ib ending or in the afferent innervation structure.

As regards the values of the discharge characteristics (DP and SD), we have already mentioned that many influences can account for their scattering (Jami and Petit, 1976). In order to overcome this difficulty, we decided to use sensitivities, and more especially, the dynamic and static sensitivities of GTOs.

The dynamic sensitivity (Davies et al., 1995) did not change in our conditions. The GTOs have a high dynamic sensitivity (Davies et al., 1995; Jami et al., 1985), which depends not only on the MU rate of force and on the GTO mechanical characteristics but also on ionic properties of the nerve terminal (Fukami and Wilkinson, 1977; Proske and Gregory, 1976).

The static sensitivity increased for the fused tetanic contraction after HH. This could be due to the decrease in MU's contractile force after a period of HH, a decrease already shown by Picquet et al. (2000). However, it is known that the GTO's SS is low and almost constant for MU tetanic forces above a level which probably depends on the muscle (Petit et al., 1994). If MU forces are below this level, which is the case for single motor units showing low force generation, the GTO SS is not constant and much higher than the SS observed with high level of contraction. These differences in SS have been ascribed to changes in unloading by the "in-parallel" muscle fibers (Petit et al., 1994). Consequently, if the unloading effect is weak for low contractions, it increases with increasing contractions and tends to saturate at high contractions. After an HH period, the forces developed by slow and fast MUs are much smaller than the forces developed by CONT MUs, as demonstrated by our data and those of Picquet et al. (2000). To explain the maintenance of static discharge after HH, we propose the following hypothesis: The "in-parallel" fibers coming from MU "in-series" with the studied GTO showed a decrease in strength level; therefore, their unloading effect could also be decreased. In these conditions, the balance between "in-series" and "in-parallel" muscle fibers within a given MU could be modified in favor of "in-series" muscle fibers. Consequently, the GTO could be more activated and its static discharge maintained. This increase could be enhanced by two main factors: firstly, a decrease in the stiffness of contractile and series elastic components (specifically actin-myosin bridges) when the muscular fibers phenotype shifts from type I (slow) to type II (fast), which results in a compliance increase (Canon and Goubel, 1995; Petit et al., 1990); and secondly, the transition in collagen isoform (from type I to type III), which also induces an increase in the compliance of series elastic components (specifically tendon) after HH (Miller et al., 2001).

In conclusion, our results suggest that, after an HH period, the afferent information provided by a GTO activated by a fused tetanic contraction is maintained despite a weak developed tension. The GTOs "become more sensitive" since they keep their high dynamic sensitivity and increase their static sensitivity. After a 14-day period of HH, the GTOs continue to complete their function, which is to signal the muscular force. The afferent message from GTO is conserved, but the isometric muscular tension is weakened, which could modify the organism interaction with its environment.

Acknowledgments

The authors wish to thank Dr. M.H. Canu, Ms. L. Cochon and Mr. A. Bous for their helpful technical support. This work was supported by grants from the Centre National d'Etudes Spatiales (CNES: 8411) and the Conseil Régional Nord-Pas-de-Calais.

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Experimental Neurology 182 (2003) 186–194

Experimental Neurology

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Compared effects of hindlimb unloading versus terrestrial deafferentation on muscular properties of the rat soleus

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Received 3 October 2002; revised 11 February 2003; accepted 25 February 2003

Abstract

Hindlimb unloading is known to induce some neuromuscular changes especially in postural muscles such as the soleus. Our goal was to determine the role of proprioceptive inputs on these modifications by comparing soleus muscle properties of rats being either hindlimb unloaded or terrestrial deafferented. Under deep anesthesia, a first group of rats were submitted to a bilateral deafferentation (DEAF group, $n = 6$) performed by section of the dorsal roots L_3 to L_5 after laminectomy. A second group of rats was submitted to a hindlimb-unloading period (HU group, $n = 6$). After 14 days, the morphological and contractile properties as well as the content in myosin heavy-chain (MHC) isoforms were studied in the right soleus muscle in HU and DEAF groups. The results were compared to those obtained in control animals (CON group). After HU versus CON group, the soleus muscle was atrophied and presented a decrease in muscle forces in relation with a slow-to-fast transition characterized by a decrease in kinetic contractile parameters and by an overexpression in the fast MHC isoforms. The DEAF soleus muscle showed both a significant muscle atrophy and a loss of forces when compared with CON rats. The comparison between DEAF and HU rats indicated that some modifications occurring after HU are purely of motor origin (i.e., slow to fast transition), whereas muscle atrophy and decrease in muscle force are partly the result of an afferent silent. Our study underlined the importance of afferent input integrity in the maintenance of muscle characteristics.

Keywords: Soleus; Hindlimb unloading; Deafferentation; Rat; Muscular atrophy

Introduction

Hindlimb unloading (HU) is a well-known model to mimic the effects of real weightlessness (Morey et al., 1979). In hindlimb muscles, an exposure to HU induces marked changes in extensor muscles such as soleus or vastus intermedius, whereas the HU impact is less important in fast muscles such as the tibialis anterior and the extensor digitorum longus (for review see Thomason and Booth, 1990; Edgerton and Roy, 1996; Fitts et al., 2000; Ohira, 2000). In the rat soleus, after HU, decreases in muscle mass and cross-sectional area of muscle fiber have been currently described. A shift of the muscle type from a slow to a fast one is reported; the contractile properties and the contractile protein isoforms evolve toward those characterizing a fast muscle (Stevens et al., 1999; Fitts et al., 2000; Ohira, 2000; Bastide et al., 2002; Stevens et al., 2002). Some muscular changes observed after HU could be attributed to modifications in EMG activity. Indeed, it has been demonstrated by Alford et al. (1987) that this experimental condition induced, in the soleus muscle, a total EMG disappearance in the 3 s following HU. However, these authors described a progressive return of EMG activity closer to normal values after only 7 days of HU (81% of control EMG activity). This result is in partial disagreement with those of Blewett and Elder (1993), who have demonstrated more recently that the EMG soleus activity remained quantitatively and qualitatively decreased even after 28 days of HU. Moreover, the EMG pattern was shifted from a tonic to a phasic-type characteristic of a fast muscle type (Riley et al., 1990). Consequently, taking into account these latter results, it was

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0014-4886/03/$ – see front matter
doi:10.1016/S0014-4886(03)00111-0

tempting to speculate that some muscle changes observed after HU are directly related to both the nature and the level of EMG activity, as suggested by Leterme and Falempin (1994). The muscle position during HU could also be a trigger to changes observed in simulated microgravity. In this situation the soleus muscle is often in a shortened position (Riley et al., 1990), and it can be supposed that muscle spindles which are stretch sensors, are probably not activated. During real or simulated microgravity this deficiency in the muscular afferent message may be considered as a functional deafferentation as previously suggested by several authors (Money and Cheung, 1991; De-Doncker et al., 2000). As the contractile properties of the soleus muscle and its composition in myosin isoforms have never been studied after deafferentation, the aims of this study were thus (1) to determine whether a chronical deafferentation induces significant modifications in the morphological and contractile properties of the soleus muscle and in its myosin heavy-chain (MHC) content and (2) to compare the effects of a terrestrial deafferentation with changes induced by a HU period in the soleus muscle. We were then able to predict whether afferent activity had a real impact on muscular modifications observed in HU.

Materials and methods

Animal groups

Male Wistar rats (Iffa Credo, l'Arbresle, France) weighing 280–300 g were divided randomly into three groups: control (CON, $n = 6$), hindlimb unloading (HU, $n = 6$), and terrestrial deafferentation (DEAF, $n = 6$). The rats were housed individually in conventional plastic cages and had free access to food and water. The rats were kept at a 25°C room temperature and under a 12:12 h light:dark cycle. All procedures described below were approved by both the Agricultural and Forest Ministry and National Education Ministry (Veterinary service of health and animal protection, authorization A 59-00980).

Hindlimb unloading protocol

The animals of the HU group were subjected to a simulated microgravity period of 14 days using the tail-suspension model of Morey (Morey et al., 1979). Briefly, an orthopedic tape-adhesive plaster, covering less than half of the cleaned and dried tail was connected to the top of the HU cage where a swivel allowed 360° rotation. The rats were unloaded at 30° head down angle to mimic fluid shift observed in real microgravity.

Deafferentation procedure

The animals of the DEAF group were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg/kg body wt). Under aseptic conditions, the midline dorsal musculature was retracted laterally and a laminectomy was performed between L_2 and S_1. After careful separation from the ventral roots with a small blunt hook, the bilateral dorsal roots L_3, L_4, and L_5 were exposed. The dorsal roots were identified after recording of afferent neurogram and after changes in this neurogram induced by ankle movements (flexion–extension). For deafferentation, these dorsal roots were sectioned from their entry into spinal cord (including the ganglion) to their entry into the vertebra to avoid regeneration of nerve fibers. Dorsal musculature and then skin incision were sutured (3-0 chronic gut). Following recovery, the rats were kept in isolated normalized cages. An antiseptic (Betadine) was applied to the cutaneous incision area once a day to prevent skin infection. Antibiotics (Trisulmix, 100 μl/kg) and analgesic (Metacam, 100 μl/kg) were orally administered for 6 days following surgery. Reflex testings on the hindlimbs were performed once a day, that is, withdrawal reflex to check the reality of the bilateral deafferentation. Throughout the study, in this group, there was no response to toe pinching. Moreover, the postoperative gait pattern presented exaggerated plantar flexion of the feet during walking, which was an evidence of deafferentation (as previously described by Kucera, 1980; Wang et al., 1997). Daily consumption of rat chow and water was verified. No debilitating problems related to the maintenance of general health were encountered in any of the rats used in this study. Contractile properties of the right deafferented soleus muscles were determined 14 days after operation. This duration was chosen with the aim to compare the effects of two experimental conditions: deafferentation and HU. Indeed, similar durations of experiments were necessary, and also it has been demonstrated that the muscular changes were maximal after 14 days of simulated microgravity (Falempin et al., 1990). A SHAM-operated group of four rats was chosen in order to monitor the effects of the trauma introduced by the surgical procedure (including all the steps of the operation and both antibiotics and analgesic administrations described above, except laminectomy and deafferentation). No difference was found between CON and SHAM groups. Consequently, the results presented in this article are induced by the deafferentation procedure. Furthermore, to maintain the animals in minimal distress, we voluntarily chose not to perform both deafferentation and hindlimb unloading on the same rat.

In situ isometric contractile properties

For the final experiments, the animals were anesthetized with sodium pentobarbital (35 mg/kg, ip) and anesthesia was prolonged with further injections of 15 mg/kg when necessary. The dissection protocol has been described in previous articles originating from our laboratory (Leterme and Falempin, 1994; Falempin and In-Albon, 1999). Contractions of the soleus muscle were induced by stimulation (Grass Model No. S 8800, Quincy, MA) of the soleus nerve

(0.2-ms duration pulses) through monopolar platinum electrodes at twice the minimum voltage required to obtain the maximal twitch response. The reference electrode was inserted into adjacent denervated muscle mass. The soleus muscles were stimulated so as to record the following parameters: (1) a single maximal twitch from which the maximal twitch tension (P_t), the time to peak (TTP), and the half-relaxation time (HRT) were measured; (2) the tension/frequency relationship (for stimulation frequencies ranging from 16 to 120 Hz), which allowed the determination of maximum tetanic tension, P_0, obtained for a 100-Hz stimulation frequency and of index P_{20}/P_0 (ratio of the tetanic tension at 20 Hz to P_0); and (3) the fatigue index (FI) defined by Burke et al. (1973).

At the end of the fatigue test, the right soleus muscles of the experimental group animals were removed, weighed (determination of the soleus muscle wet weight, MWW), frozen in isopentane precooled to its freezing point by liquid N_2, and stored at −80°C. The muscles were divided into two parts with the aim of performing both histochemical and electrophoretical determination of myosin heavy-chain isoforms on the same muscle.

Characterization of muscle fiber types by immunohistochemistry

At the midbelly, the soleus muscles of the CON, DEAF, and HU groups were cut into serial 10-μm-thick cross sections in cryostat microtome at −30°C. Myosin ATPase activity was revealed with both alkaline (pH 10.4) and acid preincubations (pH 4.3 and 4.45) according to the method of Guth and Samaha (1969). Additional sections were treated with a double preincubation (Sant'ana Pereira et al., 1995) that revealed fibers expressing MHC IIX as already described by Picquet et al. (2002). For the immunohistochemically treated sections, the protocol has been described in details (Picquet et al., 1998). The following antibodies were used: NCL-MHCs (TEBU/NOVOCASTRA), which recognizes MHC I; SC-71 (DSMZ) against MHC IIA; BF-F3 (DSMZ) against MHC IIB; and MY32 (Sigma) against MHC IIA, IIB, and IIX. These antibodies were diluted respectively at 1/20, 1/20, 1/10, and 1/2000. Muscle typing was established from a total of 300 fibers per muscle immunologically identified in serial sections. The cross-sectional area (CSA) of the fibers was also measured on ATPase sections by use of an image analyzer (Samba, Grenoble, FRANCE). A total number of 300 fibers per muscle was considered.

Determination of MHC isoforms by SDS PAGE

The MHC isoforms were studied in CON, DEAF, and HU soleus muscles. The first part of the frozen tissues was pulverized under liquid nitrogen in a small steel mortar (Ricart-Firinga et al., 2000). As already described (Toursel et al., 2000), the MHC composition was determined by polyacrylamide gel electrophoresis using a 4.5% glycerol stacking gel and on a 7.5 % glycerol separating gel. Electrophoresis was run for 18 h at 12°C (180-V constant, 13 mA/gel). After the gels were run, the gel slabs were silver stained. The relative proportion of each MHC isoform in each muscle type was established using a scanning densitometer (Quantiscan Microvial Systems, Biosoft, UK).

Table 1
Morphological characteristics of soleus muscles

	CON	DEAF	HU
BW (g)	290 ± 3	300 ± 5	284 ± 5
MWW (mg)	147 ± 6	123 ± 6*	91 ± 3*†
MWW/BW (mg · g^{-1})	0.50 ± 0.02	0.41 ± 0.02*	0.32 ± 0.01*†

Note. The results expressed as mean ± SE were obtained from six animals in each group. Abbreviations: BW, body weight; MWW, muscle wet weight.

* Statistical difference with CON group.

† Statistical difference with DEAF group.

Statistical analysis

All the results are presented as mean ± SE. After a one-way analysis of variance (ANOVA), a Bonferroni test was used to establish the intergroup difference (CON versus DEAF, CON versus HU). To compare the degree of muscle modifications after DEAF with changes classically described after HU, a Bonferroni test was also used. Statistical significance was accepted at $P < 0.05$.

Results

Muscle weight and cross-sectional area of muscle fibers

The data of each experimental group are reported in Table 1. For all three groups, the animals' body weight (BW) remained similar after 14 days of experimentation with values between 284 and 300 g. However, compared with the CON group, the DEAF and HU groups showed a significant decrease in both MWW and MWW to BW ratio. These two parameters remained significantly different when DEAF was compared to HU group. MWW and MMW/BW in the DEAF group presented intermediate values between those of CON and HU rats.

The cross-sectional area (CSA) of the fiber types are reported in Fig. 1. The CSA of the fibers were measured for slow fibers (expressing only the slow MHC I isoform), fast fibers (expressing fast MHC, i.e., MHC IIA, MHC IIX, or MHC IIB and presenting equivalent CSA values), and hybrid fibers containing more than one MHC isoform (i.e., mixture of MHC isoforms showing similar CSA values). The DEAF condition induced a significant decrease in the CSA of hybrid (mixing of MHC I + IIA + IIX) and fast

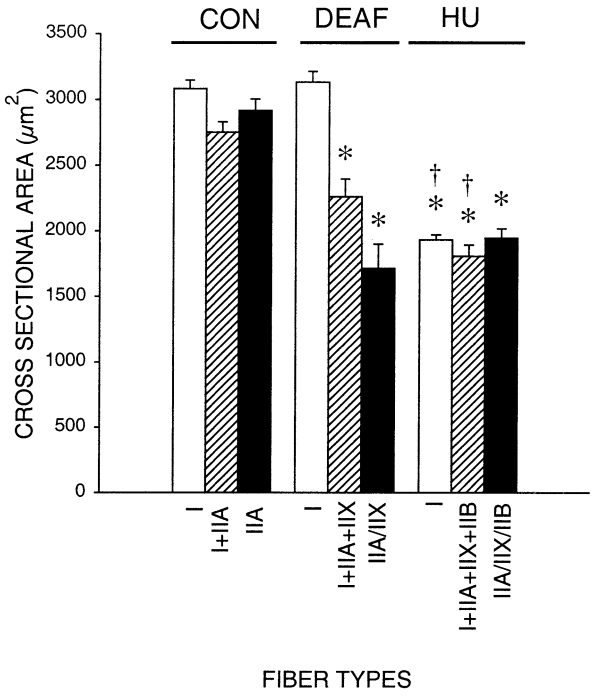

Fig. 1. Cross-sectional areas (CSA) of fiber types in CON, DEAF, and HU groups. The results were expressed as mean ± SE and were obtained from six rats in each group. The fiber types were indicated as a function of their content in MHC isoforms. To clarify the data, fibers presenting similar CSA values (fast fiber types and fibers containing mixture of MHC isoforms) were pooled. *Statistical difference with CON group. †Statistical difference with DEAF group.

(IIA or IIX) types when compared with CON group. The decrease was more marked in the HU group since all fiber types were atrophied. Moreover, the slow (expressing MHC I) and hybrid (containing mixture of MHC I + IIA + IIX + IIB) types in the HU group presented a significant atrophy versus DEAF group.

Contractile characteristics

The different values of the mechanical tests are summarized in Table 2. When compared with CON rats, DEAF condition resulted in significant reductions in both absolute and normalized to muscle mass twitch (i.e., P_t: −36% and P_t/MWW: −24%) and in tetanic tensions (P_0: −40% and P_0/MWW: −29%). The HRT value was increased by 16%. TTP, P_{20}/P_0, and FI were not affected by deafferentation. A 14-day HU period versus CON induced significant decreases in twitch and tetanic tensions (−48% and −54%, respectively), which remained persistent when tensions were normalized to muscle mass (P_t/MWW: −16% and P_0/MWW: −26%). Kinetic (TTP: −26%, HRT: −16%, P_{20}/P_0: −32%) parameters were also decreased.

The comparison between DEAF and HU groups revealed significant decreases in TTP (−30%), HRT (−28 %), and P_{20}/P_0 (−37%) in HU rats.

Histochemical and immunohistochemical identification of muscle fiber types

Histochemistry coupled with immunological staining were used to identify the MHC contents in muscles fiber types. On serial sections, we determined the presence of MHC isoforms from a total of 300 fibers per muscle. We have voluntary chosen to show the antibodies reactivity in the CON (Figs. 2A–2E), DEAF (Figs. 2F–2J), and HU (Figs. 2K–2O) soleus. The positive fibers were characterized by a dark color, whereas negative fibers showed no color, except for BF F3 antibody, in which negative fibers were slightly colored. The ATPase method that permitted identification of fibers containing MHC IIX is illustrated in Fig. 2A (CON), Fig. 2F (DEAF), and Fig. 2K (HU). The fibers that were weakly colored [fibers 1 and 2 (Fig. 2A) and fiber 3 (Fig. 2F)], did not express the MHC IIX isoform, whereas the dark fibers contained it [fibers 4 and 5 (Fig. 2K)]. Fiber 1 was type I [expression of MHC I (Fig. 2B)], this fiber being negative in all illustrated sections (Figs. 2C, 2D, and 2E). Fiber 2 expressed only MHC IIA (Figs. 2A–2E). Another fiber, named fiber 3, expressing both MHC I (Fig. 2G) and MHC IIA (Fig. 2H) but being negative in all other sections is showed in DEAF group. Fibers 4 and 5 of HU group contained mixture of MHC IIX, MHC IIA, and MHC IIB since they were positive for ATPase staining concerning MHC IIX (Fig. 2K) and for SC 71 (Fig. 2M), BF-F3 (Fig. 2N), and MY 32 (Fig. 20) labelings, but remained negative for binding against MHC I (Fig. 2L).

The distribution histograms of the different fiber types expressed in the soleus muscles are shown in Fig. 3. In the CON group, four fiber populations were identified according to their myosin coexpressions: type I which contained MHC I and was largely dominant, type IC (MHC I > IIA), type IIC (MHC IIA < I), and type IIA (MHC IIA). After

Table 2
Contractile properties of soleus muscles

	CON (6)	DEAF (6)	HU (6)
P_t (mN)	346 ± 10	220 ± 7*	180 ± 19*
P_t/MWW (mN/mg)	2.35 ± 0.05	1.78 ± 0.09*	1.97 ± 0.04*
P_0 (mN)	1713 ± 43	1019 ± 108*	788 ± 47*
P_0/MWW (mN/mg)	11.65 ± 0.6	8.28 ± 0.5*	8.65 ± 0.4*
TTP (ms)	34.1 ± 0.8	36 ± 2.5	25 ± 2*†
HRT (ms)	36 ± 1	42 ± 2*	30 ± 1*†
P_{20}/P_0 (%)	71 ± 1	77 ± 2	48 ± 4*†
FI (%)	88 ± 3	81 ± 2	82 ± 4

Note. The results, expressed as mean ± SE, were obtained from six animals in each group. The contractile parameters were reported as follows: P_t, twitch tension; P_t/MWW, normalized twitch tension to MWW; P_0, maximal tetanic tension; P_0/MWW, normalized maximal tetanic tension to MWW; TTP, time to peak; HRT, half relaxation time; P_{20}/P_0, tetanic tension obtained at 20 Hz divided by the maximal tetanic tension; FI, fatigue index.

