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Spinal TRPA1 ion channels contribute to cutaneous neurogenic inflammation in the rat

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ABSTRACT

In the spinal dorsal horn, TRPA1 ion channels on central terminals of peptidergic primary afferent nerve fibers regulate transmission to glutamatergic and GABAergic interneurons. Here we determine the cutaneous anti-inflammatory effect of a spinally administered TRPA1 channel antagonist to test the hypothesis that spinal TRPA1 channels contribute to cutaneous neurogenic inflammation induced by sustained noxious stimulation. According to the hypothesis, spinal TRPA1 channels facilitate transmission of injury discharge to GABAergic interneurons that induce a dorsal root reflex, which results in increased release of proinflammatory compounds in the skin. Intraplantar capsaicin, a TRPV1 channel agonist, was used to induce neurogenic inflammation in anesthetized rats that were pretreated intrathecally (i.t.), intraplantarly (i.pl.) or intraperitoneally (i.p.) with vehicle or Chembridge-5861526 (CHEM, a TRPA1 channel antagonist). For assessment of neurogenic inflammation, the capsaicin-induced increase of cutaneous blood flow was determined adjacent to the capsaicin-treated skin site with a laser Doppler flowmeter. Capsaicin-induced a marked increase in cutaneous blood flow. The capsaicin-induced blood flow increase was attenuated in a dose-related fashion by i.t. pretreatment with CHEM (3-10 μg). Pretreatment with CHEM at a dose of 3 mg/kg i.p. or 20 μg i.pl. failed to attenuate the capsaicin-induced increase of blood flow. The results indicate that spinal TRPA1 channels contribute to cutaneous neurogenic inflammation adjacent to the injury site, probably by facilitating a dorsal root reflex in peptidergic primary afferent nerve fibers.

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TRPA1 is a non-selective cation channel expressed on thin peptidergic primary afferent nerve fibers [12]. On peripheral terminals, it contributes to transduction of potentially harmful stimuli to nociceptive signals [2,4,6,8], and on central terminals in the spinal dorsal horn it regulates transmission to glutamatergic and GABAergic interneurons [5,18]. TRPA1 channel-mediated facilitation of transmission to glutamatergic excitatory spinal dorsal horn neurons is expected to promote nociception. In line with this, recent behavioral results have demonstrated attenuation of pain hypersensitivity by intrathecal (i.t.) treatment with a TRPA1 channel antagonist in various pathophysiological models [3,15].

We hypothesized that among consequences of the TRPA1 channel-mediated facilitation of transmission to GABAergic interneurons is enhancement of a dorsal root reflex induced by sustained nociceptive stimulation, which contributes to cutaneous neurogenic inflammation. The hypothesis is based on earlier findings demonstrating that a loop through the spinal dorsal horn contributes to cutaneous neurogenic inflammation induced by sustained activation of nociceptive primary afferent nerve fibers [16]. This proinflammatory loop involves the spinal GABAergic interneuron [7] that mediates activation of adjacent primary afferent terminals by the ascending afferent volley. Due to a high intracellular chloride concentration [9], activation of chloride channels on central terminals of primary afferent nerve fibers by GABA leads to their depolarization and induction of a dorsal root reflex [16]. This is followed by a release of neuropeptides from peripheral terminals of primary afferent nerve fibers, which promotes cutaneous vasodilatation and extravasation, hallmarks of neurogenic inflammation.

In the present study, we test the hypothesis that the spinal TRPA1 channel promotes cutaneous neurogenic inflammation by facilitating a dorsal root reflex in peptidergic primary afferent nerve fibers. Based on this hypothesis, we expected that i.t. administration of a TRPA1 channel antagonist would attenuate cutaneous neurogenic inflammation resulting in injury discharge induced by intraplantar (i.pl.) injection of capsaicin, a TRPV1 channel agonist [17].

