Physiologically adaptive changes of the L$_5$ afferent neurogram and of the rat soleus EMG activity during 14 days of hindlimb unloading and recovery

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Summary

The hindlimb unloading rat model (HU, Morey's model) is usually used to mimic and study neuromuscular changes that develop during spaceflights. This Earth-based model of microgravity induces a muscular atrophy of the slow postural muscle of hindlimbs, such as the soleus, a loss of strength, modifications of contraction kinetics, changes in histochemical and electrophoretical profiles and modifications of the tonic EMG activity. It has been suggested in the literature that some of these neuromuscular effects were due to a reduction of afferent feedback during HU. However, no direct data have confirmed this hypothesis. The aim of this study was to clearly establish if changes of the L$_5$ afferent neurogram are closely related to the soleus EMG activity during and after 14 days of HU.

Immediately after HU, the EMG activity of the soleus muscle disappeared and was associated with a decrease in the afferent neurogram. The soleus electromyographic and afferent activities remained lower than the pre-suspension levels until the sixth day of HU and were recovered between the sixth and the ninth day. On the twelfth and fourteenth days, they were increased beyond the pre-suspension levels. During the first recovery day, these activities were significantly higher than those on the fourteenth HU day and returned to the pre-suspension levels between the third and sixth recovery days.

To conclude, our study directly demonstrates that the HU conditions cannot be considered as a functional deafferentation, as suggested in the literature, but only as a reduction of afferent information at the beginning of the HU period.

Key words: rat, hindlimb unloading, neuromuscular activities.

Introduction

The importance of peripheral afferent information on the motor activity control has been assessed by spinal cord injury (Dietz, 2002; Pearson, 2000), reduced uses and deafferentation experiments (Goldberger, 1988; Hnik et al., 1981). The hindlimb unloading (HU) model (Morey et al., 1979) is an experimental animal model of simulated microgravity used in numerous laboratories to mimic some of the neuromuscular perturbations occurring in a real microgravity environment during spaceflights (Edgerton and Roy, 1996; Morey-Holton and Globus, 2002). This ground-based model is characterized by hypodynamia-hypokinesia conditions (lack of weight bearing and reduction of motor activity, respectively). These conditions have been reported to induce similar changes as in real microgravity in many muscular properties mainly in the slow postural extensor muscles such as the soleus, an ankle extensor muscle (Edgerton and Roy, 1996). Muscular changes are characterized by a muscular atrophy (loss of muscular mass), strength loss, modifications of contraction kinetics and changes in histochemical and electrophoretical profiles: the slow muscle phenotype evolves toward that of a faster muscle type (Edgerton and Roy, 1996). In real microgravity (Clement

et al., 1984; Leterme and Falempin, 1998; Recktenwald et al., 1999) or in hindlimb unloading (HU) conditions (Alford et al., 1987; Blewett and Elder, 1993; Ohira et al., 2002a; Riley et al., 1990), the electromyographic (EMG) activity of extensor postural muscles has been reported to disappear instantaneously and a redistribution of the tonic EMG activity between extensor and flexor muscles of the ankle has been observed. From a functional point of view, a lateral instability and hyper-extensions of the ankle during postural and locomotor activities have also been observed after HU (Canu and Falempin, 1996; Ohira et al., 2002a). Proprioceptive afferent information was essential to maintain EMG activities in postural ankle extensor muscles (Hiebert and Pearson, 1999). Experimentally induced and pathological deafferentation decreased the EMG activity of the ipsilateral soleus and lowered the vertical average ground reaction force (Thoumie and Do, 1996). Moreover, the hindlimb motor activity was more affected by loss of muscle proprioceptive afferents than by loss of plantar cutaneous messages (Thoumie and Do, 1996). Several studies have demonstrated the importance of afferent feedback in the postural control of the

