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Sensitization of central trigeminovascular neurons: blockade by intravenous naproxen infusion

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Abstract

We have previously observed that migraine attacks impervious to triptan therapy were readily terminated by subsequent intravenous administration of the non-steroidal anti-inflammatory drug (NSAID) ketorolac. Since such attacks were associated with periorbital allodynia – a symptom of central sensitization – we examined whether infusion of the NSAID naproxen can block sensitization of central trigeminovascular neurons in the medullary dorsal horn, using *in vivo* single-unit recording in the rat. Topical exposure of the cerebral dura to inflammatory soup (IS) for 5 min resulted in a short-term burst of activity (<8 min) and a long-lasting (>120 min) neuronal hyper-responsiveness to stimulation of the dura and periorbital skin (group 1). Infusion of naproxen (1 mg/kg) 2 h after IS (group 1) brought all measures of neuronal responsiveness back to the baseline values recorded prior to IS, and depressed ongoing spontaneous activity well below baseline. When given preemptively 1 h before IS (group 2), naproxen blocked the short-term burst of activity and every long-term measure of neuronal hyper-responsiveness that was studied in the central neurons. The same preemptive treatment, however, failed to block IS-induced short-term burst of activity in C-unit meningeal nociceptors (group 3). The results suggest that parenteral administration of naproxen, unlike triptan therapy, can exert direct inhibition over central trigeminovascular neurons in the dorsal horn. Though impractical as a routine migraine therapy, parenteral NSAID administration should be useful as a non-narcotic rescue therapy for migraine in the setting of the emergency department.

Keywords

headache; migraine; nociception; trigeminal; NSAID; pain

INTRODUCTION

Migraine headaches that are accompanied by cutaneous allodynia become increasingly resistant to triptan therapy with the progression of the attack (Burstein et al., 2004). The development of allodynia during migraine appears to be driven by sensitization of trigeminovascular neurons in the dorsal horn (Burstein and Jakubowski, 2004) which, unfortunately, are not equipped to respond to triptans directly (Potrebic et al., 2003, Levy et al., 2004). Triptans appear to act presynaptically in the dorsal horn, such as to inhibit signal transmission from peripheral (first-order) to central (second-order) trigeminovascular neurons

(Levy et al., 2004). Reining-in the central neurons using triptan treatment is possible as long as their excitability remains driven by incoming signals from the meninges, but not after they developed autonomous activity (Burstein and Jakubowski, 2004). Hitherto, no placebo-controlled studies have compared the effects of early vs late treatment with triptans or any other drug on pain-free rate in allodynic vs. non-allodynic migraineurs. Notwithstanding, attacks with allodynia can be effectively terminated, provided that the patient vigilantly resorts to triptan therapy before or soon after the onset of allodynia, but not after allodynia has become firmly established (Burstein et al., 2004). On the other hand, allodynic patients who had missed the critical window for effective triptan therapy, can still be rendered pain-free using intravenous infusion of the non-steroidal anti-inflammatory drug (NSAID) ketorolac (Jakubowski et al., 2005).

Preliminary data obtained in a single-unit recording study in the rat have suggested that infusion of ketorolac, a non-specific cyclooxygenase (COX) inhibitor, can block peripheral and central trigeminovascular neurons that were previously sensitized by stimulating the dura with a mixture of inflammatory agents (Jakubowski et al., 2005). Using the same rat model in the present study, we tested whether naproxen, another non-specific COX inhibitor, would terminate ongoing central sensitization, and also block the induction of such sensitization when given preemptively before stimulating the dura with inflammatory agents. We also compared the effects of preemptive treatment on the immediate activation of meningeal nociceptors vs. the central neurons in order to elucidate site of action of naproxen.

