Nociception and Allodynia/Hyperalgesia Induced by Intrathecal Administration of Fenvalerate

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ABSTRACT—The intrathecal injection of fenvalerate, a sodium channel activator, at doses of 0.01 to 3 μ g, dose-dependently induced the duration of a characteristic behavioral syndrome mainly consisting of reciprocal hind limb scratching directed towards caudal parts of the body and biting or licking of the hind legs in mice. Fenvalerate-induced behavior was inhibited by morphine (1–10 mg/kg, i.p.). The characteristic behavior was also inhibited by mexiletine, a sodium channel blocker; MK-801, a *N*-methyl-D-aspartate ion-channel blocker; and GR82334, a neurokinin-1-receptor antagonist. Calphostin C (3 pmol, i.t.), a protein kinase C inhibitor, inhibited fenvalerate-induced behavior. On the other hand, phorbol-12, 13-dibutyrate (50 pmol, i.t.), a protein kinase C activator, markedly enhanced the fenvalerate-induced behavior. The present results also showed that fenvalerate produced thermal allodynia and hyperalgesia in the tail-flick test. Furthermore, fenvalerate-induced thermal allodynia and hyperalgesia were inhibited by the pretreatment with calphostin C. These results suggest that the intrathecal administration of fenvalerate induces a marked nociceptive response and thermal allodynia/hyperalgesia, and they suggest that tetrodotoxin-resistant sodium channels may play an important role in this effect.

Keywords: Allodynia, Hyperalgesia, Nociception, Fenvalerate, Tetrodotoxin-resistant sodium channel

Opening of voltage-gated sodium channels causes a rapid depolarization of the membrane potential and constitutes the upstroke phase of the action potential. Most neuronal sodium channels can be blocked by tetrodotoxin (TTX) with K_d values of 1–10 nM. However, sodium channels that are resistant to the blocking action of TTX have been found in both the central and peripheral nervous systems (1–3). Dorsal root ganglion neurons express a slow activating and inactivating TTX-resistant (TTX-R) sodium channel as well as a fast activating and inactivating TTX-sensitive (TTX-S) sodium channel (4–7). The TTX-R sodium channel is predominantly expressed in the capsaicin-sensitive small neurons of the dorsal root ganglion and appears to play an important role in nociceptive transmission (8–13), and especially in allodynia and hyperalgesia (14). Furthermore, algogenic mediators such as prostaglandin, adenosine and serotonin enhance TTX-R sodium current (15–17). Null mutant mice for TTX-R sodium channels show anti-nociception in response to noxious mechanical stimuli and

a delayed development of inflammatory hyperalgesia (18). In spinal cord astrocytes, protein kinase C enhanced TTX-R sodium channels with leftward shifts in the voltage-dependence of both activation and inactivation and produced faster kinetics (19). Several studies using protein kinase C γ -knockout mice (20) and diabetic animals (21–23) have indicated that protein kinase C may play important roles in hyperalgesia.

The pyrethroid insecticides are known to modulate the gating kinetics of neuronal sodium channels to cause repetitive discharges and membrane depolarization. In TTX-R sodium channels, fenvalerate, a type II pyrethroid, irreversibly prolongs the sodium current during depolarization and greatly augments and prolongs the tail current (24).

Considering these findings, we hypothesized that the intrathecal injection of fenvalerate may cause increased nociception and allodynia/hyperalgesia in mice. Therefore, the aim of this study was to investigate the effect of fenvalerate on nociception and allodynia/hyperalgesia in mice. We also examined the effects of an *N*-methyl-D-aspartate (NMDA)-channel antagonist, neurokinin-1 (NK-1)-receptor antagonist and sodium-channel antagonist on fenvalerate-

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induced behavioral response in order to determine its mechanisms. Furthermore, we examined the possible involvement of protein kinase C on fenvalerate-induced nociception and allodynia/hyperalgesia.

MATERIALS AND METHODS

Animals

Male ICR mice (Tokyo Laboratory Animals Science Co., Ltd., Tokyo) weighing about 30 g were used. They had free access to food and water in an animal room, which was maintained at $24 \pm 1^\circ\text{C}$ with a 12-h light-dark cycle. This study was carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

Nociceptive responses

The experiment was performed according to the method described by Hylden and Wilcox (25). Each mouse was acclimated to an acrylic observation chamber ($39 \times 26 \times 24 \text{ cm}^3$) for at least 5 min before the injection of an fenvalerate. Immediately following the injection, the mice were placed in the observation chamber. The cumulative duration (s) of biting, paw licking and scratching episodes was measured for 30 min after the injection of fenvalerate.

