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Short communication

mGlu5 receptor and protein kinase C implicated in the development and induction of neuropathic pain following chronic ethanol consumption

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Abstract

The central mechanisms of neuropathic pain following chronic ethanol consumption are poorly understood. We previously reported that the levels of metabotropic glutamate 5 (mGlu5) receptor and phosphorylated-protein kinase C (PKC) were significantly increased in the spinal cord following chronic ethanol consumption. The aim of this study was to investigate whether mGlu5 receptor and PKC inhibitors directly attenuate the neuropathic pain-like state induced by chronic ethanol treatment in rats. A significant decrease in the mechanical nociceptive threshold was observed 5 weeks of chronic ethanol consumption. This hyperalgesia was significantly attenuated by repeated i.p. injection of (*S*)-2,6-diamino-*N*-[[1-(oxotridecyl)-2-piperidinyl]methyl] hexanamide dihydrochloride (NPC15437), a selective PKC inhibitor, once a day for a week after 4 weeks of ethanol treatment. Furthermore, this hyperalgesia was also significantly attenuated by repeated i.p. injection of 6-methyl-2-[phenylethynyl]-pyridine (MPEP), a selective mGlu5 receptor inhibitor, once a day for a week after 4 weeks of ethanol treatment. Furthermore, the hyperalgesia that developed after 5 weeks of ethanol treatment was significantly suppressed by a single i.p. post-injection with either NPC15437 or MPEP. These findings constitute direct evidence that spinal mGlu5 receptor and PKC play substantial roles in the development and maintenance of an ethanol-dependent neuropathic pain-like state in rats.

Keywords: Alcoholic neuropathy; Ethanol; mGlu5 receptor; PKC; Glutamate

1. Introduction

Alcoholic neuropathy has been thought to be a disorder that involves decreased nerve function caused by damage that results from long-term excessive consumption of alcohol. Although neuropathy is frequently seen among chronic alcoholics, there are few animal models of alcoholic neuropathy. We recently established a model of neuropathic pain following chronic ethanol consumption (Miyoshi et al., 2006; Narita et al., 2007a, b,c), and found that the levels of metabotropic glutamate 5 (mGlu5) receptor and phosphorylated-protein kinase C (P-PKC) were significantly increased in the spinal cord following chronic ethanol consumption (Miyoshi et al., 2006; Narita et al., 2007c). Additionally, an increased phosphorylation of NR2B subunit containing NMDA receptor was clearly observed in the spinal cord following chronic ethanol consumption (Narita et al., 2007c). However, there is still no clear direct evidence that mGlu5 receptor and PKC are involved in the development and/or maintenance of neuropathic pain following ethanol consumption.

In the spinal cord, glutamate mediates the transmission of sensory information. Recent behavioral and electrophysiological studies have shown that the administration of selective mGlu1 and mGlu5 receptor agonists enhances behavioral responses to noxious stimulation and induces activity in dorsal horn neurons (Fisher and Coderre, 1996; Neugebauer et al., 1999). Furthermore, metabotropic glutamate (mGlu) receptors have been shown to play a role in synaptic plasticity in the CNS (Conn and Pin, 1997). Accumulating evidence suggests that mGlu receptors play a pivotal role in nociceptive processing, inflammatory pain and hyperalgesia (Meller et al., 1993, 1996; Young et al., 1997; Dolan and Nolan, 2000, 2002). It has also been recognized that the up-regulation of protein kinase C activity in the spinal cord is the key factor for the induction of thermal hyperalgesia following nerve ligation. Furthermore,