EXPERIMENT PROCEDURES

Surgical preparation

All experiments were approved by the standing committee on animals of the Harvard Medical School. Male Sprague-Dawley rats (250-400 g) were anesthetized with urethane (1.2 g/kg i.p.) and fitted with (a) an intratracheal metal tube for artificial ventilation of the lungs and (b) an intravenous plastic cannula for later drug infusion through the femoral vein. The rat's head was then mounted in a stereotaxic apparatus, while keeping core temperature at 37°C using a heating blanket. End-tidal CO₂ was continuously monitored and kept within a physiological range of 3.5-4.5%. In preparation for dural stimulation later in the experiment (groups 1-3), a 5×5-mm opening was carefully carved in the parietal and occipital bones in front and behind the lambda suture, directly above the left transverse sinus. Thereafter, the exposed dura was kept moist using a modified synthetic interstitial fluid (135 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 5 mM CaCl₂, 10 mM glucose and 10 mM Hepes, pH 7.2). In preparation for single-unit recording in the spinal trigeminal nucleus (groups 1 and 2), a segment of the spinal cord between the obex and C2 was exposed, stripped of the overlying dura mater, and kept moist with mineral oil. A platinum-coated tungsten microelectrode (impedance 4-6 MΩ) was lowered repeatedly into the medullary dorsal horn in search for a target unit. At the end of the experiment, a small lesion was produced at the recording site and its localization in the dorsal horn was determined postmortem using histological analysis as described before (Burstein et al., 1998). Single-unit activity in the trigeminal ganglion (group 3) was recorded using a platinum-coated tungsten microelectrode (impedance 500 KΩ) lowered through a 2-mm 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.

Central trigeminovascular neurons

Trigeminovascular neurons in the dorsal horn were selected for the study if they proved to receive nociceptive input from the dura mater *and* from the skin around the left eye. At baseline, electrical stimulation of the exposed dura (0.1-4.0 mA, 0.5-0.8 msec, 1 Hz) evoked fast *and* slow neuronal responses corresponding to input from meningeal Aδ- and C-fibers (5-9 and 18-150 msec response latencies, respectively). Baseline threshold to mechanical indentation

of the dura was determined quantitatively using a series of calibrated von Frey monofilaments (0.07, 0.17, 0.41, 0.69, 1.20, 1.48, 2.04 and 3.63 g). Neurons were classified as wide-dynamic-range (WDR) or high-threshold according to their responses to 3 skin stimuli in the following order: brush, pressure, and pinch (using, respectively, an artist paint brush, loose arterial clip, and forceps). Each of these mechanical stimuli was delivered over 10 sec, and the inter-stimulus interval was 10 sec. Neurons were also tested for responsiveness to heat stimulation using a thermal stimulator with a 9×9 mm end surface (Yale University, New Haven) that contacted the cutaneous receptive field using a thermally-conductive paste. Skin temperature was held constant at 35 °C before stimulation, increased to 50 °C at a rate of 1 °C/sec during stimulation, held at 50°C for another 10 sec, and returned to 35 °C at a rate of 1 °C/sec. The temperature at which neuronal activity increased >25% above baseline was selected as the response threshold, and the magnitude of response was measured from the threshold point through the end of the 50 °C ramp. Each set of mechanical and heat stimuli was repeated at least 3 times every 30 min, and only neurons showing <10% variability in response magnitude were selected to be studied. Baseline firing rate (spikes/sec) was sampled over a period of 10 min.