* Statistical difference with CON group.

† Statistical difference with DEAF group.

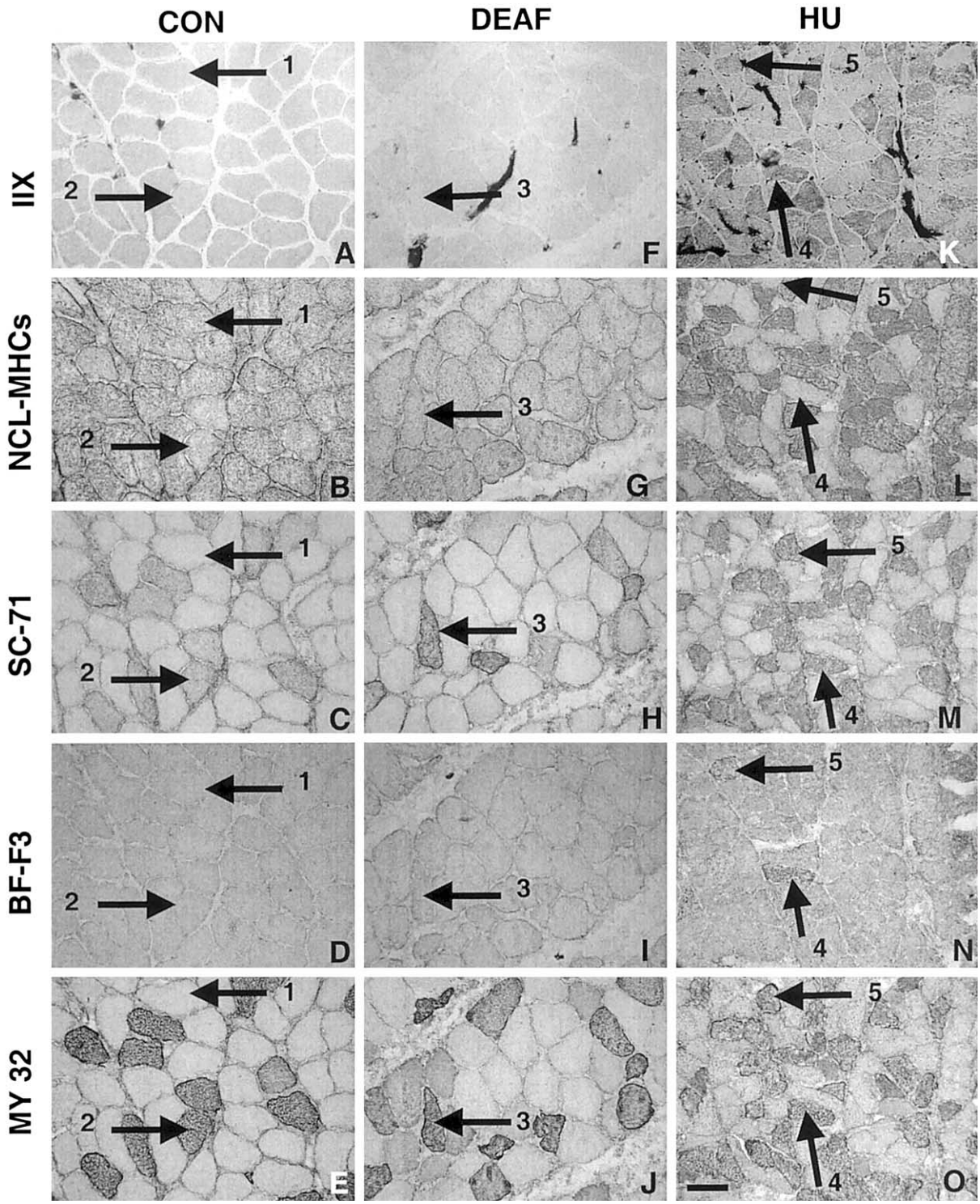

Fig. 2. Illustration of muscle typing determined by immunohisto- and histochemical methods. (A–E), (F–J), and (K–O) show the CON, DEAF, and HU soleus muscles, respectively. The ATPase staining specific of MHC IIX expression was illustrated as follows: CON group (A), DEAF group (F), and HU group (K). The reactivity of the antibodies was presented as follows: anti MHC I (B, G, and L); anti-MHC IIA (C, H, and M); anti-MHC IIB (D, I, and N), on which positive fibers were indicated by black arrows; and anti MHC II (E, J, and O). Fiber 1 expressed MHC I, fiber 2 MHC IIA, fiber 3 MHC I + IIA, and fibers 4 and 5 a mixture of MHC IIX, IIA, and IIB. Scale bar = 50 μm.

DEAF, type IC increased significantly and a new fiber group never detected in CON muscles appeared (type IIX), this type being slightly expressed. In HU condition, a decrease in type I fibers was observed (−23%) and was associated with an increase in type IIA, which was 6 times more important in HU than in CON group. Moreover, type IIX

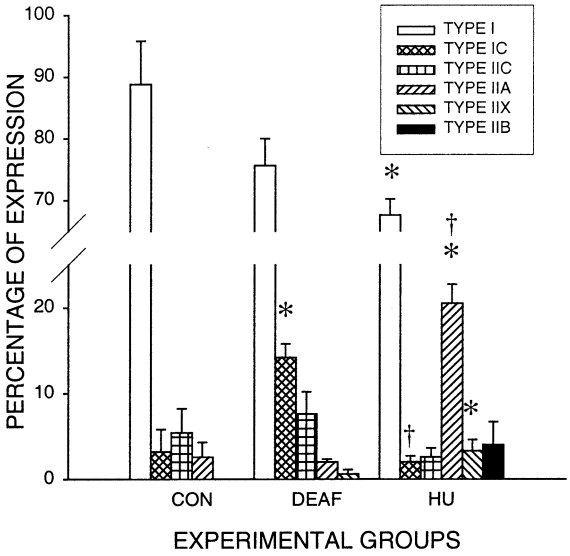

Fig. 3. Classification of fiber types according to their MHC isoform expressions. The results were expressed as means ± SE. *Statistical difference with CON group. †Statistical difference with DEAF group.

was overexpressed (5 times) and type IIB appeared. DEAF animals showed fewer type IC fibers relative to HU (−37%), which was associated with an overexpression of IIA fibers (9 times the control level).

Electrophoretical characteristics

An example of the electrophoretical profile of migration of the different MHC isoforms constituting the soleus muscle of the CON, DEAF, and HU groups is shown in Fig. 4A. The MHC isoforms were identified by comparing the electrophoretical profile of the soleus with that of a fast muscle: the EDL. Four MHC isoforms were thus determined according to their increasing order of electrophoretical mobility: MHC IIA, IIX, IIB, and I. In CON muscles, two MHC isoforms were detected, the predominant slow MHC I and the fast MHC IIA. After DEAF, a new MHC isoform was expressed: MHC IIX. HU muscles presented the four types of MHC isoforms, MHC I remaining predominant.

The relative proportion of these MHC isoforms was determined by a densitometric analysis. The results are presented in Fig. 4B. MHC I, which was the predominant isoform, decreased by 25% in the HU muscles in comparison with CON muscles. The proportion of fast MHC IIA isoform increased (+180%), whereas MHC IIX and MHC IIB were de novo expressed (respectively 5 and 10%). In the DEAF group, only the MHC IIX appeared (1%). The comparison of between DEAF and HU profiles showed a significant decrease in MHC I expression (−28%) and the appearance of MHC IIB in the HU group.

Discussion

The aim of this study was to determine whether the abolition of the afferent message induced muscular changes similar to those observed after a HU period.

Our results showed, after HU, a muscle atrophy assessed by a decrease in the CSA of all fiber types. This atrophy, correlated with a decrease in normalized muscle force, has usually been attributed to a decrease in muscle protein synthesis and to an increase in protein degradation (Thomason et al., 1987; Bock, 1998). Moreover, the muscle type evolved toward a faster one since the values of contractile kinetic parameters (TTP, HRT, P_{20}/P_0) decreased and the proportion of fast myosin increased. These results were in agreement with those already described after a period of HU (Winiarski et al., 1987; Falempin et al., 1990; Edgerton and Roy, 1996; Stevens et al., 1999; Ohira, 2000; Stevens et al., 2002).

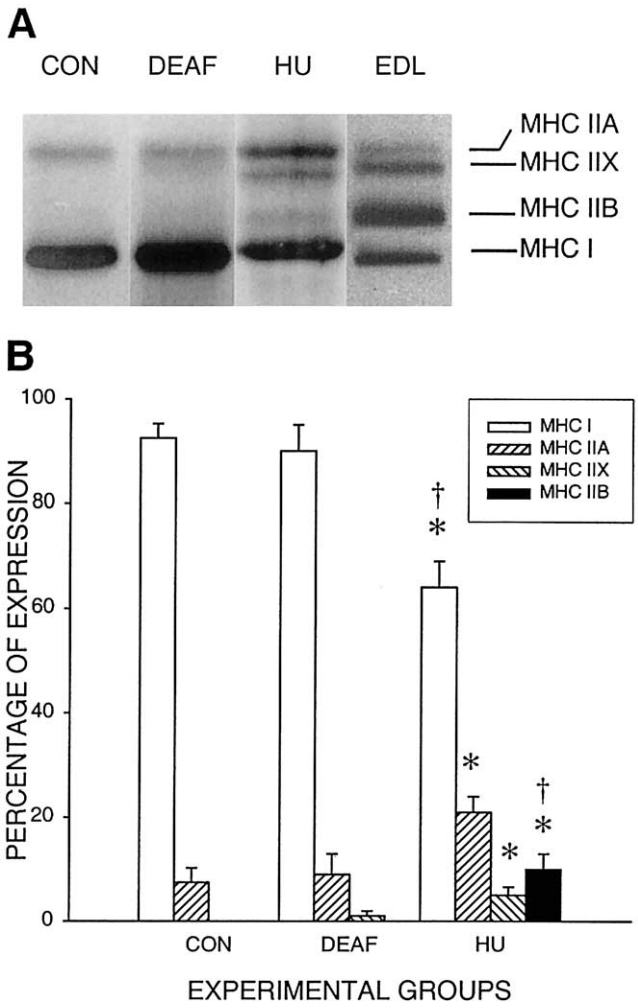

Fig. 4. (A) Electrophoretic determination of MHC isoforms according to their increasing order of electrophoretic mobility. (B) Densitometric analysis of the identified MHC isoforms in CON, DEAF, and HU groups. The results were expressed as means ± SE. *Statistical difference with CON group. †Statistical difference with DEAF group.

From our results, it can be argued that a bilateral deafferentation induces in the soleus a muscular atrophy that is less marked than after HU. Previous studies have described a significant muscle atrophy after deafferentation (Ohira, 1989). However the results we obtained from the measurement of fiber CSA demonstrated that the muscle atrophy was the consequence of a selective atrophy of hybrid and fast fiber types, whereas the CSA of slow fibers was not modified. It appeared that slow fibers were less sensitive to the lack of afferent information. Thus, deafferentation affects each fiber type differently according to its myosin content. The bilateral deafferentation also induced a significant decrease in both absolute and normalized muscle strengths. Hindlimb unloading is also characterized by significant decreases in force, these changes being attributed to a decrease in contractile proteins (Thomason et al., 1987). Thus, whatever the disuse model, the decrease in force appears related to a loss of proteins. Taking into account the MWW/BW ratio diminution (−18%) and the normalized force loss (P_t/MWW: −24% and P_0/MWW: −29%), we suggest that the force developed per cross bridge was decreased after deafferentation. However, we cannot rule out a possible decrease in the number of cross bridges and/or a modification in the filament lattice spacing. This latter hypothesis has already been put forward concerning the effects of simulated microgravity (McDonald and Fitts, 1995). When considering the contractile kinetic parameters, only the HRT value was modified after deafferentation. Therefore, the properties of the sarcoplasmic reticulum could be altered. This parameter is described as dependent on the relative content of the sarcoplasmic Ca^{2+} ATPase (SERCA) isoforms (Loukianov et al., 1998). The increase in HRT value (+16%) suggested that a bilateral deafferentation should probably increased the levels of slow SERCA isoform expression. The other contractile kinetic parameters (TTP, P_{20}/P_0) as well as the total amount of MHC isoforms were not modified after deafferentation. Considering that the MHC expression, especially the slow isoform, is dependent on motor innervation (Pette and Vrbova, 1985) and that the kinetic parameters are related to the MHC content (Leterme and Falempin, 1994), our hypothesis was that the pattern of nervous motor message would not be significantly modified by a bilateral deafferentation. In our study we did not record the EMG activity of the soleus muscle, but Hnik et al. (1982) have described that the EMG activity disappeared in the first day following a bilateral deafferentation and that, 1 or 2 days later, a spontaneous tonic EMG activity was detected. Forty-one days after the operation the EMG pattern appeared similar to that of control animals (i.e., tonic). Unfortunately this activity was not quantified and we have no idea of the level of EMG activity between 2 and 41 postoperative days. We suggest that the EMG activity was persistent after DEAF since the MHC I amount was similar to that of CON animals, this isoform being known as directly dependent on motor innervation (Narusawa et al., 1987). However, the possible changes in EMG may not have been sufficient to alter significantly both contractile properties and myosin content. Nevertheless, as reported under Material and Methods, after deafferentation the rat presented exaggerated plantar flexion, the soleus muscle being thus maintained in a shortened position. We could not discount a possible impact of the muscle position that could, in turn, have modified the muscle characteristics. Moreover, deafferentation induced an increase in the expression of the hybrid type IC, which is characteristic of a muscle in a transformation process, as already described in space flights (Allen et al., 1996; Talmadge et al., 1996), hindlimb unloading (Fauteck and Kandarian, 1995; Talmadge et al., 1996), and electrical stimulation (Staron et al., 1987).

Table 3
Comparison between DEAF and HU conditions

Muscular changes	DEAF vs CON	HU vs CON
Normalized muscle atrophy	↘	↘↘
Fiber CSA	↘	↘↘
Muscle forces	↘	↘↘
Kinetic parameters	=	↘
Fatigue index	=	=
Slow MHC isoform	=	↘
Fast MHC isoforms	=	↘

Note. The effects of deafferentation (DEAF) and hindlimb unloading (HU) situations versus control (CON) animals are synthetically reported. The decreases are distinguished according to their importance (↘ or ↘↘). The maintenance of muscle properties is indicated by =.

The comparison between rats from DEAF and HU groups is summarized in Table 3. The synthesis revealed that these two experimental conditions presented both some specific and some common consequences on the soleus muscle. For instance, deafferentation was specifically characterized by a muscle atrophy and a decrease in muscular forces, whereas the consequences of a HU period were more marked. In this latter condition, muscular atrophy was more important and all fiber types presented a decreased CSA. Moreover, the muscle phenotype evolved toward a faster type (expression of novel fast MHC isoform and decrease in kinetic values). It has already been suggested that real or simulated microgravity corresponds to a functional deafferentation, since cutaneous, proprioceptive, and vestibular information is reduced (Money and Cheung, 1991; Bock, 1998; Roll et al., 1998; De-Doncker et al., 2000). Our results demonstrated that muscle changes observed after HU were not exclusively a consequence of the absence of afferent nervous message. As a matter of fact, deafferentation did not induce changes in some muscle characteristics which are directly under motor nervous influence (MHC isoforms and kinetic parameters). Moreover, it has been reported in the literature that the qualitative EMG activity was not modified after DEAF (Hnik et al., 1982). Consequently, the muscular modifications observed in HU resulted both from an atrophy and loss of force attributed to an afferent silent and from transition slow to fast type (con-

tractile properties and myosin isoforms consecutive to a motor silent).

To conclude, our study showed that the HU condition did not induce exclusively a functional deafferentation since the muscle adaptations to HU and DEAF are very different (see Table 3). It also highlighted the significance of the afferent inputs in the maintenance of muscular properties.

Acknowledgments

This study was supported by grants from the "Centre National d'Etudes Spatiales" (3194) and the "Conseil Régional du Nord-Pas-de-Calais." The authors thank Dr. G.S. Butler-Browne for the growing of hybridomas producing SC-71 and BF-F3 antibodies initially developed by Dr. Schiaffino et al. (1989). The hybridomas are commercialized by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh, Germany).

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Volume 51(11): 1479–1489, 2003
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

ARTICLE

Expression of Myosin Heavy Chain Isoforms in Rat Soleus Muscle Spindles After 19 Days of Hypergravity

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SUMMARY The aim of this study was to determine whether a period of 19 days in hypergravity was long enough to induce changes in the expression of myosin heavy chain (MyHC) isoforms in the muscle spindles. The soleus muscle of 10 male Wistar rats (control: CONT, $n=5$; hypergravity: HG, $n=5$) was frozen, cut into serial sections, and labeled with antibodies against MyHCs: I, IIA, IIA + IIX + IIB, slow-tonic, and α-cardiac. Forty CONT and 45 HG spindles were analyzed. The results from HG spindles compared to CONT showed that there was no change in the cross-sectional area of intrafusal fibers. However, along the entire length of B1 fibers, the expression of both MyHC I and α-cardiac was increased significantly, whereas the labeling against MyHC IIA and MyHC slow-tonic was decreased. In B2 fibers, the labeling against MyHC IIA (region A), slow-tonic (region A), and fast myosins (regions A–C) was statistically decreased. In chain fibers, the labeling against both MyHC IIA and fast MyHC was reduced significantly. We conclude that hypergravity has a real impact on the MyHC content in the muscle spindles and induces some inverse changes of those observed in hypogravity for MyHCs I, α-cardiac, and slow-tonic.

(J Histochem Cytochem 51:1479–1489, 2003)

KEY WORDS
muscle spindles
intrafusal fibers
rat
soleus
myosin heavy chain isoforms
hypergravity

Muscle spindles are encapsulated stretch receptors inserted in parallel with extrafusal muscle fibers (for review see Hunt 1990). They are characterized by some typical fibers called intrafusal fibers (IFs), which are smaller than extrafusal fibers and contain some specific myosin isoforms in their contractile polar regions (Pedrosa et al. 1989; Soukup et al. 1995). Other studies have revealed that the expression of slow-tonic, α-cardiac, embryonic, and neonatal MyHC isoforms is restricted to the IFs (Maier et al. 1988; Kucera and Walro 1989,1990a,b; Pedrosa et al. 1990; Pedrosa–Domellof et al. 1991; for review see Soukup et al. 1995).

In extrafusal fibers, the modifications in myosin expression and, more precisely, of myosin heavy chain (MyHC) have been extensively studied. Many studies have established that the MyHC proportions are modified according to environmental changes. Among these factors, gravity changes have been shown to induce marked neuromuscular changes (for review see Edgerton and Roy 1996; Ohira 2000). In skeletal muscles, after a period of microgravity, slow postural muscles have been reported to present an important atrophy and to evolve towards a faster type. Their MyHC content was therefore changed: fast MyHC isoforms were expressed at the expense of slow isoforms. Conversely, when gravity was reinforced (i.e., hypergravity) it has been demonstrated that, in rats born and reared in this environment, slow postural muscles such as the soleus became slower and therefore, in this case, only the slow MyHC isoform was expressed (Picquet et al. 2002).

As for extrafusal fibers, the existence of fiber plasticity was also demontrated in IFs. The expression and the regulation of MyHC isoforms in IFs are very complex and depend on a complex interaction between inductive and suppressive influences of both sensory and motor innervations (for review see Pedrosa–Domellof et al. 1991; Soukup et al. 1995; Walro and Kucera 1999). Consequently, the IF MyHC content was mod-

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Received for publication April 14, 2003; accepted July 17, 2003 (3A6069).

0022-1554/03/$3.30

ified as soon as the neural message was changed by deefferentation, deafferentation (Wang et al. 1997), and denervation (Soukup et al. 1995). Moreover, a recent study has shown that MyHC isoforms contained in muscle spindles were also influenced by the gravitational load even though the changes appeared less important than those observed in extrafusal fibers (De-Doncker et al. 2002). Indeed, 14 days of hindlimb unloading (or simulated microgravity) were sufficient to induce both a significant decrease in the slow-twitch MyHC I content and a significant increase in slow-tonic MyHC. Moreover, the α-cardiac MyHC isoform, which is specifically expressed in IFs, was also modified, its proportion being increased along the nuclear bag fibers.

Consequently, the myosin content in the muscle spindles was modified following the level of gravity. During simulated microgravity, the hindlimbs are not in contact with the ground or floor (Riley et al. 1990). Consequently, the postural soleus muscle is often in a shortened position. Therefore, the natural stimulus of spindles, i.e., muscle stretch, is probably reduced. It has been suggested that, in this position, afferent information could be reduced (Falempin and Fodili In-Albon 1999). Because Ia fibers project via interneurons on β and γ fusimotor neurons, which innervate the contractile part of IFs, the lack of afferent activity could in turn influence the myosin content.

On the basis of these results, it was tempting to speculate that, during a period of hypergravity, the soleus muscle could be stretched and the Ia afferent activity could be increased. We therefore suggested that the MyHC content of IFs could be modified during a stay in hypergravity.

