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Research Report

Effects of simulated weightlessness on intramuscular hypertonic saline induced muscle nociception and spinal Fos expression in rats

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ABSTRACT

We assessed the effects of simulated weightlessness, hindlimb unloading (HU) by 7 days of tail suspension, on noxious mechanically and heat evoked spinal withdrawal reflexes and spinal Fos expression during muscle nociception elicited by intramuscular (i.m.) injection of hypertonic (HT; 5.8%) saline into gastrocnemius muscle in rats. In HU rats, i.m. HT saline-induced secondary mechanical hyperalgesia was enhanced, and secondary heat hypoalgesia was significantly delayed. After 7 days of HU, basal Fos expression in spinal L4-6 segments was bilaterally enhanced only in superficial (I–II) but not middle and deep laminae (III–VI) of the spinal dorsal horn, which finding was not influenced by tail denervation. Unilateral i.m. HT saline injection increased spinal Fos expression bilaterally in both the control rats and 7 days of HU rats. The HT saline-induced bilateral increase of spinal Fos occurred within 0.5 h and reached its peak within 1 h, after which it gradually returned to the control levels within 8 h. Spatial patterns of spinal Fos expression differed between the control group and 7 days of HU group. In superficial laminae, the HT saline-induced increases in Fos expression were higher and in the middle and deep laminae V–VI lower in the 7 days of HU than control rats. It is suggested that supraspinal mechanisms presumably underlie the effects of HU on spinally-organized nociception. Simulated weightlessness may enhance descending facilitation and weaken descending inhibition of nociception.

1. Introduction

Muscle atrophy is common in human lower limb eccentric anti-gravity muscles in long-term weightless environment of space flights (Fitts et al., 2001). Astronauts returning to the earth after long-term spaceflight often suffer from skeletal muscle weakness, fatigue, lack of coordination, and delayed-onset muscle soreness (Buchanan and Convertino, 1989; Riley et al., 1995; Ali et al., 2009). In particular, it is worth noting that the course and severity of muscle pain in astronauts is

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more serious than that in ordinary muscle pain patients (Ali et al., 2009). From physiological perspective, it is noteworthy that afferent barrage from muscles, particularly the muscle spindles, is significantly reduced during the weightlessness-induced condition (Ali et al., 2009). Others, in contrast, reported that motor impairments following long periods of limb disuse (Antonutto et al., 1998; Fitts et al., 2001) are not completely explained by changes of the peripheral musculoskeletal system, but rather depend on functional changes within the central nervous system (Liepert et al., 1995; Zanette et al., 1997). While the earlier findings indicate that weightlessness influences somatosensory and motor systems, they still leave open whether a change in the function of endogenous pain modulation system might contribute to muscle pain and hyperalgesia following spaceflight. It is expected that revealing the influence of weightlessness on the function of the pain regulation system may provide more insight, better understanding and thereby improved therapy of muscle pain and its concomitant phenomena, such as sensitization.

After neuronal excitation, Fos protein expression can be visualized (Hunt et al., 1987; Morgan and Curran, 1989). Fos expression has been validated as a neural marker to assess signaling as well as modulation of pain (Kaczmarek and Chaudhuri, 1997). Our recent study reported that intramuscular (i.m.) hypertonic (HT; 5.8%) saline-induced increases in Fos expression in the superficial layers (laminae I–II) and the deep layers (laminae V–VI) of the spinal DH are significantly lower and higher, respectively, in spinalized than intact rats. This observation provides evidence that activities of spinal nociceptive neurons in superficial and deep layers of the spinal DH may be modulated by endogenous descending facilitation and inhibition, respectively (Chen et al., 2013).

Regarding effects of weightlessness on spinal neuronal activities, it has been demonstrated that Fos expression in the spinal DH is significantly enhanced in rats subjected to HU for a period of e.g. 14 days (Langlet et al., 2001; Yang et al., 2008). Based on this evidence, it may be proposed that the endogenous descending controls are subject to significant functional changes during prolonged weightlessness. Here, this hypothesis was tested by determining nociception and its endogenous modulation by i.m. administration of HT saline in a rat model of simulated weightlessness induced by tail suspension. Nociception and its endogenous modulation induced by noxious conditioning muscle stimulation were assessed by behavioral assays and by determining immunohistochemically Fos expression in the spinal DH.

