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Research article

Pregabalin induces conditioned place preference in the rat during the early, but not late, stage of neuropathic pain

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ABSTRACT

The present study aimed to examine the rewarding effects of pain relief during the early and late stages of neuropathic pain using a conditioned place preference (CPP) test. Animal models of neuropathic pain were prepared by spinal nerve ligation in male Sprague–Dawley rats. Intraperitoneal and intrathecal injections of pregabalin (300 mg/kg and 100 μg/10 μL, respectively) suppressed allodynia in the von Frey test both 2 weeks (early stage) and 4 weeks (late stage) after nerve injury. Intraperitoneal and intrathecal injections of pregabalin induced CPP during the early stage of neuropathic pain, suggesting that the CPP test serves as an objective and quantifiable behavioral assay to assess the emotional aspect of pain relief. In contrast with the early stage of neuropathic pain, intraperitoneal or intrathecal injection of pregabalin did not induce CPP during the late stage of neuropathic pain. The extinguishment of the rewarding effects of pregabalin during the late stage of neuropathic pain is likely due to dysfunction of the mesolimbic reward system, although the possibility that neuronal mechanisms other than dysfunction of the mesolimbic reward system are involved in the extinguishment of pregabalin-induced CPP cannot be excluded. We previously reported that not only the dopamine release in the nucleus accumbens induced by intrathecal pregabalin injection but also that induced by sucrose intake were extinguished during the late stage of neuropathic pain. These findings, combined with the results of this study, suggest that pain chronification leads to dysfunction of the mesolimbic reward system.

1. Introduction

Comorbidity of chronic pain and depression has been recognized in the clinic [1,2], and several preclinical studies have reported depression-like behaviors in animal models of chronic pain [3]. Dopaminergic (DAergic) neurotransmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) plays important roles in the mesolimbic reward circuit. Both clinical [4,5] and preclinical studies [6] have suggested dysfunction of this neurotransmission in patients with major depressive disorders and in animal models of depression. Dysfunction of the mesolimbic DAergic pathway has also been implicated in chronic pain. Dopamine (DA) release in the NAc induced by rewarding drugs, such as morphine [7,8] and cocaine [8], was suppressed in animal models of neuropathic pain. These findings suggest that suppression of DAergic neurotransmission in the mesolimbic reward circuit may be a common neuroplastic change underlying chronic pain and depression. Our research group reported that pain relief by pregabalin induced intra-NAc DA release during the early, but not late, stage of neuropathic pain [9], suggesting that dysfunction of the mesolimbic DAergic system progresses in a time-dependent manner during chronic pain. However, the influence of such time-dependent dysfunction of the mesolimbic DAergic system on the rewarding effects of pain relief by pregabalin remains to be investigated. While a conditioned place preference (CPP) test has been widely used to assess the rewarding effects of abused drugs, such as morphine, cocaine, and ethanol [10,11], Porreca and colleagues reported that this behavioral test could be used to study the rewarding effects of pain relief in animal models of neuropathic [12,13] and post-surgical [14,15] pain. Thus, the present study aimed to examine the rewarding effects of pain relief by pregabalin during the early and late stages of neuropathic pain using the CPP test.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (Japan SLC, Hamamatsu, Japan) (240–300 g) were housed in a room with constant ambient temperature (23 ± 1 °C) under a 12 h light/dark cycle with food and water available *ad libitum*.

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All experiments were performed with the approval of the Hokkaido University Institutional Animal Care and Use Committee.

2.2. Drugs

Pregabalin (CAS number: 148553-50-8) was purchased from Carbosynth Ltd. (Compton, UK). Lidocaine hydrochloride monohydrate was purchased from Sigma Chemical Co. (St Louis, MO, USA). Drugs were dissolved in saline for intraperitoneal (i.p.) injection or in phosphate-buffered saline (PBS) for intrathecal (i.t.) injection.

2.3. Spinal nerve ligation

Neuropathic pain model animals were prepared by spinal nerve ligation (SNL) according to the methods of Li et al. [16] with slight modifications. Briefly, under pentobarbital anesthesia (50 mg/kg, i.p.), the left lumbar fifth spinal nerve (L5) was tightly ligated using a 6-0 silk suture and was cut distal to the ligature. Sham-operated control rats underwent an identical surgical procedure, but the spinal nerves were not ligated or cut. To assess tactile allodynia, a von Frey test was conducted. The rats were individually confined in wire-mesh cages, and after a habituation period of at least 30 min, calibrated von Frey filaments (0.4–15 g) were applied to the plantar surface of the ipsilateral hind paw. The 50% paw withdrawal threshold was determined using the up-down method [17]. The tests were conducted 1 day before and every 7 days after SNL surgery. Rats that showed motor impairment after surgery or did not show tactile allodynia were not used for further experiments. Four animals were excluded from the following procedures due to these exclusion criteria.

2.4. Intrathecal cannulation

Under isoflurane (2%) anesthesia, a PFA microtube (inner diameter [i.d.]: 0.1 mm, outer diameter [o.d.]: 0.3 mm; As One, Osaka, Japan) connected to a polyethylene catheter (SP10; i.d.: 0.28 mm, o.d.: 0.61 mm; Natsume Seisakusho, Tokyo, Japan) was inserted through the atlantooccipital membrane to the lumbar enlargement of the spinal cord (close to the L4–L5 segments) [18]. Rats that experienced paralysis of their hind paws after surgery were excluded from the following experiments. Catheter placement was verified by the observation of hind limb paralysis induced by i.t. administration of lidocaine (2%, 10 μL) through the catheter. Rats exhibiting lidocaine-induced transient paralysis of their hind paws were used for the following experiments. Four animals were excluded from the following procedures due to the paralysis of their hind paws or incorrect implantation of the i.t. catheter.