Meningeal nociceptors

To identify meningeal nociceptors, a recording tungsten microelectrode (platinum-coated; impedance 500 KW) was slowly lowered towards the trigeminal ganglion in search for a unit that responded to single shock stimuli (0.5 ms pulse, 5 mA, 0.5 Hz) delivered by a stimulating electrode that touched the dura overlaying the ipsilateral transverse sinus. A neuron was identified as a meningeal nociceptor if it responded with a constant latency to electrical stimuli from the dura *but not* to noxious mechanical stimuli from adjacent structures (i.e., pericranial muscles, cornea, vibrissae and ophthalmic skin). Conduction velocity was calculated using the shortest response latency to electrical stimulation of the dura which was divided by 12.5 mm – the distance between the trigeminal ganglion and the stimulation site in the dura (Burstein et al., 1998). Based on conduction velocity, neurons were classified as C-units (≤ 1.5 m/sec) or A δ -units (>1.5 m/sec). Only one neuron was studied in each animal. Spike amplitude was maximized by fine-tuning the position of the recording electrode. Spikes of the isolated unit were processed using a real-time waveform discriminator (Spike 2, Cambridge Electronic Design) and analyzed both online and offline. Throughout the experiment, ongoing activity was recorded continuously in 10-min epochs every 15 min.

Induction of sensitization

Activation and sensitization of central trigeminovascular neurons was induced using an ‘inflammatory soup’ (IS) containing 0.1 mM prostaglandin E₂, 1 mM histamine, serotonin, bradykinin, pH 5.5 (Steen et al., 1992, Steen et al., 1995, Steen et al., 1996). The exposed area of the cerebral dura was bathed in IS for 5 min as described before (Burstein et al., 1998, Levy and Strassman, 2002), and the establishment of central sensitization was verified 2 h later using any one of the following criteria: (a) increase in ongoing firing rate; (b) increase in responses magnitude to mechanical stimulation of the dura and skin; (c) shift in response threshold to heat stimulation of the skin; (d) expansion of dural or cutaneous receptive fields.

Experimental paradigm

Intravenous administration of naproxen (1 mg/kg) was given either 2 h after IS (group 1; Fig. 1A) or 1 h prior to IS (group 2; Fig. 1B). This intravenous dose is about an eighth of the initial oral dose of 500 mg recommended for treating patients with acute pain (~8 mg/kg for 60-kg body weight). In group 1 ($n=10$), ongoing activity of central trigeminovascular neurons and their responsiveness to mechanical and thermal stimulation of the facial skin were measured first at baseline, then 2 h after IS, and again 1 h after naproxen infusion (=3 h after IS). In group

2 ($n=7$), similar measurements were performed at baseline, then 1 h after naproxen infusion, and again 2 h after IS (=3 h after naproxen).

We also studied the immediate activation of central trigeminovascular neurons by IS, given either before (group 1) or after (group 2) naproxen. Since the IS-induced burst of activity observed in central trigeminovascular neurons (group 1) was blocked by naproxen (group 2), we examined whether naproxen would also inhibit the IS-induced burst of activity in the nociceptors that innervate the dura (group 3, $n=9$).

Data analysis

Raw data of individual neuronal recording are plotted graphically as mean spikes/sec calculated at bin width of either 0.5 or 1 sec; numbers in parentheses in these graphs indicate mean spikes/sec calculated for the duration of the pre-stimulus interval and for the duration of the response. Summary of the neurons in each group is presented as mean $\pm$ SEM spikes/sec.

Data analysis was performed using non-parametric statistics (Siegel, 1956, Zar, 1998). Changes in response magnitude or response threshold over the 3 time points were analyzed in each group using the Friedman test, followed by one-tailed Wilcoxon matched-pairs signed-ranks test. Level of significance was set at 0.05.

RESULTS

Effects of naproxen on sensitized neurons in the dorsal horn (group 1)

Recording sites and neuronal receptive fields in the dura and skin—Lesions marking the sites of the recorded neurons were localized to laminae IV and V of the first cervical segment (Fig. 2A). At baseline, neuronal receptive fields in the dura were restricted to the transverse sinus and adjacent areas; receptive fields in the skin were localized around the eye in all 10 cases, and spread over entire side of the face in 5 cases (Fig. 2A). Dural and cutaneous receptive fields expanded in some animals 2 h after exposing the dura to IS, and contracted 1 h after naproxen infusion (see example in Fig. 2B).