body (Duysens et al., 2000; Massion, 1992). To explain some of the effects of unloading, especially the modification of the soleus EMG activity and the development of muscular atrophy, a decrease in peripheral afferent information participating in the regulation of postural and locomotor activities is usually advanced in the literature (Bock, 1998; Dietz, 1999; Roll et al., 1998). To date, no experimental studies have directly confirmed this assertion. However, some studies have demonstrated that hindlimb unloading is a particular environment that induces afferent feedback perturbations (De-Doncker et al., 2000; Falempin and In-Albon, 1999; Kyparos et al., 2005). In normal gravity, the maintenance of muscular properties depends, in part, on the feedback exerted by specific receptors: muscle spindles, Golgi tendon organs, and otolithic, articular and cutaneous receptors (Bock, 1998). The information coming from cutaneous receptors is transmitted to the central nervous system and causes medullary reflexes that contribute to the stabilization of the feet and maintenance of postural activity (Aniss et al., 1992; Kavounoudias et al., 1998). In simulated microgravity, the patterns transmitted by these cutaneous receptors are likely to be disrupted because the soles of the feet are not in contact with the ground; consequently, the nervous motor message could be modified by the reflex pathway. The dynamic cutaneous mechanoreceptor stimulation of the plantar surface of the rat hindfeet during the 14 days of HU partially prevented soleus atrophy (De-Doncker et al., 2000; Kyparos et al., 2005). These studies demonstrated that, in HU condition, plantar cutaneous afferent information was decreased and participated in the development of muscular atrophy. Moreover, during HU, 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, the muscular stretch, being thus removed and muscle spindle discharges decreased. However, the intermittent application of brief daily tendon vibration for 14 days in HU condition (soleus stretched during application of vibrations) prevented 75% of the soleus muscular atrophy (Falempin and In-Albon, 1999). This indicated that during HU, afferent messages from muscle spindles are decreased because of plantar flexion and contribute to the development of the soleus atrophy.

Studies on the EMG activity during HU are conflicting (Alford et al., 1987; Blewett and Elder, 1993) and few works about the recovery of EMG activity after HU are present in the literature (Blewett and Elder, 1993; Ohira et al., 2002a). Moreover, there is no direct data concerning the physiologically adaptive changes of the L_5 afferent neurogram and of the soleus EMG perturbations during and after a HU period. Therefore, the aim of this work was to verify, firstly, if HU conditions can be assimilated to a functional model of deafferentation as suggested in the literature, and secondly, if physiologically adaptive changes in L_5 afferent activation level can explain changes in activation levels of the soleus EMG activity during and after (recovery) 14 days of HU.

Materials and methods

Animals

Studies were performed on male Wistar rats (C. Rivers-IFFA Credo-l'Arbresle, France) weighing 280–300 g. The rats were acclimatized to a 25°C room temperature with a 12 h:12 h L:D cycle for 1 week before the experiments began. Only one group of rats was used: the hindlimb unloading animals (HU group: N=11), each animal being its own control (pre-suspension level). The soleus EMG activity and the L_5 afferent neurogram were simultaneously recorded during and after the HU period.

The animals of the HU group were hindlimb unloaded by the tail for 14 days, using Morey's model (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 Ministry and the National Education Ministry (Veterinary Service of Health and Animal Protection, authorization 59-00980).

Electrode preparation

Electromyographic and afferent activities were recorded using bipolar electrodes made of stainless steel wire with Teflon insulation (7 strands, AM Systems, Cooner wire, Phymep, Paris, France).

For intramuscular electrodes, the recording surface was exposed by removing 2 mm of Teflon insulation 5 mm from one end of the stainless steel wires. To deactivate the electrode extremity, 1 mm of Teflon insulation was pulled over it.

For the L_5 afferent neurogram, the recording surface was exposed by removing 2 mm of Teflon insulation 5 mm from one end of the electrode. The recording surface of bipolar electrodes was introduced into silicone tubing 3 mm in length and 1 mm in inner diameter, which was cut longitudinally. The end of the electrodes was then rolled up and stuck to the silicone tubing using silicone glue. The distance between electrodes in the silicone tubing was 1 mm (Fig. 1A). To link the electrode to a connector, 5 mm of the Teflon insulation at the other end of the electrode was removed. The ground electrode was stripped of insulation for 5 mm at each end. To obtain a simultaneous recording of the soleus muscle and afferent activities, the four recording electrodes and the ground electrode were soldered to the connector and linked with dental cement (TAB 2000, Kerr, Italy) to the connector.

