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Short communication

Involvement of spinal protein kinase C in thermal hyperalgesia evoked by partial sciatic nerve ligation, but not by inflammation in the mouse

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Abstract

Activation of several protein kinases contributes to the development of hyperalgesia evoked by injuries. The present study was designed to investigate the role of protein kinase C in the spinal cord in thermal hyperalgesia evoked by sciatic nerve ligation or by intraplantar injection of complete Freund's adjuvant. The paw withdrawal latency on the ipsilateral side, but not on the contralateral side, was markedly decreased after sciatic nerve ligation. Intraplantar injection of complete Freund's adjuvant also caused markedly decreases of the paw withdrawal latency. Intrathecal pretreatment with protein kinase C inhibitor calphostin C (100 and 250 ng) attenuated the decrease of the paw withdrawal latency evoked by sciatic nerve ligation. In contrast, the decrease of the paw withdrawal latency evoked by inflammation was only slightly attenuated by intrathecal pretreatment with calphostin C. The results indicate that protein kinase C in the spinal cord is involved in the development of the thermal hyperalgesia evoked by nerve ligation and is much less involved in the thermal hyperalgesia by complete Freund's adjuvant's-induced inflammation.

Keywords: Hyperalgesia; Nerve ligation; Inflammation; Protein kinase C; Spinal cord; (Mouse)

1. Introduction

There is considerable evidence indicating that the development and maintenance of a persistent painful state after injury results from long-term changes in nociceptive processing in the spinal cord, as well as from increased activation of primary afferent nociceptors. Comparable increases in the excitability of dorsal horn neurons are produced by repetitive activation of C-fibers (Davies and Lodge, 1987; Dickenson and Sullivan, 1994). It is thought that the cascade of events leading to central sensitization starts with increased activity in C-fibers (Schouenborg and Dickenson, 1988) result in increased release of excitatory amino acids (Paleckova et al., 1992) and peptides, such as substance P (Games et al., 1979; Brodin et al., 1987) in dorsal horn. The electrophysiological and behavioral experiments indicate that activation of excitatory amino acid receptors (Davies and Lodge, 1987; Coderre and Melzack, 1991; Woolf and Thompson, 1991; Dougherty et al., 1992), neurokinin receptors (Dougherty et al., 1994, 1995) is required for central sensitization.

The sensitization of dorsal horn neurons lasts from minutes to hours, suggesting that second messenger systems activated by the release of excitatory amino acid and neuropeptides are involved. Activation of protein kinase C has been implicated in changes in pain perception. Phorbol esters, which activate protein kinase C, enhance the number of electrical impulses from knee joint afferents in response to passive joint movement (Schepelmann et al., 1993) and enhance nociceptive responses after tissue injury induced by formalin (Coderre, 1992). The level of membrane bound protein kinase C is increased in the dorsal horn neurons after noxious stimuli (Yashpal et al., 1995) and in a model of experimental peripheral neuropathy (Mao et al., 1992). Electrophysiological experiments

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have shown that activation of protein kinase C leads to long-lasting enhancement of excitatory amino acid-mediated currents in dorsal horn neurons (Gerber et al., 1989) and trigeminal neurons (Chen and Huang, 1991). Thus, activation of protein kinase C may underlie the neuronal sensitization that produces hyperalgesia.

While wild-type mice developed a severe mechanical and thermal allodynia, the protein kinase Cγ isoform knockout mice showed only a very modest expression of mechanical and thermal hyperalgesia (Malmberg et al., 1997b). Mao et al. (1992) reported increases in protein kinase Cγ isoform-like immunoreactivity in the dorsal horn of rats with nerve injury. In the inflammatory hyperalgesia model, the persistent increase in protein kinase Cγ-like immunoreactivity is seen in the ipsilateral superficial dorsal horn of the L4 and L5 segments (Martin et al., 1999). There is no experiment, however, which has examined the effect of protein kinase C inhibitors on the thermal hyperalgesia seen in the nerve-ligation and adjuvant-induced inflammation models. In the present study, we compared the effect of protein kinase C inhibitor calphostin C on nerve injury- and adjuvant-induced thermal hyperalgesia in mice.