Sensitivity to mechanical and thermal stimuli of the periorbital skin—Neuronal hypersensitivity to skin stimulation that was induced by prior exposure of the dura to IS was suppressed by subsequent infusion of naproxen. Of 10 neurons studied, 8 exhibited hyper-responsiveness to mechanical and/or thermal skin stimulation after IS application (see example in Fig. 3). In those neurons, mean response magnitude to both brush (Fig. 4A) and pressure (Fig. 4B) increased significantly after IS compared to baseline, then dropped significantly after naproxen infusion. The final brush response was similar to the initial response magnitude at baseline ($P>0.10$); the final pressure response was significantly lower than the initial response magnitude at baseline. Response threshold to heat stimulation dropped significantly by 2.46 ± 0.28 °C after IS compared to the threshold at baseline, then rebounded significantly by 3.83 ± 1.09 °C after naproxen infusion (Fig. 4C). In these neurons, the magnitude of the response to heat stimulation did not change significantly after IS compared to baseline (67.5 ± 9.1 vs. 74.8 ± 10.9 spikes/sec, respectively; $P>0.15$), but did drop significantly to 38.1 ± 6.5 spikes/sec after naproxen infusion ($P<0.01$, compared to baseline).

Sensitivity to mechanical stimulation of the dura—Naproxen infusion also suppressed neuronal hypersensitivity to mechanical stimulation of the dura that was previously induced by exposure of the dura to IS. Of 10 neurons studied, 8 exhibited hyper-responsiveness to mechanical stimulation of the dura. In those neurons, response threshold to indentation of the dura dropped significantly ($P<0.01$) from 1.93 ± 0.41 g at baseline to 0.81 ± 0.19 g after IS

application, and rebounded significantly ($P<0.01$) to 2.41 ± 0.47 g after naproxen infusion, a threshold that was comparable to the baseline value ($P>0.1$).

Spontaneous activity—Naproxen infusion suppressed ongoing neuronal activity in all 10 neurons studied (Fig. 5A, B). Exposure of the dura to IS increased the firing rate in 4 out of the 10 neurons, but subsequent naproxen infusion significantly decreased neuronal firing rate by $74\pm7\%$ below baseline in all neurons.

Effects of naproxen on non-sensitized neurons in the dorsal horn (group 2)

Recording sites and neuronal receptive fields in the dura and skin—Lesions marking the sites of the recorded neurons were localized to the ventrolateral region of lamina V at the level of C1-2 (Fig. 6A). At baseline, neuronal receptive fields in the dura were restricted to the transverse sinus and adjacent areas; receptive fields in the skin were restricted to the area around the eye in 3 cases, and spread over entire side of the face in 4 cases (Fig. 6A). Dural and cutaneous receptive fields contracted 1 h after naproxen infusion, and remained contracted after exposing the dura to IS (see example in Fig. 6B).

Sensitivity to mechanical and thermal stimuli of the periorbital skin—Naproxen infusion suppressed the responsiveness to skin stimulation in naïve (i.e., non-sensitized) trigeminovascular neurons, and prevented the induction of hyper-responsiveness by subsequent exposure of the dura to IS (see example in Fig. 7). Responses to brush (Fig. 8A) and pressure (Fig. 8B) stimuli were significantly smaller in magnitude 1 after naproxen and 2 h after IS (=3 h after naproxen) compared to baseline responses. Response threshold to heat stimulation was significantly higher by 2.5 ± 1.2 °C 1 h after naproxen infusion and by 3.6 ± 1.3 °C 2 h after IS compared to baseline values (Fig. 8C). In these neurons, the magnitude of response to heat stimulation was $47\pm11\%$ below baseline ($P<0.02$) 1 h after naproxen, and $77\pm10\%$ below baseline 2 h after IS ($P<0.01$).