Electrode implantation

The implantation was performed on anaesthetized rats (sodium pentobarbital, 30 mg kg^{-1}, intraperitoneal injection). Supplementary injections (15 mg kg^{-1}) were provided when necessary. A laminectomy was done between L_3 and L_6. The skin of the skull and of the right leg was incised. The connector (Pedestal 2298 6 pin white del, Phymep, Paris, France) was

held on to the skull with three screws, which were introduced into the bony part and held in place by dental cement. The intramuscular electrodes were then guided to the right hindlimb under the skin. Under the dissecting microscope, the soleus recording electrodes were introduced in parallel into the right soleus muscle, and placed 2 mm apart, using a 23-gauge, 1.5-hypodermic needle. The position of the electrodes and their sensitivity were tested by electrical stimulation (Grass Instruments S88, Quincy, MA, USA). These electrodes were then ligatured and secured at the muscle entry and exit. To record the afferent neurogram, the intact right L_5 dorsal root was carefully introduced into the silicon tubing. After surgery, a cutaneous application of betadine (betadine, polyvidone iodée, Viatris, Merignac, France) was necessary to prevent infection. The animals were placed in individual cages to recover and were maintained under analgesic (Metacam: 100 μl kg^{-1}, Boehringer Ingelheim, Germany) for 3 days, and under antibiotic (Trisulmix liquid: 100 μl kg^{-1}, Coophavet, France) for 7 days.

Recording and analysis

Although the EMG activity was specific to the soleus muscle, the L_5 afferent neurogram also reflected activities of neurons innervating other hindlimb muscles (gastrocnemius, for example) affected or not by the microgravity environment (Peyronnard et al., 1986). During the experimental period (pre-suspension and reloading), the animals were placed in small cages and left alone to limit movements and to record neuromuscular activities under postural conditions. In this study, analyzed recordings (80–90% of daily recordings) were obtained when rats were quiet. Activity bursts produced by rat movements were not taken into account. Within each 30 min period, only phasic-like bursts (corresponding to the progressive recovery of the tonic EMG activity of the soleus muscle) have been averaged, except at the beginning (D0) of HU when the hypoactivity periods predominated over few phasic-like bursts, which were very short in duration. The control and HU recordings began 2 and 7 days, respectively, after surgery. The rats were recorded as control for 5 days (mean ± S.D.) and during the first, third, sixth, ninth, twelfth and fourteenth days of HU (D0, D3, D6, D9, D12, D14) and recovery (RD0, RD3, RD6, RD9, RD12, RD14). The soleus EMG activity and the L_5 afferent neurogram were simultaneously recorded twice a day for 30 min each (10:00 h and 17:00 h). The raw EMG and the afferent neurogram were amplified (pre-amplifier model P 511, Grass Instruments, Quincy, MA, USA; Gain 10,000, band pass 30 Hz to 3 kHz), recorded and analyzed on a personal computer through interactive software (Spike 2, Cambridge Electronic Design, UK). This program rectified the EMG and neurogram signals throughout the recording periods. The mean EMG (mEMG) and the afferent neurogram were expressed as V s^{-1} and averaged.
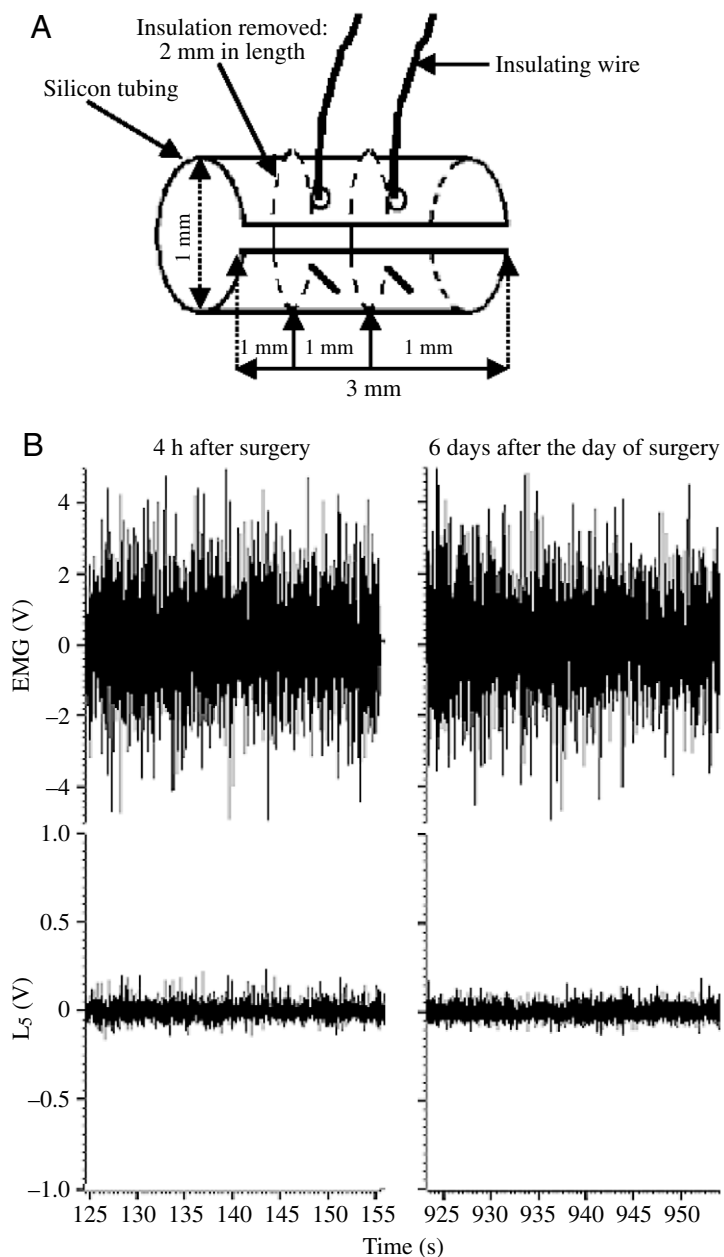

