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Modulation of meningeal nociceptors mechanosensitivity by peripheral proteinase-activated receptor-2: the role of mast cells

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Abstract

Inflammatory-related activation and sensitization of meningeal nociceptors is believed to play a key role in promoting the intracranial throbbing pain of migraine. We have shown recently that mast cell activation and various mast cell-derived inflammatory mediators can promote activation and sensitization of meningeal nociceptors. Mast cell tryptase has also been proposed to promote pain hypersensitivity by activating the proteinase-activated receptor 2 (PAR2) that is expressed on nociceptive neurons. In this study using in vivo single-unit recording in the trigeminal ganglion of anaesthetized rats, we found that local meningeal activation of PAR2 using the specific agonist SLIGRL-NH₂ promoted sensitization of the threshold response while provoking desensitization of the suprathreshold responses. SLIGRL-NH₂ also excited a subpopulation of meningeal nociceptors. Chronic mast cell depletion enhanced the sensitizing effects of PAR2 activation while curbing its desensitizing effects. Mast cell depletion did not change the PAR2-mediated excitatory effect. We propose that by enhancing the mechanical sensitivity of meningeal nociceptors local PAR2 activation could play a role in promoting the throbbing pain of migraine and that local mast cell degranulation may modulate such an effect.

Keywords

Headache; mast cells; meningeal nociceptor; migraine; PAR2

Introduction

The throbbing pain of migraine is generally believed to ensue as a result of an inflammatory-related excitation and enhanced responsiveness (i.e. sensitization) of trigeminal mechanosensitive nociceptive afferents that supply large intracranial blood vessels and the meninges (1,2). In recent years we have focused on delineating some of the inflammatory cells and mediators that could play a role in promoting such activation and sensitization of meningeal nociceptors. We have found that local activation of mast cells (MCs), granulated immune cells that populate the intracranial dura mater, can promote persistent excitation of meningeal nociceptors, and that numerous MC mediators, including histamine, serotonin and prostacyclin, can also enhance the mechanosensitivity of these neurons (3,4).

Previous studies have suggested that yet another inflammatory MC constituent, the protease tryptase, could play an important role in promoting pain hypersensitivity by activating a G-protein coupled receptor known as proteinase-activated receptor-2 (PAR2) (5), which is expressed on the cell body of primary afferent nociceptive neurons in dorsal root ganglia (6–

8). PAR2 is also expressed by trigeminal ganglion (TG) cells (9,10) and, similar to dorsal root ganglia cells, its activation has been shown to promote increased neuronal excitability in vitro (10). Such PAR2-mediated trigeminal hyperexcitability suggests its potential involvement also in trigeminal pain syndromes that involve MCs and inflammation such as migraine. To explore a potential role for PAR2 activation in migraine pain we sought to examine whether its local activation can either excite or promote mechanical sensitization of trigeminal meningeal nociceptors.

Although direct activation of neuronal PAR2 is believed to contribute to increased nociceptor excitability and the resultant pain hypersensitivity, PAR2 is also expressed on MCs and its activation can enhance their degranulation and mediator release (11–13). Furthermore, some of the inflammatory responses elicited by PAR2 activation, such as increased vascular permeability, are partially mediated by local MC degranulation (14,15). Given that MC degranulation per se can activate meningeal nociceptors (3), we also examined the effect of local application of a PAR2 agonist on meningeal nociceptors recorded from rats that were chronically depleted of their MCs.

Methods

Animals

Sprague-Dawley male rats (200–300 g) were used. All experiments were in compliance with the experimental protocol approved by the institutional Animal Care and Use Committee of the Harvard Medical School.

Animal preparation

Rats were deeply anaesthetized with an initial intraperitoneal dose of 1.8 g/kg urethane and maintained under anaesthesia throughout the experiment with 0.2 g/kg supplemental doses given as needed. Core temperature was kept within physiological range using a rectal probe and a feedback-controlled thermal blanket.