2. Materials and methods

2.1. Animals

Male ICR mice weighing 23–27 g (Charles River Breeding Laboratories, Wilmington, MA) were used. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Animals were used only once. All experiments were approved by and conformed to the guidelines of the Medical College of Wisconsin Animal Care Committee.

2.2. Nerve injury and inflammatory pain models

The mice were anesthetized with intraperitoneal (i.p.) sodium pentobarbital (60 mg/kg). A partial nerve ligation of sciatic nerve was made by tying a tight ligature with 7-0 silk suture around approximately 1/3 to 1/2 dorsal portion of the sciatic nerve, similar to the procedure described in rats by Seltzer et al. (1990) and in mice by Malmberg and Basbaum (1998). In sham-operated mice, the nerve was exposed without ligation. For producing an unilateral inflammation, mice were injected with 0.05 ml of complete Freund's adjuvant (*Mycobacterium tuberculosis*; Sigma, St. Louis, MO) subcutaneously in the plantar surface of the right hindpaw (i.pl.). This dose of complete Freund's adjuvant produced significant hindpaw swelling, but the animals exhibited normal behavior. Nerve-ligated and complete Freund's adjuvant-injected mice appeared healthy and were well groomed. When not moving, the mice occasionally held the injured paw or injected paw in a protected position under the body. In both groups, mice did not show foot droop and autotomy.

2.3. Hind paw withdrawal response induced by thermal stimulus

To measure withdrawal latency to radiant heat, the mice were placed on a glass plate preheated to a constant temperature surrounded by a clear plastic chamber (model 336 Analgesia Meter; IITX Life Science Instruments, Woodland Hills, CA) (Mansikka et al., 1999). A radiant heat stimulus was applied from underneath the glass floor with a high-intensity projector lamp bulb and the withdrawal latency was measured using an electronic timer (Hargreaves et al., 1988). The heat stimulus was focused on the plantar surface of each hind paw. The intensity of the heat stimulus was adjusted to derive an average baseline latency of paw withdrawal latency of approximately 9 s in naive mice. A 20-s cut-off was used to prevent tissue damage. Paw withdrawal latency was determined as the average of two measurements per paw. Left and right hind paws were tested alternately in no less than 2 min. Paw withdrawal latency to radiant heat were tested before surgery (day 0) and 1 h after every injection of calphostin C (days 1 to 7).

2.4. Drugs and intrathecal (i.t.) injection

The drugs used were calphostin C (Calbiochem-Novabiochem International, San Diego, CA) and complete Freund's adjuvant (Sigma). Calphostin C was dissolved in 0.1% dimethylsulfoxide in saline (0.9% NaCl). Calphostin C was injected 10 min prior to surgery and 1 h prior to stimulation of each paw. The doses of calphostin C were chosen based upon the data that they selectively blocked the protein kinase C (Kobayashi et al., 1989; Narita et al., 1995).

Intrathecal injections were performed as described by Hylden and Wilcox (1980) with a 25-μl Hamilton syringe with a 30-gauge needle. Injection volumes were 5 μl for i.t. injection.

2.5. Statistical analysis

The behavioral data are presented as the mean ± S.E.M. at different time points after the nerve injury or adjuvant injection. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Newman–Keuls test. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effect of calphostin C on the thermal hyperalgesia induced by nerve-ligation and adjuvant-injection in mice

Partial ligation of the sciatic nerve produced a profound and prolonged decrease of thermal paw withdrawal latency of the ipsilateral side (Fig. 1). The reduction of thermal threshold developed at day 1, reached a maximum at day 3 after nerve ligation and remained decreased for more than 7 days (Fig. 1). Intrathecal pretreatment with calphostin C (100 or 250 ng) attenuated the decrease of the paw withdrawal latency on the ipsilateral side of the sciatic nerve ligation. The same treatment did not affect the paw withdrawal latency of the contralateral side (Fig. 1).