Fig. 1. (A) Diagram of the silicon tubing used to record the L_5 afferent neurogram. (B) Test of the electrode sensitivity by recording the EMG and L_5 afferent activities 4 h after surgery and 6 days after the day of surgery.

We have not included in this study a separate sham-operated group of rats to monitor the evolution of the signals over the full time period. However, before recording control levels of the soleus EMG activity and of the L_5 afferent neurogram, we also recorded these activities four hours (wide awake animals) after the electrode implantation. These recordings are illustrated in the Fig. 1B. No significant difference can be observed between EMG and afferent activities on the day of the surgery and 6 days later. Therefore, at least for this time period, the electrode sensitivity is not altered. We have not tested the electrode sensitivity over this time period. However,

the possible decrease in the electrode sensitivity to detect signals during HU or the recovery, could account to some degree for the decreased activities during HU. Nevertheless, this decrease is not involved and cannot explain the increases in EMG and L_5 afferent activities during HU and recovery. Consequently, physiologically adaptive changes of these activities cannot be attributed to alterations triggered by electrode sensitivity changes. Moreover, at the end of the experiments, the position of each electrode was checked under the dissecting microscope and was found to be as originally placed, spaced out 2 mm between intramuscular electrodes and 1 mm between electrodes into the silicon tubing. The electrode sensitivity was also verified by using electrical stimulation and was found to be the same as just after electrode implantation.

Statistical analysis

All results are expressed as means ± S.D. Significant differences ($P<0.05$) between HU and its own control were established by using a paired Student's t-test. No significant difference in neuromuscular activities was observed between the two recording periods (10:00 h and 17:00 h). Therefore, results have been pooled.

Results

Hindlimb unloading

Qualitative and quantitative physiologically adaptive changes of the soleus EMG activity and L_5 afferent neurogram during 14 days of HU are illustrated in Figs 2A and 3.

