Sensitization and Activation of Intracranial Meningeal Nociceptors by Mast Cell Mediators

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Received March 30, 2007; accepted May 3, 2007

ABSTRACT

Intracranial headaches such as migraine are thought to result from activation of sensory trigeminal pain neurons that supply intracranial blood vessels and the meninges, also known as meningeal nociceptors. Although the mechanism underlying the triggering of such activation is not completely understood, our previous work indicates that the local activation of the inflammatory dural mast cells can provoke a persistent sensitization of meningeal nociceptors. Given the potential importance of mast cells to the pain of migraine it is important to understand which mast cell-derived mediators interact with meningeal nociceptors to promote their activation and sensitization. In the present study, we have used in vivo electrophysiological single-unit recording of meningeal nociceptors in the trigeminal ganglion of anesthetized rats to examine the effect of a number of mast cell mediators on the activity level and mechanosensitivity of meningeal nociceptors. We have found that serotonin (5-HT), prostaglandin I2 (PGI2), and to a lesser extent histamine can promote a robust sensitization and activation of meningeal nociceptors, whereas the inflammatory eicosanoids PGD2 and leukotriene C4 are largely ineffective. We propose that dural mast cells could promote headache by releasing 5-HT, PGI2, and histamine.

ABBREVIATIONS: MC, mast cell; LT, leukotriene; PG, prostaglandin; SIF, synthetic interstitial fluid; CV, conduction velocity; 5-HT, 5-hydroxytryptamine (serotonin); IQR, interquartile range.
pain pathway underlying migraine headache (Levy et al., 2007). Given the potential excitatory effects of MCs on meningeal nociceptors, in this study we used in vivo electrophysiological single-unit recording of meningeal nociceptors in anesthetized rats to examine the potential activating and sensitizing effects of a number of MC-related inflammatory mediators. This study reports the effects of the MC-derived bioamines histamine and serotonin, the stable analog of prostaglandin \( I_2 \) (iloprost), and the major MC leukotriene, LTC\(_4\) (Roberts et al., 1979; Metcalfe et al., 1997).

**Materials and Methods**

**Animals.** Sprague-Dawley male rats (250–300 g) were used in compliance with the experimental protocol approved by the institutional Animal Care and Use Committee of the Harvard Medical School.

**Surgery and Electrophysiological Recording.** Rats were deeply anesthetized with an initial intraperitoneal dose of 1.8 g/kg urethane with 0.2 g/kg supplemental doses as needed. Single-unit recordings of meningeal nociceptors in the trigeminal ganglion were obtained as described previously (Strassman et al., 1996; Levy et al., 2005). In brief, the head of the rat was mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and a 2 × 2-mm craniotomy was performed to expose the dura over the mid sagittal and left transverse sinuses. The exposed dura was bathed with a modified synthetic interstitial fluid (SIF: 135 mM NaCl, 5 mM KCl, 1 mM MgCl\(_2\), 5 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH 7.2). A platinum-coated tungsten microelectrode (impedance 500 KΩ; FHC Inc., Bowdoinham, ME) was lowered into the trigeminal ganglion through a small circular opening (2 mm in width) that was drilled in the left parietal bone, approximately 2 mm caudal to the Bregma suture and 2 mm left of the midline. Meningeal nociceptors were then identified by their constant latency response to single shock stimulation (0.5-ms pulse; 5 mA; 0.5 Hz). Response latencies were used to calculate conduction velocity (CV) based on a 12.5-mm distance to the trigeminal ganglion (Strassman et al., 1996), and nociceptors were classified as either C-units (CV ≤ 1.5 m/s) or A\(\delta\) units (CV > 1.5 m/s). A waveform of the action potential evoked by the electrical stimuli was stored as a template using a real-time waveform discriminator (Spike 2; CED, Cambridge, UK), which was used to acquire experimental data and perform on- and off-line analyses. In all experiments, only one unit was tested in each animal.