Unilateral injection of complete Freund's adjuvant into the plantar surface of mouse hindpaw produced a profound decrease of paw withdrawal latency of the injected paw. The decrease of the paw withdrawal latency already reached maximum day 1 after the complete Freund's adjuvant injection and remained decreased for up to 7 days. The paw withdrawal latency on the contralateral paw was not changed (Fig. 2). Intrathecal pretreatment with calphostin C 250 ng only slightly but significantly attenuated the decrease of the paw withdrawal latency on the complete Freund's adjuvant injected paw. The same pretreatment with calphostin C did not affect the latency of the paw withdrawal response of the contralateral side (Figs. 1 and 2).

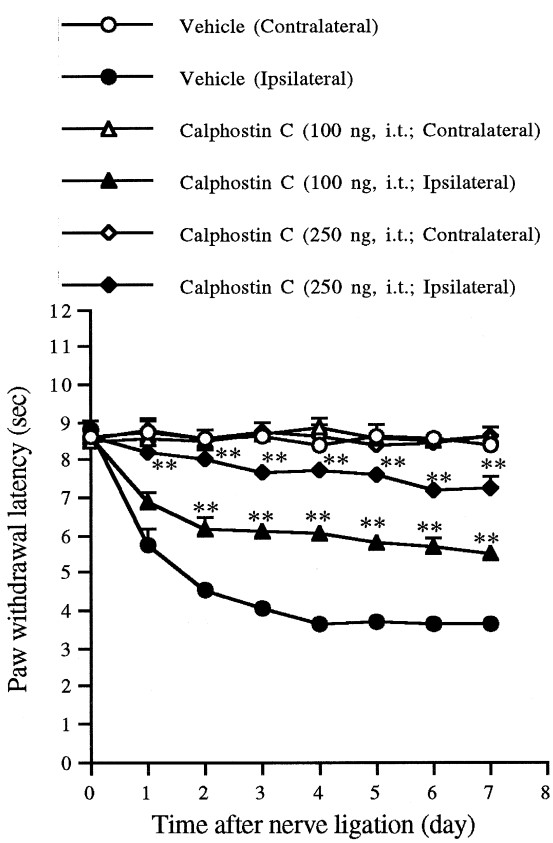

Fig. 1. Effect of calphostin C (100 or 250 ng, i.t.) on partial nerve ligation-induced thermal hyperalgesia. Calphostin C or its vehicle were administered intrathecally 10 and 60 min before surgery and testing, respectively. Each point represents the mean ± S.E.M. for 7–8 mice. The error bars of are obscured by the symbol. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the corresponding vehicle-treated group.

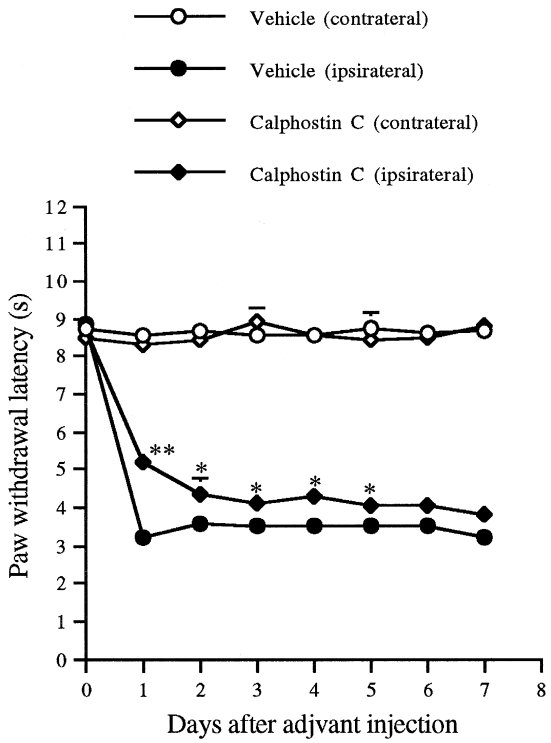

Fig. 2. Effect of calphostin C (250 ng) on complete Freund's adjuvant-induced thermal hyperalgesia. Calphostin C or its vehicle were administered intrathecally 10 and 60 min before the injection of complete Freund's adjuvant and testing, respectively. Each point represents the mean ± S.E.M. for 7–8 mice. The error bars of are obscured by the symbol. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the corresponding vehicle-treated group.

