Capsazepine Inhibits Thermal Hyperalgesia but Not Nociception Triggered by Protease-Activated Receptor-2 in Rats

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ABSTRACT—Protease-activated receptor-2 (PAR-2), expressed in sensory neurons, triggers thermal hyperalgesia, nociceptive behavior and spinal Fos expression in rats. In the present study, we examined if the nociceptive processing by PAR-2 is mediated by trans-activation of capsaicin receptors. The thermal hyperalgesia following an intraplantar (i.pl.) administration of the PAR-2-activating peptide SLIGRL-NH$_2$ was completely abolished by the capsaicin receptor antagonist capsazepine. In contrast, neither the nociceptive behavior nor spinal Fos expression in response to i.pl. SLIGRL-NH$_2$ were attenuated by capsazepine. Our data imply that trans-activation of capsaicin receptors by PAR-2 might be involved in the PAR-2-triggered thermal hyperalgesia, but not nociception.

Keywords: Protease-activated receptor-2, Capsazepine, Pain

Protease-activated receptor-2 (PAR-2) is a G protein-coupled receptor activated by certain serine proteases such as trypsin, trypase, and coagulation factors VIIa and Xa (1 – 3), playing multiple roles throughout the body including in the nervous systems (4, 5). PAR-2 is also expressed in sensory neurons and its activation produces neurogenic inflammation in rat paw (6). We have shown that peripheral PAR-2 activation induced thermal, but not mechanical, hyperalgesia and triggered nociceptive behavior (licking/biting) in a manner independent of mast cells (7). Specific expression of spinal Fos, a marker of nociception, was induced by peripheral PAR-2 activation in mast cell-depleted rats (8). Most recently, the nociceptive role of peripheral PAR-2 was also demonstrated by two independent studies (9, 10).

Vanilloid receptor-1 (VR1), a capsaicin receptor, is a cation channel activated by heat or proton (11). Interestingly, the capsaicin receptor can be sensitized or trans-activated by multiple agonists of distinct receptors such as bradykinin, ATP, and nerve growth factor that stimulate phospholipase C (PLC) (12, 13). This sensitization/trans-activation appears to occur in either a protein kinase C (PKC)-dependent (12) or -independent (13) manner. Given that activation of the PLC-PKC pathway appears to occur in either a protein kinase C (PKC)-dependent (12) or -independent (13) manner. Given that activation of the PLC-PKC pathway via Gq is downstream of PAR-2 (1,4), it is likely that PAR-2 might sensitize or trans-activate capsaicin receptors, resulting in hyperalgesia/nociception. We thus examined if capsazepine, an antagonist of capsaicin receptors, could inhibit the PAR-2-mediated thermal hyperalgesia and nociceptive behavior followed by expression of spinal Fos.

Male Wistar rats (7-week-old) were purchased from Japan SLC, Inc. (Shizuoka). To determine the thermal nociceptive threshold, the rat right hindpaw was tested for responsiveness to radiant heat, using a thermal nociception meter (MK-330; Muromachi Kikai, Co., Tokyo). The intensity of the thermal stimulus was adjusted to obtain baseline latencies of about 10 s. A cut-off latency of 20 s was used to avoid damage to the paw (7). The time-course of the latency was examined after intraplantar (i.pl.) administration of the PAR-2-activating peptide SLIGRL-NH$_2$ at 0.5 μmol/paw, and the area under the time-latency curve for 30 min (AUC$_{0–30}$) was also calculated. The PAR-2-mediated nociceptive behavior was assessed as described previously (7). Briefly, the time spent in licking and biting toward the treated paw was measured for 10 min immediately after i.pl. administration of SLIGRL-NH$_2$ at 0.5 μmol/paw (7). In the experiments to evaluate thermal hyperalgesia, the PAR-2-mediated nociceptive behavior itself did not affect the measurement of thermal nociceptive threshold, because the nociceptive responses that peaked 2 – 3 min after the i.pl. challenge almost disappeared at 5 min, a time point for the measurement of thermal nociceptive threshold.
The analysis of neurons expressing immunoreactive Fos in spinal dorsal horn following peripheral PAR-2 activation was performed by the previously described method (8). Three hours after i.pl. administration of SLIGRL-NH$_2$ at 0.5 μmol/paw, the rat was anesthetized with i.p. administration of urethane at 1.35 g/kg and perfused transcardially with 4% paraformaldehyde in a phosphate buffer (pH 7.4). The fifth lumbar (L5) segment was serially sectioned (40-μm thickness) on a freezing microtome, and immunoreactive Fos was detected by using a kit (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) as described previously (8). The labeled cells in the dorsal horn were observed and counted under a microscope (×400).

Previously, we could detect the PAR-2-triggered specific nociceptive behavior and spinal Fos expression in mast cell-depleted rats where the effect of non-specific degranulation due to peptides was excluded (7, 8). We thus employed mast cell-depleted rats for these experiments, while the PAR-2-mediated thermal hyperalgesia was investigated in naïve rats. Depletion of mast cells was achieved by repeated administration of compound 48/80 (Sigma, St. Louis, MO, USA) as described previously (7, 8).