**Mechanical Stimulation.** Mechanical receptive fields of meningeal nociceptors were mapped initially by stroking the dura with blunt forceps. The lowest threshold point was determined using a series of calibrated von Frey monofilaments ranging from 0.39 to 58.82 mN (38–443 kPa). Response magnitudes to mechanical stimulation were determined quantitatively, using a servo force-controlled mechanical stimulator (Aurora Scientific, Aurora, ON, Canada) fitted with a flat-ended plastic cylinder (0.5 mm in diameter) aimed at the lowest threshold point on the dura. Stimulus trials for testing changes in mechanical sensitivity consisted of a graded series of three square-wave stimuli (100-ms rise time, 2-s width, 60-s interstimulus interval) delivered in ascending order, which included a threshold and two suprathreshold stimuli. Stimuli that evoked 1–2 Hz afferent discharge were considered as threshold. Suprathreshold stimuli were usually two and four times greater than threshold. Such stimulus trials consisting of these three stimuli were then delivered repeatedly at 15-min intervals. This intertrial interval was not changed once baseline testing started. A 30-s interval preceding the threshold stimulus was used for measurement of baseline spontaneous activity. The response to each mechanical stimulus was calculated by subtracting the spontaneous firing rate from the mean firing rate during the stimulus. In all experimental protocols, baseline measurements of spontaneous and mechanically evoked activity were obtained before drug administration. Only units that exhibited consistent responses at all stimulus intensities in at least three consecutive baseline trials were tested further. These trials also served as vehicle controls, because the receptive field was bathed in SIF, which was the vehicle for all drugs.

**Drugs and Solutions.** The following drugs were used: histamine (1–1000 \( \mu \)M), serotonin (5-HT; 0.1–100 nM); PGD\(_2\) (1–1000 \( \mu \)M), the PGF\(_2\alpha\) stable analog iloprost (0.1–100 \( \mu \)M), and LTC\(_4\) (2–200 \( \mu \)M). Histamine and 5-HT were obtained from Sigma-Aldrich (St. Louis, MO), PGD\(_2\) was from EMD Biosciences (San Diego, CA), and iloprost and LTC\(_4\) from Cayman Chemicals (Ann Arbor, MI). Stock solutions for all drugs were prepared by dissolving the drugs in either distilled water (histamine and 5-HT) or 100% ethanol (for the eicosanoids), and they were kept frozen (−20°C). Drugs were further diluted on the day of the experiments with fresh SIF. Final concentration of ethanol never exceeded 0.1%.

**Experimental Paradigm.** The effects of the MC-related mediators on the activity and mechanosensitivity of meningeal nociceptors was tested by applying the agents topically to the dural receptive field using a small piece of cotton soaked with approximately 40 \( \mu \)l of the test agent. Individual neurons were tested with ascending doses of a specific MC mediator, with each dose applied for one trial (~15 min). In cases in which a certain dose was found to affect at least one parameter (threshold, suprathreshold, or ongoing activity), the dura was washed for 15 min before the next dose was applied. To examine the duration of the response, in sensitized neurons, recording was maintained for up to 60 min during the last wash period. In cases when the highest dose was found ineffective in one trial, it was left for up to 1 h to examine potential indirect effects of the agent due to an inflammatory action in the dura. In most neurons, only one agent was tested, whereas in a few neurons two agents were tested. A second agent was tested only if no response was elicited by the first agent, and it was applied to the dura following at least 2 h of a wash period. At the end of experiment, rats were euthanized with an intravenous bolus of 1 M KCl.

**Data Analysis.** Data are displayed as the median and interquartile range (IQR). For each neuron, an increase in threshold or suprathreshold responses, or ongoing discharge level was defined as an increase in firing rate that exceeded the mean plus 2 times the S.D. of the baseline. Group comparisons were made between baseline and the various doses using the Friedman test. Post hoc paired comparisons between the different doses and baseline were performed using a one-tailed Wilcoxon matched pairs signed ranks test. Effects were analyzed separately for A\(\delta\) and C-units. The level of significance was set at 0.05. The Mann-Whitney \( U \) test was used to analyze differences between the baseline mechanical threshold and spontaneous activity of the A\(\delta\) and C-units tested.

**Results**

**Population of Units Tested.** Extracellular unit recordings were obtained from 69 mechanosensitive meningeal nociceptors in the trigeminal ganglion that were identified by their response to single shock stimulation of the dura overlying the ipsilateral transverse sinus. All neurons in this study exhibited mechanical receptive fields on the dura overlying or immediately adjacent to the ipsilateral transverse sinus or the caudal-most part of the superior sagittal sinus. The baseline response properties of the neurons tested are depicted in Table 1.