Sensitivity to mechanical stimulation of the dura—Naproxen infusion suppressed responsiveness to mechanical stimulation of the dura in 5 out of the 7 naïve neurons, and prevented the development of hyper-responsiveness by subsequent exposure of the dura to IS. In those neurons, response threshold to indentation of the dura increased from 1.97 ± 0.57 g at baseline to 2.54 ± 0.44 g at 1 h after naproxen infusion ($P>0.02$) and to 5.78 ± 1.24 g 2 h after IS (=3 h after naproxen), a threshold that was significantly higher than the baseline value ($P<0.01$).

Spontaneous activity—Naproxen administration suppressed ongoing firing rate in all 7 naïve neurons (Fig. 9A, B). Compared to baseline values, ongoing firing rate was significantly lower by $51\pm34\%$ at 1 h after naproxen infusion, and by $96\pm3\%$ at 2 h after IS (=3 h after naproxen).

Acute neuronal activation by IS—The brief exposure of the dura to IS induced rapid increase in neuronal firing in naïve animals (group 1) but not in those pre-treated with naproxen (group 2). In group 1 (example in Fig 10A), neuronal firing rate increased significantly from 19.4 ± 5.1 spikes/sec at baseline to 41.0 ± 3.6 during the first 8 min after IS ($P<0.01$), and remained elevated for a period of 47.0 ± 13.4 min. In group 2 (example in Fig. 10B), neuronal firing rate also increased significantly from 1.8 ± 0.7 spikes/sec (i.e., a level already depressed by prior naproxen infusion) to 3.5 ± 1.2 spikes/sec during the first 8 min after IS ($P<0.02$); this small response, however, was short-lived, lasting only 2.6 ± 1.3 min.

Effects of naproxen on activation of non-sensitized meningeal nociceptors (group 3)

Having observed that preemptive naproxen infusion prevented central trigeminovascular neurons from exhibiting acute activation in response to IS stimulation, we examined if the same

paradigm would result in a similar blockade in the primary afferents. The results varied with class of afferents studied. A δ units ($n=5$) – like the central neurons – did not exhibit an IS-induced burst of activity after naproxen pretreatment: mean firing rate of the A δ units was 0.27 ± 0.21 , 0.39 ± 0.20 , 0.35 ± 0.22 and 0.41 ± 0.21 spikes/sec at 0, 15, 30 and 45 min after IS, respectively ($P>0.1$). C-fiber units ($n=4$) – unlike the central neurons or A δ units – did exhibit an IS-induced burst of activity after naproxen pretreatment (Fig 11): mean firing rate of the C-units increased significantly from 0.46 ± 0.27 at baseline to 1.26 ± 0.52 , 1.93 ± 0.98 and 1.85 ± 0.93 spikes/sec at 15, 30 and 45 min after IS, respectively ($P<0.04$).

DISCUSSION

Intravenous naproxen administration counteracted both the induction and maintenance of sensitization in trigeminovascular neurons of the spinal trigeminal nucleus. Given 2 h *after* stimulating the dura with IS (group 1), naproxen infusion reversed the following measures of IS-induced central sensitization: (a) neuronal receptive fields in the dura and skin, which expanded in response to IS, contracted back to normal after naproxen; (b) response magnitude to mechanical and thermal stimulation of the skin, which became exaggerated after IS, returned to baseline or below after naproxen; (c) response threshold to thermal skin stimuli, which dropped after IS, rose back to baseline after naproxen; (d) ongoing neuronal firing, which increased in some cases after IS, dropped after naproxen below baseline in all cases. Given preemptively 1 h *before* stimulating the dura with IS (group 2), naproxen infusion blocked the initiation of each of the IS-induced measures of central sensitization that we studied. The findings that the immediate burst of neuronal activity upon placement of IS on the dura (group 1) was blocked by preemptive naproxen infusion in central trigeminovascular neurons (group 2), but not in C-unit meningeal nociceptors (group 3), suggests that naproxen acted in the dorsal horn to inhibit the central neurons directly or/and reduce the synaptic input from the peripheral to the central trigeminovascular neuron.