The PAR-2-activating peptide SLIGRL-NH$_2$ was kindly provided from Sumitomo Pharmaceutical Co., Ltd. (Osaka). Capsazepine was obtained from Sigma. SLIGRL-NH$_2$ and capsazepine were dissolved in saline and 50% dimethyl sulfoxide (DMSO), respectively. Capsazepine at 20 mg/kg (14) was administrated s.c. 30 min before i.pl. administration of the SLIGRL-NH$_2$. Control animals received each vehicle only.

Data are shown as the mean ± S.E.M. Statistical significance was evaluated by ANOVA followed by Tukey’s multiple comparison test at $P<0.05$.

An i.pl. administration of the PAR-2-activating peptide SLIGRL-NH$_2$ at 0.5 μmol/paw rapidly produced significant decrease in the latency as assessed by the thermal nociception test in naïve rats (Fig. 1A), as reported previously (7). This PAR-2-mediated thermal hyperalgesia was clearly abolished by the capsaicin-receptor antagonist capsazepine at 20 mg/kg (Fig. 1A). The data expressed as AUC$_{0–30}$ also show complete inhibition by capsazepine of the PAR-2-mediated thermal hyperalgesia (Fig. 1B). It is of note that capsazepine itself at the dose employed did not affect the thermal nociceptive threshold.

SLIGRL-NH$_2$, administered i.pl. at 0.5 μmol/paw produced prompt nociceptive behavior and delayed expression of spinal Fos in mast cell-depleted rats (Fig. 2), in agreement with our previous study (7, 8). Capsazepine, when administered s.c. at 20 mg/kg, attenuated neither nociceptive behavior nor expression of spinal Fos following peripheral PAR-2 activation (Fig. 2).

In the present study, we demonstrated that the capsaicin receptor antagonist capsazepine blocked the PAR-2-mediated thermal hyperalgesia, but not nociceptive behavior or expression of spinal Fos, implying that sensitization/trans-activation of capsaicin receptors by PAR-2 might be involved in the evoked hyperalgesia, but not nociception.

Recently, it has been shown that capsaicin receptors, ion channels activated by heat and proton, can be activated even at body temperature and neutral pH by PKC that is activated through generation of diacylglycerol by PLC via Gq proteins (12). An independent study has revealed that...
diminution of plasma membrane phosphatidylinositol-4,5-bisphosphate levels following PLC-mediated hydrolysis plays a critical role in the trans-activation of capsaicin receptors by bradykinin and nerve growth factor (13). Taken together with that the PLC-PKC pathway is activated by PAR-2 via Gq (1, 4), our data suggest that the PAR-2-mediated thermal hyperalgesia might result from an enhancement of capsaicin receptor activity through the PAR-2-triggered intracellular signals. This hypothesis is consistent with our previous findings that peripheral PAR-2 activation triggered thermal, but not mechanical, hyperalgesia (7).

Of surprise was that capsazepine failed to block the nociceptive behavior and spinal Fos expression in response to the PAR-2 agonist in rats. This means that PAR-2 could induce sufficient excitation of nociceptive neurons in a manner independent of capsaicin receptors. In other words, capsaicin receptors could be sensitized by activation of PAR-2 and become sensitive to thermal stimuli, resulting in thermal hyperalgesia, whereas the full sensitization of capsaicin receptors (activation of the ion channels at body temperature without application of thermal stimuli) could not be achieved by activation of PAR-2. Other mechanisms downstream of the PLC-PKC pathway activated by PAR-2, responsible for the PAR-2-triggered nociception, remain to be elucidated. Among sensory neurons, capsaicin-sensitive neurons are particularly abundant in PAR-2 (6), whereas specific distribution of PAR-2 in sensory neurons has yet to be analyzed in detail. Distinct distribution of capsaicin receptors and PAR-2, if any, might provide an alternative explanation. It is noteworthy that Davis et al. have shown that capsaicin receptors are essential for the development of sensitization to thermal stimuli during inflammation but not for the normal sensation of noxious heat (15).

The possibility that capsazepine, administered systemically, might inhibit the PAR-2-mediated thermal hyperalgesia by acting at supraspinal levels cannot be completely excluded, although capsaicin receptors are, in general, known to be poorly expressed in the brain. We could not examine the effect of topical administration of capsazepine, because i.pl. administration of vehicle (50% DMSO) for capsazepine itself produced potent antinociception in our preliminary experiments. Alternative approaches may be necessary to exclude the possibility, although there is, to our knowledge, no available evidence that capsaicin receptors present in regions other than sensory neurons modulate nociceptive processing.

In summary, capsaicin receptors would thus appear to play a distinct role in nociceptive processing by PAR-2, being essential for development of thermal hyperalgesia, but not nociception itself, caused by peripheral PAR-2 stimulation.

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