Consequently, the aims of our study were to determine whether the expression level of MyHC isoforms was changed after a period of hypergravity and to compare the effects on muscle spindles of hypergravity with those of microgravity.

Materials and Methods

Animal Groups

Ten male Wistar rats with initial body weights of 200 g were used for this study. They were randomly divided into two groups: a control (CONT, $n=5$) and a 2-g-centrifugated group (HG: $n=5$) for a duration of 19 days. The experimentations were approved by both the Ministry of Agriculture and the Ministry of Education in France (Veterinary Service of Health and Animal Protection, authorization 59-00980) and by the Animal Care Committee of the Institute of Biomedical Problems in Moscow, CEI.

Centrifugation Apparatus

The centrifugation was performed in Moscow at the Institute of Biomedical Problems. The apparatus consisted of a velocity-controlled direct-current motor located in the vertical axis of the apparatus and driving two horizontal crossarms (total length 4 m) at a constant rotation speed. Four free-swinging gondolas were jointed at the four extremities of the horizontal arms. Each gondola contained five rats allowed to maintain a natural tetrapod position. For the centrifugation, the gondolas were tilted at a constant angle of 60° from vertical. The rotations were performed at a constant velocity of 21 rad/min. Taking into account the mass and the inertia of the gondolas and the cage and rat masses, this velocity corresponded to a 2-g resultant force. Each gondola was equipped with an aeration system and with lights that reproduced a circadian rhythm (12-hr light:12-hr dark). The centrifugation was stopped daily for 15 min at 1100 hr for animal care (feeding and cleaning of the cages). Food and tapwater were available ad libitum. For the entire duration of the experiments, the control animals were maintained in the centrifuge room in cages similar to those contained in the gondolas. They were therefore submitted to the same noise, light, and temperature (20 ± 1C).

At the end of the 19 experimental days, both CONT and HG rats were anesthetized with an IP injection of sodium pentobarbital (35 $mg.kg^{-1}$). The right soleus muscles were excised, weighed, and frozen in isopentane precooled with liquid nitrogen. At the end of the experimental procedure, the animals received a lethal dose of anesthetic (100 $mg.kg^{-1}$).

Muscle Processing

The soleus muscles of the CONT and HG groups were cut into serial frozen transverse sections (10 μm thick) using a cryostat microtome (Leica CM 1800; Heidelberg, Germany) set at –20C. This specific protocol has already been described by De-Doncker et al. (2002). Briefly, every 280 μm along the muscle, eight sections were made perpendicular to the muscle longitudinal axis. The first section was used as a negative control in the IHC analysis. The next two sections were processed for myofibrillar adenosine 5′-triphosphatase (ATPase) with acid (pH 4.3) and alkaline (pH 10.4) preincubations according to the method of Guth and Samaha (1969). On these sections, we measured the cross-sectional area (CSA) of IFs with an image analyzer (SAMBA 2005; Grenoble, France) in the regions A (equatorial and juxtaequatorial), B (from the end of the periaxial space to the end of the capsule), and C (extracapsular region), these regions being described by Kucera et al. (1978). The remaining sections were labeled with antibodies against myosin heavy chain (MyHC) isoforms.

Antibodies and Labeling

Five primary monoclonal antibodies (MAbs) were used in this study: NCL-MHCs (against MyHC I); SC71 (against MyHC IIA); MY32 (anti-fast MyHC: IIA, IIX, IIB); ALD58 (against slow-tonic MyHC); and F88 (anti-α-cardiac MyHC). The dilutions of these antibodies already reported in De-Doncker et al. (2002) were 1:20, 1:20, 1:2000, 1:5, and 1:1, respectively. NCL-MHCs is commercialized by Tebu-Novocastra (Le Perray en Yvelines, France), SC71 by DSMZ (Braunschweig, Germany), MY32 by Sigma–Aldrich (Saint Quentin Fallavier, France), ALD58 by DSHB (Iowa City,

IA), and F88 by Coger (Paris, France). The binding of the primary antibodies was localized by an immunoperoxidase reaction using the Novostain Universal Quick Kit (Tebu-Novocastra). Serial cross-sections were incubated in prediluted blocking serum (normal horse serum) for about 10 min. The excess serum was blotted and sections were incubated with primary antibodies diluted in PBS for 2 hr. Serial cross-sections were washed for 5 min in PBS. Then, they were incubated for 30 min in prediluted biotinylated universal secondary antibody. At the end of the 30 min, sections were washed with PBS for 5 min and incubated in ready-to-use streptavidin–peroxidase complex reagent for 30 min. To label the serial cross-sections, the peroxidase substrate solution (diaminobenzidine, DAB; Sigma–Aldrich) was added after the sections had been washed for 5 min with PBS. Finally, after dehydration with alcohol and toluene, the slides were mounted in Eukitt resin. Positive fibers were characterized by a brown color and negative fibers remained unlabeled. To estimate the coloration intensities, we have developed a densitometric approach previously described in detail (De-Doncker et al. 2002). The IHC-labeled slides were examined with an image analyzer (SAMBA 2005). To avoid a disparity in the labeling, some experimental precautions were taken. For each antibody, all the sections were processed with the same diluted solution. Moreover, the slides were incubated in a common DAB solution. The zero of the densitometer was adjusted on the negative fibers in a given section; the slight variations of labeling between each slide were therefore discarded. The IHC-labeling slides were analyzed with the image analyzer, which was made up of a digital camera fixed to a standard optical light microscope. The camera was coupled to a computer with video monitor and image acquisition and storage modules. The measures were quantified to 256 gray levels (level 1 corresponding to black color and to 0% of light transmission, and level 256 to white color and 100% of light transmission), which were then converted automatically into optical density, taking into account the studied area. The densitometric values were expressed in percentage ($OD = \log_{10}(1/\text{light transmission})$). According to Nibbering et al. (1986), the formation of the reaction product after DAB/H_2O_2 staining for immunoperoxidase-labeled cells indicated a linear relationship between the amount of enzyme-coupled antibodies bound to cells and the amount of enzyme reaction product. This densitometric analysis has already been used as a semiquantitative approach in rat muscle fibers (Sant'Ana Pereira et al. 1995). The immunoreactivity in muscle spindle IHC is usually scored as absent (−), weak to moderate (+), and strong (++), which does not allow detection of fine modifications in the expression level of myosin isoforms. Consequently, we intentionally chose to express our results as values obtained using densitometric measurements.

As previously described by Kucera (1981), in the most nucleated equatorial region of IFs, the labeling was localized around the IF. In this case, the densitometric measurement was performed only on the colored part. The IF center, which was not stained, was discarded.

Identification of IF Types

The muscle spindles were identified in both CONT and HG soleus muscle groups as encapsulated structures containing small-diameter fibers and presenting three regions (A,B, and C) classically described (Kucera et al. 1978). IFs were classified as B1 (nuclear bag1), B2 (nuclear bag2), and chain (nuclear chain) fibers according to their histochemical labeling (Ovalle and Smith 1972; Kucera et al. 1978) and IHC profiles, as previously reported (Pedrosa–Domellof et al. 1991; Kucera et al. 1992; Soukup et al. 1995; Walro and Kucera 1999). Rat muscle spindles usually contain four IFs: one B1, one B2, and two chain fibers (Kucera and Walro 1988b). The diameter of these IFs decreases in the following order: nuclear B2 > B1 > chain fibers. Nuclear B1 fibers exhibit low acid and alkaline ATPase activity along their intracapsular region but have high acid ATPase activity at their poles. Nuclear B2 fibers have an acid-stable ATPase activity along their length and an intracapsular alkaline-stable ATPase activity, which is lost beyond the capsular sleeve. Nuclear chain fibers have low acid and high-alkaline ATPase activities along their entire length (Kucera et al. 1978). The nuclear B1 and B2 fibers were labeled with both ALD58 antibody, specific for the slow-tonic MyHC isoform, and F88 antibody, specific for the α-cardiac MyHC isoform. Nuclear chain fibers did not express these two MyHC isoforms except in a very short equatorial length where the slow-tonic isoform was detected (Pedrosa et al. 1989; Pedrosa–Domellof et al. 1991).

Statistical Analysis

All results were expressed as means ± SD. After an ANOVA, Student's *t*-test was used to establish the intergroup comparisons and statistical significance was accepted at $p<0.05$. *Indicates a significant difference with the A region, †with the B region, and §with the control group.

Results

Morphological Characteristics

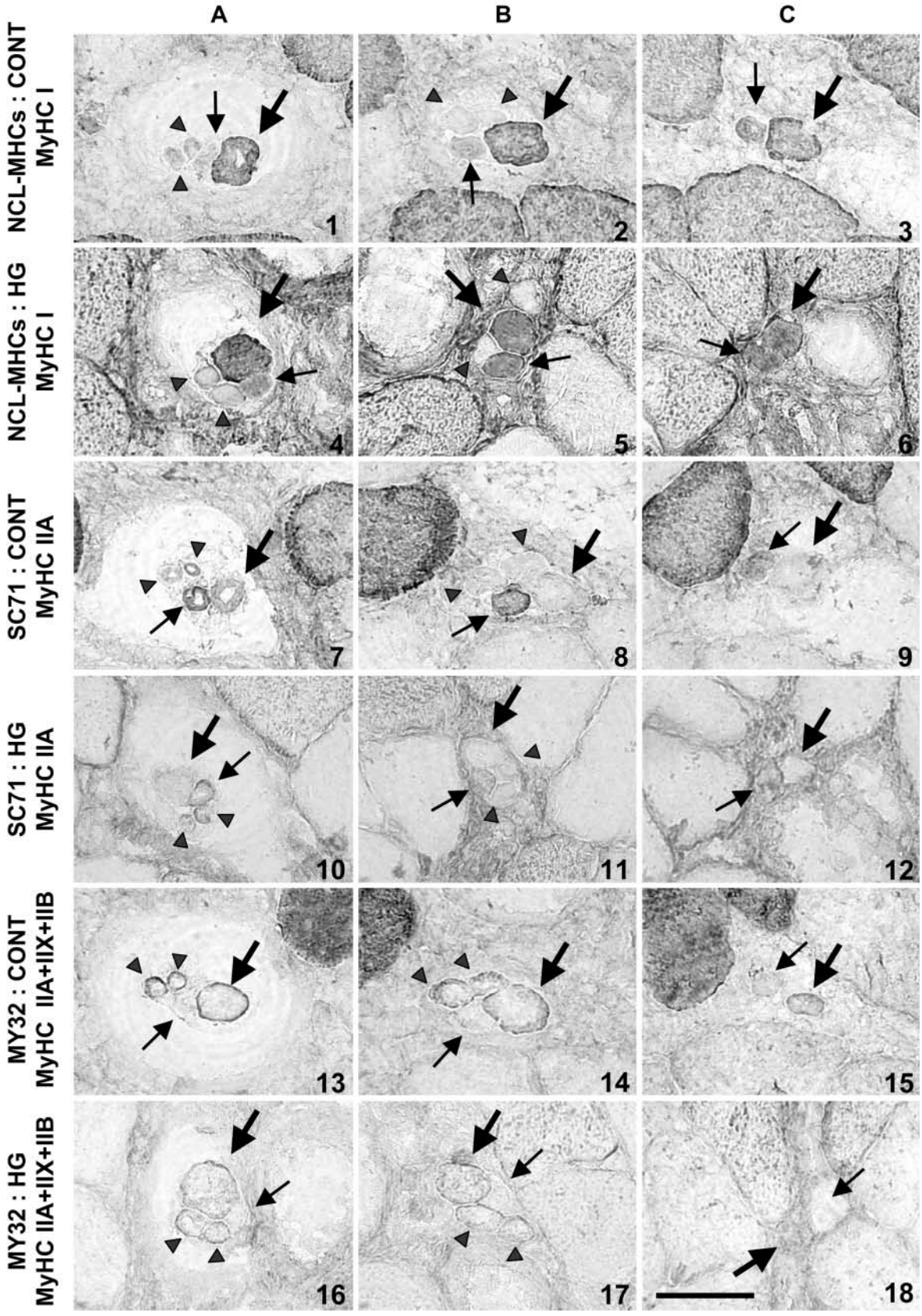
Table 1 reports the body weight (BW), the muscle wet weight (MWW), and the ratio MWW:BW after 19 days of experiment for CONT and HG groups. The values of BW remained similar, whereas both the MWW and the ratio MWW:BW were significantly increased (+21% and +27%, respectively).

Concerning the muscle spindle sample, we studied 40 spindles in the CONT group and 45 in the HG group. The rat spindles usually contained one B1, one B2, and two chain fibers. However, some spindles showed three chain fibers in both CONT and HG groups. This was the case for three of the 40 CONT spindles and two of the 45 HG spindles. Consequently, in the CONT group the total number of stud-

Table 1 Mean values of morphological parameters[a]

	CONT	HG
BW, g (5)	279 ± 27	266 ± 14
MWW, mg (5)	112 ± 12	136 ± 14
MWW:BW, mg/g (5)	0.40 ± 0.02	0.51 ± 0.03

[a]Values are expressed as means ± SD. Morphological parameters are reported as follows: body weight (BW), muscle wet weight (MWW), and ratio MWW:BW of CONT and HG groups, the sample appearing in brackets.


A
B
C
NCL-MHCs : CONT
MyHC I
NCL-MHCs : HG
MyHC I
SC71 : CONT
MyHC IIA
SC71 : HG
MyHC IIA
MY32 : CONT
MyHC IIA+IIX+IIB
MY32 : HG
MyHC IIA+IIX+IIB
1
2
3
4
5
6
7
8
9
10
11
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17
18

ied fibers was for B1 40, for B2 40, and for chain 83. In the HG group, these samples were, respectively, 45, 45, and 92. The CSA of IFs were examined in CONT and HG rats, from A to C regions. There was no statistical difference between the two experimental groups. The ATPase labeling revealed no difference between CONT and HG groups.

Identification of MyHC Isoforms in IFs

The MyHC content was identified by antibodies along the IF length. The results illustrated in Figures 1 and 2 as follows.

NCL-MHCs Antibody (Against MyHC I Isoform). In the CONT group, B2 fibers were labeled and expressed the MyHC I isoform from A to C regions, whereas B1 fibers were labeled only at the end of the B region and in the C region, and chain fibers were negative (Figures 1A1–1A3 for the CONT group). In the HG group (Figures 1A4–1A6), B2 and chain labeling remained similar, whereas B1 fibers appeared reactive against the NCL-MHCs antibody from the A to the C region.

SC71 Antibody (Against MyHC IIA Isoform). B1 fibers of CONT and HG rats were labeled in the A, B, and C regions, B2 and chain fibers being stained only in the A region (Figures 1A7, 1B8, and 1C9, CONT spindles; HG spindles Figures 1A10, 1B11, and 1C12).

MY32 Antibody (Against MyHC IIA + IIX + IIB Isoforms). In both experimental groups, B2 and chain fibers were reactive against this antibody from A to C regions, whereas B1 fibers were never labeled (Figure 1: CONT Figures 1A13, 1B14, and 1B15; HG Figures 1A16, 1B17, and 1C19).

ALD58 Antibody (Against Slow-tonic MyHC Isoform). In both CONT and HG groups, only B1 and B2 fibers contained the slow tonic MyHC isoform in A, B and C regions, and only in the A and inner B regions, respectively (Figure 2: Figures 2A1, 2B2, and 2C3 for CONT rats; Figures 2A4, 2B5, and 2C6 for HG rats).

F88 Antibody (Against α-cardiac MyHC Isoform). In CONT rats, the α-cardiac MyHC was expressed in B2 fibers from A to C regions, the chain fibers being absolutely negative. However, B1 fibers were reactive against F88 only from outer B to C regions (Figures 2A7, 2B8, and 2B9). For HG rats, B2 and chain labeling remained identical, but the α-cardiac MyHC was now expressed from A to C regions in B1 fibers (Figures 2A10, 2B11, and 2C12).

Densitometric Data

Because slight variations in MyHC content after HG were not clearly visible with a single antibody labeling, we refined the data using densitometric measurements. The densitometric values are reported in Table 2 (CONT group) and Table 3 (HG group). The results are expressed as percentage of optical density (OD) with OD = log (1/light transmission) considering that 0% of light transmission corresponded to black color and 100% of light transmission to white color. Consequently, the more elevated the OD value, the more important the myosin content. However, the results were not directly quantitative but rather were semiquantitative.

In the CONT group, the densitometric data showed that, for B1 fibers, labeling against NCL-MHCs (anti-MyHC I), SC71 (anti-MyHC IIA), ALD58 (anti-slow-tonic MyHC), and F88 (anti-α-cardiac MyHC) antibodies was significantly decreased in the C region compared to the A region (NCL-MHCs and F88) and compared to both A and B regions (SC71 and ALD58). In B2 fibers, labeling against MY32 (anti-MyHC IIA + IIX + IIB) and F88 was significantly reduced in the C region vs the A and B regions.

In the HG group, B1 fibers exhibited a significantly increased labeling against both NCL-MHCs and F88 antibodies in the B and C regions compared with the A region. Moreover, the densitometric results revealed marked changes in labeling amounts. After HG, these fibers became reactive from A to C regions against NCL-MHCs and F88 antibodies ($p<0.05$), the labeling being increased. Conversely, the binding intensity was statistically decreased for SC71 in the A and B regions and for ALD58 in the A to C regions.

In HG spindles, B2 fibers showed a decreased reactivity against SC71 (A region) and MY32 and ALD58 (A and B regions) antibodies ($p<0.05$). In chain fibers, the labeling intensity was significantly decreased for SC71 and MY32 antibodies in the A region and in the A and B regions, respectively.

Discussion

The aim of the present study was to determine by IHC the effects of a hypergravity environment on MyHC isoform expressions among the three types of IFs in rat soleus muscle.

Figure 1 Transverse sections of CONT and HG soleus muscles (10-μm thick) containing a spindle with one B1 (thin arrow), one B2 (thick arrow), and two chain fibers (triangle). The illustrated sections were processed with the following antibodies: NCL-MHCs (anti-MyHC I), SC71 (anti-MyHC IIA), and MY32 (anti-fast MyHC: IIA + IIX + IIB) from **A**, **B**, and **C** regions. Bar = 32 μm.

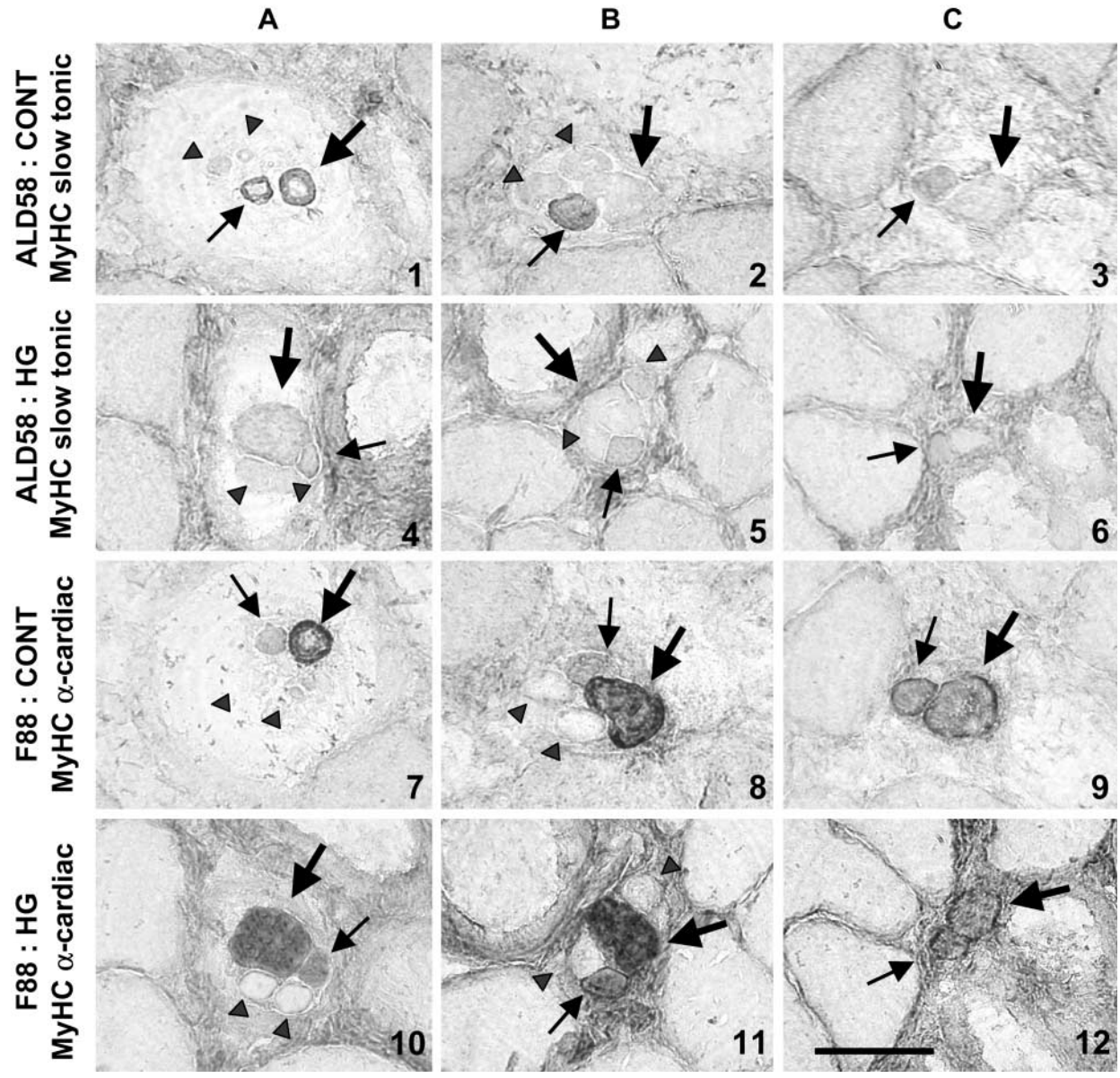

Figure 2 Soleus sections of CONT and HG groups showing a spindle from the **A** to **C** regions. The following antibodies were used: ALD58 (anti-slow-tonic MyHC) and F88 (anti-α-cardiac MyHC). B1, thin arrow; B2, thick arrow; chain, triangle. Bar = 32 μm.