Electrophysiological recording

The method for recording the activity of meningeal nociceptors has been described previously (16,17). Briefly, anaesthetized rats were placed in a stereotaxic head-holder (David Kopf, Tujunga, CA, USA) and a 2×2 -mm craniotomy was performed to expose the left transverse sinus as well as the adjacent dura extending approximately 4–6 mm rostral and 2 mm caudal to the sinus. The transverse sinus was exposed from the midline to approximately 5 mm laterally. Following exposure, the dura was bathed in a modified synthetic interstitial fluid (SIF, pH 7.2) containing 135 mm NaCl, 5 mm KCl, 1 mm MgCl₂, 5 mm CaCl₂, 10 mm glucose and 10 mm HEPES. For single-unit recordings of meningeal nociceptors a platinum-coated tungsten microelectrode (impedance 500 K Ω ; FHC, Bowdoin, ME, USA) was advanced into the left TG through an additional, smaller circular opening that was drilled in the left parietal bone, about 2 mm caudal to the Bregma suture and 2 mm left to the midline. Meningeal nociceptors in the TG were identified by their constant latency response to single-shock stimulation of the dura overlying the ipsilateral transverse sinus (0.5 ms pulse, 0.1–5 mA, 0.5 Hz). Response latencies were used to calculate conduction velocity (CV), based on a conduction distance to the TG of 12.5 mm. Neurons were classified as either C units ($CV \leq 1.5$ m/s) or A δ units ($1.5 < CV \leq 5$ m/s). Action potentials were filtered, amplified and acquired for on-line and off-line analysis using spike 2 (CED, Cambridge, UK). Only one neuron was studied in each animal. At the end of the experiments, animals were euthanized with an intravenous bolus injection of 1 m KCl.

Mechanical stimulation

Mechanical receptive fields of meningeal nociceptors were initially mapped using a set of calibrated von Frey monofilaments ranging from 0.39 to 58.82 mN (exerting pressure stimuli in the range of 38–443 kPa). For quantitative determination of the neurons' mechanical responsiveness, graded stimuli were applied to the dural surface using a servo force-controlled mechanical stimulator (Series 300B; Aurora Scientific, Aurora, ON, Canada) that was fitted with a flat-ended plastic cylinder (0.5 mm diameter) and aimed at the lowest threshold point on the dura (18). 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. A 30-s interval preceding the threshold stimulus was used for measurement of baseline spontaneous activity. To limit nociceptor fatigue (19), responses to these stimuli, as well as ongoing spontaneous activity, were recorded every 15 min throughout the experiment. The neuronal response to each mechanical stimulus was calculated by subtracting the spontaneous firing rate from the mean firing rate during the stimulus.

Experimental design

In all experiments, baseline measurements of spontaneous and mechanically evoked activity were obtained prior to drug administration. Only units that exhibited consistent responses at all stimulus intensities (i.e. changes of < 0.5 and < 2.5 Hz for the threshold and suprathreshold responses, respectively, and 0.3 Hz for the ongoing activity level) 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 the PAR2 agonist. To examine the effect of PAR2 activation on the activity and mechanosensitivity of meningeal nociceptors the selective PAR2 agonist peptide having the following sequence Ser-Leu-Ile-Gly-Arg-Leu-amide (SLIGRL-NH₂; Sigma, St Louis, MO, USA) was applied topically to the dural receptive field using a small piece of cotton soaked with approximately 40 µl of the agent. Individual neurons were tested with ascending doses (5–500 µg/cc, equal to approximately 0.3–30 nmole) of the agonist with each dose applied for one trial (around 15 min). To examine the duration of the response, the highest dose was maintained on the dura for 60 min (four trials). Given that the PAR2 agonist was most effective at the highest dose tested (30 nmole, see Results), only this dose was used to examine potential changes in meningeal nociceptors recorded in chronically MC-depleted animals. In these animals, MCs were depleted of their granules using repeated pretreatments with the secretagogue compound 48/80 using a method adapted from Coderre et al. (20). Briefly, the polycationic secretagogue agent compound 48/80 (0.1% w/v in sterile saline; Sigma-Aldrich) was injected intraperitoneally twice a day (morning and afternoon) for a total of eight doses, starting with 0.5 mg/kg injections for the first day, 1 mg/kg for the second and 2 and 4 mg/kg for the third and fourth days, respectively. Animals were used 3–4 days later.

Data analysis

Data are presented as mean ± S.E.M. For each neuron, a change in threshold and suprathreshold responses, or ongoing discharge level was defined as a change in firing rate that exceeded the mean plus two times the standard deviation of the baseline. Dose effectiveness was tested using paired Student's t-test. Differences between the effect of PAR2 activation on meningeal nociceptors recorded from MC-depleted and non-depleted groups were analysed over five time points (baseline, 15, 30, 45 and 60 min during application of the PAR2 agonist) using Repeated Measures Analysis of Variance. Differences in the frequency of responders between the MC-depleted and non-depleted groups were analysed using the χ^2 test. Baseline von Frey hair

mechanical threshold and spontaneous activity between groups were analysed using unpaired Student's t-test. Analyses were conducted separately for the A δ and C-units. The level of significance was set at 0.05.

