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Short communication

Effects of glucocorticoid receptor antagonists on allodynia and hyperalgesia in mouse model of neuropathic pain

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Abstract

Injury to the spinal nerves of mice induces mechanical allodynia and thermal hyperalgesia. In the injured spinal cord, the expression of glucocorticoid receptor mRNA was increased, whereas it was decreased in N-type Ca^{2+}-channel-deficient mice, in which neuropathic pain is eliminated. Intrathecal and intraperitoneal injection of the glucocorticoid receptor antagonist RU486 produced antinociceptive effects, whereas intracerebroventricular injection was without effect. The more selective antagonist dexamethasone 21-mesylate suppressed both mechanical allodynia and thermal hyperalgesia. These results suggest that spinal glucocorticoid receptors play an important role in neuropathic pain, and that controlling the activity of glucocorticoid receptors may be of great importance in the treatment of neuropathic pain.

Keywords: Glucocorticoid receptor; Neuropathic pain; N-type calcium channel; cDNA microarray

1. Introduction

Injury to peripheral nerves induces neuropathic pain-like responses in rodents. We have previously demonstrated that mice lacking N-type voltage-dependent Ca^{2+} channels (VDCC) show markedly reduced symptoms of neuropathic pain-related behavior induced by spinal nerve injury (Saegusa et al., 2001), suggesting a critical role of N-type VDCC in the development of neuropathic pain. Although several studies have demonstrated that blockade of this channel is effective against neuropathic pain in rodents (Bowersox et al., 1996; Scott et al., 2002), it has been reported that clinical application of an N-type VDCC blocker is limited by its serious side effects (Penn and Paice, 2000). Therefore, it is desirable to develop a new drug that alleviates neuropathic pain without having severe side effects.

To search for new molecules involved in the signaling cascade from N-type VDCC to neuropathic pain, we investigated the genes that are differentially expressed in the spinal cord following nerve injury using microarray techniques and compared the gene expression profiles with those of N-type VDCC-deficient mice. Our present study of cDNA microarray analysis identifies the glucocorticoid receptor as a candidate target for the management of neuropathic pain. Therefore, we examined the effects of glucocorticoid receptor antagonists on neuropathic pain-like responses in mice.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (Clea Japan, Inc., Tokyo, Japan) and N-type VDCC-deficient mice (Saegusa et al., 2001) weighing 22–26 g were used. They were housed three per cage under controlled temperature (22 ± 1 °C) and humidity (55 ± 10%) with a 12-h light/dark cycle with food and water freely available. All behavioral experiments were performed in a sound-proof room during the light cycle (7:00 AM–7: 00 PM). Experiments were conducted with the approval of the Animal Care Committee of Tokyo Medical and Dental University, and according to the guidelines for investigations of experimental pain in animals published by the International Association for the Study of Pain (Zimmermann, 1983).

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2.2. Animal model of spinal nerve injury

Spinal nerve ligation (SNL) was carried out as described previously (Kim and Chung, 1992; Saegusa et al., 2001). Briefly, under sodium pentobarbital anesthesia, the right L5 and L6 spinal nerves were exposed by removing a small piece of the paravertebral muscles and a part of the right spinous process of the L5 lumbar vertebra. The L5 and L6 spinal nerves were then carefully isolated and tightly ligated with 8-0 silk thread. After nerve ligation, the muscle, the adjacent fascia and the skin were closed with sutures.

2.3. cDNA microarray analysis

Two weeks after spinal nerve injury or sham operation of wild-type (WT) and N-type VDCC-deficient mice (KO), L5/6 spinal cords were dissected and collected for RNA preparation. cDNA microarray analysis was performed using the CodeLink™ UniSet Mouse 10K I (Amersham Biosciences, NJ) following the protocol provided by the manufacturer. Four data sets (Sham-WT, SNL-WT, Sham-KO and SNL-KO) were compared using the CodeLink™ System Software.