Activation of nociceptors has been shown to increase biosynthesis of prostaglandins in the spinal dorsal horn [Yang, 1996 #16143; Dirig, 1999 #16145] through COX2 upregulation [Beiche, 1996 #16146; Beiche, 1998 #16147]. In the dorsal horn, prostaglandins bind pre-synaptically to the central terminals of primary afferents, and post-synaptically to central nociceptive neurons [Matsumura, 1995 #16148]. Evidence for pre-synaptic prostaglandin action includes PGE₂-induced increased glutamate release from C-fiber terminals [Malmberg, 1995 #16149; Ferreira, 1996 #10656] and neuropeptide release from spinal cord slices [Andreeva and Rang, 1993, Vasko, 1995]. Evidence for post-synaptic prostaglandin action includes the preservation of PGE₂-induced inward (depolarizing) current in the presence of synaptic transmission blockade (Baba et al., 2001). Accordingly, attenuation of PGE₂-induced allodynia and hyperalgesia [Uda, 1990 #16150; Minami, 1994 #16151] by intrathecal administration of COX1/COX2 inhibitors [Malmberg, 1992 #16154; Malmberg, 1995 #16155] is likely to involve both pre- and post-synaptic mechanisms. On the pre-synaptic side, decreased PGE₂ level would lead to reduced neurotransmitter release from the primary afferents [Andreeva, 1993 #8832; Vasko, 1995 #7935; Malmberg, 1995 #16149; Ferreira, 1996 #10656]. On the post-synaptic side, decreased PGE₂ level would result in diminished hyperexcitability of the central nociceptive neurons [Chapman, 1992 #16156; Pitcher, 1999 #13930].

That naproxen, a non-selective COX1/COX2 inhibitor, blocked both evoked hyper-responsiveness and ongoing activity of sensitized trigeminovascular neurons of the medullary dorsal horn (group 1) is consistent with inhibition of sensitized WDR neurons in the spinal dorsal horn by both selective [You, 2003 #16157] and non-selective COX inhibitors [Neugebauer, 1995 #16158]. The inhibition of ongoing and evoked activity in the *non-sensitized* trigeminovascular neurons by naproxen (group 2) is in agreement with previous

studies on non-sensitized trigeminovascular neurons (Kaube et al., 1993, Ellrich et al., 1999), but at variance with studies on non-sensitized WDR neurons in the lumbar spinal cord in which selective or non-selective COX inhibitors did not suppress ongoing neuronal activity [Pitcher, 2001 #16159; Pitcher, 1999 #13930] or mechanically-induced activity [You, 2003 #16157; Willingale, 1997 #8568]. Assuming that the surgical procedure did not induce long-lasting sensitization in the studied neurons, it would appear that dura-sensitive WDR neurons are suppressible by COX inhibitors in the sensitized *and* the non-sensitized state. However, we cannot rule out the possibility that the surgical procedure might have resulted in mild neuronal sensitization in the seemingly naïve state.

Prolonged barrage of incoming signals from nociceptors causes activation and sensitization of nociceptive neurons (Ji et al., 2003) and activation of glial cells (Samad et al., 2001, Ghilardi et al., 2004) in the spinal dorsal horn. With time, local prostaglandin release from the activated neurons and glia becomes essential for the maintenance of sensitization of adjacent nociceptive neurons [You, 2003 #16157; Watkins, 2001 #8080; Watkins, 2001 #8081]. Central trigeminovascular neurons exhibit a similar shift: at first they become activated by incoming signals from the meninges; later on, they remain hyperactive independent of those incoming signals [Burstein, 2006 #16160]. Thus, as inhibitors of COX activities that regulate prostaglandin biosynthesis, naproxen (present study) and ketorolac (Jakubowski et al., 2005) are well positioned to counteract the sensitization of central trigeminovascular neurons that mediate migraine with allodynia. That activity-independent central sensitization can be terminated using NSAID infusion but not triptan therapy is compatible with our earlier observation that patients having migraine with periorbital allodynia (a sign of central sensitization) became pain-free within 1 h of ketorolac infusion after failing to respond to triptan therapy earlier in the same migraine attack (Jakubowski et al., 2005).