Results

Meningeal nociceptor population

The effect of local meningeal PAR2 activation was recorded in 40 meningeal nociceptors; 21 were A δ units (mean CV 3.01 ± 0.21 m/s, range 1.79–5.00) and 20 were C-units (mean CV 0.71 ± 0.06 m/s, range 0.26–1.32). All units responded to punctuate mechanical stimulation of the dura and had receptive fields located on, or in the vicinity of the left transverse sinus.

Effect on threshold responses

Local application of the PAR2 agonist promoted increases in the threshold responses of a subpopulation of the A δ and C-units. Among the A δ units tested, enhanced threshold responses occurred primarily following application of the highest dose (Fig. 1a) and were observed in 6/11 units (Fig. 1b). This effect had a short duration with an onset in most neurons within 15 min, followed by a rapid decline (Fig. 1d). Among the C-units tested, PAR2 activation was similarly associated with an increase in threshold responses primarily following application of the higher dose (Fig. 1a) and was detected in 5/8 units (Fig. 1c). However, unlike its effect on the A δ population, PAR2-mediated threshold sensitization of the C-units persisted throughout the application time (Fig. 1e).

In A δ units recorded from chronically MC-depleted rats, the mean baseline von Frey hair threshold (108.1 ± 24.9 kPa) was not statistically different than that observed in units recorded from the non-depleted animals (113.5 ± 18.3 kPa). Topical application of the PAR2 agonist produced sensitization in 4/10 A δ units, a proportion which was not statistically different from that observed in the non-depleted animals. However, in neurons recorded from chronically MC-depleted rats PAR2 activation resulted in sustained sensitization having a larger magnitude (Fig. 1d), an effect that was statistically different from that observed in neurons recorded from non-depleted animals ($P = 0.024$).

In C-units recorded from chronically MC-depleted rats, the mean baseline von Frey hair threshold was 149.4 ± 27.3 kPa and was not statistically different from that observed in units recorded from non-depleted animals (105.7 ± 18.6 kPa). Topical application of the PAR2 agonist produced sensitization in 5/12 of the C-units, a similar proportion to that seen in the non-depleted animals. In these units, chronic MC depletion affected neither the magnitude nor the duration of sensitization.

Effect on suprathreshold responses

Local PAR2 activation had an entirely opposite effect on the suprathreshold mechanical responses of meningeal nociceptors. Overall, the PAR2 agonist produced a dose-dependent desensitizing effect in most units tested (Fig. 2a). Among the A δ units tested the highest dose produced a clear desensitization in 8/11 units (Fig. 2b), an effect that was observed already at 15 min and did not resolve over time (Fig. 2d). Among the C-units tested, PAR2 activation had a similar desensitizing effect beginning at the 50 $\mu\text{g}/\text{cc}$ dose. Application of the highest dose was associated with a marked reduction in the responses of 5/8 units tested (Fig. 2c) that also did not improve during prolonged exposure (Fig. 2e).

In A δ units recorded from chronically MC-depleted animals, PAR2 activation was associated with a desensitizing effect only in 2/10 of the units tested. The response of the remaining units was unchanged. The number of units affected was statistically lower than that observed in the

non-depleted animals ($P = 0.008$). Overall, in the A δ nociceptor population chronic MC depletion prevented the reduction in the suprathreshold responses throughout the duration of the study (Fig. 2d, $P = 0.024$). Although chronic MC depletion did not reduce the overall number of C-units that became desensitized (7/12) following local PAR2 activation, the overall time course of such effect was slower, with significant inhibition attained only 45 min following application of the PAR2 agonist (Fig. 2e).

Effect on ongoing discharge rate

Overall, PAR2 activation resulted in a brief excitatory effect that was recorded in a small number of meningeal nociceptors. Among the A δ units tested, 3/11 units were excited following application of both the 50 and 500 $\mu\text{g/cc}$ doses (Fig. 3a,b). In these neurons such effect was brief and never exceeded 15 min in duration, although the drug was maintained on the dura mater for up to 60 min. Overall, for the population local PAR2 activation did not promote excitation. Among the C-unit tested, only 1/8 units was excited at the 50 $\mu\text{g/cc}$ dose, whereas 5/8 units were excited when the highest dose was applied to their receptive field (Fig. 3c). This excitatory effect was also short-lived and lasted only for 15 min. Overall, for the population PAR2 activation did not promote excitation.