**Effects of Histamine.** The effect of topical application of histamine was examined in nine A\(\delta\) units and 10 C-units. Among the A\(\delta\) units tested, only two of nine units showed an increase in their threshold and suprathreshold responses, whereas among the C-units tested, 7 of 10 units were me-
Mechanically sensitized, and both threshold and suprathreshold responses increased (Fig. 1). Overall, histamine significantly increased threshold and suprathreshold responses only at the two highest doses tested. Histamine-induced mechanical sensitization was brief, with mechanical sensitivity returning to baseline values 15 min after wash with SIF (Fig. 1). Histamine induced an increase in the ongoing activity rate only in one of nine A\textsubscript{a} units tested, but it affected 6 of 10 C-units. Overall, the increase in ongoing discharge rate within the C-unit population was achieved only at the two highest histamine doses tested, it was brief, and it always returned to baseline after 15-min wash with SIF.

**Effect of 5-HT.** The effect of topical application of 5-HT was examined in six A\textsubscript{a} units and eight C-units. Topical application of 5-HT for 15 min affected the mechanosensitivity of both the A\textsubscript{a} unit and C-unit populations. Among the A\textsubscript{a} units tested, 5-HT increased threshold responses in four of six units and suprathreshold responses in five of six units. Among the C-units tested, 5-HT increased threshold responses in seven of eight units and suprathreshold responses in five of eight units. Overall, 5-HT induced a significant mechanical sensitization already at 0.1 \( \mu \)M within the C-units and at 1 \( \mu \)M within the A\textsubscript{a} units (Fig. 2). The lowest effective dose that promoted increases in threshold responses was 0.1 \( \mu \)M for the A\textsubscript{a} units and 1 \( \mu \)M for the C-units. The sensitizing effect of iloprost was dose-dependent within both the A\textsubscript{a} and C-unit populations. In most sensitized neurons, threshold responses remained elevated for at least 30 min during the wash period. Iloprost also significantly increased the level of ongoing discharge in three of six A\textsubscript{a} units and five of seven C-units. The minimal dose that elicited this response was 100 \( \mu \)M for the

### TABLE 1

Properties of the A\textsubscript{a} unit and C-unit meningeal nociceptors used in the current study

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>CV ( m/s )</th>
<th>von Frey Threshold ( kPa )</th>
<th>Baseline Ongoing Discharge ( \text{spikes/s} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{a} unit</td>
<td>33</td>
<td>3.13 (1.27, 1.56–4.17)</td>
<td>132 (81, 38–263)</td>
<td>0.1 (0.50, 0–1.15)</td>
</tr>
<tr>
<td>C-unit</td>
<td>36</td>
<td>0.70 (0.39, 0.27–1.49)</td>
<td>133 (81, 63–372)</td>
<td>0.3 (0.62, 0–3.24)*</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \), Mann-Whitney U test between A\textsubscript{a} units and C-units.

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![Fig. 1. Effects of histamine of the activity and mechanosensitivity of meningeal nociceptors.](image-url)

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Aβ units and 0.1 μM for the C-units. In all neurons in which iloprost promoted activation, the increased ongoing discharge rate, similar to the mechanical sensitization, persisted for at least 30 min during the last wash period.

Effect of PGD₂. The effect of PGD₂ was tested in six Aβ units and six C-units. Topical application of PGD₂ evoked minimal mechanical sensitization in one of six Aβ units and one of six C-units, which lasted for only one trial (~15 min). PGD₂ also had a minimal effect on the level of spontaneous activity, in one of six C-units with spontaneous activity returning to baseline levels 15 min after wash. Overall, there was no significant effect of PGD₂ on either the Aβ units or the C-units tested (Fig. 4, left).

Effect of LTC₄. The effect of LTC₄ was tested in six Aβ units and five C-units. Topical application of LTC₄ at all doses tested, and up to 1 h of treatment, did not affect the mechanosensitivity or the level of spontaneous discharges in any of the meningeal nociceptors tested (Fig. 4, right).
Historically, histamine is considered the main effector molecule underlying MC-related inflammatory actions (Metcalfe et al., 1997). Clinical evidence suggests the involvement of histamine in migraine based on findings showing elevated histamine levels during migraine (Heatley et al., 1982; Haimart et al., 1987), the ability of histamine infusion to trigger migraine-like headache by activating the H1 receptor (Lassen et al., 1995), and the prophylactic effect of antihis-
tamines in a subset of migraine patients (Rossi et al., 2003; Lewis et al., 2004; Togha et al., 2006).