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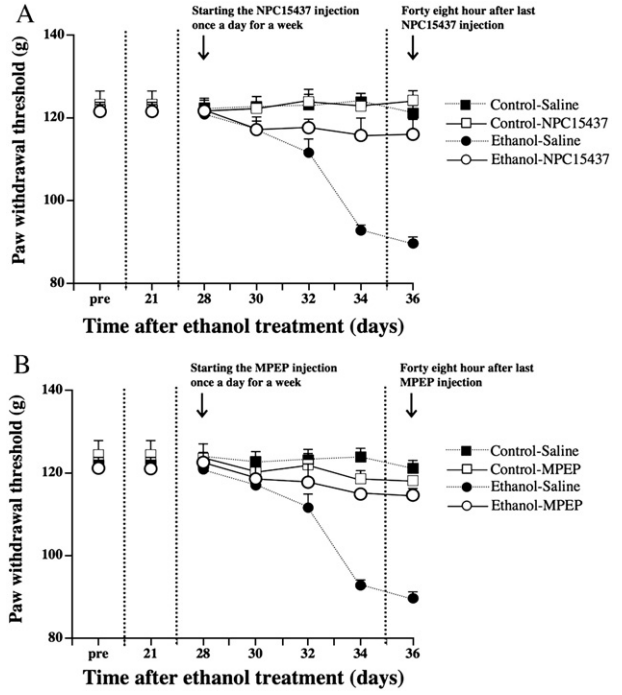

Fig. 1. Effects of NPC15437 and MPEP on the development of mechanical hyperalgesia following chronic ethanol consumption. Rats were treated with ethanol (0–5 w/v% in a liquid diet) for 5 weeks (see Materials and methods). After 4 weeks of ethanol, i.p. treatment with NPC15437 (2 mg/kg) (A), MPEP (2 mg/kg) (B) or saline was started once a day for a week. Four weeks after ethanol treatment was started, an analgesic assay was performed every other day in both ethanol-treated and control rats. The paw-withdrawal threshold was defined as the mean of the measurements. Each test was performed just before drug injection. Each point represents the mean ± S.E.M. of 6–10 rats. Ethanol-saline vs. ethanol-NPC15437, F (1, 70) = 19.9, P < 0.001. Ethanol-saline vs. ethanol-MPEP, F (1, 70) = 22.775, P < 0.001.

mice that lack the PKCγ isoform gene exhibit only a very modest expression of mechanical and thermal hyperalgesia after nerve injury (Malmberg et al., 1997; Ohsawa et al., 2001).

The goal of the present study was to seek direct evidence that mGlu5 receptor and PKC are involved in the development and/or maintenance of neuropathic pain following ethanol consumption.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Every effort was made to minimize the number and any suffering of animals used in the following experiments.

Male Fischer 344 rats (250–300 g) were obtained from Charles River Japan (Atsugi, Kanagawa, Japan). The animals were housed at a room temperature of 23 ± 1 °C with a 12-hr light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were available ad libitum.

Fischer 344 rats were repeatedly treated with a liquid diet containing ethanol. Using an escalating ethanol dosage schedule, the rats were gradually introduced to an ethanol diet as follows: days 1–3: 2.5 w/v%; days 4–6: 4 w/v%; days 7–16: 4.5 w/v% and days 17–35: 5 w/v% ethanol diet. Every 24 h, the amount of diet consumed was measured and replaced with fresh ethanol-containing or control liquid diet. Pair-fed control rats were given the same volume of ethanol-free liquid diet with isocaloric quantities of sucrose substituted for ethanol.

Four weeks after ethanol treatment was started, (*S*)-2,6-diamino-*N*-[[1-(oxotridecyl)-2-piperidinyl]methyl]hexanamide dihydrochloride (NPC15437) or 6-methyl-2-[phenylethynyl]-pyridine (MPEP) was injected i.p. daily for 7 days. MPEP and NPC15437 (Sigma-Aldrich, Inc., MO, USA) were dissolved in saline. Drugs were administered in a volume of 1.0 ml/kg.

The nociceptive flexion reflex was quantified using the Randall–Selitto paw pressure device (Muromachi Kikai, Tokyo, Japan), which applies a linearly increasing mechanical force to the dorsum of the rat hindpaw (Taiwo et al., 1989). The mechanical nociceptive threshold was defined as the force in grams at which the rat withdrew its paw. Briefly, the training procedure consisted of repeated paw-withdrawal tests at 5-min intervals for 1 h per day. The paw-withdrawal threshold was defined as the mean of the measurements. Paw-withdrawal testing was performed for both ethanol-treated rats and control rats once every two days just before drug injection, and each group was tested on the same day. After drug injection was