For the CONT group, the depth and the regional variation of labeling with NCL-MHCs, MY32, ALD58, and F88 antibodies were in agreement with other studies (Pedrosa et al. 1990; Pedrosa–Domellof et al. 1991; Kucera et al. 1992; McWhorter et al. 1995; Soukup et al. 1995; De-Doncker et al. 2002). However, some differences were observed with SC71 antibody labeling: a low level of labeling was seen in the B2 fibers. This point has been discussed in a previous article (De-Doncker et al. 2002) and was attributed to a different dilution used by other authors (Kucera et al. 1992). A discrepancy in labeling was observed in the nuclear B1 fibers with MY32 and SC71 antibodies. Nuclear B1 fibers were labeled by SC71 along their entire length, whereas MY32 antibody never labeled these fibers. This was surprising because the MY32 antibody is supposed to react with all fast-twitch MyHC isoforms (Schiaffino et al. 1989). SC71 antibody is specific for MyHC IIA isoform and never crossreacts with other MyHC isoforms. Therefore, the fast-twitch MyHC isoform, recognized in nuclear B1 fibers by the SC71 antibody but not by MY32, could be a specific muscle spindle MyHC isoform whose epitope resembles that recognized by SC71 on the

Table 2 Densitometric analysis of the IHC labeling in intrafusal fibers of the control group[a]

Fibers (*n*)	Region	NCL-MHCs	SC71	MY32	ALD58	F88
B1 (40)	A	NL	29.6 ± 8.7	NL	23.4 ± 5.8	NL
	B	14.4 ± 5.5	30.7 ± 5.1	NL	22.9 ± 5.2	26.8 ± 7.3
	C	21.5 ± 4.6†	22.2 ± 3.7*†	NL	17.1 ± 5*†	23.1 ± 7.5†
B2 (40)	A	22.2 ± 4.1	20.8 ± 6.2	23.3 ± 6.6	20.8 ± 4.5	30.6 ± 7.9
	B	20.9 ± 4.7	NL	26.1 ± 7.7	NL	28.4 ± 8.5
	C	22.5 ± 6	NL	19.9 ± 6*†	NL	23.9 ± 7.1*†
Chain (83)	A	NL	24.9 ± 6	28.5 ± 9.7	NL	NL
	B	NL	NL	27.8 ± 8.7	NL	NL

[a]The densitometric analysis is presented as a percentage of B1, B2, and chain fibers in the CONT group. Values are given for each antibody in the three different muscle spindle regions (A,B,C).
*Significant difference of labeling with the A region and †with the B region. NL, no labeling.

MyHC IIA isoform. This possible existence of other specific muscle spindle MyHC isoforms, not expressed in extrafusal fibers, has previously been suggested by other authors (Kucera et al. 1992; Pedrosa–Domellof et al. 1993). Kucera et al. (1992) suggested that the labeling intensity and the regional variation with MY32 were higher and broader than those with SC71 (MyHC IIA) and BF-F3 (MyHC IIB) antibodies, indicating the possible existence of a specific fast-twitch MyHC isoform expressed by the IFs but not by the extrafusal fibers.

After 19 days of hypergravity, labeling with NCL-MHCs, SC71, MY32, ALD58, and F88 antibodies was modified. The expression of both MyHC I and α-cardiac appeared in the A region and increased in B and C regions of B1 fibers. The novel expression of MyHC I and MyHC α-cardiac in the A region of B1 fibers was not the result of a crossreaction between NCL-MHCs and F88 antibodies. Indeed, the epitope detected by the F88 in the MyHC α-cardiac was not present in embryonic, neonatal, slow-twitch, fast-twitch, and slow-tonic isoforms (Pedrosa et al. 1990). Labeling against SC71 and ALD58 antibodies was decreased along the full length of B1 fibers and in the A region of B2 fibers. For chain fibers, a significant decrease in SC71 labeling was also observed in their A region. Moreover, MY32 labeling was decreased along the entire length of B2 fibers and in the A and B regions of chain fibers.

Two major points are discussed here: (a) the impact of neural regulation on expression of the MyHC isoform in IFs in both developing and adult rats, and (b) the influence of the gravitational load on expression of these myosin isoforms.

Neural Regulation of IF MyHC Isoform Expression in Developing Rat

Several studies have shown that sensory innervation is required for the development of muscle spindles and for the expression of spindle-specific MyHC isoforms. The motor innervation contributed to the regional variation of the different MyHC isoforms along the length of the nuclear bag fibers (Kucera and Walro 1988a; Zelena 1994; Soukup et al. 1995). The regulation of MyHC isoform expression along IFs was very complex and reflected interactions between inductive and/or suppressive effects of both motor and sensory innervation, as well as the intrinsic properties of specific myoblast lineages (Kucera and Walro 1990a,c; Pedrosa–Domellof et al. 1991; Soukup et al. 1995; Walro and Kucera 1999). Moreover, the afferent influence diminished and motor influence increased with

Table 3 Densitometric analysis of the IHC labeling in IFs of the HG group[a]

Fibers (*n*)	Region	NCL-MHCs	SC71	MY32	ALD58	F88
B1 (45)	A	16.9 ± 2.9§	17.3 ± 4.1§	NL	16 ± 5.7§	17.1 ± 4.1§
	B	19 ± 4*§	17.7 ± 5.4§	NL	15.8 ± 7.1§	29.8 ± 5*§
	C	23.8 ± 3*†§	15.4 ± 4.1§*†	NL	12.1 ± 5*†§	26 ± 3.9*†§
B2 (45)	A	22.6 ± 3.4	12.1 ± 3.3§	15.9 ± 6§	16.9 ± 2.8§	28 ± 4.9
	B	21.5 ± 2.9	NL	16.1 ± 6.8§	NL	26 ± 6
	C	21.8 ± 3.9	NL	13 ± 4.2*†§	NL	22.6 ± 4.8*†
Chain (92)	A	NL	14 ± 4.1§	15.5 ± 6.3§	NL	NL
	B	NL	NL	14.6 ± 6.6§	NL	NL

[a]Densitometric analysis is presented as a percentage of B1, B2, and chain fibers in the HG group. Values are given for each antibody in the A, B, C muscle spindle regions.
§Significant difference of labeling with control group, *with the A region, and †with the B region. NL, no labeling.

increasing distance from the equator of IFs (Pedrosa et al. 1989; Kucera et al. 1992).

Sensory innervation has a specific modulatory key role in slow-tonic MyHC isoform expression. Fetal rat denervation using bungarotoxin (a non-selective neurotoxin) (Kucera and Walro 1990b), neonatal denervation (Thornell et al. 1989; Soukup et al. 1994), and deafferentation (Kucera and Walro 1988a) prevented the expression of the slow-tonic MyHC isoform in nuclear bag fibers. However, after neonatal de-efferentation, the diversity in slow-tonic MyHC distribution along the intrafusal B2 fibers was modified and this MyHC isoform was expressed more intensely and over most of the B2 fiber length (Soukup et al. 1990a; Pedrosa–Domellof et al. 1991). Conversely, efferents rather than afferents might induce and maintain expression of the α-cardiac MyHC isoform (Pedrosa et al. 1990; Soukup et al. 1999). On the one hand, during normal development the expression of the α-cardiac MyHC isoform appeared in nuclear bag fibers 1 day after the arrival of γ motor innervation (Pedrosa et al. 1990; Pedrosa and Thornell 1990). On the other hand, neonatal de-efferentation induced a decrease in the expression of this MyHC isoform in B2 fibers and its removal in B1 fibers (Pedrosa et al. 1990; Soukup et al. 1999).

Neural Regulation of IF MyHC Isoform Expression in Adult Rat

Perturbations in spindle sensory and motor innervations of adult rat produced less severe alterations in MyHC isoform expression than did similar lesions in developing IFs (Wang et al. 1997). Contrary to fetal and neonatal rat, adult rat deafferentation never induced degeneration of muscle spindles, but altered the stereotypical MyHC profiles of intrafusal fibers (Wang et al. 1997). Indeed, IFs in adult chronically deafferented muscles upregulated fast MyHC isoforms, particularly MyHC IIA, and to a much lesser degree the MyHC I isoform at the expense of developmental MyHC isoforms. The activity imposed on nuclear bag fibers by efferents or muscle stretch might modify the MyHC I expression along these IFs (Kucera et al. 1992), as is the case for extrafusal fibers deprived of motor innervation (Harris et al. 1989). In the presence of motor innervation and in the absence of sensory innervation, the MyHC content of IFs assumed more extrafusal-like features (Kucera and Walro 1988a,b; Walro et al. 1989). Therefore, as suggested by Wang et al. (1997) and Walro et al. (1997), the upregulation of MyHC isoforms expressed by extrafusal fibers reflected a de-repression of these isoforms. After adult de-efferentation of the rat extensor digitorum longus muscle, Wang et al. (1997) have shown that B1 fibers that normally expressed the α-cardiac MyHC isoform moderately in the outer B region ceased to express this MyHC isoform. Moreover, B2 fibers continued to express this isoform but less intensely than normal, and this MyHC expression was limited to a shorter region of B2 fibers, as described in the studies of Pedrosa et al. (1990) and Wang et al. (1997).

Finally, according to these data, it appeared that MyHC expression in muscle spindles was directly under neural influence, both sensory and motor. However, the impact of the gravitational environment on neural activity is now understood, and recent data have demonstrated that this environment may also regulate the MyHC expression in muscle spindles.

Influence of the Gravitational Load on Muscle Spindle MyHC Isoforms

De-Doncker et al. (2002) have reported that expression of some MyHC isoforms along intrafusal fibers of rat soleus muscle was modified after a 14-day period of hypogravity. These authors observed that, whereas histochemical profiles of IFs were maintained, there was a decrease in slow type 1 and an increase in slow-tonic MyHC isoform expressions in B and C regions of B1 fibers. Moreover, the α-cardiac MyHC expression was significantly decreased along the entire length of B2 fibers and in the B and C regions of B1 fibers. Furthermore, some chain fibers expressed the slow type I and α-cardiac MyHC isoforms over a short distance of the A region, although these isoforms were never expressed in the control intrafusal fibers. De-Doncker et al. (2002) suggested that the decrease in α-cardiac and the increase in slow-tonic MyHC isoforms in nuclear bag fibers probably reflected a decrease in the activity pattern of the motor nerves during a hypogravity period, considering the results of several studies on denervation or de-efferentation experiments (Pedrosa et al. 1990; Soukup et al. 1990a,1999; Wang et al. 1997). The structure of rat muscle spindle was not modified by a hypogravity period. Indeed, IFs were not atrophied and their number per muscle spindle was not changed by hypogravity conditions, as previously described by Soukup et al. (1990b) and De-Doncker et al. (2002). These authors concluded that IFs were more resistant to myogenic atrophy than extrafusal fibers, as suggested earlier by Yellin and Eldred (1970) and Maier et al. (1972). In a previous report (De-Doncker et al. 2002), we hypothesized that during a 14-day period of hypogravity (HH), muscle spindles were little or not at all stimulated, considering the shortened position of the rat soleus muscle. Therefore, muscle spindle afferent activities were probably reduced (Ohira et al. 1992). An indirect proof of this statement was provided by the fact that the reactivation of Ia fibers by tendinous vibrations constituted an effective countermeasure to prevent muscle atrophy developed during hindlimb unloading conditions (Falempin and Fodili In-Albon

1999). It is known that Ia afferents project in the spinal cord onto α-skeletomotor and β-skeletofusimotor neurons, and onto interneurons which, in turn, project onto γ-fusimotor neurons (Bernstein and Goldberg 1995). We suggest that this reduction in muscle afferent activity could modify MyHC isoform expression by altering the pattern discharge of β- and γ-fusimotor neurons that innervate the contractile portion of the IF. Another factor should also be implied in the modifications of motor neuron activity. It has been demonstrated that simulated weightlessness induced a decrease in acetylcholinesterase activity in neuromuscular junctions of both extrafusal and intrafusal fibers, which might be linked with a decrease in α- and γ-motor neuron activities (Tang et al. 2002). This study refuted our first work (De-Doncker et al. 2002), which suggested that changes in MyHC expressions of intrafusal fibers after 14 days of weightlessness could be due to a decreased influence of γ- and/or β-motor innervation during the simulated microgravity environment. To complete the preceding results, we studied the effects of a hypergravity environment on MyHC isoform expression along the three types of IFs of rat soleus muscle. However, contrary to what occurred in a hypogravity environment, the soleus muscle in hypergravity was probably stretched and, consequently, the muscle spindle afferent activities were increased. By a reflex pathway, considering the study of Bernstein and Goldberg (1995), the muscle spindle motor activity was also increased. The MyHC α-cardiac and MyHC I increases in B1 fibers and MyHC slow-tonic decrease in nuclear bag fibers reflected a reinforced motor innervation influence during a hypergravity period, contrary to the hypogravity situation (De-Doncker et al. 2002). Two hypotheses might explain the discrepancy between hypogravity and hypergravity concerning F88 labeling in B2 fibers: the duration of exposure to a modified gravitational load (14 vs. 19 days) and/or different myogenic lineages (Kucera and Walro 1990a; Walro and Kucera 1999). Moreover, after a hypergravity situation, it has been demonstrated that the proportion of slow-type extrafusal fibers expressing the MyHC I isoform was increased up to 100% (Martin and Romond 1975; Picquet et al. 2002). After adult rat deafferentation (Wang et al. 1997), MyHC I isoform was slightly overexpressed in muscle spindles. In our study, we suggested that afferent activity from muscle spindles was increased. Consequently, MyHC I isoform expression should be decreased. However, the expression of this MyHC isoform was increased along the full length of B1 fibers after a hypergravity period. We therefore supposed that the excitatory motor influence prevailed on the inhibitory afferent influence, at least for the MyHC I isoform regulation. Wang et al. (1997) have also demonstrated that IFs upregulated fast MyHC isoforms after adult rat deafferentation. They concluded that excitatory motor influence was de-repressed by the lack of afferent activity. In our study we considered that both afferent and motor innervation were similarly increased after hypergravity. Therefore, given the conclusions of Wang et al. (1997), the increase in motor innervation influence should upregulate the fast MyHC isoforms. However, our results showed a decrease in fast MyHC isoform expression along IFs. Consequently, contrary to MyHC I regulation, the inhibitory influence prevailed on the excitatory motor influence for fast MyHC isoform expression. At least for these MyHC isoforms (slow and fast), the hypergravity induced some inverse changes of those observed in a hypogravity environment. Conversely, this was not the case for fast MyHC isoform expression. Indeed, the fast MyHC isoforms were not changed after a hypogravity period, whereas they were downregulated in IFs after hypergravity. It therefore appears that IFs were more sensitive to a hypergravity environment. Because chronically deafferented adult muscle spindles overexpressed fast MyHC isoforms along IFs (Walro et al. 1997; Wang et al. 1997) and because these MyHC isoforms appeared downregulated in our study, we can therefore suggest that muscle spindle afferent activities were increased. It is well known that the γ-motor innervation (dynamic and static) of IFs play a major role in control of the muscle spindle sensitivity during different positions

Table 4 Comparison between hypergravity (HG) and hypogravity (HH) conditions vs control rats on the immunolabeling of IFs[a]

Antibody labeling	Intrafusal fiber types	Experimental condition	Intrafusal fiber regions		
			A	B	C
NCL-MHCs	B1	HG	NE	+	+
	B1	HH	NL	—	—
	B2	HG	NC	NC	NC
	B2	HH	NC	NC	NC
	Chain	HG	NL	NL	
	Chain	HH	NE	NL	
SC71	B1	HG	—	—	—
	B1	HH	NC	NC	NC
	B2	HG	—	NL	NL
	B2	HH	NC	NL	NL
	Chain	HG	—	NL	
	Chain	HH	NC	NL	
MY32	B1	HG	NL	NL	NL
	B1	HH	NL	NL	NL
	B2	HG	—	—	—
	B2	HH	NC	NC	NC
	Chain	HG	—	—	
	Chain	HH	NC	NC	

[a]Effects of gravity changes on intrafusal MyHC isoforms are synthetically reported. The decrease, the increase, or the maintenance of MyHC content are respectively indicated by —, +, and NC (no change). A de novo expression is indicated by NE (novel expression). NL, no labeling.

Table 5 Comparison between hypergravity (HG) and hypogravity (HH) conditions vs control rats on the immunolabeling of IFs[a]

Antibody labeling	Intrafusal fiber types	Experimental condition	Intrafusal fiber regions		
			A	B	C
ALD58	B1	HG	—	—	—
	B1	HH	NC	+	+
	B2	HG	—	NL	NL
	B2	HH	NC	NL	NL
	Chain	HG	NL	NL	
	Chain	HH	NL	NL	
F88	B1	HG	NE	+	+
	B1	HH	NL	—	—
	B2	HG	NC	NC	NC
	B2	HH	—	—	—
	Chain	HG	NL	NL	
	Chain	HH	NE	NL	

[a]Effects of gravity changes on intrafusal MyHC isoforms are synthetically reported. The decrease, the increase, or the maintenance of MyHC content are respectively indicated by —, +, and NC (no change). A de novo expression is indicated by NE (novel expression). NL, no labeling.

and activities (for reviews see Hulliger 1984; Prochazka et al. 1988; Banks 1994). After 14 days of HH or 19 days of HG, it is possible that subtle modifications in MyHC isoform expression along IFs would result in very little functional adaptation. However, these modifications would change the contraction of IF extremities and consequently would affect the control of the muscle spindle sensitivity. Moreover, fast extrafusal type II fibers containing MyHC II isoforms are less stiff than the slow extrafusal type I fibers containing the MyHC I isoform (Petit et al. 1990). Therefore, changes in MyHC isoform expression by IFs would modify the viscoelastic properties of IFs, which would affect the detection of the muscle stretching by muscle spindles. Consequently, the discharge of muscle spindle afferents would be modified after 19 days of HG, as has been demonstrated after 14 days of HH (De-Doncker et al. 2003), and muscle proprioception would be disrupted.

Our conclusion is that IFs are more resistant than extrafusal fibers to changes in the gravitational load. Moreover, the balance between afferent and motor innervation influences was probably modified during both hypogravity and hypergravity (see Tables 4 and 5). This imbalance differently regulated the MyHC isoform expression along the three types of IFs. Contrary to Kucera et al. (1993) and Walro et al. (1997), who attributed the mechanism of afferent influence only to neurotrophically mediated substances, our data suggest that afferent nerve activity and an intact reflex arc are also required to determine levels and regional variations of MyHC isoform expression along intrafusal fibers.

Acknowledgments

Supported by grants from the Centre National d'Etudes Spatiales (8411) and the Conseil Régional du Nord-Pas-de-Calais.

We wish to thank Dr G.S. Butler–Browne for growing of hybridomas producing SC-71 antibodies initially developed by Dr Schiaffino et al. (1989). The hybridomas are commercialized by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

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Contractile properties and myosin expression in rats born and reared in hypergravity

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Received 31 October 2001; accepted in final form 22 January 2002

Picquet, F., V. Bouet, M. H. Canu, L. Stevens, Y. Mounier, M. Lacour, and M. Falempin. Contractile properties and myosin expression in rats born and reared in hypergravity. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R1687–R1695, 2002; 10.1152/ajpregu.00643.2001.—The effects of hypergravity (HG) on soleus and plantaris muscles were studied in Long Evans rats aged 100 days, born and reared in 2-*g* conditions (HG group). The morphological and contractile properties and the myosin heavy chain (MHC) content were examined in whole muscles and compared with terrestrial control (Cont) age-paired rats. The growth of HG rats was slowed compared with Cont rats. A decrease in absolute muscle weight was observed. An increase in fiber cross-sectional area/muscle wet weight was demonstrated, associated with an increase in relative maximal tension. The soleus muscle changed into a slower type both in contractile parameters and in MHC content, since HG soleus contained only the MHC I isoform. The HG plantaris muscle presented a faster contractile behavior. Moreover, the diversity of hybrid fiber types expressing multiple MHC isoforms (including MHC IIB and MHC IIX isoforms) was increased in plantaris muscle after HG. Thus the HG environment appears as an important inductor of muscular plasticity both in slow and fast muscle types.