EMG activity

At rest on the floor (pre-suspension level), the EMG activity was tonic and the mean EMG activity (mEMG) was 0.56 ± 0.09 (V s^{-1}). Immediately after animal hindlimbs were elevated (D0), the mEMG activity of the soleus muscle significantly decreased by 81% (0.11 ± 0.03 V s^{-1}) in comparison with the pre-suspension level (Figs 2A, 3) and remained lower until the sixth HU day (Fig. 3). Moreover, the EMG activity of the soleus muscle was shifted from 'tonic' to 'phasic-like' activity during HU (Fig. 2A). The number, amplitude and duration of EMG bursts increased with the duration of the HU period. Although the tonic EMG activity did not totally recover its pre-suspension level between the sixth and ninth day of HU (Fig. 2A), mEMG bursts were quantitatively comparable to the tonic EMG activity of the pre-suspension level (Fig. 3). Afterwards, the mEMG activity gradually increased until the fourteenth day of HU ($P<0.05$). In comparison with the pre-suspension values, the mEMG activity significantly increased by 23% (0.69 ± 0.09 V s^{-1}) on the twelfth and by 32% (0.74 ± 0.1 V s^{-1}) on the fourteenth day of HU (Figs 2A, 3). However, on the fourteenth day of HU, during the 60 min period of recording in HU, EMG bursts represented only 76% of this period vs 24% for hypo-activity periods.
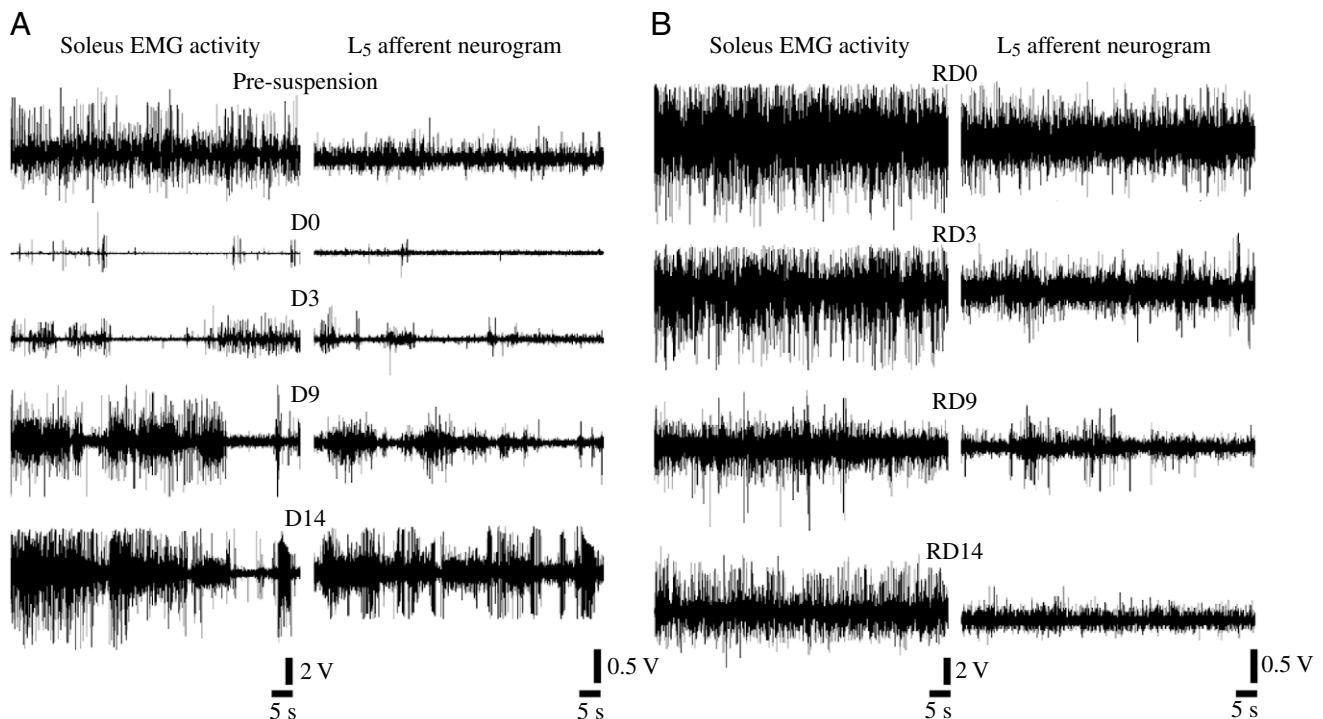