2.4. Behavioral studies

Behavioral studies were conducted 2–3 weeks after spinal nerve injury. To assess mechanical allodynia, paw withdrawal thresholds were measured using the Dynamic Plantar Aesthesiometer (Ugo Basile, Italy). Mice were placed individually in plastic cages with a wire mesh bottom and allowed to acclimatize for at least 2 h. Increasing mechanical stimulation (0.25 g/s, cut-off force: 5 g) was applied to the plantar surface of a hind paw. In each test session, each mouse was tested in three sequential trials with an interval of 2–3 min. When a withdrawal response occurred, the stimulus was terminated and the response threshold was measured electronically. Paw withdrawal threshold was calculated as the mean of the threshold of three trials.

Thermal hyperalgesia was evaluated using the Paw Thermal Stimulator (UCSD, San Diego, CA). Mice were placed individually in plexiglass cubicles mounted on a glass surface maintained at 30 °C and allowed to acclimatize for at least 2 h. A thermal stimulus was then applied to the plantar surface of each hind paw. In each test session, each mouse was tested in three sequential trials with an interval of 2–3 min. Paw withdrawal latencies were calculated as the mean of the latencies of three trials. An assay cut-off was set at 20.5 s to avoid tissue damage.

2.5. Intrathecal and intracerebroventricular injections

Intrathecal injection was given in a volume of 5 μl by percutaneous puncture through an intervertebral space at the level of the 5th or 6th lumbar vertebra, according to a previously reported procedure (Hylden and Wilcox, 1980; Yokoyama et al., 2004) using a 25-μl Hamilton microsyringe with a 30-gauge needle. The mouse was not anesthetized during the i.t. injection.

Intracerebroventricular administration was performed as described previously (Haley and McCormick, 1957; Yokoyama et al., 2004). In brief, mice were anesthetized with ether and a 27-gauge needle attached to a microsyringe was inserted into the lateral ventricle. The volume for i.c.v. injection was 5 μl per mouse.

2.6. Agents

RU486 (mifepristone, 11β-(4-Dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) (Sigma, St. Louis, MO) was dissolved in physiological saline and was administered intraperitoneally, intrathecally or intracerebroventricularly. Dexamethasone 21-mesylate (Steraloids Inc., Newport, RI) was dissolved in physiological saline and was administered intrathecally. The effects of these agents on mechanical allodynia and thermal hyperalgesia were tested 2 weeks after nerve ligation.

2.7. Statistical analysis

Experimental data are expressed as means±S.E.M. and analyzed using one-way analysis of variance and post hoc Dunnett's tests. A P value less than 0.05 was considered statistically significant.

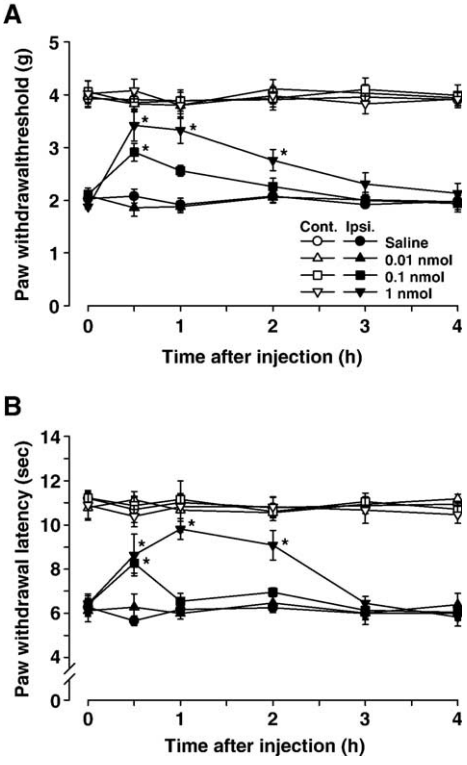

Fig. 1. The effects of intrathecal injection of RU486 on (A) mechanical allodynia and (B) thermal hyperalgesia in a mouse model of neuropathic pain. Paw withdrawal threshold to mechanical stimulation and paw withdrawal latency to thermal stimuli are plotted against the time after intrathecal injection of RU486. The data are presented as means and S.E.M. for 6–7 mice. *P<0.05 when compared with pre-drug (at 0 h) data.