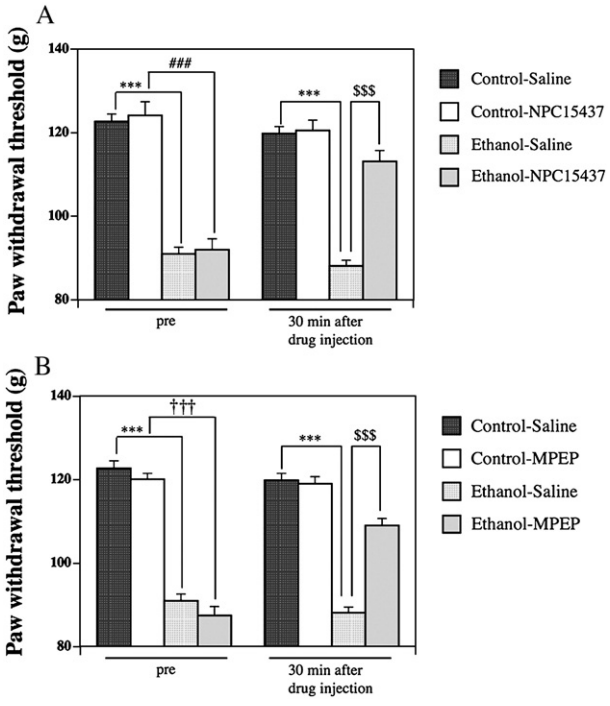

Fig. 2. Effects of NPC15437 and MPEP on the maintenance of mechanical hyperalgesia following chronic ethanol consumption. Rats were treated with ethanol (0–5 w/v% in a liquid diet) for 5 weeks (see Materials and methods). After ethanol treatment for 5 weeks, NPC15437 (2 mg/kg) (A), MPEP (2 mg/kg) (B) or saline was injected i.p. Thirty minutes after drug injection, the paw-withdrawal threshold was measured. The paw-withdrawal threshold was defined as the mean of the measurements. Each point represents the mean ± S.E.M. of 5–12 rats. ***P < 0.001 vs. control-saline. $^{###}P$ < 0.001 vs. control-NPC15437. $^{†††}P$ < 0.001 vs. control-MPEP. $^{$$$}P$ < 0.001 vs. ethanol-saline.

started, testing was performed once every two days in both ethanol-treated and control rats.

All data are presented as the mean±S.E.M. The statistical significance of differences between groups was assessed by two-way analysis of variance (ANOVA) or one-way ANOVA followed by the Bonferroni/Dunn multiple comparison test.

3. Results

Before administration of the liquid diet, the paw-withdrawal threshold was not significantly different between the control and ethanol-diet group. A significant decrease in the mechanical nociceptive threshold was observed at 5 weeks of chronic ethanol consumption. Rats were administered NPC15437 i.p. at 2 mg/kg once a day for 7 days starting 4 weeks after ethanol treatment. The hyperalgesia due to chronic ethanol treatment was significantly reduced by repeated administration of NPC15437 after 5 weeks of chronic ethanol consumption ($P<0.001$. Ethanol-saline vs. ethanol-NPC15437, F (1, 70)= 19.9, Fig. 1A).

Rats were administered MPEP i.p. at 2 mg/kg once a day for 7 days starting 4 weeks after ethanol treatment. The hyperalgesia induced by chronic ethanol treatment was significantly reduced by repeated administration of MPEP after 5 weeks of chronic ethanol consumption ($P<0.001$. Ethanol-saline vs. ethanol-MPEP, F (1, 70)=22.775, Fig. 1B).

Five weeks after ethanol treatment, rats were administered NPC15437 i.p. at 2 mg/kg. The hyperalgesia induced by chronic ethanol treatment was significantly reduced by a single i.p. administration of NPC15437 ($P<0.001$ vs. ethanol-saline, Fig. 2A).

Five weeks after ethanol treatment, rats were administered MPEP i.p. at 2 mg/kg. The hyperalgesia induced by chronic ethanol treatment was significantly reduced by a single i.p. administration of MPEP ($P<0.001$ vs. ethanol-saline, Fig. 2B).