2. Results

2.1. *Variations of spinal withdrawal reflexes in i.m. HT saline-induced muscle nociception in rats with or without exposure to simulated weightlessness*

To assess effects of HU on spinally-organized nociception, variations of noxious mechanically and heat evoked spinal withdrawal reflexes during i.m. HT saline-induced muscle nociception with or without exposure to 7 days of simulated weightlessness were investigated. Fig. 1 shows the mean bilaterally determined paw withdrawal threshold to mechanical and heat stimuli evaluated 30 min prior to, and 30 min, 1–4 h, 1–7 d after the i.m. injection of HT saline into the (left) GS muscle in rats not exposed to HU and rats exposed for 7 days to HU (7 days' HU). Mechanically and heat evoked paw withdrawal reflexes in rats exposed to neither HU nor i.m. injection of HT saline during the above observation period served as controls.

Following the unilateral i.m. HT saline injection, a significant decrease of the mechanical paw withdrawal threshold was found bilaterally (i.e. secondary mechanical hyperalgesia) in rats without or with exposure to 7 days' HU ($P<0.05$, Two-way ANOVA, Fig. 1a). A significant difference in the magnitude of the mechanically-evoked withdrawal threshold decrease was observed between animals not exposed to HU and HU animals ($P<0.05$, Two-way ANOVA, Fig. 1a). Within 1 day after the i.m. injection of HT saline, the thresholds of the mechanically-evoked responses in HU rats were significantly lower than in the group of rats not exposed to HU (ipsilateral to HT saline administration: $P<0.05$; contralateral to HT saline administration: $P<0.05$) (Fig. 1a). One day after the i.m. injection of HT saline, the magnitude of mechanical hyperalgesia in 7 days' HU rats declined promptly, and the withdrawal threshold returned to the same level as in rats not exposed to HU 3 days after the i.m. HT saline injection.

In contrast to the rapid occurrence of bilateral mechanical hyperalgesia, we did not find any significant variations of noxious heat-evoked paw withdrawal reflexes during the initial 4 h following the i.m. HT saline injection in the rats not exposed to HU or HU rats ($P>0.05$, Two-way ANOVA, Fig. 1b). One day after the unilateral i.m. injection of HT saline, the latency of the heat-evoked paw withdrawal reflex in rats was significantly prolonged bilaterally, from the baseline latencies of 10.4 ± 1.0 s (ipsilaterally) and 10.4 ± 0.9 s (contralaterally) to 16.4 ± 1.1 s and 16.2 ± 0.8 s, respectively. This latency increase lasted to the end of the 7 day observation period indicating a long-lasting i.m. HT saline-induced heat hypoalgesia (Fig. 1b, $P<0.001$, Two-way ANOVA). In the 7 days' HU group, the onset of secondary heat hypoalgesia after the i.m. HT saline injection was delayed as indicated by the finding that significant heat hypoalgesia was not found until 3 days after the i.m. injection of HT saline ($P<0.05$). After that the secondary heat hypoalgesia declined promptly to the baseline level by the fifth day after the i.m. HT saline injection. In untreated control animals (without exposure to HU or HT saline), neither the mechanically evoked withdrawal threshold (Fig. 1a) nor the heat-evoked withdrawal latency (Fig. 1b) were significantly changed during 1-week observation period.

2.2. *Spinal Fos expression with or without exposure to 7 days of HU in untreated animals versus animals with S1/S2 neurectomy*

Spinal Fos expression within the lumbar spinal cord in rats with or without exposure to 7 days of HU was studied to determine spinal neuronal correlate for the behavioral findings. To exclude the influence of tail stimulation on spinal Fos expression during 7 days of HU, denervation of the tail was performed in some of the rats. Fig. 2 shows the basal Fos expression of the spinal DH in rats exposed to four experimental conditions: (i) untreated