Previous electrophysiological studies conducted in visceral tissues, including testis (Koda et al., 1996), heart (Nishi et al., 1977), and airways (Matsumoto et al., 1992, 1993; Riccio et al., 1996), have shown that histamine can excite polymodal nociceptors having both Aδ unit and C-unit CVs, probably through activation of the H1 receptor. The results of our studies, however, suggest that histamine excites mainly C-unit meningeal nociceptors. Such preferential activation is reminiscent of the effect of MC degranulation on C-unit meningeal nociceptors (Levy et al., 2007). We found that histamine also preferentially promotes mechanical sensitization of C-units, findings that are in agreement with its effect on testicular nociceptors (Koda and Mizumura, 2002). Our study further showed that both the activation and sensitization mediated by histamine are short-lived, suggesting that histamine alone may not be able to sustain the prolonged activation of meningeal nociceptors evoked by MCs degranulation. Such a sustained effect, therefore, may require the action of histamine in combination with other MC mediators.

Although platelets are a major source of peripheral 5-HT, significant quantities are also present in the granules of MCs, in both rodent and human (Metcalfe et al., 1997; Kushnir-Sukhov et al., 2007). Previous studies conducted in humans and rats have shown that direct administration of 5-HT promotes mechanical hyperalgesia (Jensen et al., 1990a,b; Taiwo and Levine, 1992; Taiwo et al., 1992), an effect that is thought to be mediated by the activation and sensitization of nociceptors. However, previous electrophysiological studies have shown that although 5-HT is capable of promoting relatively short-duration excitation of high threshold Aδ and C nociceptive afferents innervating skin (Beck and Handwerker, 1974), knee joint (Herbert and Schmidt, 1992), jejenum (Brunsden and Grundy, 1999), and colon (Blackshaw and Grundy, 1993a; Coldwell et al., 2007), it does not promote mechanical sensitization (Blackshaw and Grundy, 1993a; Coldwell et al., 2007), unless the application of 5-HT is preceded by local inflammation (Herbert and Schmidt, 1992; Coldwell et al., 2007). In our study, 5-HT, being the most potent of all the MC mediators tested, promoted both activation and mechanosensitization in both the C- and Aδ meningeal nociceptor populations. Overall, 5-HT was slightly more potent in the C-unit population, eliciting effects at a lower dose. The ability of 5-HT to promote mechanical sensitization of meningeal nociceptors may reflect a different repertoire of 5-HT receptors on trigeminal nociceptors, in particular meningeal nociceptors, which could mediate mechanical sensitization. Alternatively, the induction of mechanical sensitization may be related to a dural irritation induced by the craniotomy needed to expose the receptive field. Such irritation may also explain the presence of baseline spontaneous activity seen frequently in this preparation (Levy and Strassman, 2002a,b).

Activation of at least four types of 5-HT receptors, including 5-HT_{1A} (Taiwo and Levine, 1992; Cardenas et al., 1997b), 5-HT_{2A} (Wei et al., 2005; Sasaki et al., 2006), 5-HT_{3} (Blackshaw and Grundy, 1993b; Zeitz et al., 2002), and 5-HT_{4} (Cardenas et al., 1997a) were implicated in the peripheral nociceptive actions of 5-HT. However, it is unclear which receptor system mediates neuronal activation and which mediates mechanosensitization. Activation of the 5-HT_{1A} and
5-HT₄ receptors that are coupled to activation of the cAMP-protein kinase A signaling cascade has been suggested to promote sensitization by enhancing tetrodotoxin-resistant Na⁺ currents (Gold et al., 1996; Cardenas et al., 2001). However, we have shown that activation of this cascade promotes only mechanical sensitization in meningeal nociceptors without activation (Levy and Strassman, 2002a), suggesting that 5-HT promotes excitation of meningeal nociceptors through a different receptor and/or signaling cascade. A recent study has shown that activation of the 5-HT₁₅D receptor promotes an increase in firing rate in both Aδ units and C-units (Zeitz et al., 2002). However, 5-HT₃ agonist does not promote mechanical sensitization (Taiwo and Levine, 1992). Further studies are needed to examine which 5-HT receptors promote activation and sensitization in meningeal nociceptors.