Long Evans rats; soleus; plantaris; myosin heavy chain; contractile parameters

MANY STUDIES HAVE INVESTIGATED the remarkable capacity of the muscular tissue to adapt to environmental stimuli. Among many factors, gravity has been shown to induce neuromuscular changes. For instance, skeletal muscles of rats are atrophied under unloading conditions during spaceflight or simulated microgravity, and the degree of the modifications is differential according to the muscle type and muscle function (7, 10). The changes are greater in slow extensor muscles than in fast ones. In the soleus, which is a slow postural muscle, several changes have classically been described in the literature (7) after episodes of microgravity (real or simulated) as follows: *1*) a muscular atrophy characterized by decreases in absolute muscle weight and fiber cross-sectional area (CSA); *2*) a decrease in absolute and relative strength; and *3*) a decrease in twitch contraction time confirmed by changes in histochemical (ATPase staining) and electrophoretic properties [contents in myosin heavy chain (MHC) isoforms; see Ref. 36] during which the soleus muscle changed into a faster type. In the plantaris muscle, which is the fast agonist of the soleus muscle, the following less drastic effects have been reported: *1*) a slight muscular atrophy after simulated microgravity (4, 8, 39); *2*) no change in the relative tetanic tension (4, 8), and *3*) no change (8) or an increase in time to peak (TTP; see Ref. 4).

On the contrary, the impacts of a hypergravity (HG) environment on the muscular system were less substantiated. Until now, studies performed on cockerels or rats (young or adult) during short or long periods of HG exposure have described modifications in the body and muscular masses, protein synthesis rate, and RNA and enzyme activities (3, 6, 15, 21, 31).

In most of the studies performed in HG, the animals were conceived and born in normal gravity and then exposed to HG. Presently, there are no data relative to the morphological, contractile, and biochemical behavior of muscles from rats born and reared in HG. Therefore, the aim of this work was to study the morphological muscle characteristics, contractile properties, and MHC content in two hindlimb extensor muscles [a slow one (the soleus) and its fast agonist (the plantaris)]. According to the function of these two muscles, we predicted that these muscles were more activated during HG and, probably, the soleus muscle became slower and the plantaris muscle became faster.

METHODS

Animal groups. Experiments were performed on 55 male Long Evans rats aged 100 days and divided into the following two groups: control rats (Cont, $n = 25$) and HG rats ($n = 30$). The 30 HG animals were selected from a population of the

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first generation derived from couples mated in the chronic centrifuge apparatus. After 15 days, males were taken off, and females stayed until weaning. We studied male rats descending from different littermates. The Cont rats were reared in conditions similar to those of the centrifuge, i.e., the same room, same dark-light cycle (12:12 h), and same temperature inside a standard home cage contained in a gondola. The contractile and morphological properties were determined in right soleus muscles of 11 Cont and 15 HG rats and in right plantaris muscles of 14 Cont and 15 HG rats.

The experiments and the maintenance conditions of the animals were approved by both the Agricultural and Forest Ministry and National Education Ministry (authorization 03805).

Centrifugation apparatus. The apparatus consisted of a velocity-controlled direct-current motor (3.5 kW) located in the vertical axis of the apparatus and driving two horizontal cross-arms (total length 165 cm) at constant rotation speed. Four free-swinging gondolas were jointed at the four extremities of the horizontal arms, 76.5 cm away from the axis of rotation; the gondolas were equipped with an aeration system, lights that reproduced a 12:12-h light-dark cycle, and standard home cages for rats. One gondola was equipped with a video system composed of a wide-angle video camera connected to a video monitor, which allowed knowledge of the exact birth date of litters and to allow control of the movement ability of the animals. During centrifugation, the gondolas were tilted at a constant angle of 60° from vertical, depending on the chosen speed. Counterclockwise rotations were done at a constant velocity of 3.81 rad/s. Given the mass and the inertia of the gondolas, including the home cages and the rat, this angular velocity led to 2-*g* resultant force. The resultant force was directly measured using a standardized force sensor at the center of the floor of the gondola.

During centrifugation, rats were subjected to a gravitoinertial force vector (**Z** vector) whose direction was always similar to that exerted in normal gravity (parallel to the dorsoventral axis of the animal), and magnitude was two times the normal magnitude exerted on Earth. Consequently, the rats were also subjected to Coriolis accelerations. The amplitude of Coriolis accelerations depended on the speed of animal locomotion within the home cage and led to a deviation of the rat movement trajectory.

For animal care (cleaning and feeding), the centrifugation was stopped for 15 min every week. Food and water were available ad libitum. Food intake was measured. No statistical difference was found between Cont and HG rats except during 7 days after weaning. At this period, the HG rats showed a slight decrease in food intake. However, after acclimation, the HG rats gained body mass at a rate similar to Cont, but their mass remained lower than Cont animals.

Surgical techniques and physiological measurements. The physiological studies were performed *1*) in the right soleus of 11 Cont rats and 15 HG rats and *2*) in the right plantaris muscles of 14 Cont rats and 15 HG rats. Animals were anesthetized with pentobarbital sodium (30 μg/g). Supplementary doses were injected if necessary (15 μg/g). Under deep anesthesia, assessed by the absence of blink reflexes, all of the muscles of the right thigh and lower limb were denervated, except the studied muscle (either the soleus or the plantaris). The dissected limb was fixed to maintain the isometric conditions and was immersed in a paraffin oil bath thermostatically controlled (37°C). The limb was stabilized by a combination of pins, clamps, and bars so that the muscle was maintained in a horizontal position. The muscle (soleus or plantaris) was isolated from surrounding tissues, and its tendon was sutured by a short 3.0 silk suture around a force transducer (FT 10; Grass). Its length was adjusted to produce a maximal twitch tension. The stimulating (Teflon-coated platinum, ø: 125 μm) and reference (Teflon-coated platinum, ø: 250 μm) electrodes were maintained by micromanipulators. Muscle contractions were induced through a stimulating monopolar electrode put under the soleus or the plantaris nerves. The reference electrode was inserted in the adjacent denervated muscle mass. The TTP and the half-relaxation time (HRT) were measured from the maximal twitch tension (P_t) recording. A series of stimulation frequencies, from 20 to 120 Hz, was also applied and permitted to establish the force/frequency relationship from which several parameters could be determined, such as the maximal tetanic tension (P_0) obtained at 100 Hz and the ratios between unfused tetanic tension and fused tetanic tension: P_{20}/P_0 (tetanic tension at 20 Hz divided by P_0) for the soleus muscle and P_{40}/P_0 (tetanic tension at 40 Hz divided by P_0) for the plantaris muscle, which are indicators of the muscle type (44).

After the measurements of contractile properties, the soleus and plantaris muscles were excised, weighed, and divided into two parts that were frozen in isopentane precooled in liquid nitrogen. One of the two parts was used to determine the MHC isoform composition by SDS-PAGE analysis. On the second part, the muscle typing was established with immunohistochemical methods. After the experiments, animals were killed with a lethal dose of pentobarbital sodium.

Determination of MHC isoforms by SDS-PAGE. The MHC isoforms were determined in five Cont and five HG soleus muscles and in five Cont and five HG plantaris muscles. The first part of the frozen tissues was pulverized under liquid nitrogen in a small steer mortar (30). As already described (40), the MHC composition in plantaris and soleus muscles was determined on a 4.5% glycerol stacking gel and on a 7.5% glycerol separating gel. Electrophoresis was run for 18 h at 12°C (180 volts constant, 13 mA/gel). After the gels were run, the gel slabs were silver stained. The relative proportion of each MHC isoform in each muscle type was determined using a scanning densitometer (Quantiscan Microvial Systems; Biosoft).

Characterization of muscle fiber types by immunohistochemistry. The fiber types were determined in five Cont and five HG soleus and in five Cont and in five HG plantaris muscles by ATPase staining, on which the muscle fiber types were assessed by immunolabeling of MHC isoforms. Frozen serial sections (10 μm thick) were obtained in the second part of soleus and plantaris muscles. The myosin ATPase activity was revealed both with alkaline (pH 10.4) and acid preincubations (pH 4.3 and 4.45) according to the method of Guth and Samaha (13). Additional sections were treated with a double preincubation (32) that differentiated, in a fast muscle, only fibers expressing MHC IIX. The sections were preincubated for 15 min at room temperature in a 20 mM glycine buffer solution containing 20 mM $CaCl_2$ (pH 10.4). They were fixed for 3 min in a 2% methanol-free paraformaldehyde solution containing 0.2 M sodium cacodylate and 75 mM $CaCl_2$ with 11.5% sucrose (pH 7.2) and then further preincubated for 15 min (room temperature) in a solution containing 0.1 M sodium acetate and 0.1 M potassium chloride (pH 4.5, adjusted with acetic acid). Washings with a solution containing 0.1 M Tris and 18 mM $CaCl_2$ (pH 7.2) were made between preincubations and the fixation. Incubation was performed for 25 min at room temperature in a medium containing 40 mM glycine buffer, 20 mM $CaCl_2$, and 2.5 mM ATP (pH 9.4). The sections were then rinsed with 1% $CaCl_2$ and were stained with 2% $CoCl_2$ and 5% $(NH_4)_2S$. For the immunohistochemically treated sections, the protocol has already been described in detail (29). The following antibodies were used:

Table 1. *Mean values of morphological parameters*

	Soleus		Plantaris	
	Cont (n = 11)	HG (n = 15)	Cont (n = 14)	HG (n = 15)
BW, g	364 ± 19	219 ± 22*	370 ± 15	221 ± 20*
MWW, mg	198 ± 19	115 ± 13*	430 ± 18	231 ± 47*
MWW/BW mg/g	0.54 ± 0.03	0.53 ± 0.05	1.16 ± 0.04	1.04 ± 0.14
CSA				
Slow fibers, μm^2	4,742 ± 62	3,298 ± 56*	3,291 ± 216	1,935 ± 108*
Int + fast fibers, μm^2	3,662 ± 36		3,083 ± 63	2,553 ± 35*
Slow fibers/MWW, μm^2/mg	23.94 ± 0.31	28.68 ± 0.48*	7.65 ± 0.50	8.37 ± 0.46
Int + fast fibers/MWW, μm^2/mg	18.50 ± 0.18		7.16 ± 0.14	11.05 ± 0.15*

Values are means ± SE; n, no. of rats. Morphological parameters were reported as follows: body weights (BW), muscle wet weights (MWW), ratio MWW/BW of control (Cont) and hypergravity (HG) groups. In each group, the absolute cross-sectional area (CSA) and the CSA normalized to MWW (CSA/MWW) were determined for the pure slow fiber type and for the regrouped intermediate (int) + fast fiber types. *Statistical difference ($P < 0.05$) between HG and its Cont paired group.

NCL-MHCs (Tebu/Novocastra), which recognize MHC I; SC-71 (DSMZ) against MHC IIA; BF-F3 (DSMZ) against MHC IIB; and MY32 (Sigma) against MHC IIA, IIB, and IIX. These antibodies were diluted, respectively, at 1:20, 1:20, 1:10, and 1:2,000. The muscle typing was established on a total of 300 fibers/muscle immunologically identified in serial sections. The CSA of the fibers was also measured on ATPase sections using an image analyzer (Samba, Grenoble, France) on a minimum of 300 fibers/muscle (11 Cont and 15 HG soleus and 14 Cont and 15 HG plantaris).

Statistical analysis. All results are expressed as means ± SE. For the two muscles, after a one-way ANOVA, the intergroup comparisons (soleus HG vs. Cont, plantaris HG vs. Cont) were performed using Student's t-test with a significance level of $P < 0.05$.

RESULTS

Morphological data. The morphological parameters are reported in Table 1. The values of body weights (BW), absolute [muscle wet weight (MWW)], and relative (MWW/BW) muscle weights showed that, after 100 days of HG, the rats presented BW (−40%) and MWW (soleus −42%, plantaris −46%) significantly smaller than their age-paired Cont groups. However, when the results were expressed in terms of MWW/BW, the ratios were not significantly different compared with Cont data. The CSA of the fibers were measured on a minimum of 300 fibers/muscle: on slow fibers (expressing only the slow MHC I isoform) and on intermediate (int) + fast fibers (expressing a combination of slow MHC I isoform with one or more fast MHC isoforms). With the aim to clarify the data and to perform a statistical analysis, these populations were expressed in terms of "slow type" and "int + fast types." The absolute CSA of slow and int + fast fiber types decreased significantly in each HG muscle when compared with paired Cont muscles. In the soleus muscle, the decrease reached 30% for the CSA of slow type fibers, whereas in plantaris muscle this decrease was 41 and 17% for the CSA of slow and int + fast type fibers, respectively. On the contrary, when normalized to MWW, the CSA were increased significantly by 20% for the slow fibers in the HG soleus muscle and by 54% for int + fast fibers in the HG plantaris muscle.

Contractile characteristics. The contractile parameters of Cont and HG soleus and Cont and HG plantaris are reported in Table 2. After HG, the soleus muscles showed significantly increased values in kinetic parameters (TTP: +51%, HRT: +76%, P_{20}/P_0: +19%). The absolute twitch and maximal tetanic forces were reduced significantly (−23 and −31%, respectively). On the contrary, when normalized to MWW, twitch and maximal tetanic forces increased (+44 and +9%, respectively). The plantaris muscle was differently af-

Table 2. *Mean values of studied contractile parameters for the soleus and plantaris muscles in cont and HG groups*

	Soleus		Plantaris	
Parameters	Cont (n = 11)	HG (n = 15)	Cont (n = 14)	HG (n = 15)
TTP, ms	29.60 ± 0.95	44.62 ± 2.71*	17.22 ± 0.46	15.40 ± 0.26*
HRT, ms	35.48 ± 3.41	62.62 ± 7.15*	13.46 ± 1.18	7.73 ± 0.93*
P_{20}/P_0, %	65.21 ± 1.03	77.33 ± 1.02*		
P_{40}/P_0, %			44.02 ± 0.56	35.38 ± 1.11*
P_t, mN	323 ± 23	248 ± 11*	728 ± 63	525 ± 29*
P_0, mN	1,790 ± 81	1,235 ± 37*	3,305 ± 178	1,838 ± 282*
P_t/MWW, mN/mg	1.46 ± 0.10	2.10 ± 0.07*	1.56 ± 0.15	2.21 ± 0.14*
P_0/MWW, mN/mg	9.80 ± 0.37	10.70 ± 0.20*	6.61 ± 0.30	7.01 ± 0.94

Values are means ± SE; n, no. of rats. Contractile parameters were reported as follows: TTP, time to peak; HRT, half-relaxation time; P_{20}/P_0 (P_{40}/P_0), tetanic tension obtained at 20 Hz (40 Hz) divided by the maximal tetanic tension; P_t/MWW, normalized twitch tension to MWW; P_0/MWW, normalized maximal tetanic tension to MWW. *Statistical difference ($P < 0.05$) between HG and Cont group in a given muscle.

fected by an HG period, since TTP, HRT, and P_{40}/P_0 were decreased (by −10, −42, and −20%, respectively). The absolute twitch and maximal tetanic forces were decreased (−28 and −44%), whereas normalized maximal twitch force increased significantly by 40% and maximal tetanic force remained unchanged.

Electrophoretic determination of MHC isoforms. An example of electrophoretic migration profiles is given in Fig. 1*A*. Four MHC isoforms were determined according to their increasing order of electrophoretic mobility (MHC IIA, IIX, IIB, and I). In a previous study (30), we established the level of migration of these isoforms in our gels. Two MHC isoforms were detected in the Cont soleus [the predominant slow MHC I and the fast MHC IIA isoform (Fig. 1, *lane 1*)]. After HG, the MHC IIA band disappeared (Fig. 1, *lane 2*). Figure 1, *lanes 3* and *4*, show plantaris muscles for Cont and HG groups, respectively. The four MHC isoforms were expressed both in the Cont and HG plantaris muscles.

A densitometer analysis, presented in Fig. 1*B*, permitted determination of the relative proportion of each MHC isoform. The Cont soleus was composed of 63.8% MHC I and 36.2% MHC IIA. The HG situation induced the disappearance of MHC IIA, with HG soleus muscles containing only the MHC I isoform (100%). The Cont and HG plantaris muscles expressed the four MHC isoforms (IIA, IIX, IIB, and I) in proportions that were not significantly different between Cont and HG groups.

Immunological identification of muscle fiber types by their MHC content. The identification of muscle fiber types was performed by histochemistry associated with immunological staining. On serial sections, the fiber MHC isoform expression was determined in a total of 300 fibers/muscle. Figure 2 shows the antibody reactivity in Cont muscles [soleus (Fig. 2, *2* and *3*) and plantaris (Fig. 2, *7–10*)] and in the HG muscles [soleus (Fig. 2, *4* and *5*) and plantaris (Fig. 2, *12–15*)]. Positive fibers were characterized by a dark color; negative fibers showed no color, except for the antibody against MHC IIB in which negative fibers were slightly colored. The ATPase method, which allowed differentiation of fibers containing the MHC IIX isoform, is shown in Fig. *2*, *1* (Cont soleus), *6* (Cont plantaris), and *11* (HG plantaris). The fibers that were weakly colored (light staining) did not express the MHC IIX isoform, whereas the dark fibers contained the isoform.

The Cont soleus muscles contained fibers that expressed the MHC I and IIA isoforms, whereas, after HG, the slow MHC I was only expressed. In these two groups, no type IIX was detected. In the Cont and HG plantaris muscles, the four MHC isoforms were expressed, since positive fibers were identified in all sections. Indeed, some fibers of the plantaris muscles showed a dark staining indicating the presence of MHC IIX.

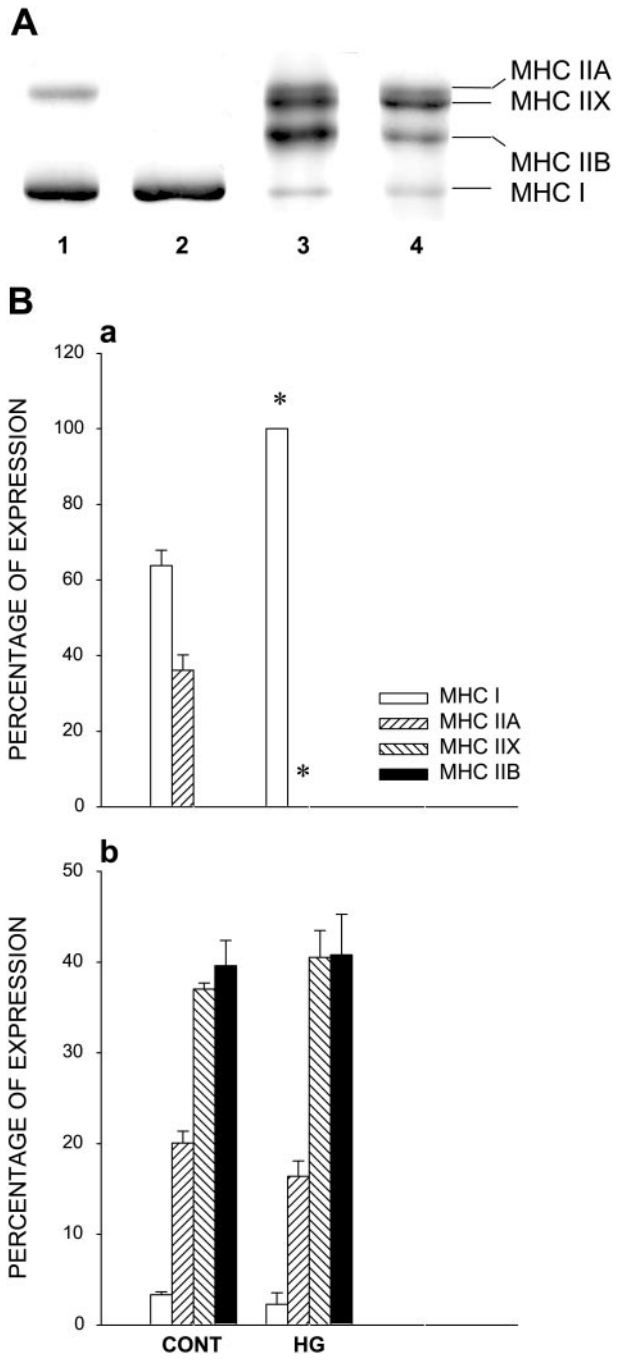

Fig. 1. *A*: electrophoretic determination of myosin heavy chain (MHC) isoforms. The MHC isoforms were identified in increasing order of their electrophoretic mobility. *Lane 1*, control (Cont) soleus; *lane 2*, hypergravity (HG) soleus; *lane 3*, Cont plantaris; *lane 4*, HG plantaris. *B*: densitometric analysis of the identified MHC isoforms. MHC isoform composition, measured by densitometry, was determined for soleus and plantaris muscles. Results are expressed as means ± SE. *Statistical difference between HG and Cont paired groups.