4. Discussion

In the present study, ethanol-induced hyperalgesia was clearly observed after 5 weeks of chronic ethanol consumption. Under these conditions, the hyperalgesia induced by chronic ethanol treatment was significantly reduced by repeated i.p. administration of MPEP, a selective mGlu5 receptor inhibitor, once a day for 7 days starting 4 weeks after ethanol treatment. The mGlu5 receptor has been shown to modulate inflammatory pain at the dorsal horn of the spinal cord via activation of its second messenger and protein kinases (Coderre, 1992; Fisher and Coderre, 1996; Young et al., 1997; Karim et al., 2001). It has been well documented that activation of the mGlu5 receptor results in phospholipase C-catalyzed phosphoinositide hydrolysis, which leads to the release of Ca^{2+} from intracellular sources and the stimulation of PKC (Kawabata et al., 1998). Additionally, i.t. administration of the group I mGlu receptor agonist DHPG leads to spontaneous nociceptive behaviors, such as biting and licking, as well as thermal hyperalgesia and tactile allodynia (Fisher and Coderre, 1996; Karim et al., 2001; Dolan and Nolan, 2002). Interestingly, our recent study showed that mGlu5 receptor-like immunoreactivity was dramatically increased in the superficial spinal dorsal horn following chronic ethanol consumption (Miyoshi et al., 2006). Taken together, these findings strongly support the idea that mGlu5 receptor in the spinal cord is increased and activated under the neuropathic pain-like state following chronic ethanol consumption and the activated mGlu5 receptor directly contributes to the development of the neuropathic pain-like state following chronic ethanol consumption.

We also investigated whether the hyperalgesia induced by chronic ethanol treatment was significantly reduced by repeated i.p. administration of NPC15437, a selective PKC inhibitor, once a day for 7 days starting 4 weeks after ethanol treatment. A substantial body of evidence supports the notion that PKC expressed in dorsal horn neurons plays a role in regulating pain hypersensitivity in several different models of neuropathic pain (Mao et al., 1992; Malmberg et al., 1997; Ohsawa et al., 2000, 2001). Consistent with those reports, we previously found that mice with nerve ligation showed a spinal PKC-dependent neuropathic pain-like behavior (Ohsawa et al., 2000; Yajima et al., 2003) and rats that had been subjected to chronic ethanol consumption exhibited a long-lasting hyperalgesia associated with activated spinal PKC (Narita et al., 2007c). Interestingly, direct activation of spinal PKC by a single intrathecal (i.t.) injection of the specific PKC activator phorbol 12,13-dibutyrate caused a persistent thermal hyperalgesia in normal mice (Narita et al., 2004; Oe et al., 2004). The present data strongly support the idea that PKC in the spinal cord is phosphorylated and activated under the neuropathic pain-like state following chronic ethanol consumption and this activated PKC directly contributes to the development of the neuropathic pain-like state following chronic ethanol consumption.

Our recent study suggested that the NR2B subunit containing NMDA receptor exhibited increased phosphorylation following chronic ethanol consumption (Narita et al., 2007c). Furthermore, we also reported that post-treatment with ifenprodil dramatically reduced the hyperalgesia induced by chronic ethanol consumption (Narita et al., 2007c). It has been well documented that activation of mGlu5 receptor results in phospholipase C-catalyzed phosphoinositide hydrolysis, which leads to the release of Ca^{2+} from intracellular sources and the stimulation of PKC (Kawabata et al., 1998). Moreover, it has been reported that Ser1303 in the C terminus of NR2B-Rs can be directly phosphorylated and activated by PKC (Chen and Huang, 1992; Liao et al., 2001). The present results constitute direct evidence that the mGlu5 receptor and PKC are involved in the development of neuropathic pain following chronic ethanol consumption. These results suggest that NR2B subunit phosphorylation mediated by mGlu5 receptor-dependent PKC activation in the rat spinal cord following chronic ethanol consumption may contribute to ethanol-induced neuropathic pain-like behavior in rats.

Similar to the suppression of the development of hyperalgesia by repeated i.p. administration of either MPEP or NPC15437, the hyperalgesia induced by chronic ethanol treatment was significantly reduced by a single i.p. post-administration of either MPEP or NPC15437. The present data

strongly support the idea that activation of either mGlu5 receptor or PKC in the spinal cord directly contributes to the expression and/or induction of the neuropathic pain-like state following chronic ethanol consumption.

In conclusion, selective inhibitors of the mGlu5 receptor and PKC effectively reversed the induction of the neuropathic pain-like state by chronic ethanol consumption. The present data suggest that mGlu5 receptor and PKC are important drug targets for the ethanol-induced neuropathy.

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