4. Discussion

It is well known that the development of neuropathies may involve an increase in the spontaneous activity of the injured nerves and subsequent development of a state of "central sensitization". The key factors for central sensitization are activation of peripheral C-fiber afferents, with the release of excitatory amino acid and activation of NMDA receptors (Dickenson and Besson, 1997). Events downstream of the activation of NMDA receptors, including activation of protein kinases, have been implicated. Previously, it has been reported that treatment with GM1 ganglioside, which inhibits the translocation and activation of protein kinase C (Vaccarino et al., 1987), attenuated

thermal hyperalgesia in a model of peripheral nerve injury (Hayes et al., 1992). Thermal hyperalgesia in rats chronic nerve injury was related to an increase in spinal cord membrane-bound protein kinase C (Hayes et al., 1992; Mao et al., 1992). Activation of protein kinase C by i.t. administration of the protein kinase C activator phorbol 12,13-dibutyrate produces thermal hyperalgesia in mice (Ohsawa and Kamei, 1999). Thermal hyperalgesia and several neural changes evoked by partial nerve ligation in wild-type mice were not produced in protein kinase Cγ knockout mice (Malmberg et al., 1997b). We found in the present study that the inhibition of protein kinase C by i.t. pretreatment with a protein kinase C inhibitor, calphostin C, markedly attenuated the thermal hyperalgesia evoked by partial sciatic nerve ligation. Our finding provide additional evidence to support the notion that activation of protein kinase C in the spinal cord is involved in the development of thermal hyperalgesia in neuropathic pain.

In contrast to the hyperalgesia induced by sciatic nerve ligation, which was profoundly blocked by the i.t. pretreatment with colphostin C, the hyperalgesia induced by intraplantar injection of complete Freund's adjuvant was only slightly, but significantly, attenuated by the i.t. pretreatment with calphostin C. The development of inflammation-evoked hyperalgesia may involve both peripheral and central sites for changes and the blockade of central protein kinase C alone may not be sufficient to reverse the hyperalgesia. Alternatively, the hyperalgesia induced by inflammation may also involve other protein kinases. A selective deficit was found in the development of inflammation and tissue injury-induced nociceptive pain in mice that lack the neuron-specific isoform of the type I regulatory subunit (RIβ) of protein kinase A, although these mice showed no change in the neuropathic pain behavior induced by partial nerve injury (Malmberg et al., 1997a). In contrast to protein kinase A RIβ subunit knockout mice, protein kinase Cγ isoform knockout mice did not show thermal hyperalgesia and the neurochemical changes evoked by nerve injury and inflammation (Malmberg et al., 1997b). These studies suggest that inflammation-evoked thermal hyperalgesia may involve several protein kinases, including protein kinase Cγ and protein kinase A, but nerve ligation-induced thermal hyperalgesia may be modulated by the protein kinase Cγ isoform. These findings strongly indicate that the mechanisms underlying nerve ligation-evoked thermal hyperalgesia are different from those of inflammation-evoked thermal hyperalgesia.

In conclusion, spinal protein kinase C may play an important role in the development and maintenance of the thermal hyperalgesia evoked by nerve ligation. In contrast to nerve ligation, thermal hyperalgesia evoked by inflammation is partially modulated by spinal protein kinase C. The differential effects of the protein kinase C inhibitor between nerve ligation- and inflammation-evoked thermal hyperalgesia may contribute to the differences of their development processes.

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