Regardless of its effectiveness in suppressing central sensitization, parenteral NSAID therapy can only be used as rescue treatment for migraine in the setting of the emergency department. Allodynic patients from our earlier study (Jakubowski et al., 2005) who were rendered pain-free using parenteral ketorolac – the only intravenous formulation of NSAID currently approved in the US – testified that ingestion of oral NSAID formulations did nothing in the way of aborting their migraine attacks. Given that oral and intravenous administration of COX inhibitors result in similar profiles of plasma concentration (Jung et al., 1988, Pasloske et al., 1999, McAleer et al., 2007), and that intrathecal doses that block pain caused by peripheral inflammation are less than 100th of the doses required systemically (Malmberg and Yaksh, 1992), we suggest that non-parenteral administration somehow leads to sub-optimal concentration of NSAIDs in the dorsal horn. It may prove useful to investigate whether the eventual concentration attained in the dorsal horn using parenteral *vs.* non-parenteral NSAID formulations is related to their efficacy in aborting migraine with allodynia.

CONCLUSION

The results suggest that parenteral administration of naproxen, unlike triptan therapy, can exert direct inhibition over central trigeminovascular neurons in the dorsal horn. Though impractical as a routine migraine therapy, parenteral NSAID administration should be useful as a non-narcotic rescue therapy for migraine in the setting of the emergency department.

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List of abbreviations

COX, cyclooxygenase; IS, inflammatory soup; NSAID, non-steroidal anti-inflammatory drug.

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Fig. 1.

Experimental design. Group 1 examined whether naproxen infusion terminates ongoing sensitization that was induced earlier by a 5-min exposure of the dura to IS. Group 2 examined whether naproxen infusion prevents activation and induction of sensitization by subsequent exposure of the dura to IS. Green – baseline; red – after IS; Blue – after naproxen.

Fig. 2.

Recording sites and receptive fields – group 1. (A) Lesions marking individual recording sites in the spinal trigeminal nucleus and the respective neuronal receptive fields in the dura and facial skin. (B) an example of expansion and contraction of dural and cutaneous receptive fields in response to IS and naproxen, respectively. Green – baseline; red – after IS; Blue – after naproxen. Numbers identify individual rats.

Fig. 3.

Effects of naproxen infusion on responsiveness of sensitized central trigeminovascular neurons to stimulation of their cutaneous receptive field – group 1. Shown are examples of responses to brush and pressure (A) and heat (B) in a neuron studied before (baseline/green) and after IS (red), and again after naproxen infusion (blue). Bin width: 0.5 sec. Numbers in parentheses indicate mean spikes/sec during the pre-stimulus intervals and during the response intervals. Notice that the magnitude of all responses decreased below baseline values, and that heat response threshold returned to baseline after naproxen infusion.

A. Response magnitude to brush ($p < 0.003$) B. Response magnitude to pressure ($p < 0.001$) C. Response threshold to heat ($p < 0.001$) **Fig. 4.**

Summary of the effects of naproxen infusion on responsiveness of sensitized central trigeminovascular neurons to skin stimulation – group 1. Shown are mean $\pm$ SEM response magnitude to brush (A) and pressure (B), and response threshold to heat (C) before (baseline/green) and after IS (red), and again after naproxen infusion (blue). Gray bars (A, B) indicate spontaneous activity in the 10-sec interval that preceded the stimulus. Probability values indicate significant overall differences across green, red and blue bars (Friedman test). ^a $p < 0.02$ and ^b $p < 0.03$ vs. respective green bars; ^c $p < 0.02$ vs. respective red bars (Wilcoxon test).