2.5. CPP

CPP tests were conducted as described previously [19,20]. The CPP chambers consisted of two equal-sized compartments (30 × 30 × 30 cm) with distinct tactile and visual cues (one compartment had a black floor and walls with an equally spaced stainless steel stripe-like grid on the floor, and the other had a white floor and walls with a stainless steel grid on the floor), which were separated by a removable partition. The CPP chambers were set in sound-attenuating boxes equipped with a ventilating fan (Muromachi Kikai, Tokyo, Japan). On days 1 (habituation) and 2 (pre-conditioning test session), rats freely explored the two compartments for 900 s, and the time spent in each compartment during the exploratory period was measured using infrared sensors (Supermex; Muromachi Kikai) positioned on the top cover of each compartment. Rats that spent > 80% (> 720 s) of the total time (900 s) in one side on day 2 or showed a difference of > 200 s in the time spent in one side between days 1 and 2 were eliminated from subsequent procedures. Nine animals were excluded from the statistical analyses due to these exclusion criteria. We used a biased design for conditioning [10] and designated the compartment in which each rat spent less time on day 2 (pre-conditioning test session) as their drug-paired compartment. The conditioning session was conducted during six consecutive days (days 4–9). In the first series of experiments, rats were given vehicle (saline, i.p.) and confined to the non-drug-paired compartment during a 60-min period that began 60 min after i.p. injection on days 4, 6, and 8, and given pregabalin (300 mg/kg, i.p.), and confined to the drug-paired compartment during the same period on days 5, 7 and 9. In the second series of experiments, rats were given vehicle (PBS, i.t.) and confined to the non-drug-paired compartment during a 90 min period that began 30 min after i.t. injection on days 4, 6, and 8, and given pregabalin (100 μg/10 μL, i.t.), and confined to the drug-paired compartment during the same period on days 5, 7, and 9. On day 11 (post-conditioning test session), rats were allowed to explore the two compartments freely for 900 s, and the time spent in each compartment was measured. The CPP scores were calculated by subtracting the time spent in the drug-paired compartment during the pre-conditioning test session from the time spent in the same compartment during the post-conditioning test session.

2.6. Statistical analyses

All data are expressed as the means ± standard error of the mean. Statistical analyses were performed using GraphPad Prism® v. 6.00 (GraphPad Software, San Diego, CA, USA). Data from the von Frey tests were analyzed using one-way repeated measures analysis of variance followed by Sidak's multiple comparisons *post hoc* test. Paired *t*-tests were conducted to compare the time spent in the drug-paired compartment between the pre-conditioning and post-conditioning test sessions in the CPP test. Two-tailed unpaired *t*-tests were conducted to compare the CPP scores between the SNL and sham groups. Differences with $p < 0.05$ were considered significant.

3. Results

The experimental schedules are shown in Fig. 1. CPP tests were conducted during the early (Fig. 1A) and late (Fig. 1B) stages of neuropathic pain. Pain relief by pregabalin was used as a reward stimulus in the conditioning session.

In the first series of experiments, we examined the effects of i.p. administration of pregabalin on CPP during the early and late stages of neuropathic pain. Using a von Frey test, we determined the time course of the analgesic effects of i.p. injection of pregabalin (300 mg/kg) (Fig. 2A and D). In both stages of neuropathic pain, significant analgesic effects were observed at 60 min after i.p. injection and lasted until at least 120 min. The i.p. injection of pregabalin did not affect the pain threshold in the sham group. Thus, in the CPP test, the duration of conditioning was set to 60 min, which began 60 min after injection. During the early stage of neuropathic pain, the time spent in the drug-paired compartment was increased in the post-conditioning (post) session compared to that in the pre-conditioning (pre) session (SNL [pre]: 320.6 ± 15.2 s; SNL [post]: 576.9 ± 20.3 s; $t = 12.36$, $df = 14$, $p < 0.0001$, $n = 15$) (Fig. 2B). In the sham group, there was no significant difference in the time spent in the drug-paired compartment between the pre and post sessions (sham [pre]: 315.0 ± 13.0 s; sham [post]: 354.8 ± 31.0 s; $t = 1.275$, $df = 14$, $p = 0.223$, $n = 15$). The CPP score of the SNL group was significantly larger than that of the sham group (SNL: 256.3 ± 20.7 s; sham: 39.8 ± 31.2 s; $t = 5.783$, $df = 28$, $p < 0.0001$) (Fig. 2C). In contrast with the early stage of neuropathic pain, during the late stage, i.p. injection of pregabalin did not affect the time spent in the drug-paired compartment in the SNL group (SNL [pre]: 354.2 ± 16.1 s; SNL [post]: 414.0 ± 42.8 s; $t = 1.541$, $df = 11$, $p = 0.1516$, $n = 12$) or in the sham group (sham [pre]: 325.4 ± 35.5 s; sham [post]: 396.4 ± 32.1 s; $t = 1.828$, $df = 10$, $p = 0.0975$, $n = 11$) (Fig. 2E). There was no significant difference in the CPP score between the SNL (60.0 ± 41.8 s) and sham (71.0 ± 58.6 s) groups ($t = 0.2038$, $df = 21$, $p = 0.8405$) (Fig. 2F).