3. Results

3.1. cDNA microarray analysis

Using cDNA microarray techniques, we found that the level of expression of 934 genes out of 10,000 genes in the spinal cord was increased more than 1.2-fold by spinal nerve injury as compared with the level in sham-operated mice. Most of these 934 genes were also up-regulated in the spinal cord from nerve-injured N-type VDCC-deficient mice, but the expression of 70 genes was reduced more than 1.2-fold compared with that in sham-operated N-type VDCC-deficient mice. The glucocorticoid receptor was one of these genes: its expression was modestly increased by 1.6-fold by nerve injury in wild-type mice but was down-regulated by 1.7-fold in N-type VDCC-deficient mice.

3.2. Effects of glucocorticoid receptor antagonist

Intrathecal injection of the glucocorticoid receptor antagonist RU486 (0.01–1 nmol) produced a significant, dose-dependent inhibition of mechanical allodynia and thermal hyperalgesia induced by spinal nerve injury (Fig. 1A, B). RU486 did not change mechanical and thermal nociceptive thresholds of the contralateral hind paw (Fig. 1A, B) and did not induce any aberrant behaviors (such as decrease or increase of locomotor activity) at the doses tested. Intraperitoneal injection of RU486 (0.3–30 mg/kg) dose dependently alleviated both mechanical allodynia and thermal hyperalgesia (Fig. 2A, B). In contrast, intracerebroventricular injection of RU486 (1 nmol) had no effect on mechanical allodynia and thermal hyperalgesia (Fig. 2C, D). Intrathecal injection of dexamethasone 21-mesylate (1 nmol), a more selective glucocorticoid receptor antagonist, inhibited mechanical allodynia and thermal hyperalgesia (Fig. 2E, F). No abnormal behaviors, including increase or decrease of locomotor activity, were observed after local injection of these agents.

4. Discussion

We previously demonstrated that the neuropathic pain-like behavior induced by spinal nerve injury is eliminated in mice lacking N-type VDCC (Saegusa et al., 2001). Using cDNA microarray techniques, we found that the level of expression of several genes in the spinal cord is increased by nerve injury. The glucocorticoid receptor gene is one of the genes whose expression level was modestly increased in the wild-type spinal cord. It is of particular interest that the glucocorticoid receptor is down-regulated in the spinal cord of N-type VDCC-deficient mice, in which neuropathic pain-like behaviors are eliminated (Saegusa et al., 2001). These results suggest that the glucocorticoid receptor is located downstream in the signal transduction mechanism governed by the N-type VDCC at a spinal level and that spinal glucocorticoid receptors contribute to the induction of neuropathic pain.

Glucocorticoid receptors play crucial roles in the anti-inflammatory effects of glucocorticoids in the periphery (Barnes, 1998). Glucocorticoid receptors are widely expressed