We have shown recently that the migraine drug sumatriptan, an agonist at the 5-HT₁D/₁F₃ receptors, can also activate and sensitize meningeal nociceptors at micromolar concentrations (Strassman and Levy, 2004; Burstein et al., 2005). Because activation of the 5-HT₁D receptor does not produce hyperalgesia (Taiwo and Levine, 1992), sumatriptan may promote enhanced excitability of meningeal nociceptors via an alternative excitatory 5-HT receptor. Activation of both the 5-HT₁A and 5-HT₇ receptors, both of which are positively coupled to cAMP and which sumatriptan is capable of activating at a similar dose range (Schoeffter and Hoyer, 1989; Bard et al., 1993), may play a role in promoting mechanical sensitization.

Previous studies indicated that PGI₂, which can be secreted from MCs (Metcalfe et al., 1997), is capable of promoting activation and sensitization of articular mechanonociceptors (Birrell et al., 1991, 1993), but it is capable of only weakly activating visceral testicular nociceptors (Mizumura et al., 1991). Our results suggest that the PGI₂ sensitivity of meningeal nociceptors is similar to that of the knee joint with PGI₂-mediated activation and sensitization of both Aδ units and C-units. Given that the prostacyclin receptor is primarily coupled to activation of adenyl cyclase, increased cAMP may play a role in the mechanical sensitization elicited by the PGI₂ analog iloprost (Pitchford and Levine, 1991; Nakae et al., 2005). However, the prostacyclin receptor may also be coupled to activation of phospholipase C and increased intracellular calcium (Hayes et al., 1999; Lawler et al., 2001), which may play a role in eliciting meningeal nociceptor activation.

PGD₂, synthesized by the prostaglandin D₂ synthase, is the major prostanoid secreted by MCs (Metcalfe et al., 1997). Activation of visceral MCs has been shown to promote enhanced excitability of sensory vagal afferents, in part by promoting inhibition of a Ca²⁺-dependent K⁺ current underlying postspike hyperpolarization (afterhyperpolarization, slow), which controls repetitive spike firing (Weinreich and Wonderlin, 1987; Greene et al., 1988; Cordoba-Rodriguez et al., 1999). The ability of PGD₂ to promote inhibition of postspike hyperpolarization afterhyperpolarization, slow, in vagal afferents (Greene et al., 1988; Cordoba-Rodriguez et al., 1999) suggests a potential role for this MC mediator in promoting sensitization of visceral sensory neurons. However, in our in vivo preparation of meningeal nociceptors, we found that PGD₂ had no effect on the response properties of most meningeal nociceptors. Although our results are in discrepancy to those found in vagal afferents, they are in agreement with other electrophysiological studies showing no, or minimal, effect of PGD₂ on sensory dorsal root ganglion neurons (Rueff and Dray, 1993; Bley et al., 1998; Hwang et al., 2000). Although currently it is unknown whether nociceptive neurons expresses functional PGD₂ receptors (i.e., DP₁, DP₂), the lack of increase in cAMP levels in dorsal root ganglion neurons after exposure to PGD₂ (Smith et al., 1998) suggests that dorsal root and trigeminal ganglion nociceptors may not express such receptors.

Although PGD₂ may be devoid of direct action on most meningeal nociceptors, it has been shown to promote a rapid increase in vascular permeability (Woodward et al., 1993; Nishimura et al., 2001) and vasodilation of meningeal arterioles (Ellis et al., 1979), both of which have been suggested as putative mechanisms underlying vascular headaches as putative mechanisms underlying vascular headaches such as migraine. Given that topical application of PGD₂ for up to 1 h did not affect the responsiveness or ongoing activity of meningeal nociceptors, it is questionable whether such PGD₂-related inflammatory responses may promote headache.

Similar to PGD₂, the cysteinyl leukotriene LTC₄, another major MC constituent, has been shown to play a role in the excitatory action of MCs on vagal afferent neurons (Undem et al., 1993; Cordoba-Rodriguez et al., 1999) together with promoting plasma extravasation in rat skin (Morimoto et al., 1989). LTC₄, however, does not affect the mechanosensitivity of airway afferents (Riccio et al., 1996). In our current study, we found that LTC₄ did not affect meningeal nociceptors even when applied at the highest dose and for up to 1 h. Although there is evidence to suggest that leukotriene receptor modifiers, such as montelukast, which blocks signal transduction through the leukotriene receptor CysLT1 (Funk, 2005), can serve as prophylactic migraine drugs (Sheftell et al., 2000), our results suggest that if LTC₄ or its metabolites play a role in migraine precipitation, their action may not be mediated by promoting the activation or sensitization of meningeal nociceptors.