Fig. 5.

Effects of naproxen infusion on spontaneous activity of sensitized central trigeminovascular neurons – group 1. (A) mean firing rate of a single neuron studied before (baseline/green) and after IS (red), and again after naproxen infusion (blue). Bin width: 1 sec; numbers in parentheses indicate mean spikes/sec during the entire 600-msec intervals. (B) mean $\pm$ SEM firing rate of all neurons (color coded as above). Significant overall differences across green, blue and red bars ($p < 0.003$, Friedman test). ^a $p < 0.01$ vs. green bar; ^b $p < 0.01$ vs. red bar (Wilcoxon test). Notice that ongoing firing rate was suppressed below baseline values after naproxen infusion.

Fig. 6. Recording sites and receptive fields – group 2. (A) Lesions marking individual recording sites in the spinal trigeminal nucleus and the respective neuronal receptive fields in the dura and facial skin. (B) an example of contraction of dural and cutaneous receptive fields in response to naproxen, and failure of IS to induce expansion of the receptive fields. Green – baseline; blue – after naproxen; red – after IS;. Numbers identify individual rats.

Fig. 7.

Effects of naproxen infusion on responsiveness of non-sensitized central trigeminovascular neurons to stimulation of their cutaneous receptive field – group 2. Shown are examples of responses to brush and pressure (A) and heat (B) in a neuron studied before (baseline/green) and after naproxen infusion (blue), and again after IS (red). Bin width: 0.5 sec. Numbers in parentheses indicate mean spikes/sec during the pre-stimulus intervals and during the response intervals. Notice that naproxen infusion markedly suppressed the magnitude of all responses below baseline values, shifted heat response threshold above baseline, and blocked induction of hyper-responsiveness by IS.

Fig. 8.

Summary of the effects of naproxen infusion on responsiveness of non-sensitized central trigeminovascular neurons to skin stimulation – group 2. Shown are mean $\pm$ SEM response magnitude to brush (A) and pressure (B), and response threshold to heat (C) before (baseline/green) and after naproxen infusion (blue), and again after IS (red). Gray bars (A, B) indicate spontaneous activity in the 10-sec interval that preceded the stimulus. Probability values indicate significant overall differences across green, blue and red bars (Friedman test). ^a $p < 0.02$ and ^b $p < 0.05$ vs. respective green bars; ^c $p < 0.05$ vs. respective blue bar (Wilcoxon test).

Fig. 9.

Effects of naproxen infusion on spontaneous activity of non-sensitized central trigeminovascular neurons – group 2. (A) mean firing rate of a single neuron studied before (baseline/green) and after IS (red), and again after naproxen infusion (blue). Bin width: 1 sec; numbers in parentheses indicate mean spikes/sec during the entire 600-msec intervals. (B) mean $\pm$ SEM firing rate of all neurons (color coded as above). Notice that naproxen infusion markedly suppressed ongoing firing rate below baseline values. Significant overall differences across green, blue and red bars ($p < 0.004$, Friedman test). ^a $p < 0.03$ vs. green bar; ^b $p < 0.05$ vs. blue bar (Wilcoxon test).

Fig. 10.

Naproxen inhibition of acute response to IS in a central trigeminovascular neuron. Shown are examples of IS-induced increase in mean firing rate of a naïve neuron (A) and the suppression of IS-induced discharge in a neuron previously exposed to naproxen infusion (B). Green – baseline; blue – after naproxen; red – after IS. Bin width: 1 sec. Numbers in parentheses indicate mean spikes/sec during the pre-stimulus intervals and during the response intervals. Exposure of the dura to IS for 5 min is indicated by black line.

Fig. 11.

Example of an acute response to IS in a C-unit meningeal nociceptor previously exposed to naproxen infusion. Blue – after naproxen and before IS; red – after IS. Bin width: 0.5 sec.