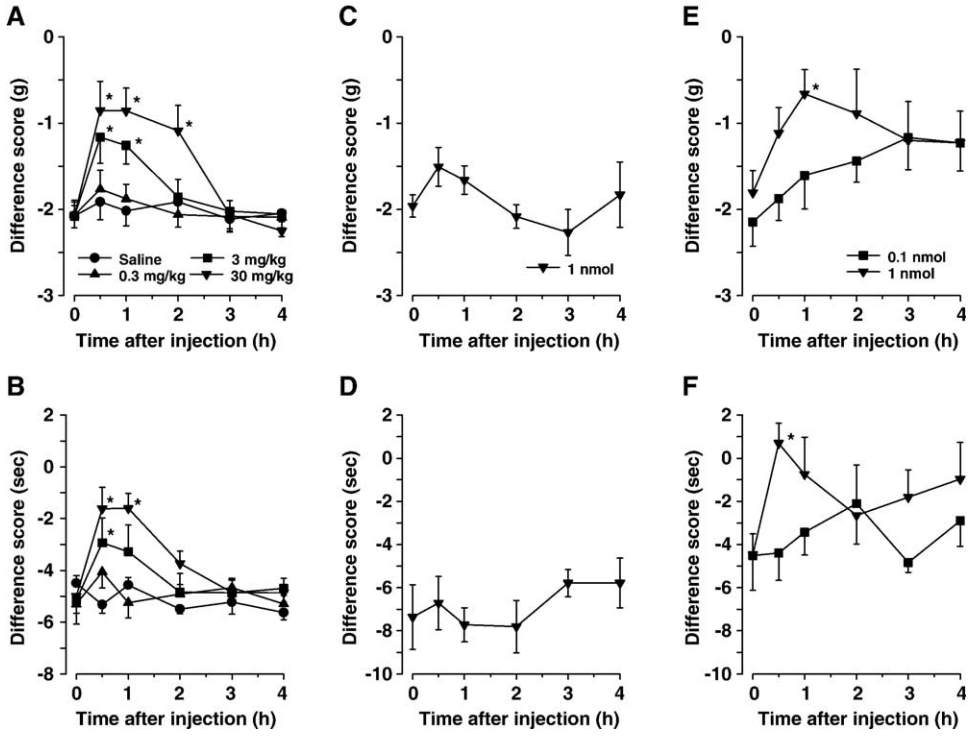

Fig. 2. The effects of (A, B) intraperitoneal and (C, D) intracerebroventricular injection of RU486, and (E, F) intrathecal dexamethasone 21-mesylate on mechanical allodynia and thermal hyperalgesia. Time course of the difference in paw withdrawal threshold (upper panels) and paw withdrawal latency (lower panels) for the ipsilateral versus the contralateral hind paws (i.e., the difference score) after injection of the agents. The data are presented as means and S.E.M. for 6–7 mice. *$P<0.05$ when compared with pre-drug (at 0 h) data.

in the central nervous system. In the spinal cord, glucocorticoid receptors are located in dorsal horn neurons, the region involved in the control of pain transmission (Cintra et al., 1993). However, the contribution of glucocorticoid receptors to neuropathic pain remains unknown. In the present study, intrathecal injection of the glucocorticoid receptor antagonists RU486 and dexamethasone 21-mesylate produced a potent inhibition of the mechanical allodynia and thermal hyperalgesia induced by spinal nerve injury in mice. Our results strongly suggest that the glucocorticoid receptor plays an important role in the maintenance of neuropathic pain.

Our present results are consistent with recent reports that repeated and single administration of RU486 alleviates mechanical allodynia and thermal hyperalgesia in rat models of chronic constriction nerve injury and spinal nerve injury (Wang et al., 2004, 2005). Although RU486 is known to have an antagonistic action at the glucocorticoid receptor, it also acts as an antiprogesterone (Mao et al., 1992). In the present study, intrathecal injection of dexamethasone 21-mesylate, a more selective glucocorticoid receptor antagonist, inhibited neuropathic pain-related responses. Although we cannot deny the possible contribution of the antiprogesterone effect of RU486 to antinociception, RU486 is likely to exert its antinociceptive effects via an antagonistic action at glucocorticoid receptors in the spinal cord.

In the present study, systemic (intraperitoneal) administration of RU486 also suppressed mechanical allodynia and thermal hyperalgesia. Although intrathecal injection of RU486 alleviated neuropathic pain-like responses, intracerebroventricular injection failed to inhibit them. These results suggest that the antinociceptive effects of RU486 administered systemically are mediated mainly by actions on the spinal cord, but not on the brain. Indeed, spinal nerve (L5 and L6) injury did not affect the level of expression of glucocorticoid receptor mRNA in the brain (our unpublished data).

The molecular details of the contribution of glucocorticoid receptors to neuropathic pain remain unclear. However, some insights can be gained from the following findings. It has been reported that plasma corticosterone levels increase after peripheral nerve injury, and that nerve injury-induced neuropathic pain-like behaviors are eliminated in adrenalectomized rats (Wang et al., 2004). Corticosterone prolongs the NMDA receptor-mediated Ca^{2+} elevation in hippocampal neurons (Takahashi et al., 2002). Considering these findings, we speculate that prolonged Ca^{2+} elevation in the spinal dorsal horn neurons, which is induced by the increased level of corticosterone, may contribute to the induction of neuropathic pain-like behaviors.

Although the molecular details of the contribution of glucocorticoid receptors to neuropathic pain and how the expression of this receptor is regulated by N-type VDCC remain unclear, glucocorticoid receptors in the spinal cord may be a useful target in the management of neuropathic pain. Thus, it may be worth testing the potency of glucocorticoid receptor antagonists to inhibit pain in patients with neuropathic pain.

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