Fig. 2. Qualitative physiologically adaptive changes of EMG and L_5 afferent activities during 14 days of hindlimb unloading (HU) and recovery. (A) Qualitative representations of the right soleus EMG activity and the right L_5 afferent neurogram at the pre-suspension level, at the beginning of the HU period (D0) and on the third (D3), ninth (D9) and fourteenth (D14) days of HU. (B) Qualitative representations of the right soleus EMG activity and the right L_5 afferent neurogram on the first (RD0), third (RD3), ninth (RD9) and fourteenth (RD14) day of recovery.
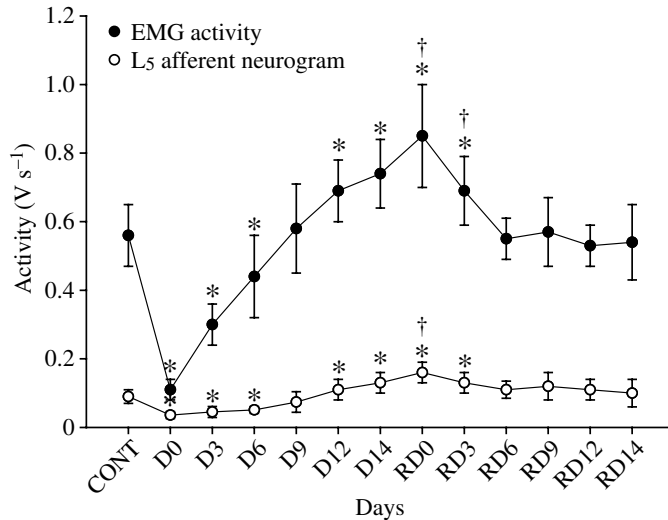
Fig. 3. Physiologically adaptive changes of neuromuscular activities during 14 days of hindlimb unloading (HU) and recovery. Changes of the amplified soleus EMG and L_5 afferent activity values (V s^{-1}) are expressed as mean ± S.D. (N=11). *Significant difference from the control (CONT) values; †significant difference from the fourteenth day of HU.

L_5 afferent neurogram

Physiologically adaptive changes of the quantitative and qualitative afferent neurogram during HU are illustrated in Figs 2A, 3. The mean afferent neurogram (0.09±0.02 V s^{-1}) was decreased by 60% (0.036±0.01 V s^{-1}) on the day of HU ($P<0.05$) in comparison with the pre-suspension level (Fig. 3). Moreover, the L_5 afferent activity was shifted from 'tonic' to 'phasic-like' during HU (Fig. 2A). Between the sixth and ninth day of HU, afferent bursts increased in amplitude and duration to reach tonic activity of the pre-suspension level (Fig. 3). Afterwards, they increased by 22% (0.11±0.03 V s^{-1}) on the twelfth day of HU ($P<0.05$) and by 44% (0.13±0.03 V s^{-1}) on the fourteenth day (Fig. 3).

Recovery

Qualitative and quantitative physiologically adaptive changes of the soleus EMG activity and the L_5 afferent neurogram during 14 days of reloading are illustrated in Figs 2B, 3.

EMG activity

At the end of the fourteenth HU day, immediately after reloading (RD0), the phasic-like EMG activity disappeared and the tonic EMG activity of the pre-suspension level was recovered (Fig. 2B). However, the mEMG activity significantly increased by 51% (0.85±0.15 V s^{-1}) on the day of reloading and by 23% (0.69±0.1 V s^{-1}) 3 days later, in comparison with the pre-suspension level (0.56±0.09 V s^{-1}) (Fig. 3). Moreover, the soleus EMG activity was increased by 15% ($P<0.05$) in comparison with the EMG value of the fourteenth day of HU (0.74±0.1 V s^{-1}). Between the third and sixth day of reloading, the quantitative EMG activity regained the value of the pre-suspension level (Figs 2B, 3).

L_5 afferent neurogram

The L_5 afferent activity presented an adaptive change similar to that of the EMG activity (Fig. 2B). The mean afferent neurogram was increased by 78% (0.16±0.04 V s^{-1}) on the day of reloading ($P<0.05$) and by 45% (0.13±0.03 V s^{-1}) on the third day of reloading ($P<0.05$) in comparison with the pre-suspension levels (Fig. 3). Moreover, the L_5 afferent activity was increased by 23% (0.16±0.03 V s^{-1}) in compared with the fourteenth day of HU ($P<0.05$). Between the third and sixth day of ground support, the L_5 afferent neurogram recovered the pre-suspension level (Figs 2B, 3).

