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Hyperalgesia in an immobilized rat hindlimb: Effect of treadmill exercise using non-immobilized limbs

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HIGHLIGHTS

- Immobilization of limb induced mechanical hyperalgesia in rats.
- The hyperalgesia could be reduced by treadmill running during immobilization period.
- This effect might be based on an activation of descending pain modulatory system in the brain.

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ABSTRACT

Cast immobilization of limbs causes hyperalgesia, which is a decline of the threshold of mechanical and thermal mechanical stimuli. The immobilization-induced hyperalgesia (IIH) can disturb rehabilitation and activities of daily living in patients with orthopedic disorders. However, it is unclear what therapeutic and preventive approaches can be used to alleviate IIH. Exercise that activates the descending pain modulatory system may be effective for IIH. The purpose of this study was to investigate the effects of treadmill exercise during the immobilization period, using the non-immobilized limbs, on IIH. Thirty-six 8-week-old Wistar rats were randomly divided into (1) control, (2) immobilization (Im), and (3) immobilization and treadmill exercise (Im + Ex) groups. In the Im and Im + Ex groups, the right ankle joints of each rat were immobilized in full plantar flexion with a plaster cast for an 8-week period. In the Im + Ex group, treadmill exercise (15 m/min, 30 min/day, 5 days/week) was administered during the immobilization period while the right hindlimb was kept immobilized. Mechanical hyperalgesia was measured using von Frey filaments every week. To investigate possible activation of the descending pain modulatory system, beta-endorphin expression levels in hypothalamus and midbrain periaqueductal gray were analyzed. Although IIH clearly occurred in the Im group, the hyperalgesia was partially but significantly reduced in the Im + Ex group. Beta-endorphin, which is one of the endogenous opioids, was selectively increased in the hypothalamus and midbrain periaqueductal gray of the Im + Ex group. Our data suggest that treadmill running using the non-immobilized limbs reduces the amount of hyperalgesia induced in the immobilized limb even if it is not freed. This ameliorating effect might be due to the descending pain modulatory system being activated by upregulation of beta-endorphin in the brain.

1. Introduction

In experimental studies using animal, cast immobilization of limbs causes hyperalgesia, which is a decline of the threshold of mechanical and thermal mechanical stimuli [1,2], and limb immobilization for long durations may induce chronic pain associated with central sensitization [3]. The hyperalgesia induced by cast immobilization was also observed in humans [4]. The immobilization-induced hyperalgesia (IIH) can disturb rehabilitation and activities of daily living in patients. Cast immobilization is carried out for restoring damaged tissue and is widely used in the medical treatment of orthopedic disorders such as fracture and

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sprain. During the immobilization period, exercise is prescribed to prevent muscular disuse atrophy mainly in the non-immobilized limbs, and is not prescribed for prevention of IIH in the immobilized limb. Although exercise may alleviate IIH, the immobilized limb is not moved directly, and the mechanism by which IIH of the affected limb can be treated remains unknown.

Clinical studies suggest that exercise decreases pain symptoms and improves function in patients with chronic pain due to various diseases such as rheumatoid arthritis, fibromyalgia, and complex regional pain syndrome [5–7]. Recent studies using experimental animals have shown that beta-endorphin is involved in the mechanism by which exercise affects pain. Beta-endorphin is an endogenous opioid with analgesic properties and is distributed throughout the brain by axons emanating from the hypothalamus. Stagg et al. [8] reported that exercise training on a treadmill ameliorated thermal and tactile hyperalgesia in spinal nerve-ligated animals; the effects of exercise were reversed by systemically administered opioid receptor antagonists. Moreover, exercise increased beta-endorphin content in the rostral ventromedial medulla and in the midbrain periaqueductal gray (PAG). Thus, exercise can activate the descending pain modulatory system via upregulation of beta-endorphin in the central nerve system.

Considering the therapeutic effect of beta-endorphin on pain, one is led to the hypothesis that though the immobilized limb is not moved, the upregulation of endorphins induced by the exercise of the non-immobilized limbs may suppress IIH in the immobilized limb via activation of the descending pain modulatory system. To test this hypothesis, treadmill exercise was administered to rats in which the right hindlimb was immobilized by casting, and any resulting changes in IIH and content of endorphins in the midbrain relative to cast-treated but unexercised rats were examined.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n = 36$; 8 weeks old; Kudo Laboratories, Tokyo, Japan) were randomly divided into three groups: immobilization for 8 weeks (Im, $n = 10$); immobilization for 8 weeks and administered treadmill exercise (Im + Ex, $n = 16$); and age-matched controls (Con, $n = 10$). A flow diagram of the experimental design is presented in Fig. 1. All rats were housed in 2s or 3s in plastic cages at 22–24 °C with a 12-h light/dark cycle. Water and food were available ad libitum. All procedures received approved by the Nagasaki University Animal Care Committee (approval number: 1305201061) and complied with the recommendations of the International Association for the Study of Pain.