Thermal allodynia and hyperalgesia

The nociceptive response was evaluated by recording the latency to withdrawal of the tail in response to several different rates of noxious skin heating as previously described (22). Briefly, the tails of mice were exposed to the focused beam of light from a 50-W projection bulb. The heat intensity was set to one of five values by adjusting the source of voltage for the bulb from 20 to 80 V. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30 s), the trial was terminated to prevent tissue damage. The tail-flick latency was determined before and 60 min after the injection of fenvalerate. The heat intensities at 20, 25, 35, 50, 65 and 80 V produced surface skin heating rates of 0.1°C/s , 0.2°C/s , 0.4°C/s , 0.9°C/s , 3.0°C/s and 7.3°C/s , respectively.

Intrathecal injection

Intrathecal (i.t.) administration was performed following the method described by Hylden and Wilcox (26). Each i.t. injection was administered using a 30-gauge needle directly through the intact skin between the L5 and L6 vertebrae. Drugs were given in a volume of $5 \mu\text{l}$ /mouse.

Drugs

Fenvalerate, calphostin C and phorbol-12,13-dibutyrate (PDBu) were purchased from Calbiochem-Novabiochem Corporation (La Jolla, CA, USA). (5*R*,10*S*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5,10-imine hydrogen maleate (MK-801) and D-Pro⁹-[spiro- γ -lactam]-Leu¹⁰-Trp¹¹-physalaemin(1–11) (GR82334) were purchased from Research Biochemical International, Natick, MA, USA. Mexiletine hydrochloride was generously supplied by Boehringer Ingelheim KG (Ingelheim, Germany). Fenvalerate was dissolved in 0.4% DMSO in saline. Calphostin C and PDBu were dissolved in 0.1% ethanol in saline. Calphostin C and PDBu were injected i.t. 60 min prior to the injection of fenvalerate. Morphine and mexiletine were dissolved in saline and administered i.p. 30 min prior to the injection of fenvalerate. MK-801 and GR82334 were dissolved in saline and administered i.t. 30 min prior to the injection of fenvalerate. The dose and schedule for calphostin C and PDBu in this study were determined as described previously (27, 28).

Data analyses

The data are expressed as means $\pm$ S.E.M. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni/Dunnett test.

RESULTS

Behavioral response induced by intrathecally administered fenvalerate

Intrathecal injection of fenvalerate elicited a characteristic behavioral syndrome mainly consisting of reciprocal hind limb scratching directed towards caudal parts of the body and biting or licking of the hind legs in mice. The behavior response induced by fenvalerate at a dose of $3 \mu\text{g}$ peaked at 10 to 20 min and almost disappeared 30 min after injection. Therefore, in subsequent experiments, the mice were observed for 30 min after injection of fenvalerate ($3 \mu\text{g}$). As shown in Fig. 1, fenvalerate at doses of 0.01 to $3 \mu\text{g}$, i.t. dose-dependently enhanced the duration of these nociceptive responses. To determine whether fenvalerate-induced behavior is related to nociception, the effect of morphine on fenvalerate-induced behavior was examined. As shown in Fig. 2A, pretreatment with morphine (1, 3 and 10 mg/kg) given i.p. dose-dependently inhibited fenvalerate-induced behavior, suggesting that the behavioral response is related to nociception.

Effects of mexiletine, MK-801 and GR82334 on fenvalerate-induced nociceptive response

Figure 2B shows the effect of mexiletine, a sodium channel blocker, on fenvalerate-induced behavior. Pretreatment

Fig. 1. Duration of the nociceptive response induced by the intrathecal administration of fenvalerate in mice. Data are expressed as the total duration of the response during the 30-min period after injection. Each column represents the mean with S.E.M. ($n = 10$). * $P < 0.05$ vs vehicle-treated group (Vehi).

with mexiletine (10 and 30 mg/kg, i.p.) dose-dependently inhibited fenvalerate-induced nociceptive behavior.

As shown in Fig. 2C, pretreatment with MK-801 (0.3 – 3 µg, i.t.), an NMDA channel blocker, caused a dose-dependent inhibition of fenvalerate-induced nociceptive response. Furthermore, as shown in Fig. 2D, GR82334 (0.3 – 3 µg, i.t.), a non-peptidic NK-1-receptor antagonist, yielded results similar to that observed for MK-801.

Effects of protein kinase C modulators on fenvalerate-induced nociceptive behavior

Calphostin C (3 pmol, i.t.), a selective protein kinase C inhibitor, when administered before fenvalerate, caused a significant ($F(1,55) = 6.78$, $P < 0.05$) inhibition of fenvalerate-induced nociceptive behavior with a rightward shift of the dose-response curve for fenvalerate-induced nociceptive behavior (Fig. 3). On the other hand, PDBu (50 pmol, i.t.), a protein kinase C activator, significantly ($F(1,56) = 9.60$, $P < 0.01$) enhanced the fenvalerate-induced nociceptive behavior (Fig. 3). Indeed, when mice were pretreated with PDBu, the dose-response curve for fenvalerate-induced nociceptive behavior was shifted to the left. Either calphostin C or PDBu, by itself, did not produce any significant behavioral changes.