Discussion

The aim of this work was to determine firstly, if HU conditions can be assimilated and used as a model of functional deafferentation and secondly, if changes in the L_5 afferent activation level can explain changes in activation levels of the soleus EMG activity during and after (recovery) 14 days of HU.

According to Alford et al. (1987), the soleus EMG activity was immediately reduced after HU. This activity remained lower than that of the pre-suspension level until the sixth HU day and was recovered between the sixth and ninth days. In our study, after the ninth HU day, the soleus EMG activity gradually increased until the fourteenth HU day. Alford et al. (1987) observed that the soleus EMG activity was maintained at near normal levels after 7–10 HU days. In the present work, the soleus EMG activity was higher on the first day of recovery than at the pre-suspension level and on the fourteenth day of HU. After the third recovery day, we observed that the soleus EMG activity regained the pre-suspension level. Similar physiologically adaptive changes were observed in the L_5 afferent neurogram during both HU and recovery.

Therefore, this study demonstrates that HU conditions cannot be considered as a functional deafferentation, as suggested in the literature, but only as a reduction of afferent information at the beginning of the HU period (between D0 and D6).

As already stated in our results, the soleus EMG activity disappeared immediately after hindlimb unloading. Later, the tonic activity was gradually recovered in phasic-like activity form, the amplitude and duration of which increased progressively to reach the basal tonic activity level. The term 'phasic-activity' does not characterize phasic high-amplitude events due to hindlimb movements; indeed, in our study, phasic-like bursts occurred whereas no visible movement of the hindlimbs was observed. Our results did not permitted the determination of the mechanisms implied in this phenomenon.

Immediately after HU

The soleus EMG reduction resulting from HU observed in this work, may be closely related to the passive shortening of the muscle caused by plantar flexion (Alford et al., 1987; Ohira et al., 2002b; Riley et al., 1990), which can inhibit tension development and decreases in afferent input. In fact, during the

0G phase of a parabolic flight, Kawano et al. (2002) observed reductions in the rat soleus EMG and efferent activities concomitant with a decrease in afferent information. However, it is very difficult to determine the precise nature of the afferent input responsible for alterations in the L_5 afferent neurogram and EMG activity during the HU period.

The vestibular system is reported to control head and eye movements in space. Vestibular and cervical reflexes stabilize the head and neck by exciting the neck muscles in opposition to head and body movements (Keshner and Cohen, 1989). On the one hand, the vestibulocollic reflex (vestibular-neck reflex) aligns the head with respect to the gravitational vertical. On the other hand, the cervicocollic reflex aligns the head with respect to the position of the body. These two reflexes work jointly to stabilize the head (Peterson et al., 1985). In the normal terrestrial situation, the labyrinth detects the gravity vector, and the otolithic messages project on to the α motoneurons via the lateral vestibular spinal fasciculus. In real microgravity, these afferent messages are altered or absent (Correia, 1998). Immediately after a bilateral labyrinthectomy, the soleus postural activity has been reported to decrease but to return to control level after a short period. However, after 17 days, a hyperactivity of the soleus muscle has been observed (Kasri et al., 2004). In our study, using Morey's model (hindlimb unloading), the gravitational load was maintained and thus, labyrinthine inputs remained persistent. Moreover, the vestibulocollic and cervicocollic reflexes can occur thanks to the 30° head down of the rat: the head position was left in HU conditions and was similar to the control head position. Consequently, in order to maintain the gravitational force and head position during HU, labyrinthine inputs could not be significantly modified. Finally, the vestibulospinal information on hindlimb motoneurons was probably not altered. For this reason, modifications of vestibular inputs during HU have been discounted.

Although it has not been directly demonstrated in the literature, some studies have suggested that a limited amount of afferent information, particularly provided by plantar cutaneous mechanoreceptors and muscle spindles, is involved in HU conditions. Indeed, as already stated in the Introduction, in normal conditions, the information coming from cutaneous receptors is transmitted to the central nervous system and causes medullary reflexes that contribute to the stabilization of the feet and maintenance of postural activity (Aniss et al., 1992; Kavounoudias et al., 1998). In HU conditions, the patterns transmitted by these cutaneous receptors are very probably disrupted because the soles of the feet are not in contact with the ground. Consequently, the nervous motor message could be modified. This hypothesis has been confirmed by experiments that used the stimulation of plantar cutaneous mechanoreceptors, since the stimulation of these receptors during the HU period prevents 53% muscular atrophy (De-Doncker et al., 2000) indicating that plantar cutaneous information is greatly decreased during HU. Concerning the implication of proprioceptive information, Riley et al. (1990) have observed that in HU conditions, the soleus was often in