Using the combination of enzyme histochemistry and immunochemistry, we were able to distinguish several MHC isoform coexpressions (Fig. 3). The fiber types were classified according to their MHC expressions in *1*) pure types, corresponding to the expression of only one MHC isoform, slow (I) or fast (IIA, IIX, or IIB), *2*) hybrid types presenting two, three, or four MHC isoforms according to a coexpression order based on the "next-neighbor rule" i.e., I→IIA→IIX→IIB, and *3*) atypic hybrid types, corresponding to a coexpression

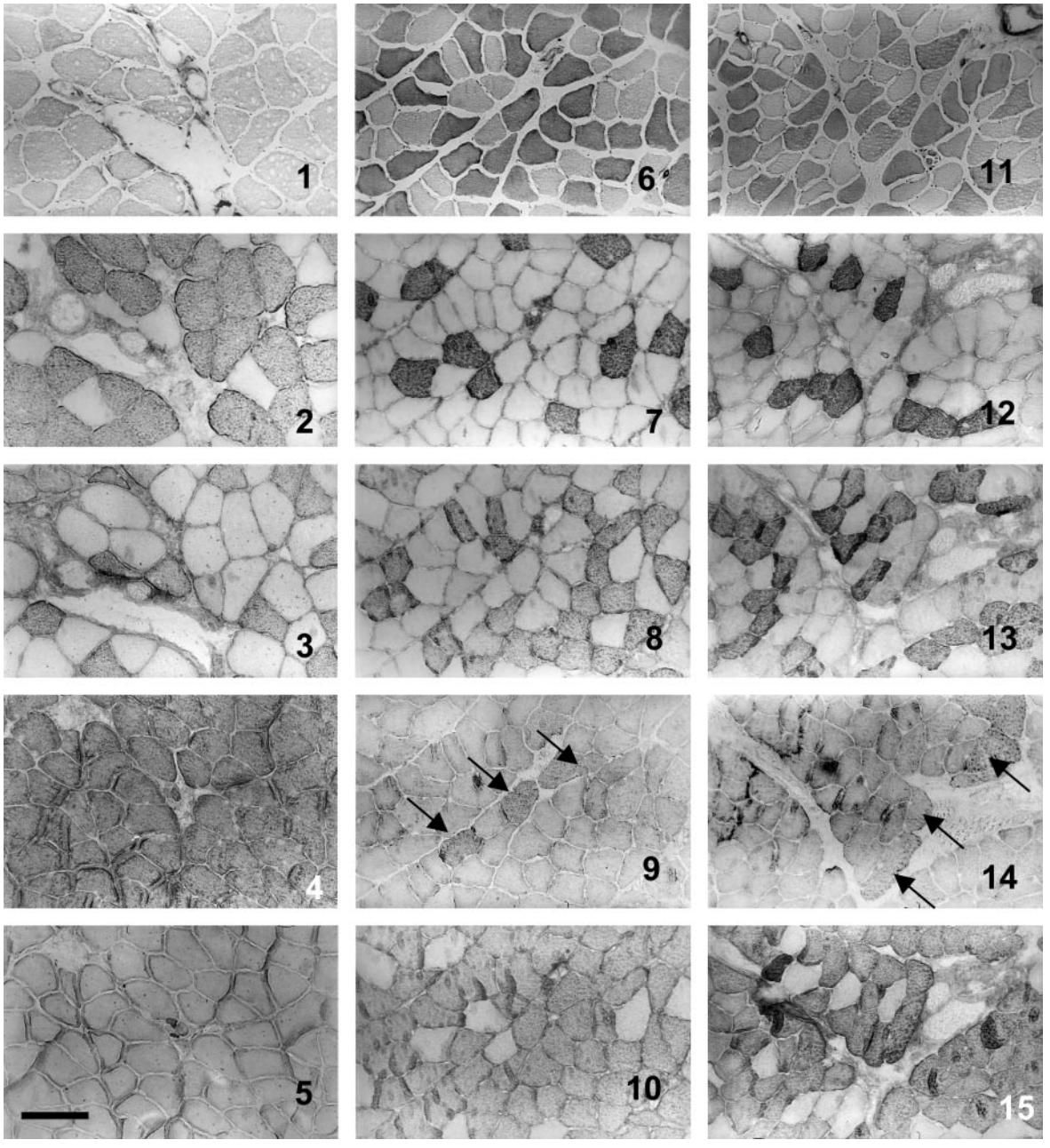

Fig. 2. Muscle typing determined by immunohisto- and histochemical methods. *1–3* and *4–5*, Cont and HG soleus muscles, respectively; *6–10* and *11–15*, Cont and HG plantaris muscles, respectively. Reactivity of the antibodies is presented as follows: anti-MHC I (*2*, *4*, *7*, and *12*), anti-MHC IIA (*3*, *8*, and *13*), anti-MHC IIB (*9* and *14*, on which positive fibers are indicated by black arrows), and anti MHC II (*5*, *10*, and *15*); *1*, *6*, and *11* show the histochemical method that differentiates, by a dark color, fibers expressing MHC IIX. Scale bar = 140 μm.

of two, three, or four MHC isoforms with gaps in the transition order.

In the Cont soleus muscle, the following three fiber populations could be detected: two pure fiber types, the predominant type I (68%) and the type IIA (27%); and one hybrid population (5%) with MHC I and MHC IIA being coexpressed. After HG, only the pure slow type (100% MHC I) was identified in the soleus muscle.

The plantaris muscle showed multiple MHC isoform combinations. In the Cont plantaris, we distinguished four pure populations of fibers expressing either the MHC I isoform (11%), the MHC IIA isoform (32%), the MHC IIX isoform (2.6%), or the MHC IIB isoform (2.7%). Five hybrid types coexpressed multiple MHC isoforms according to the next-neighbor rule as follows: I→IIA→IIX→IIB, i.e., I + IIA→2%, IIA + IIX→29%, IIX + IIB→6%, I + IIA + IIX→0.25%, and IIA + IIX + IIB→12.5%. Three atypic hybrid types were also detected as follows: I + IIX→0.25%, IIA + IIB→1.5%, and I + IIA + IIB→0.2%.

In the HG plantaris, the pure fiber types represented a similar content as in Cont muscles (~48%). However,

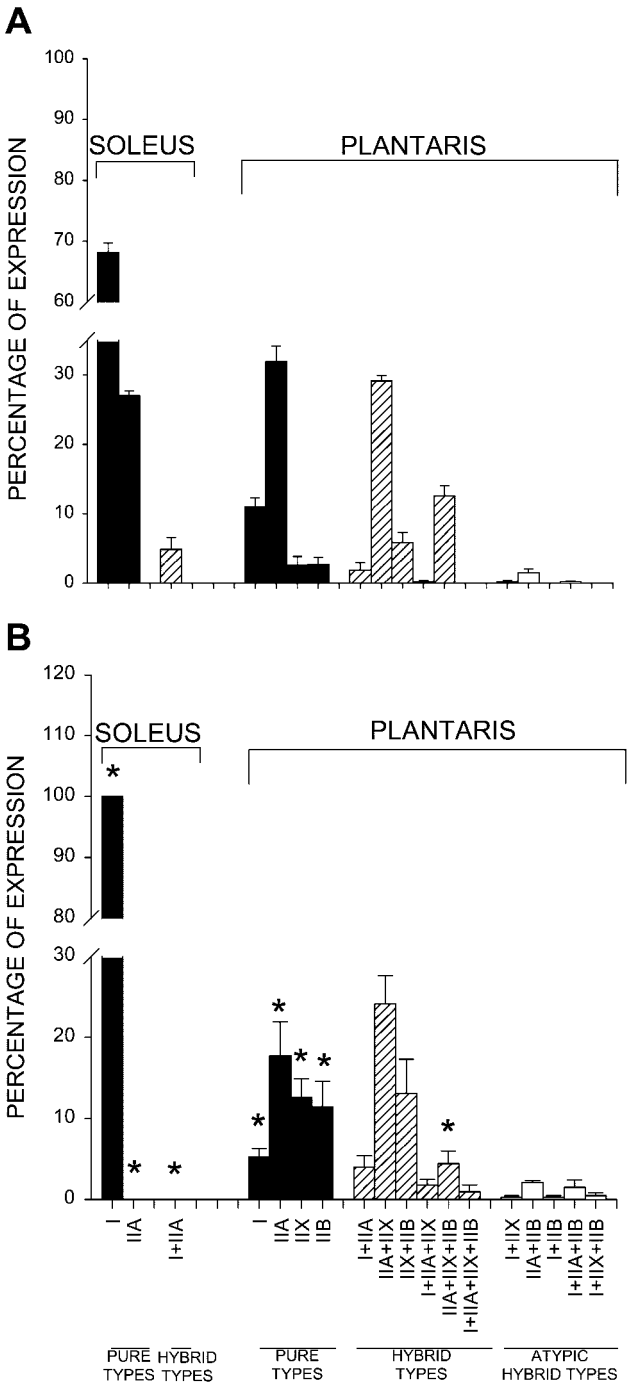

Fig. 3. Repartition of fiber types within each muscle. Fibers were classified according to their MHC isoform coexpressions. Results are expressed as means ± SE. *Statistical difference between HG (*A*) and Cont (*B*) paired group.

the fibers containing MHC I and MHC IIA isoforms were decreased by 5.2 and 17.7%, respectively, whereas the fibers containing MHC IIX or MHC IIB were overexpressed by 12.6 and 11.5%, respectively. Concerning the hybrid types, a slight redistribution between the proportion in the different fiber types and an increase in the number of hybrid groups appeared. Moreover, the atypic hybrid fiber types appeared increased, and the proportion of such fibers reached 5% after HG.

DISCUSSION

This study presents, for the first time, the impacts of a long stay in an HG environment on rats submitted from their conception until 100 days postbirth to HG conditions. We chose to compare the effects of this long HG duration on two ankle extensor muscles [a slow one (the soleus) and its fast agonist (the plantaris muscle)]. Thus morphological, contractile properties and MHC contents of these muscles were analyzed. The primary findings showed that *1*) the animal growth was slowed down, with associated decreases in the two muscle weights being observed, *2*) the relative maximal tensions of the two muscles were increased after HG, and *3*) the contractile properties of each muscle were modified according to changes in their phenotypes. Thus, after HG, the slow soleus became slower and the fast plantaris presented a higher proportion of pure fibers that expressed the fastest MHC isoforms (IIX and IIB). These main results were discussed and compared with those obtained in a real or simulated microgravity situations.

Effects of HG on morphological properties and muscle strength. The body weight of growing rats was significantly lower (−40%) under 2-*g* loading, and the mean absolute masses for soleus and plantaris muscles were also decreased by 42 and 46%, respectively. The effects of a 2-*g* environment on growing rats could appear relatively surprising. In fact, the physiological function of these two agonist muscles is an antigravity function (20), and one may expect a hypergrowth under an HG situation. Our results showed a decrease in muscle weights. This may be the consequence of a decrease in protein synthesis resulting from the HG environment. Indeed, previous studies have demonstrated that HG induced, in growing rats, a marked decrease in the weight and muscular protein content (21). However, our data showed that the ratio MWW/BW was unchanged after HG both in soleus and plantaris muscles. This result supported the hypothesis that HG induced a slowing down in the growth of the entire animal. This might be a consequence of a nutritional deficiency already described in HG conditions (11, 41) and/or a decrease in the muscle body fat reserves (11, 14). Moreover, a transient elevation of circulating corticosterone that returns to control levels after 5 days was demonstrated during HG (27). This increase in corticosteone triggered the lipolysis (for review, see Ref. 24). Taking into account other data previously published, it also appeared that HG may have differential effects, depending on the species, age, and sex of the animals (rats) and the duration of HG. Indeed, Roy et al. (31) described in adult male Wistar rats submitted 2 wk at 2 *g* *1*) no change in the absolute soleus and medial gastrocnemius muscle mass and *2*) a significant increase of the relative muscle mass. On the contrary, Amtmann and Oyama (3) described, in female Sprague-Dawley rats, a decrease in body mass

after a 810-day exposition at 2.76 *g*. These latter results are in agreement with our data.

Our results showed that, after HG, absolute values of CSA were significantly lower than age-matched Cont. Indeed, the absolute CSA of the slow fibers decreased in the soleus by 30%. In the plantaris, the CSA of slow and fast fibers decreased by 41 and 17%, respectively. However, the analysis of the normalized CSA (CSA/MWW) revealed, for each fiber type, an increase by 20% in the soleus (100% slow fibers) and by 54% in the fast fibers of the plantaris (95% of the total fiber content) after HG. Therefore, the fiber atrophy was exceeded by total body weight loss (and MWW) so that the ratio was increased. This could indicate that protein in the muscle was slightly spared compared with protein in other tissues. Moreover, it is not clear whether the water content was changed by 2-*g* conditions. The relative CSA would increase if muscle water was reduced, thus inducing an MWW decrease. Unfortunately, we have no measurement of the water content in our conditions. However, it has been reported that the water balance in rats exposed to a chronic centrifugation was not modified after 12 days of HG (26). Finally, it should be pointed out that HG affected slow and fast fibers, whereas, in microgravity, atrophy was more developed in slow than in fast fibers (7, 10).

Another aim of this study was to determine the effects of HG on the force level developed by the soleus and the plantaris muscles. Our results showed that absolute P_t and P_0 significantly decreased in the soleus (-23 and -31%, respectively) and in the plantaris (-28 and -44%, respectively). When normalized to MWW, the maximal twitch forces increased in both muscles, and the maximal tetanic tension increased significantly only in the soleus. Previous studies (23) have suggested that cross-bridge formation involves intermediate steps in which the cross-bridge transforms from a low- to a high-force state. In microgravity, a decline in force per CSA could reflect a drop in the force level per cross-bridge and/or a reduction in the number of cross-bridges consecutive to a loss of myofibrillar protein (22). Taking into account the increase in force after HG, we suggested that the force developed per cross-bridge was reinforced in HG conditions. However, we cannot rule out a supplementary hypothesis, i.e., a possible increase in the number of cross-bridges, perhaps because of an increased intracellular Ca^{2+} concentration or because of a link to a modification in the filament lattice spacing, an hypothesis already proposed for microgravity (22). These results thus clearly demonstrate the reverse of observations in microgravity, which first demonstrate either losses of forces (soleus muscle; see Ref. 7) or no change (plantaris; see Ref. 4) and, second, a much larger effect in slow than in fast muscles (44).

Effects of HG on kinetics and fiber typing. In the soleus muscle, our results showed that the kinetic parameters were modified after a 100-day period of HG. TTP, HRT, and the P_{20}-to-P_0 ratio significantly increased ($+51$, $+77$, and $+19$%, respectively) and changed into those of a slower muscle type. The acquisition of slower contractile parameters was confirmed by the electrophoretic and histochemical profiles. Indeed, after this 2-*g* period, only the MHC I isoform was detected in all of the studied fibers. MHC IIA and type IIA fibers identified by SDS-PAGE and ATPase staining, respectively, and normally expressed in the Cont rats had disappeared in the HG soleus muscle. These results were in agreement with those of Martin (19) who observed that HG resulted in a progressive increase in the proportion of slow oxidative fibers, up to 100% in developing Sprague-Dawley rats, when the centrifugation was initiated at 30 days of age. On the contrary, using 60-day-old rats, Roy et al. (31) demonstrated that, after 2 wk at 2 *g*, the soleus of centrifuged rats had a slightly lower percentage of fibers expressing the MHC I isoform and a higher percentage of fibers (15 vs. 10%) that coexpressed type I and IIA MHC isoforms. Therefore, these differential effects might again be linked to the animals and to their developmental state when HG was applied. It has been demonstrated that, in the soleus muscle, the MHC isoforms and their mRNA expression were not modified during postnatal undernutrition (43).

In the plantaris muscle, HG conditions induced decreases in TTP (-11%), HRT (-42%), and the P_{40}-to-P_0 ratio (-20%). This acquisition of faster contractile properties could be related to the reorganization in the proportions of pure fibers, especially the increase in fibers expressing the fastest MHC isoforms (IIX and IIB). The HRT parameter has classically been described as dependent on the relative expression of the different sarco(endo)plasmic reticulum Ca^{2+} (SERCA) isoforms (18). The changes observed in HRT values, i.e., increase in the soleus muscle and decrease in the plantaris muscle, suggested that, after HG, the levels in the slow SERCA 2a isoform and the fast SERCA 1a isoform were overexpressed in the two muscles.

Consequently, both slow and fast muscles were affected by HG, whereas, in simulated or real microgravity, fast muscles were less modified than slow muscles. Indeed, in soleus muscles, a decrease in normalized force concomitant with a decrease in slow MHC and with an increase in fast MHC isoforms has been commonly described after microgravity (7, 10, 44). In the unloaded soleus muscle, the decreases in TTP, HRT, and force were correlated with changes in SERCA kinetics and with a de novo appearance of fibers coexpressing MHC I and/or various MHC II isoforms (for review, see Ref. 25). On the contrary, normalized tensions of fast muscles such as gastrocnemius, tibialis anterior, or extensor digitorum longus were maintained after microgravity (for review, see Ref. 7). Moreover, the myosin distribution was not modified in the medial portion of the gastrocnemius and tibialis anterior (25). Thus it appears that HG had a more global impact than microgravity on the muscular system.

Effects of HG on the MHC repartition and their regulation. Our results demonstrated that the HG environment had a real impact on MHC isoform transitions. After HG, the soleus muscle became slower, after the transition MHC IIA→MHC I. Indeed, only the

pure fiber type remained, expressing the MHC I isoform. On the contrary, in plantaris muscles, HG induced a subtle reorganization of the MHC expression within the pure and hybrid fibers without modification of the total content in each MHC isoform. We suggested that generally the transitions followed the next-neighbor rule as proposed by Pette and Staron (28). Interestingly, in plantaris muscles submitted to HG, a larger amount of fibers (5% in HG vs. 1.5% in Cont conditions) presented atypic MHC expressions. The increase in fibers that coexpressed multiple MHC isoforms was characteristic of a muscle presenting a state of transition, as already described in spaceflight (2, 38), after hindlimb unloading (9, 38) and electrical stimulation (34). Thus all of the results indicated that HG induced changes in the expression of the MHC isoforms, since it was also described in microgravity. However, the results were the opposite. In fact, in real or simulated microgravity, the soleus muscle acquired fast MHC isoforms (36), and few or no changes appeared in fast muscles (35). Conversely, it is interesting to note that HG induced changes in the fast plantaris. The changes in MHC isoforms resulting from microgravity have been proven to be related to their different mRNA expression (36). Therefore, we can suggest that the alterations in the MHC isoform content after HG could result from a modification in mRNA expression levels.

It has been well established that mechanical properties (17) and the myosin expression pattern (5) were dependent on motor innervation. It has been demonstrated that, in rat soleus muscle, the suppression of motor innervation induces an increase in fiber diversity (37). Moreover, a neonatal denervation blunted the upregulation of MHC IIB in the plantaris muscle and of MHC I in the soleus muscle and shifted the pattern to greater expression of MHC IIA and IIX in both muscles (1). In our conditions, the new myosin repartition after HG, in soleus and plantaris muscles, should be a direct consequence of a modification and/or an increase in the motor nervous message during HG. To support this hypothesis, it has been previously demonstrated that, during the two HG phases of parabolic flight, the electromyographic activity of the rat soleus muscle was immediately strongly increased (16). This increase originated from the recruitment of new motor neurons, with a shift toward higher-amplitude units being observed. It can therefore be argued that, during the 100-day period of the 2-*g* environment, an increase in the recruitment of soleus motor neurons was achieved and influenced the myosin expression during development. Moreover, in terrestrial conditions, the extensor muscle activity of hindlimb is regulated by information originating from vestibular receptors. It has been demonstrated that, during spaceflight, this information is reduced (42). Inversely, it is tempting to suppose that, during HG, the vestibular information was increased and therefore hindlimb muscles received an increased motor discharge. Moreover, it has been demonstrated in the rat, by using the Fos protein expression, that the otolith-vestibulo-olivar pathways are strongly activated by an increase of the gravitoinertial forces (12).

Conclusion

Our results show that HG produced some inverse effects to those observed in real or simulated microgravity. Indeed, both soleus and plantaris muscles were affected by HG. The soleus muscle presented an increase in relative maximal tension and a change to a slower type, as a function of both kinetic parameters and MHC isoform expression. The fast plantaris became a faster muscle (kinetic parameters increased). Moreover, a new diversified repartition of the MHC isoforms was revealed in this fast muscle. Our results thus demonstrated that the slow and fast extensor muscles presented a specific adaptation to a chronic HG. Finally, in the soleus muscle, the changes seemed to be the result of a qualitative continuity from micro- to hypergravitational effects; for instance, microgravity induces decreases in force, TTP, and MHC I expression; additionally, 1 *g* represents normality and 2 *g* induces increases in force, TTP, and MHC I expression. In the plantaris muscle, this continuity rule did not fully apply since this fast muscle was slightly affected by microgravity.

Gathered together with data obtained in microgravity conditions, our results underline the importance of the gravity factor in muscle physiology.

We thank Dr. G. S. Butler-Browne for the growing of hybridomas producing SC-71 and BF-F3 antibodies initially developed by Schiaffino et al. (33) and Laetitia Cochon for technical assistance. The hybridomas are commercialized by Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany.

The Laboratoire de Plasticité Neuromusculaire was supported by grants from the Centre National d'Etudes Spatiales (3194), the Conseil Régional du Nord-Pas-de-Calais, and the Fonds Européen de Développement Régional (F 007), and the Laboratoire de Neurobiologie des Restaurations Fonctionnelles was supported by UMR 6562 Centre National de la Recherche Scientifique/Université de Provence and by grants from the Centre National d'Etudes Spatiales.

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Am J Physiol Regul Integr Comp Physiol 289: R217–R224, 2005.
First published March 17, 2005; doi:10.1152/ajpregu.00596.2004.