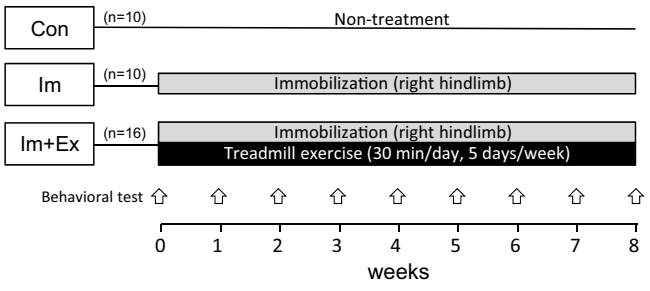

Fig. 1. Experimental design. Treadmill exercise was performed without release of immobilization of the right hindlimb; rats ran using both the forelimbs and the left hindlimb. After the last behavioral test, the rats were killed for brain tissue sampling.

2.2. Immobilization

Rats in the Im and Im + Ex groups were anesthetized with sodium pentobarbital (40 mg/kg). Subsequently, their right ankle joints were fixed in full plantar flexion by using plaster casts (ipsilateral hindlimb). Left ankle joints were not immobilized (contralateral hindlimb). The plaster cast was replaced at least every 2 or 3 days to prevent loosening, and to prevent edema in the hind paw. The period of cast immobilization was 8 weeks.

2.3. Treadmill exercise

Before the experiment, rats of the Im + Ex group were acclimated to the treadmill lanes without running (30 min/day, 1 week). During the immobilization period, rats of the Im + Ex group were administered forced treadmill running (15 m/min, 30 min/day, 5 days/week). The immobilization of the right hindlimb continued during treadmill running. Thus, these rats ran using their three non-immobilized limbs: forelimbs bilaterally and left hindlimb.

2.4. Behavioral test

All rats were tested for mechanical sensitivity before the immobilization period and once every week following application of the cast. The tests were performed using a home-made restrainer made of cloth, as described previously [1]; this technique was employed because the experimental ankle joint contracture prevented the immobilized rats from placing their right hind paws on the ground. The rats were placed individually in the restrainer after cast removal and allowed to acclimate for 20 min in a quiet room from 10:00 a.m. to 6:00 p.m.; room temperature was maintained at 22–24 °C. The glabrous skin of the hind paw was probed 10 times with 4- and 15-g von Frey filaments (VFF; North Coast Medical, Morgan Hill, CA, USA) every 10 s. Lifting or pulling back of the paw was counted as a paw withdrawal response (PWR) [9]. Where applicable, measurements were performed 23 h after exercise training. Immediately after the behavioral test, a plaster cast was again applied to rats in the immobilization groups to continue the immobilization period.

2.5. Sampling and preparation

At the end of the immobilization period, 23 h after the final period of treadmill running in the Im + Ex group, all rats were deeply anesthetized with sodium pentobarbital (40 mg/kg). Rats were transcardially perfused with saline for blood removal and the whole brain was extracted. The hypothalamus was immediately dissected from the brain and stored in a deep freezer (−80 °C) until enzyme-linked immunosorbent assaying for beta-endorphin was performed. Next, the midbrain was dissected out and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h. After fixation, the midbrains were soaked successively in 10% and 20% sucrose in 0.01 M phosphate buffer saline (PBS, pH 7.4) for 24 h. The tissues were then embedded in tragacanth gum and frozen by immersion in liquid nitrogen-cooled isopentane.

2.5.1. Enzyme-linked immunosorbent assay for beta-endorphin in hypothalamus

All rat hypothalami were homogenized in 10 mM Tris–HCl buffer (pH 7.4) at 4 °C. The homogenate was centrifuged (10,000 rpm, 10 min), and the supernatant stored at −80 °C. The level of beta-endorphin in a hypothalamus sample was examined using an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., USA, EK-022-33). The range of validity of the kit was 0–100 ng/mL of beta-endorphin. The protein content of each tissue supernatant was determined with a BCA Protein Assay Kit (Pierce, Rockford, IL,