The experiments were performed with male Hannover–Wistar rats (220–260 g; Harlan, Horst, Netherland) in Biomedicum Helsinki. All experiments were approved by the ethical commit-

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tee for experimental animals studies of the State Provincial Office of Southern Finland (Hämeenlinna, Finland) and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available. The animals were housed in polycarbonate cages with a deep layer of saw dust, one to three animals in each cage, in a thermostatically controlled room at 24.0 ± 0.5 °C. The room was artificially illuminated from 8.30 A.M. to 8.30 P.M. The animals received commercial pelleted rat feed (CRM-P pellets, Special Diets Services, Witham, Essex, England) and tap water *ad libitum*.

I.t. catheter was administered at least one week before experiments under pentobarbitone anesthesia (50 mg/kg i.p.; Orion Pharma, Espoo, Finland) using a method described by Størkson and colleagues [11]. Briefly, a polyethylene (PE-10; Becton Dickinson and Company, Sparks, MD) tube was inserted into the lumbar subarachnoid space for i.t. drug administrations. The catheter was then fixed through a layer of superficial muscles, tunneled rostrally and made to appear through the skin in the occipital region. Upon recovery from anesthesia, 7–10 μl of 2% lidocaine hydrochloride, followed by 15 μl of saline was given through the catheter- with the help of a 50-μl Hamilton microsyringe- to verify if it was indeed intraspinally located. An immediate onset of a temporary hind limb paralysis (lasting 15–30 min) was considered to be indicative of correct intraspinal location of the catheter. Only rats with catheters located intraspinally and with no neurological deficits from the catheter were included in the study.

Chembridge-5861526, (CHEM; a TRPA1 channel antagonist, a derivative of HC-030031) was synthesized by ChemBridge Corporation (San Diego, CA). Our previous *in vitro* results show that CHEM is a selective TRPA1 channel antagonist that shows no TRPA1 channel agonism and no TRPV1 channel antagonism at doses up to 100 μM [14,15]. With i.p. administrations, CHEM was dissolved in 0.5% methylcellulose. With i.t. and i.pl. administrations, CHEM was dissolved in physiological saline. Vehicle was used for control injections.

Analysis of capsaicin-induced neurogenic inflammation via laser Doppler flowmeter is a validated method [13]. In the present study, blood flow in the plantar skin was monitored by Periflux Pf2 laser Doppler flowmeter (Perimed AB, Sweden). Electrical calibration for zero blood flow was made in all recordings. The analogue output of this equipment gives no absolute values but relative changes of cutaneous blood flow. The gain of the flowmeter was kept the same in all experiments. The output signal was sampled using CED micro 1401 (Cambridge Electronic Design, Cambridge, U.K.) and analyzed using Spike 2 software (Cambridge Electronic Design). When measuring the cutaneous blood flow, a probe holder was attached by a double-sided adhesive to the skin.

In the actual experiments, anesthesia was induced by i.p. administration of pentobarbitone (50 mg/kg). When needed, further doses of pentobarbitone (15–20 mg/kg) were given to keep the level of anesthesia constant during the experiment, total duration of which was less than one hour. CHEM (10 μg i.t. or 3 mg i.p.) or vehicle was administered 20 min before i.pl. administration of capsaicin. When animals were pretreated with i.pl. CHEM (20 μg/20 μl), CHEM was administered 10 min before i.pl. administration of capsaicin. Time intervals between CHEM and capsaicin treatments were chosen based on our earlier results on the time course of CHEM-induced effects following i.p., i.t. and i.pl. administrations [14,15]. Capsaicin (10 μg/20 μl; Sigma–Aldrich, St. Louis, MO) that induces, via action on the TRPV1 ion channel, nociception and neurogenic inflammation [17], was administered at a site that was 1–2 cm distal to probe of the laser Doppler flowmeter (Fig. 1). With ipl. pretreatment, CHEM was applied in the same plantar skin area as capsaicin 10 min later. Blood flow values measured