Changes in rat soleus muscle phenotype consecutive to a growth in hypergravity followed by normogravity

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Submitted 1 September 2004; accepted in final form 15 March 2005

Picquet, F., V. Bouet, L. Cochon, M. Lacour, and M. Falempin. Changes in rat soleus muscle phenotype consecutive to a growth in hypergravity followed by normogravity. *Am J Physiol Regul Integr Comp Physiol* 289: R217–R224, 2005. First published March 17, 2005; doi:10.1152/ajpregu.00596.2004.—It has been demonstrated that a long-term stay in hypergravity (HG: 2G) modified the phenotype and the contractile properties of rat soleus muscle. The ability of this muscle to contract was drastically reduced, which is a sign of anticipated aging. Consequently, our aim was to determine whether rats conceived, born, and reared in hypergravity showed adaptative capacities in normogravity (NG: 1G). This study was performed on rats divided into two series: the first was reared in HG until 100 days and was submitted to normogravity until 115 to 220 postnatal days (HG-NG rats); the second was made up of age paired groups reared in normogravity (NG rats). The contractile, morphological, and phenotypical properties of soleus muscle were studied. Our results showed that the NG rats were characterized by coexpressions of slow and fast myosin, respectively, 76.5 and 23.5% at 115 days. During their postnatal maturation, the fast isoform was gradually replaced by slow myosin. At 220 days, the relative proportions were respectively 91.05% and 8.95%. From 115 to 220 days, the HG-NG rats expressed 100% of slow myosin isoform and they presented a slower contractile behavior compared with their age-matched groups; at 115 days, the whole muscle contraction time was increased by 35%, and by 15%, at 220 days. Our study underlined the importance of gravity in the muscular development and suggested the existence of critical periods in muscle phenotype installation.

muscular properties; myosin transition; gravity change; hindlimb muscle

ALL LIVING ORGANISMS ARE DIRECTLY under the influence of a common and constant factor, gravity. It is well known that the development and the maturation of numerous life systems such as cell division, axonal growth, posture, and body movements are directly regulated by this factor (6, 14, 15, 16).

The effects of real (spaceflight) and simulated (hypodynamia-hypokinesia model, HH) microgravity on the neuromuscular system are well documented. In hindlimb muscles, an exposure to a HH period induces more marked effects in slow extensor muscles such as the soleus than in fast muscles (for a review, see Refs. 23 and 24). Most studies have been carried out in adult rats. Indeed, in soleus muscle from rats submitted to real and simulated microgravity, a muscular atrophy correlated to a decrease both in fiber and muscle cross-sectional area and to a decrease in muscle strength, which has been commonly described (for review, see Ref. 13). A slow-to-fast phenotype transition, characterized by changes in contractile protein isoforms such as myosin heavy and light chains (3, 32) and regulatory proteins (2), has also been reported. Moreover, the proprioceptive information could be disturbed (9), and a cortical reorganization has been demonstrated (5, 10). Furthermore, it has been reported that the effects of microgravity are reversible, as muscle properties are recovered afer reloading (23). Consequently, gravity appears to be a key element necessary to maintain neuromuscular integrity.

Another way to study the importance of gravity was to increase it by centrifugation to obtain hypergravity (HG). Compared with microgravity, this condition offers the advantage of studying long-term changes. It has been established that the GABA immunoreactivity was decreased in the cortex of HG rats, suggesting changes in sensory feedback information from muscle receptors (7). Furthermore, it has been reported that the behavior of rats born and raised in HG was altered compared with control animals (36). In a recent publication, we have established that contractile properties and the phenotype of hindlimb muscles of rats conceived, born, and reared in HG now aged 100 days were modified after a long-term HG period (26). Muscle characteristics appeared reinforced, as slow muscle, such as the soleus, became slower (100% slow myosin) and fast muscle, such as the plantaris, became faster, regarding its kinetic properties. The soleus phenotype was completely unusual, because, in control animals, the appearance of a pure slow phenotype is characteristic of more aged rats (1, 8). Considering these results, it was tempting to determine the adaptive capacities of rats reared in HG and then submitted to normogravity.

The aim of the present study was thus to establish whether soleus muscle phenotype from rats conceived, born, and reared in HG was a stilted structure or if it could be remodeled; that is, express other myosin than slow isoforms during a stay in normogravity. For these animals, normogravity was similar to microgravity, compared with the gravity level from their conception.

MATERIAL AND METHODS

Animal groups. The study was performed on a total of 58 male Long-Evans rats divided into two series. The first one was made up of rats conceived, born, and reared in hypergravity until postnatal *day 100*. The centrifugation apparatus was then stopped and the animals continued their growth in normogravity (HG-NG groups). The HG-NG rats were selected from a population of the first generation derived from couples mated in the centrifugation apparatus. After 15 days, males were removed, whereas females stayed on until weaning.

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The studied rats descended from different littermates. The second series consisted of terrestrial rats conceived, born, and reared in normogravity (NG groups). The NG rats were reared in conditions similar to those of the centrifuge, that is, same room, same dark-light cycle (12:12 h), and same temperature inside a standard home cage contained in a gondola until postnatal *day 100*. Both NG and HG-NG rats were then transferred in a thermoregulated room (25°C) with circadian rhythm (12:12 h dark-light) but without centrifugation noise for a maximal duration of 120 days. The HG-NG group comprised four subgroups. HG-NG rats aged 100 days continued their postnatal growth until postcentrifugal days: *1)* 15 (group HG-NG-115, $n = 6$), *2)* 30 (group HG-NG-130, $n = 6$), *3)* 60 (group HG-NG-160, $n = 8$) and *4)* 120 (group HG-NG-220, $n = 10$). Age-paired groups consisted, for NG rats, of NG-115 ($n = 6$), NG-130 ($n = 6$), NG-160 ($n = 8$), and NG-220 ($n = 8$). Consequently, in any group, the values 115, 130, 160, and 220 indicate the postnatal age of rats, whereas NG and HG-NG represent the growth in normogravity or in hypergravity + normogravity, respectively.

The contractile, morphological, and phenotypical properties were determined in the right soleus muscle of both NG and HG-NG rats. All experimental procedures described below were approved by both the French Agricultural and Forest Ministry and French National Education Ministry (Veterinary Service of Health and Animal Protection, authorization A 59–00980).

Centrifugation apparatus. The apparatus has already been described in detail (26). It consisted of a velocity-controlled DC motor (3.5 kW), located in the vertical axis of the apparatus and driving two horizontal cross-arms (total length: 165 cm) at constant rotation speed. Four free-swinging gondolas were jointed at the 4 extremities of the horizontal arms, 76.5 cm away from the axis of rotation; the gondolas were equipped with standard home cages for rats (30 × 40 cm, each cage containing three rats) with an aeration system and lights that reproduced a 12:12-h light-dark cycle. A video system in the gondolas allowed us to know the exact birth date of litters and to control the movement ability of the animals. During centrifugation, the gondolas were tilted at a constant angle of 60° from the vertical. Counterclockwise rotations were done at a constant velocity of 3.81 rad/s. Given the mass and the inertia of the gondolas, including the home cages and the rats, this angular velocity led to 2 G resultant force. The resultant force was directly measured using a standardized force sensor placed at the center of the floor of the gondola. During centrifugation, rats were subjected to a gravito-inertial force vector whose direction was always similar to that exerted in normal gravity (parallel to the dorso-ventral axis of the animal), and magnitude was twice the normal magnitude exerted on Earth. Consequently, the rats were also subjected to Coriolis force accelerations.

After weaning, female pups and their mothers were removed from the centrifuge. For animal care (cleaning and feeding), the centrifugation was stopped for 15 min every week. Food and water were available ad libitum. The centrifugal rats gained body mass at a rate similar to controls but their mass remained lower than control animals, whereas no statistical difference of food intake was observed between the two populations. After 100 days of centrifugation, the rats were taken off the apparatus and were maintained in normogravity for a maximal duration of 120 days.

In situ isometric contractile properties. The animals were anesthetized with pentobarbital sodium (40 mg/kg ip), and anesthesia was prolonged with further injections of 20 mg/kg, when necessary. The dissection protocol has been described in previous papers originating from our laboratory (12, 19). Briefly, under deep anesthesia, assessed by the absence of blink reflexes, all the muscles of the right thigh and lower limb were denervated, except the soleus muscle. The dissected limb was fixed to maintain the isometric conditions and was immersed in a paraffin oil bath thermostatically controlled (37°C). The limb was stabilized by a combination of pins, clamps, and bars, so that the muscle was maintained in a horizontal position. The soleus muscle was isolated from surrounding tissues, and its tendon was stitched up by a short 3.0 silk suture around a force transducer (Grass, FT 10, USA). Its length was adjusted to produce a maximal twitch tension. The stimulating [Teflon-coated platinum, outside diameter (OD) : 125 μm] and reference (teflon-coated platinum, OD : 250 μm) electrodes were maintained by micromanipulators. Contractions of the soleus muscle were induced by stimulation (Grass model S 8800, Quincy, MA) of the soleus nerve (0.2-ms duration pulses) through monopolar platinum electrodes at twice the minimum voltage required to obtain the maximal twitch response. The reference electrode was inserted into adjacent denervated muscle mass. The soleus muscles were stimulated so as to record the following parameters: *1)* a single maximal twitch from which the maximal twitch tension (P_t), the time to peak (TTP), and the half-relaxation time (HRT) were measured; *2)* the tension/frequency relationship (for stimulation frequencies ranging from 16 to 120 Hz), which allowed the determination of maximum tetanic tension P_0 obtained for a 100-Hz stimulation frequency and of index P_{20}/P_0 (ratio of the tetanic tension at 20 Hz to P_0); and *3)* the fatigue index (FI), previously defined by Winiarski et al. (35).

At the end of the fatigue index, the right soleus muscles of the experimental animals were removed, weighed (determination of the soleus muscle wet weight, MWW), frozen in isopentane precooled to its freezing point by liquid N_2, and stored at −80°C. The muscles were divided into two parts to perform both immunohistochemical and electrophoretical determinations of myosin heavy-chain isoforms on the same muscle.

Characterization of muscle fiber types by immunohistochemistry. At the midbelly, the soleus muscles of the NG and HG-NG groups were cut into serial 10-μm-thick cross sections in cryostat microtome at −20°C. The protocol relative to immunohistochemically treated sections has been described in detail (25). The following antibodies were used: NCL-MHCs (Tebu-Novocastra), which recognizes MHC I, SC-71 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ) against MHC IIA, BF-F3 (DSMZ) against MHC IIB and MY32 (Sigma) against MHC IIA, IIB, and IIX. These antibodies were diluted at 1/20, 1/20, 1/10 and 1/2,000, respectively. The binding of the primary antibodies was localized by an immunoperoxidase reaction using the Novostain Universal Quick Kit (Tebu/Novocastra). Serial cross sections were incubated in prediluted blocking serum (normal horse serum) for about 10 min. The excess serum was blotted, and sections were incubated with primary antibodies diluted in PBS solution for 2 h at room temperature. Serial cross sections were washed for 5 min in PBS solution. Then, they were incubated for 30 min in prediluted biotinylated universal secondary antibody. At the end of the 30 min, sections were washed with PBS for 5 min and incubated in ready-to-use streptavidin/peroxidase complex reagent for 30 min. To label the serial cross sections, the peroxidase substrate solution (diaminobenzidine, DAB; Sigma-Aldrich) was added after the sections had been washed for 5 min with PBS. Finally, after dehydration with alcohol and toluene, the slides were mounted in Eukitt resin. Positive fibers were characterized by a brown color, while negative fibers remained unlabeled. Muscle typing was established from a total of 300 fibers per muscle, immunologically identified in serial sections. In our laboratory, we have previously verified that such a sample is representative of all muscle fiber types contained in the muscle. This approximation is conceivable because Kugelberg (17), in a previous paper, has reported that, in soleus rat muscle, the fibers are more evenly distributed throughout the muscle cross section than in the other hindlimb muscles. Indeed, the muscle fiber distribution in the soleus muscle is homogenous.

Determination of MHC isoforms by SDS PAGE. The MHC isoforms were studied in NG and HG-NG soleus muscles. The first part of the frozen tissues was pulverized under liquid nitrogen in a small steel mortar (30). As already described (33), the MHC composition was determined by polyacrylamide gel electrophoresis using a 4.5% glycerol stacking gel and a 7.5% glycerol separating gel. Electrophoresis was run for 18 h at 12°C (180 V constant, 13 mA per gel). After the gels were run, the gel slabs were silver-stained. Briefly, they

Table 1. *Distribution in body weights, muscle wet weights, and ratio of rats reared in normogravity and of rats reared in hypergravity for 100 days followed by normogravity*

Groups	BW, g	MWW, mg	MWW/BW, mg/g
NG-115 (6)	390±6	160±5	0.41±0.01
HG-NG-115 (6)	253±12*	114±6*	0.45±0.01*
NG-130 (6)	395±2	173±5	0.44±0.01
HG-NG-130 (6)	304±6*†	116±5*	0.38±0.01*†
NG-160 (8)	462±7[a,b]	209±8[a,b]	0.45±0.02
HG-NG-160 (8)	425±7*†‡	153±6*†‡	0.36±0.01*†
NG-220 (8)	537±6[a,b,c]	229±5[a,b]	0.43±0.01
HG-NG-220 (10)	456±10*†‡	177±3*†‡§	0.39±0.01*†

The results are reported as means ± SE; *n* is provided in parentheses. The numbers following the group names (NG or HG-NG) correspond to the age of the rats. *Statistical difference between age-paired groups using Student's *t*-test. [a,b,c] Statistically significant difference between NG-115, NG-130, and NG-160, respectively. †‡§, statistically significant difference between HG-NG-115, HG-NG-130, and HG-NG-160, respectively by using a Bonferroni test. For all statistical tests, statistical significance was accepted at $P < 0.05$. BW, body weight; MWW, muscle wet weight; MWW/BW, ratio of muscle wet weight to body weight; NG, normogravity; HG-NG, hypergravity for 100 days followed by normogravity.

were postfixed for 20 min in a solution containing 50% methanol and 5% acetic acid and for 10 min in a 50% methanol solution. Between each fixation, the gels were rinsed with distilled water. They were thus silver-stained in a mix of 0.25% silver nitrate and 0.02% of formaldehyde (from a 35% stock solution). They were developed with 0.04% formaldehyde (35%) and 2% sodium carbonate. The staining was stopped with 5% acetic acid. The relative proportion of each MHC isoform in each muscle type was established using a scanning densitometer (Multi analyst, Biorad).

Statistical analysis. All of the results are presented as means ± SE. After a one-way ANOVA was performed, a Bonferroni test was used to establish the intergroup difference within an identical experimental condition (i.e., between NG rats, from 115 to 220 days and between HG-NG rats from 115 to 220 days). This test allowed us to compare the temporal evolution of NG and HG-NG rats during their adult maturation. To compare the degree of muscle modifications after growth in NG and HG-NG rats, a Student's *t*-test was used between age-paired groups. Statistical significance was accepted at $P < 0.05$.

RESULTS

Morphological data. The evolution of body weights (BW), MWW, and normalized MWW to body weight (MWW/BW) are reported in Table 1. In NG rats, as a function of age, from 115 to 220 days, the results showed a gradual increase both in BW (+37%) and in MWW (+43%), whereas the ratio MWW/BW remained unchanged. During the same period, the HG-NG group presented increases in BW (+80%) and MWW (+55%), whereas MWW/BW was decreased (−13%). Consequently, the difference between values of NG and HG-NG groups decreased from 115 to 220 days. Indeed, at 115 days, the BW of HG-NG animals represented 64% of BW in NG rats (vs. 71% for MWW), whereas at 220 days, these differences were 84% for BW and 77% for MWW, respectively, compared with age-paired groups. The comparison between NG and HG-NG age-paired groups revealed that the BW and MWW of HG-NG rats were significantly smaller than those of NG rats. The MWW/BW ratios were also significantly lower, except for rats aged 115 days, in which the tendency was reversed.

Contractile properties. The contractile properties in both NG and HG-NG rats are summarized in Table 2. The muscle maturation between *days 115* and *220* in NG groups revealed that both TTP and HRT parameters increased and stabilized after 130 days. The ratio P_{20}/P_0 did not significantly change except at 160 days when its value surprisingly became lower than those obtained for both NG-115 and NG-130 groups. The normalized maximal tetanic force presented identical values through all studied groups. Similar results were observed regarding the normalized twitch strength data, except in NG-160 rats, which presented an increased mean value compared with NG-115 group.

The growth in normogravity of HG rats (HG-NG groups) showed that both TTP and HRT parameters remained unchanged between 115 and 220 days. The ratio P_{20}/P_0 only showed a decreased value in HG-NG-220 rats when compared with HG-NG-160 animals. The normalized muscle force decreased from 115 to 220 days, the values becoming significantly lower after 160 days for the twitch strength and after 220 days for the maximal tetanic force.

The comparison between age-paired groups in NG and HG-NG rats revealed that at 115 days, HG-NG rats presented significantly higher kinetic (TTP and HRT) values. Furthermore, they showed an increase in muscle force both in normalized twitch and maximal tetanic strengths. At 130 days, the soleus muscle in HG-NG rats remained significantly slower (except for HRT parameter) and stronger than in NG rats. At 160 days, the muscle showed only higher values of TTP and P_{20}/P_0, the muscle force being similar to control values. At 220

Table 2. *Evolution of contractile parameters in NG and HG-NG groups*

Groups	TTP, ms	HRT, ms	P_{20}/P_0, %	P_t/MWW, mN/mg	P_0/MWW, mN/mg	FI, %
NG-115 (6)	29.5±1.3	32.4±3.1	81±0.8	1.46±0.12	9.8±0.6	81±1.4
HG-NG-115 (6)	39.8±2.2*	58.7±5.8*	78.8±0.5*	2.5±0.13*	11.81±0.51*	88.5±2.53
NG-130 (6)	40.5±2.2[a]	55.5±1.2[a]	82.5±1.3	1.55±0.07	10.25±0.7	84.3±6.3
HG-NG-130 (6)	46.5±1.3*	56.8±1.1	78.7±0.5*	2.16±0.06*	11.9±0.2*	81.72±2.55
NG-160 (8)	40.1±0.74[a]	54.5±3[a]	73.2±1.9[a,b]	1.86±0.12[a]	10.39±0.4	86.7±0.5
HG-NG-160 (8)	48±2.9*	66±5.3	82.8±0.8*	1.78±0.09[†‡]	10.59±0.22	83.5±4.9
NG-220 (8)	41.9±0.9[a]	59±5.1[a]	77.1±1.1	1.75±0.06	10.19±0.27	83.6±2.5
HG-NG-220 (10)	48.5±1.7*	62.4±4.7	75.6±1.4§	1.66±0.06†‡	10.55±0.26†‡	81.6±1.02

The results are reported as means ± SE (number of studied muscles). The values following the group names (NG or HG-NG) correspond to the age of the rats. *Statistically significant difference between age-paired groups using the Student's *t*-test. [a,b] Statistically significant difference between NG-115 and NG-130, respectively. †‡§, Statistically significant difference between HG-NG-115, HG-NG-130 and HG-NG-160, respectively by using a Bonferroni test. For all statistical tests, statistical significance was accepted at $P < 0.05$. TTP, time to peak; HRT, half relaxation time; P_{20}/P_0, ratio of tetanic tension at 20 Hz to maximal tetanic tension; P_t/MWW, ratio of maximal twitch tension to muscle wet weight; P_0/MWW, ratio of maximal tetanic tension to muscle wet weight; FI: fatigue index

days, only the kinetic TTP parameter was significantly greater in HG-NG rats. For both NG and HG-NG series of rats, the FI was unchanged.

Identification of muscle fiber type by immunohistochemistry. Figure 1 shows representative serial cross sections from NG and HG-NG rats, in which muscle fiber types were identified by immunoreactivity. The MHC expressions were determined on a total of 300 fibers per muscle. Figure 1 illustrates the antibody reactivity in NG (Fig. 1: parts 1, 2, 5, 6, 9, 10, 13, 14) and in HG-NG (Fig. 1: parts 3, 4, 7, 8, 11, 12, 15, 16) rats. We have chosen to present the staining with NCL-MHCs (Fig. 1: parts 1, 3, 5, 7, 9, 11, 13, 15) and SC-71 (Fig. 1: parts 2, 4, 6, 8, 10, 12, 16) antibodies, respectively, directed against MHC I and MHC IIA isoforms. In Fig. 1, the positive fibers were characterized by a dark color, whereas negative fibers remained uncolored. In NG rats, from 115 to 220 days, fibers expressing MHC I and/or MHC IIA could be identified, the number of type I fibers being largely predominant. In HG-NG rats at any age, the soleus muscle presented only fibers containing MHC I isoform, as all sections incubated with SC-71 antibody remained uncolored. For both NG and HG-NG rats, the reactivity with MY32 antibody was strictly identical to that observed with SC-71. Moreover, in any of the NG and HG-NG groups, sections treated with BF-F3 antibody remained negative, indicating that soleus muscle did not express MHC IIB isoform.