In rats chronically depleted of their MCs, the baseline ongoing discharge level of the A δ units was 0.29 ± 0.1 Hz and was not statistically different from that observed in the non-depleted animals (0.41 ± 0.1 Hz). Within the C-unit population, the baseline ongoing discharge level was 0.38 ± 0.1 Hz and was also not statistically different than that observed in the non-depleted animals (0.60 ± 0.17 Hz). Following chronic MC depletion, PAR2 activation was not different from that observed in the non-depleted group; an increase in the ongoing discharge level was observed in 2/10 and 4/12 of the A δ and C-units, respectively, proportions that were not statistically different between the MC-depleted and non-depleted groups. The magnitude and duration of the excitatory effect of PAR2 activation were also not statistically different between the groups (Fig. 3d,e).

Discussion

Previous studies have characterized the effect of PAR2 activation on the electrophysiological properties of dorsal root (6–8,21,22) and TG (10) in vitro. To the best of our knowledge, the present study is the first to record in vivo the effect of PAR2 activation on the mechanosensitivity and ongoing discharge level of A δ and C-unit mechanosensitive nociceptors, and particularly those innervating the intracranial meninges.

Peripheral PAR2 activation has been shown to promote mechanical hypersensitivity in behavioural studies (6,8,23). However, a recent in vitro electrophysiological study using the skin-nerve preparation in rats has failed to detect PAR2-mediated sensitization of mechanosensitive nociceptors, albeit it was able to show PAR2-mediated nociceptor excitation and thermal sensitization (24) and thus implicated a more central mechanism in the emergence of PAR2-related mechanical hypersensitivity. Our in vivo findings provide the first evidence that peripheral PAR2 activation is capable of promoting sensitization of mechanonociceptors, and suggest that peripheral mechanosensitization is likely to be one of the neural substrates of PAR2-mediated behavioural mechanical hypersensitivity.

We found that the sensitization mediated by local PAR2 activation was limited to increases of threshold responses with both A δ and C-units showing similar propensities (55% and 63%, respectively) to become sensitized following PAR2 activation. However, the time course of such sensitization differed between the two nociceptor populations: in A δ units it was brisk, whereas in C-units it had a similar onset but persisted throughout the course of the study. These propensities may reflect the percentage of primary afferent neurons that express the PAR2

receptor (15) or are affected by its activation (7,25). The difference in time course between the A δ and C-unit populations, however, may be explained by a neuronal mechanism such as lower chemosensitivity or reduced ability to maintain sensitization of A δ units.

The exact mechanism by which PAR2 promotes threshold sensitization in meningeal as well as other mechanosensitive nociceptors is currently unknown. PAR2 has been suggested to enhance the excitability of nociceptive neurons through mechanisms that involve sensitization of TRPV1 (7,10,25,26), TRPV4 (8), TRPA1 (27) and activation of the protein kinase C (8, 10,21,22,25,26), protein kinase A (8,26), phospholipase C β (8,21) and extracellular related kinase (22) signalling cascades. Whether activation of a single or combination of second messenger cascades is involved in the PAR2-evoked threshold changes needs to be evaluated further.

Although it is likely that direct activation of the neuronal PAR2 plays a role in promoting changes in threshold response, our finding that chronic MC depletion prolonged the PAR2-mediated sensitization of the A δ population suggests the additional involvement of MCs especially in limiting, or inhibiting A δ sensitization (see also below). Given that PAR2-mediated mechanical hypersensitivity persists beyond 1 h (6), our results suggest that although both A δ and C-units could play a role in the initiation of the hypersensitivity, C-units are likely to maintain it. During a migraine attack, such PAR2-mediated C-unit mechanosensitization is therefore likely to play a role in promoting the throbbing sensation and the pain exacerbation associated with innocuous activities such as coughing or sneezing.