Intrathecal administration of fenvalerate-induced thermal allodynia and hyperalgesia

As shown in Fig. 4, in vehicle-treated mice, the heat intensity at 35 V did not cause a tail-flick response within the 30-s limit. However, when the voltage of the bulb was increased to 50 V, the mean tail-flick latency was significantly less than 30 s. Furthermore, when the voltage was increased to 65 and 80 V, the mean tail-flick latency decreased to approximately 8.1 and 3.3 s, respectively. When

Fig. 2. Effects of morphine (A), mexiletine (B), MK-801 (C) and GR82334 (D) on the fenvalerate (3 µg, i.t.)-induced nociceptive response in mice. Data are expressed as the total duration of the response during the 30-min period after injection. Morphine (1, 3 and 10 mg/kg) and mexiletine (10 and 30 mg/kg) were injected i.p. 30 min before the injection of fenvalerate. MK-801 (0.3, 1 and 3 µg) and GR82334 (0.3, 1 and 3 µg) were injected i.t. 30 min before the injection of fenvalerate. Each column represents the mean with S.E.M. ($n = 10$). * $P < 0.05$ vs saline-treated group (open column).

mice were pretreated with fenvalerate, at a dose of 1 µg, the mean tail-flick latencies at 35, 50 and 65 V were significantly reduced. However, fenvalerate failed to reduce the mean tail-flick latency at 80 V.

As shown in Fig. 4, i.t. pretreatment with calphostin C

Fig. 3. Effects of i.t. administration of calphostin C and phorbol 12,13-dibutyrate (PDBu) on the fenvalerate-induced nociceptive response in mice. Calphostin C (3 pmol, open circle) or PDBu (50 pmol, closed circle) was injected i.t. 60 min before testing. Each point or column represents the mean with S.E.M. ($n = 9 - 12$).

Fig. 4. Effect of calphostin C on the fenvalerate-induced thermal allodynia and hyperalgesia in mice. Calphostin C (10 pmol, triangles) and its vehicle (circles) were injected i.t. 60 min before the injection of fenvalerate. Fenvalerate (1 μg, closed symbols) and its vehicle (open circle, closed circle and open triangle) were injected i.t. 60 min before testing. Each point represents the mean with S.E.M. for 9–10 mice in each group. * $P < 0.05$ vs vehicle-alone-treated group (open circle).

(10 pmol) reversed the fenvalerate-induced reduction of tail-flick latencies at 35, 50 and 65 V. Fenvalerate-induced reduction of the tail-flick latencies at 50 and 65 V were not observed in calphostin C-treated mice. However, calphostin C (10 pmol), by itself, had no effect on the tail-flick latencies (Fig. 4).

DISCUSSION

In the present study, we found that i.t.-administered fenvalerate produced a characteristic behavioral response mainly consisting of hind limb scratching directed towards caudal parts of the body and biting or licking of the hind

legs, which peaked at 10 to 20 min and almost disappeared 30 min after injection. Pretreatment with morphine (1–10 mg/kg) given i.p. reduced fenvalerate-induced behavior at 3 μg, i.t. in a dose-dependent manner. Therefore, behavior induced by fenvalerate seems to be related to nociception.

The present study also showed that fenvalerate-induced behavior was dose-dependently reduced by mexiletine (10 and 30 mg/kg, i.p.), a lidocaine-like sodium channel blocker. We previously reported that mexiletine produced marked antinociception in hyperalgesia and allodynia in diabetic mice (29–31). However, since mexiletine (30 mg/kg, i.p.) had no significant effect on the tail-flick latency after heating the tail at 80 V (without mexiletine, 3.4 ± 0.2 s, $n = 10$; with mexiletine, 3.9 ± 0.2 s, $n = 10$), it is possible that mexiletine by itself did not produce the antinociception in naive mice. Therefore, fenvalerate-induced behavior seems to be related to nociception caused by the activation of sodium channels. Furthermore, we also showed that MK-801, an NMDA-channel antagonist, pretreated i.t. with fenvalerate caused a dose-dependent inhibition of fenvalerate-induced nociceptive responses. Moreover, the fenvalerate-induced nociceptive response was found to be inhibited by GR82334, a non-peptidic NK-1-receptor antagonist. These results lead us to suggest that fenvalerate-induced nociceptive response may be mediated through the release of glutamate and neurokinins, which caused the activation of NMDA and NK-1 receptors, by acting on the sodium channel.