The relative proportions of fiber types within each group are reported in Fig. 2. The quantitative data revealed that the soleus muscle of NG-115 rats contained ~76% of type I fibers, ~14% of fast type IIA fibers, and ~10% of fibers coexpressing the two MHC isoforms, MHC I + MHC IIA. During muscle maturation in normogravity, the amount of fibers expressing MHC I significantly increased between 115 and 130 days, remained stable until 160 days, and showed again a significant increase at 220 days, when data were compared with those obtained at 115 days. Hybrid fibers containing both MHC I +

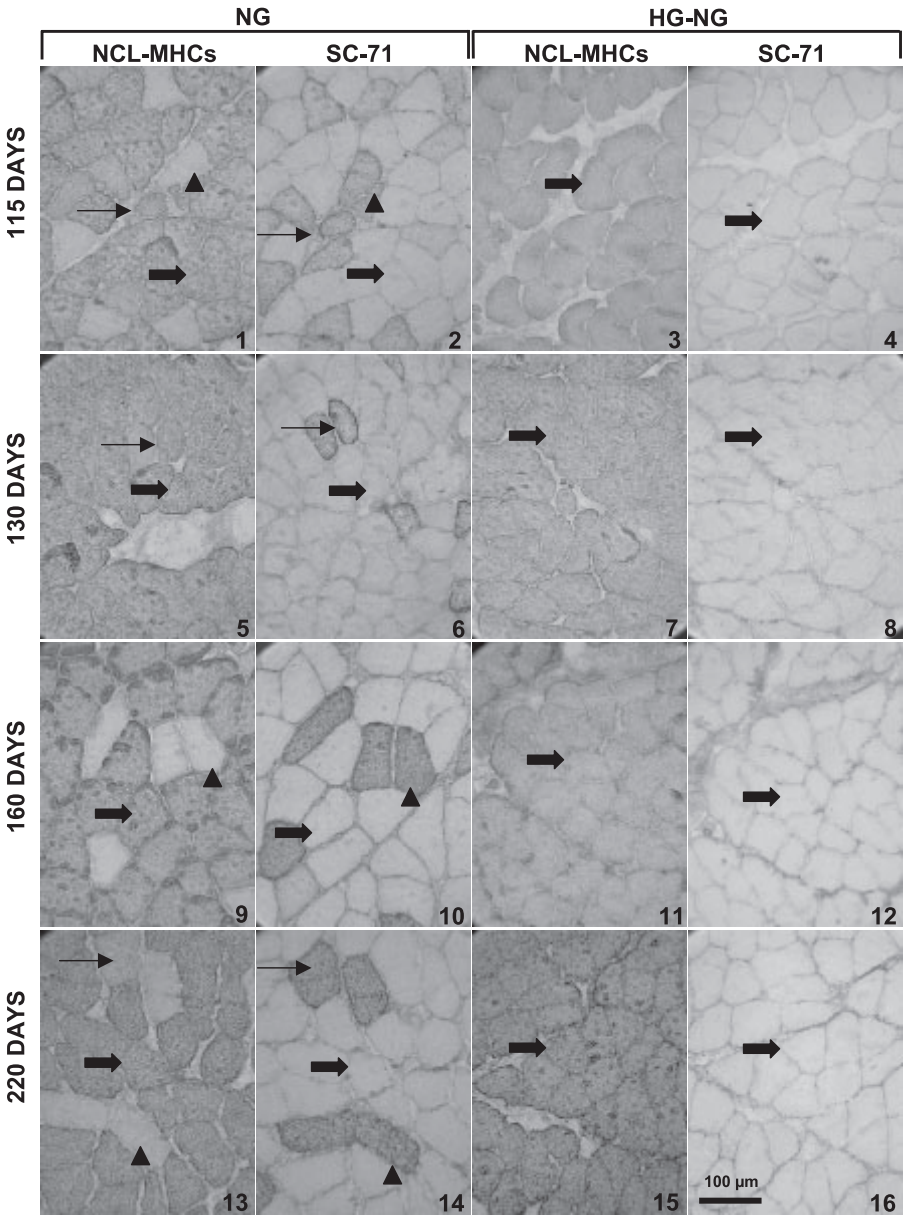

Fig 1 Serial sections illustrating muscle typing determined by immunohistochemistry in normogravity (NG) and hypergravity-normogravity (HG-NG) rats. The experimental conditions NG or HG-NG and the antibodies used are indicated at the top of the figure. The age of the animals is reported on the left of the photos: 115 days, 1–4; 130 days, 5–8; 160 days, 9–12; and 220 days, 13–16. Examples of fiber types are indicated by ▲, type IIA fiber; a thin solid arrow, fiber expressing the antibodies MHC I + MHC IIA; and a thick arrow, type I fiber.

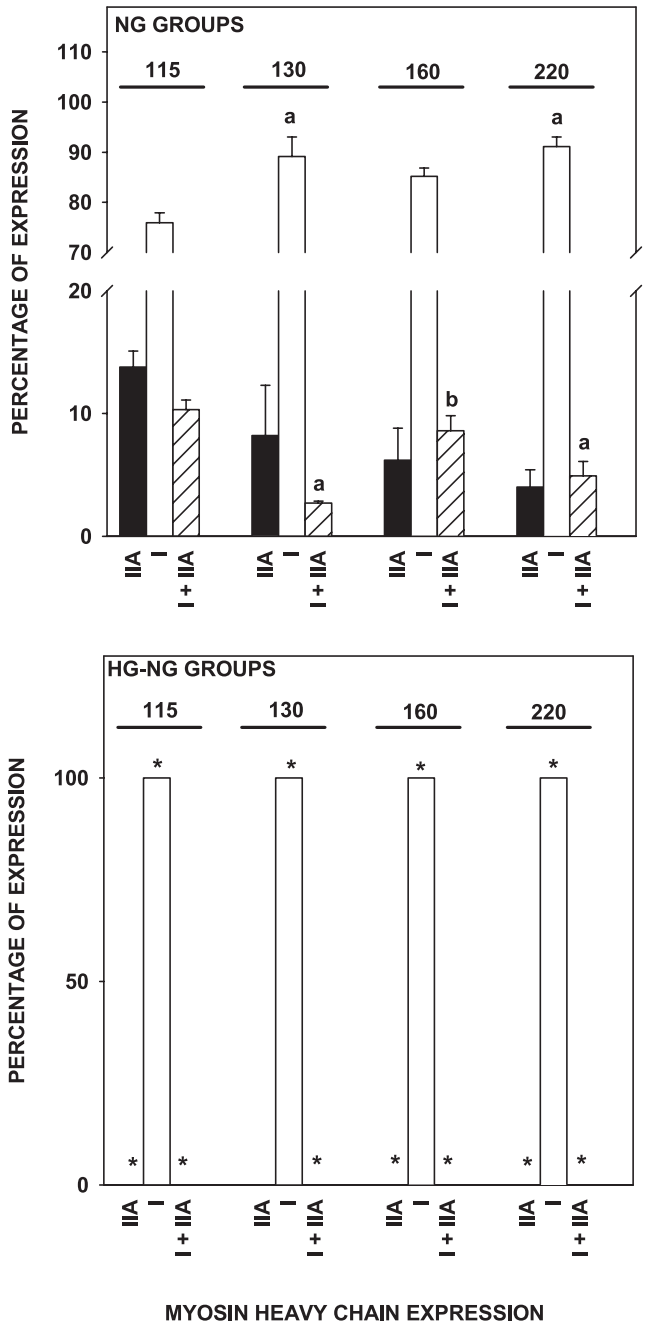

Fig 2 Repartition of fiber types within each muscle group. The fibers were classified according to their MHC isoform coexpressions.The results were expressed as means ± SE. *Statistical difference between age-paired groups using the Student's *t*-test. Letters a and b, indicate statistically significant difference between NG-115 and NG-130, respectively, by using the Bonferroni test. For all statistical tests, statistical significance was accepted at $P < 0.05$.

MHC IIA were decreased *1)* between 115 and 130 days (respectively from 10% to 2.8%) and *2)* at 220 days (~5%). They then showed an unexpected increase at 160 days. The fast IIA fiber did not show any variation along the studied period. The muscle fiber types of HG-NG rats remained identical from 115 to 220 days; the entire muscle presented only type I fibers. The examination of muscle fiber types in HG-NG groups revealed that the soleus muscle only presented slow-type fibers whose composition remained unchanged along the studied period.

Muscle myosin heavy-chain composition. The different MHC isoforms were determined in soleus muscle by electrophoresis. The gel migrations are presented in Fig. 3. The MHC isoforms contained in the soleus muscles were identified by comparing their electrophoretic migration with that of a mix of fast (extensor digitorum longus) and slow (soleus) muscles (Fig. 3: lane 5). We were able to determine four MHC isoforms already described in our previous studies (26, 27). According to their increasing order of electrophoretic mobility, in lane 5, fast MHC isoforms were identified as being MHC IIA, MHC IIX, MHC IIB, and slow MHC isoform as MHC I. In Fig. 3, lanes 1 and 2 present examples of soleus muscle electrophoretic migration at 115 days (lane 1: NG rat, lane 2: HG-NG rat). Examples of profiles at 220 days are illustrated in lane 3 (NG rats) and in lane 4 (HG-NG rats). In NG rats, we identified two different MHC isoforms (MHC I, which was predominant, and MHC IIA) between 115 and 220 days. During this period, MHC I gradually increased at the expense of MHC IIA. In HG-NG rats at both 115 and 220 days, only the slow MHC I isoform could be determined (lanes 3 and 4, respectively).

The total amount of MHC isoforms was then obtained by densitometry. The results are summarized in Fig. 3*B*. In NG groups, two MHC isoforms were identified, MHC I and MHC IIA. The densitometric measurements revealed the marked predominance of MHC I isoform at the expense of MHC IIA. During muscle maturation in normogravity, MHC I became significantly more expressed: ~76% at 115 days to ~90% at 220 days contrary to MHC IIA that declined (from ~24% at 115 days to 10% at 220 days). In HG-NG groups, the MHC expression was significantly modified, as only MHC I isoform was detected. Consequently, it could be deduced that the soleus muscle was made up of 100% MHC I from 115 to 220 days.

DISCUSSION

This study presents for the first time on a timescale of 120 days from the 100th postnatal day the evolution of phenotypical and contractile muscle behavior in terrestrial rats and in rats conceived, born, and reared in HG, and then submitted to normogravity. In a previous paper, we have demonstrated that soleus muscle from rats conceived, born, and reared in HG presented a complete slow phenotype (26). Consequently, our aim was to determine whether these animals were able to express fast myosin in a situation of normogravity corresponding for these animals to hypogravity or whether their protein expression potential was durably modified by hypergravity. Our results proved that soleus muscle appeared unable to express fast myosin, although some muscle contractile properties changed with increasing to age.

Morphological data and muscle strength. Our data showed that for both NG and HG-NG rats, body and muscle weights increased with age. The comparison between the two experimental series suggested a differential maturation. The ratio MWW/BW remained lower in HG-NG rats compared with age-paired controls, this latter result indicating that the growth of BW was proportionately bigger than the growth of MWW. Nevertheless, BW and MWW in HG-NG rats always remained lower than their age-matched controls.

Consequently, two relevant findings could be deduced from our results: first, the growth of HG-NG rats remained slow even after a long stay in normogravity and second, from the

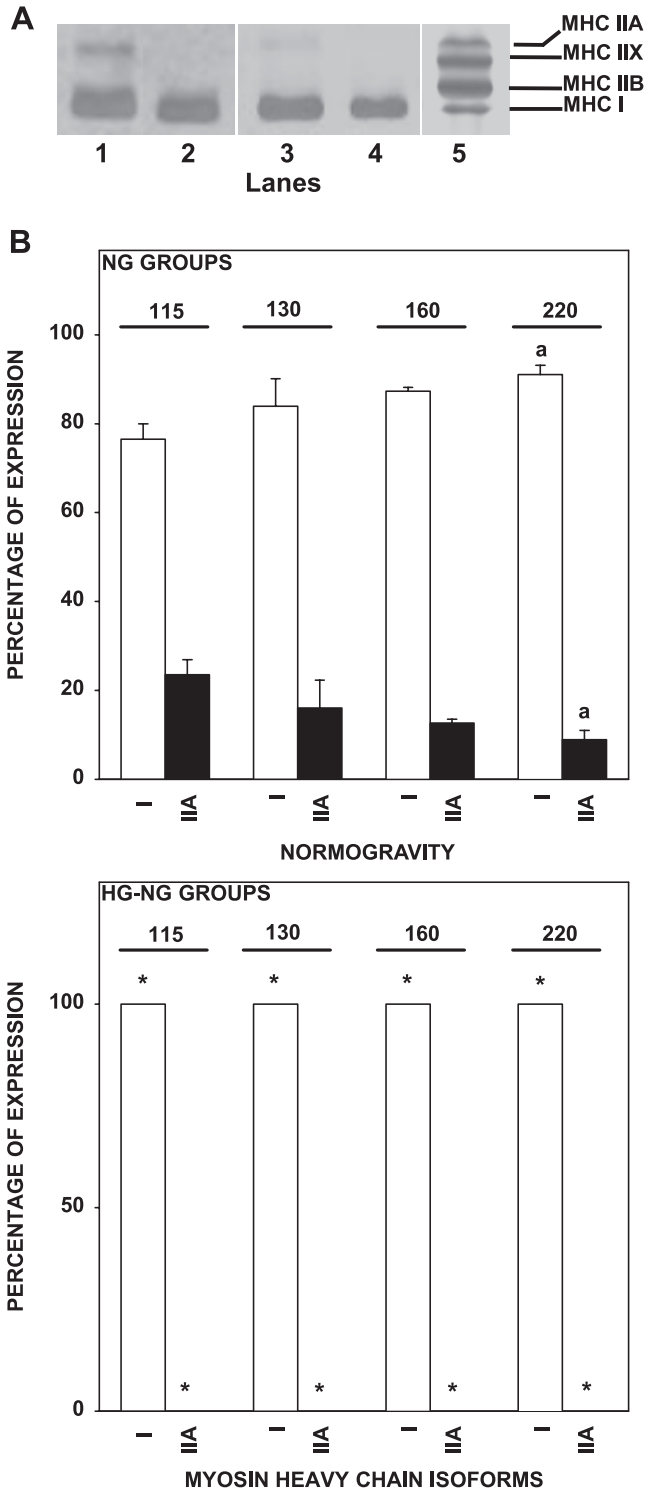

Fig 3 *A*: electrophoretic migration of myosin heavy-chain isoforms. The MHC isoforms were identified according to their electrophoretic mobility. Lane 1, NG-115 soleus muscle; lane 2, HG-NG-115 soleus muscle; lane 3, NG-220 soleus muscle; lane 4, HG-NG-220 soleus muscle; lane 5, mix of soleus and extensor digitorum longus muscles. *B*: densitometric determination of MHC isoforms expressed in NG and HG-NG rats. The results were reported as means ± SE. *Statistical difference between age-paired groups using the Student's *t*-test. Letter a indicates statistical difference with NG-115, by using the Bonferroni test. For all statistical tests, statistical significance was accepted at $P < 0.05$.

MWW/BW data, the growth of soleus muscles of these animals was delayed compared with NG rats. This latter point could be explained by a decrease in food intake of rats reared in HG. However, no statistical difference was found between HG rats and their age-matched group, except during 7 days postweaning. Only at that time, the HG rats showed a slight decrease in food intake (26). After acclimation, the HG rats gained body mass at a rate similar to controls, but their mass remained lower than control animals. Consequently, after a long stay in HG, the rats could be considered acclimated to HG environment. A durable modification in the balance of anabolism/catabolism proteins could also explain the mass differences between NG and HG-NG rats caused by a decrease in protein synthesis due to the hypergravity environment. Indeed, previous studies have demonstrated that hypergravity induced, in growing rats, a marked decrease in the weight and muscular protein content (22). A final hypothesis was that normogravity must have been perceived as hypogravity by the HG-NG rats, since the soleus muscles were involved in an antigravity function. In such a case, the soleus muscles would be less solicited and muscular growth could be slowed.

The comparison of the strength data between NG and HG-NG groups revealed that HG-NG rats were stronger until 30 days in normogravity. Afterward, there was no difference in strength between NG and HG-NG rats. Since results were expressed in terms of normalized muscle force, they indicated that the stay in normogravity had induced in HG-NG rats an increase in muscle mass coupled with an increase in muscle force. These increases might also be due to a rise in the proportion of contractile myofibrillar proteins in relation with motor activity. Indeed, it has been previously demonstrated that in HG, the locomotor activity is reduced compared with control animals (34). HG rats, which are suddenly submitted to normogravity, present an increased sensorimotor activity (15). This locomotor activity could be more intense during the first days in normogravity, the animals later adapting to their new environment and thus decreasing their exploratory capacity. Basically, this increased locomotor activity could be the cause of *1)* an increase only within muscle mass (in the 15 days after being placed into normogravity), and *2)* an increase in muscle force (from 30 days in normogravity), later coupled with an increase in muscle mass resulting from natural growth.

Myosin expression and kinetic parameters. In NG rats, basically, parameters such as TTP, HRT, and to a lower extent P_{20}/P_0 revealed increased values from 115 to 130 days. Afterward, the values stabilized until 220 days. These data were in relation with the percentages of fiber expression since from 130 days, the relative amounts of fiber containing slow MHC I and both MHC I and MHC IIA were increased and decreased, respectively, before they stabilized at 220 days. A previous study focusing on the myosin changes during postnatal life has suggested that the mature muscle phenotype was achieved from the 125th day in the soleus muscle (18). However, the changes do not usually take on that importance. Moreover, in this study, the rats that were used did not belong to Long-Evans species. One hypothesis is the existence of a key period in the motor message maturation. It is possible that there was an increase in the electromyographical activity (EMG) activity, which became more tonic and reinforced the MHC I expression. The increase in slow MHC could be sufficient to induce an increase in contractile parameters. Unfortunately, there is no

data in the literature relative to an EMG maturation, specific to Long-Evans rats.

In a previous paper (26), we have already reported that, surprisingly, our control rat soleus presented contractile parameters (TTP, HRT, and P_{20}/P_0) less slow than those usually observed in Wistar control rats, although the experimental setup such as as force transducer was strictly identical. Similar results have already been reported by Elder and Vasallo (11), in young Long-Evans rats. Our explanation is, thus, the existence of interspecies variability.

The comparison of HRT values between NG and HG-NG rats demonstrated that they were significantly lower in NG-115 rats than in the age-matched animals. From *day 130*, HRT values of HG-NG rats became similar to those of NG rats. This parameter has been proved to be dependent on calcium ATPase isoform in the sarcoplasmic reticulum (21). Consequently, we postulate that ATPase isoforms became identical in NG and HG-NG rats, from 130 days.

In a previous paper (26), we have established that the soleus muscle of rats born and reared in HG became slow. In our study, when applied to such animals, normogravity seemed unable to transform the muscle phenotype. In hypogravity, simulated or real, a slow-to-fast transition for contractile protein is commonly described, which was not the case in our experiments. In fact, HG-NG rats could be considered in "hypogravity" from the very moment they were installed in normogravity. Their disability to express fast myosin remains unclear. It could be the result of a change in the nervous motor message during HG. Indeed, it has been demonstrated that, during HG, slow muscles became slower. Then, we hypothetized that the nervous message was reinforced (from tonic to more tonic) and was sufficient to alter the muscle phenotype. Unfortunately, it is difficult to perform the measurement of EMG in the centrifugation apparatus, but we have some data on animal behavior. It has been reported that during HG, the locomotor activity of the rats is reduced (7, 15). Animals exposed to 2 G walked more slowly than off-centrifuge control animals. Moreover, they adopted a different form of locomotion that resulted from a four-footed stance which increased the postural stability (7). These authors therefore suggested that, in HG, the locomotion is differently controlled, compared with normogravity.

As the body weight was increased by a factor of 2 during HG, we argue that, to support this increased body charge, the EMG is increased too. Unfortunately, we have no information concerning the future of EMG during prolonged stay in HG. Nevertheless, a previous study has demonstrated that during 2 G phases of parabolic flight, this activity was transiently increased (20). According to our hypothesis, the EMG activity could, in turn, modify the afferent message coming from muscle receptor, such as neuromuscular spindles. Indeed, it has been reported that 19 days in HG are sufficient to induce some intrafusal myosin changes (28). Considering that afferent fibers coming from intrafusal fiber are projected onto α and γ motoneurons, they could also modify, by reflex pathway, the EMG activity during HG.

HG rats which are submitted to normogravity present an increased locomotor activity (15), whereas they perceive normogravity as hypogravity. We postulate that the existence of a contact of hindlimb plantar soles with the ground together with locomotor movements, which are usually nonexistent in microgravity, could contribute to the maintenance of slow myosin despite gravity change.

To sum up, when gathering the data, the soleus muscle appears deeply modified by a long stay in HG. Our study underlines the importance of gravity in the maintenance of neuromuscular integrity. However, at present, several questions remain unanswered. Further studies could be undertaken to clarify these issues. Moreover, by using combinations of different ages in relation with different alternating periods of HG and normogravity, critical periods for gene or protein expressions both in the embryonic and postnatal developments could be determined.

ACKNOWLEDGMENTS

The authors wish to thank Dr. G. S. Butler-Browne for the growing of hybridomas producing SC 71 and BF F3 antibodies initially developed by Dr. Schiaffino (31). The hybridomas were produced by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany.

GRANTS

This study was supported by grants from the Centre National d'Etudes Spatiales (8411) and the Conseil Régional du Nord-Pas-de-Calais.

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