In contrast to the increases in threshold responses, dural PAR2 activation was associated with pronounced desensitization of the suprathreshold responses in both the A δ and C-unit populations. The behavioural manifestation of such an effect has never been addressed, since most of the behavioural studies that examined the acute effect of PAR2 activation have focused only on changes in threshold (6,8,28) rather than examining hyperalgesic effect, which reflects enhanced responses to suprathreshold stimuli. Although our previous studies suggest that threshold and suprathreshold responses are not necessarily mutually affected when meningeal nociceptors are exposed to sensitizing agents (29), it is unclear at present whether the reduction in the suprathreshold responses following PAR2 activation is mediated by direct neuronal PAR2 action. Given that the sensitizing effect of PAR2 agonists has been shown to be associated with activation of various intracellular signalling cascades, in particular protein kinase A (8,26), which promotes increased suprathreshold responses of meningeal nociceptors (29), it is clear that other mechanisms play a role in promoting the desensitizing effect of PAR2 activation. The finding that chronic depletion of dural MCs virtually prevented such an inhibitory effect in the A δ units while postponing it within the C-unit population points to potential involvement of a MC-derived mediator in promoting such an inhibitory effect. Further studies are needed for a better understanding of this phenomenon.

In addition to promoting changes in the mechanosensitivity of meningeal nociceptors, PAR2 activation was also associated with a brief increase in the ongoing discharge rate in a small number of both A δ and C-units. Such PAR2-mediated excitation was reported to occur in vitro in about 20% of cutaneous C-units (24). Our study, however, is the first to report also the activation of some A δ mechanonociceptors. Nevertheless, given that overall the magnitude of this excitatory effect was not statistically significant for either of the nociceptor populations, the physiological relevance of such activation may be limited. Although activation of a single nociceptor can evoke an elementary painful sensation (30,31), we propose that the brief and limited excitatory effect induced by PAR2 activation, although unlikely to promote directly the sensation of headache, may contribute to the development of central sensitization in brainstem trigeminovascular neurons.

We have previously reported that other MC constituents, including histamine, serotonin and prostacyclin, have the ability to promote both activation and mechanical sensitization of meningeal nociceptors (4). Compared with these mediators, the effect of PAR2 activation on meningeal nociceptors was limited and required a much higher dose of the agonist to elicit similar sensitization effects. Although it is possible that natural PAR2 ligands, such as MC tryptase, may be more potent in eliciting a nociceptive response (6,15), the relative importance of PAR2 activation in mediating the neuronal nociceptive responses evoked by dural MC degranulation will need to be evaluated further.

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Figure 1.

Topical activation of proteinase-activated receptor (PAR) 2 promotes increases in mechanical threshold responses of meningeal nociceptors. (a) Threshold responses of A δ and C-unit meningeal nociceptors following application of vehicle [synthetic interstitial fluid (SIF)] and subsequent ascending doses of the PAR2 agonist SLIGRL-NH₂. Individual neuronal responses to threshold mechanical stimulation of A δ -units (b) and C-units (c) 15 min following topical application of 500 $\mu\text{g/cc}$ SLIGRL-NH₂. The effect of chronic mast cell (MC) depletion using 48/80 on the duration of PAR2-mediated threshold sensitization of A δ (d) and C-units (e). * $P < 0.05$ Student's t-test SLIGRL-NH₂ vs. vehicle.

Figure 2.

Topical activation of proteinase-activated receptor (PAR) 2 promotes desensitization of mechanical suprathreshold responses of meningeal nociceptors. (a) Suprathreshold responses of A δ and C-unit meningeal nociceptors following application of vehicle [synthetic interstitial fluid (SIF)] and subsequent ascending doses of the PAR2 agonist. Individual neuronal responses to suprathreshold stimulation of A δ -units (b) and C-units (c) 15 min following topical application of 500 μ g/cc SLIGRL-NH₂. The effect of chronic mast cell (MC) depletion using 48/80 on the duration of PAR2-mediated suprathreshold desensitization of A δ (d) and C-units (e). * $P < 0.05$ Student's t-test SLIGRL-NH₂ vs. vehicle.

Figure 3.

Proteinase-activated receptor (PAR) 2-mediated excitation of meningeal nociceptors. (a) Changes in ongoing discharge rate of A δ and C-unit meningeal nociceptors following application of vehicle [synthetic interstitial fluid (SIF)] and subsequent ascending doses of the PAR2 agonist. Individual changes in neuronal ongoing discharge rate of A δ -units (b) and C-units (c) 15 min following topical application of 500 $\mu\text{g/cc}$ SLIGRL-NH₂. The effect of chronic mast cell (MC) depletion using 48/80 on the duration of PAR2-mediated activation of A δ (d) and C-units (e). * $P < 0.05$ Student's *t*-test SLIGRL-NH₂ vs. vehicle.