a shortened position as after tenotomy and immobilization in a plantar flexion position. In these latter conditions, the natural physiological stimulus of muscle spindles, the muscular stretch (Hunt, 1990), was removed. Consequently, during HU, the soleus muscle spindles were probably little, or not at all, stimulated and the afferent activity of Ia and II fibers originating from these stretch receptors was reduced. Indeed, the reactivation of Ia fibers by tendinous vibrations has been reported as an effective countermeasure preventing muscle atrophy developed during HU, thus indicating that proprioceptive information was greatly disrupted in this condition (Falempin and In-Albon, 1999).

During HU

Although the plantar flexion occurred during the major part of the HU period, the soleus EMG activity was gradually recovered to the pre-suspension level after 7–10 days. These data may imply intramuscular reorganization. A reorganization in the number and length of sarcomeres could occur in HU conditions, resulting in the shortening of the soleus fiber length, similarly to a shortened position immobilization (Heslinga et al., 1995). Some muscle passive tension (stretching) could therefore be restored (Gillette and Fell, 1996) and consequently, muscle spindle discharges could reappear. However, this mechanism could participate in the early recovery of neuromuscular activities, but does not explain the increases in the soleus EMG activity on the twelfth and fourteenth days of HU beyond the pre-suspension levels. Intrafusal fibers are more resistant to myogenic atrophy and to structure modifications than extrafusal fibers. This is probably due to the presence of the capsule surrounding intrafusal fibers (Yellin and Eldred, 1970; Maier et al., 1972). In a study on the morphological and histochemical properties of intrafusal fibers after HU (De-Doncker et al., 2002), we have demonstrated that there was no significant difference in number, cross-sectional area and histochemical properties of intrafusal fibers in comparison with soleus muscle spindles of control rats. Moreover, in the rat soleus muscle, the number (HU: 13.5±1.3; CONT: 14.3±1.5) and length (HU: 2.75±0.2 mm; CONT: 2.82±0.3 mm) of muscle spindles were not changed after a HU period. Therefore, no intrafusal fiber reorganization seemed to occur during HU.

A decrease in the GABA immunoreactivity of the sensorimotor cortex has been observed by D'Amelio et al. (1996) after 14 days in HU. Consequently, we can also suggest that during HU, an increase in descending excitatory inputs on soleus motoneurons could be enhanced and contributed to the soleus EMG increase observed after the HU period. In addition, Hnik et al. (1981, 1982) previously suggested that EMG activity reappearance after 1–2 days deafferentation was apparently due to hypersensitivity of spinal neurons to supraspinal influences.

Recovery

During HU reloading, recovery effects could be due to increased levels of afferent input from muscle spindles and

plantar cutaneous receptors above control levels; some studies support this hypothesis. Indeed, after 14-day HU, we have previously demonstrated in the rat that Ia and II fiber discharges from the soleus during ramp-and-hold (3 and 4 mm) and sinusoidal stretches were increased because of changes in passive mechanical properties of the soleus during HU (De-Doncker et al., 2003). Consequently, the stretches could be better transmitted to the muscle spindles after a HU period and could be related to an increase in the connective tissue, as previously proposed by authors in tenotomized (Hnik and Lessler, 1973) and shortened immobilized muscles (Gioux and Petit, 1993; Maier et al., 1972; Tardieu et al., 1982). The sensitivity of plantar cutaneous receptors, participating in the maintenance of the postural activity (Aniss et al., 1992), could also be increased after HU. Indeed, a 14-day period of hindpaw sensory deprivation decreased cutaneous thresholds and enhanced the responsiveness of rat cortical somatosensory neurons (Dupont et al., 2003). These increases in afferent inputs could explain why the EMG activity was higher on the first day of reloading than on the fourteenth day of HU.

To summarize, our data directly showed that contrary to suggestions in the literature, this study demonstrates that HU conditions cannot be considered as a model of functional deafferentation, but only as a reduction of afferent information at the beginning of the HU period.

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