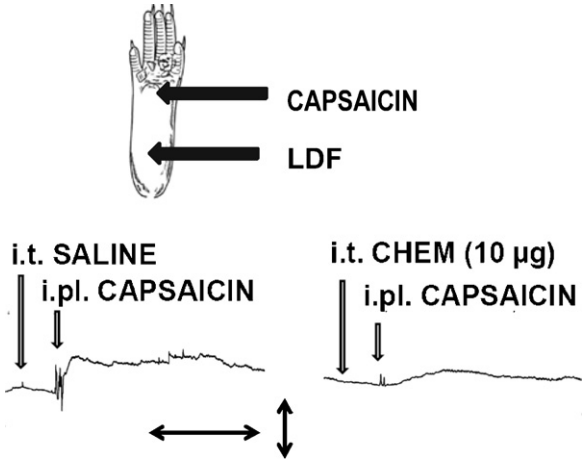

Fig. 1. Examples of cutaneous blood flow recordings in the plantar skin of two rats. Capsaicin (10 μg/20 μl), a TRPV1 channel agonist, was administered intraplantarly (i.pl.) at a site adjacent to the probe of the Laser Doppler Flowmeter (LDF), as shown in the upper illustration. The animal was pretreated 20 min before capsaicin treatment with intrathecal (i.t.) saline or Chembridge-5861526 (CHEM, 10 μg; a TRPA1 channel antagonist). Capsaicin-induced blood flow increase, and its suppression by pretreatment with CHEM, is shown in the lower graphs. The horizontal calibration bar represents 10 min, and the vertical one represents 1 arbitrary unit. Note that for illustrative purposes, i.t. pretreatments are shown at a later time point than they were actually performed. Note also, that the sharp deflections in the blood flow curves reflect movement-related artifacts that were excluded from the data analysis.

in arbitrary units for 5 min before and for 15 min after capsaicin injections were considered in the data analysis. Based on our earlier studies, this observation period overlaps with the duration of the effect by CHEM, independent of the route of administration [14,15]. Movement-related artifacts (sharp deflections in the blood flow measurement curve), such as induced by i.pl. injections *per se*, were excluded from the data analysis. In each experiment, the capsaicin-induced increase in blood flow was determined by subtracting the mean blood flow level measured for 5 min before capsaicin treatment from that measured for 15 min after capsaicin treatment. Thus, positive values represent increase of blood flow by capsaicin treatment. The effects by pretreatments both with CHEM and vehicle control were tested in each animal; the interval between testing sessions with the same animal was at least three days, the test side (left or right hind limb) was varied within the animals, and the order of testing vehicle and CHEM was varied between the animals. After completion of the study, the animals were sacrificed by administering a lethal dose of pentobarbitone (150 mg/kg i.p.).

Data were analyzed using one-way analysis of variance (1-w-ANOVA) followed by t-test with a Bonferroni correction for multiple comparisons. $P < 0.05$ was considered to represent significant difference.

In animals pretreated with vehicle, i.pl. administration of capsaicin (10 μg) produced a marked increase of cutaneous blood flow adjacent to the capsaicin-treated site, while i.t. pretreatment with CHEM produced a dose-related (3-10 μg) suppression in the capsaicin-induced blood flow increase ($F_{2,19} = 3.77$, $P = 0.042$; Fig. 2 A). Pretreatment with CHEM at a dose of 3 mg/kg i.p. or at a dose of 20 μg i.pl., however, failed to influence the capsaicin-induced increase in cutaneous blood flow ($F_{2,17} = 0.28$; Fig. 2 B).

Our results indicate that a TRPA1 channel antagonist significantly attenuates cutaneous neurogenic inflammation adjacent to the injury site, as revealed by attenuation of the cutaneous blood flow increase. This effect was due to a block of spinal rather than peripheral TRPA1 channels, since the cutaneous anti-inflammatory effect was induced by i.t. pretreatment with a TRPA1 antagonist at

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