The present results also showed that fenvalerate produced thermal hyperalgesia and allodynia in the tail-flick test. In vehicle-treated mice, the heat intensity at bulb voltages of 65 and 80 V evoked a rapid tail-flick response, whereas that at 50 V evoked an intermediate tail-flick latency and that at 35 V evoked no tail-flick response within 30 s. In fenvalerate-treated mice, the tail-flick latency after heating the tail at 50 and 65 V was significantly shorter than that in vehicle-treated mice, indicating that fenvalerate-treated mice exhibit thermal hyperalgesia. In addition, a lower voltage bulb (35 V), which did not evoke a tail-flick response in vehicle-treated mice, did evoke a tail-flick response in fenvalerate-treated mice, indicating that fenvalerate-treated mice exhibit thermal allodynia.

A TTX-R sodium channel current appears to be primarily responsible for action potential generation in the cell body and terminals of nociceptive afferents (32–36). Furthermore, nerve terminals in distal limb neuromas and skin from patients with chronic local hyperalgesia and allodynia all show marked increases in TTX-R sodium channel-immunoreactive fibers, suggesting that a TTX-R sodium channel may be related to persistent nociceptive hypersensitivity. Moreover, a TTX-R sodium channel is predominantly expressed in the capsaicin-sensitive small neurons of the dorsal root ganglion. Therefore, a TTX-R sodium

channel appears to play an important role in nociceptive transmission (8–13, 33, 35), and especially in allodynia and hyperalgesia (14). Based on these results, it is possible that the fenvalerate-induced nociceptive response and thermal allodynia/hyperalgesia may be mediated through the activation of TTX-R sodium channels. However, although the mode of action is different, fenvalerate is known to modulate both TTX-R and TTX-S sodium channels in a similar direction (24). Thus, it remains unclear whether TTX-R or TTX-S sodium channels are involved in the fenvalerate-induced nociceptive response and thermal allodynia/hyperalgesia. In the present study, we demonstrated that the fenvalerate-induced nociceptive response and thermal allodynia/hyperalgesia were inhibited by the pre-treatment with calphostin C, a selective protein kinase C inhibitor. In contrast, the fenvalerate-induced nociceptive response was enhanced when protein kinase C was activated by PDBu. These results suggest that the fenvalerate-induced nociceptive response and thermal allodynia/hyperalgesia may be modulated by the protein kinase C activity. In this regard, Thio and Sontheimer (19) reported that the activation of protein kinase C by phorbol 12-myristate 13-acetate had different effects on TTX-S and TTX-R sodium currents. In their paper, phorbol 12-myristate 13-acetate reduced peak TTX-S sodium currents by 25–60% and potentiated peak TTX-R sodium currents by 60–150% (19). It has also been reported that treatment with phorbol 12-myristate 13-acetate changes sodium current kinetics (19). TTX-R current activation was faster and current inactivation changed from a single- to a bi-exponential after exposure to phorbol 12-myristate 13-acetate, suggesting that protein kinase C phosphorylation may have activated formerly quiescent sodium channels (19). In contrast, TTX-S current activation was unchanged, and current inactivation decreased by an average of 50% following exposure to phorbol 12-myristate 13-acetate (19). Similarly, it has been reported that the activation of protein kinase C increased TTX-R sodium current, whereas inhibitors of protein kinase C decreased TTX-R sodium current (37). Moreover, epinephrine-induced mechanical and thermal hyperalgesia, and epinephrine-induced enhancement of TTX-R sodium current in cultured rat dorsal root ganglion neurons, are reportedly inhibited by a PKC inhibitor (38). On the other hand, Scholz and Vogel (39) recently demonstrated that TTX-R sodium channel-related action potentials in dorsal root ganglion neurons were suppressed by local anesthetics, such as lidocaine and bupivacaine, in a concentration-dependent manner. In the present study, we observed that mexiletine, a lidocaine-like sodium channel blocker, inhibited the fenvalerate-induced nociceptive response. Therefore, these results and our present study strongly suggest that the activation of TTX-R sodium channels is necessary to produce a fenvalerate-induced nociceptive response and

thermal allodynia/hyperalgesia.

In the present study, we observed that although the fenvalerate-induced nociceptive response was inhibited by morphine, the effective dose of morphine was relatively high. Neuropathic pain is usually difficult to treat because it includes spontaneous pain syndromes, allodynia and hyperalgesia (40). It is particularly insensitive to opioid analgesics (41). Therefore, low efficacy of morphine on fenvalerate-induced nociceptive response suggest that fenvalerate-induced nociceptive response may be a neuropathic pain model.

In conclusion, our findings indicate that the intrathecal administration of fenvalerate, a type II pyrethroid, induces a marked nociceptive response and thermal allodynia/hyperalgesia, and suggest that TTX-R sodium channels may play an important role in this effect. Furthermore, it is suggested that nociception and allodynia/hyperalgesia induced by intrathecal administration of fenvalerate might be useful for studying mechanisms and the treatment of neuropathic pain.

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