Comparative Effects of the Nonsteroidal Anti-inflammatory Drug Nepafenac on Corneal Sensory Nerve Fibers Responding to Chemical Irritation

M. Carmen Acosta,1 Carolina Luna,1 Gustav Graff,2 Victor M. Meseguer,1 Felix Viana,1 Juana Gallar,1 and Carlos Belmonte1

Purpose. To compare the corneal analgesic efficacy of the nonsteroidal anti-inflammatory drugs (NSAIDs) nepafenac, diclofenac, and ketorolac, and to evaluate the possibility that their inhibitory effects on corneal polymodal nociceptor fiber activity are partly mediated by a decrease in sodium currents.

Methods. Corneal sensory afferent units were recorded in the anesthetized cat. The response of thin myelinated polymodal nociceptor fibers to mechanical and acidic stimulation (98.5% CO2) was recorded before and at various times after topical application of the vehicle or of nepafenac 0.1% (Nevanac; Alcon Laboratories, Ltd., Fort Worth, TX), diclofenac 0.1% (Voltaren; Novartis, Basel, Switzerland), and ketorolac 0.4% (Aculair LS; Allergan, Irvine, CA). Voltage-clamp recordings were performed in cultured trigeminal ganglion neurons.

Results. Nepafenac, diclofenac, and ketorolac reduced the mean frequency of the impulse response evoked by repeated CO2 stimuli in polymodal nociceptor fibers. The progressive increase in ongoing activity, observed in vehicle-treated eyes after repeated acidic stimulation was also prevented. Nepafenac exhibited a more rapid and a slightly more pronounced effect on spontaneous and CO2-evoked activity than did diclofenac and ketorolac and did not affect the responsiveness of corneal mechanonociceptor or cold receptor fibers. In cultured mice trigeminal ganglion neurons, diclofenac significantly suppressed sodium currents, whereas nepafenac or its metabolite, amfenac, exhibited only minimal inhibitory effects.

Conclusions. The inhibition of polymodal nociceptor activity by nepafenac, a weak inhibitor of cyclooxygenase, is most likely due to its greater lipophilicity compared with diclofenac and ketorolac, leading to a rapid saturation of the corneal epithelium where nociceptor terminals are located. In contrast to diclofenac, nepafenac does not exhibit local anesthetic effects. (Invest Ophthalmol Vis Sci. 2007;48:182–188) DOI: 10.1167/iovs.06-0710

Injury to the cornea leads to activation of trigeminal nociceptors that innervate the ocular surface, thereby evoking pain.1,2 Corneal nociceptor terminals are excited by exogenous noxious stimuli and also by inflammatory substances released by damaged cells. These include arachidonic acid metabolites, neuropeptides, biogenic amines, and kinins.3 Inflammatory mediators may directly activate nociceptive terminals or induce sensitization.4 Sensitized nociceptors exhibit a decreased threshold; an increased response to noxious stimuli; and spontaneous, ongoing impulse activity.5–7 This altered responsiveness of corneal nociceptors after injury contributes to the sustained ocular pain and hyperalgesia that follow corneal damage.8

Prostaglandins (PGs) have a well-established role as mediators of inflammatory pain9 because they excite and sensitize nociceptors.10–15 Accordingly, nonsteroidal anti-inflammatory drugs (NSAIDs), which are extensively used as anti-inflammatory agents in ocular surgery are also prescribed as analgesic drugs to reduce postoperative pain after photorefractive surgery.16–18 Their anti-inflammatory effect is due to an inhibition of the cyclooxygenases (COX-1 and -2), which convert arachidonic acid into biologically active PGs.19 The analgesic action of NSAIDs has also been attributed, at least in part, to this reduction of PG production.17 However, different NSAIDs affect ocular sensitivity to varying degrees in humans18,19 and also have different effects on nerve impulse discharges evoked by chemical irritation of the cornea in anesthetized cats.20 Thus, it has been suggested that in addition to the decreased nociceptor sensitization secondary to reduced PG production common to all NSAIDs, some of these drugs may directly affect the excitability of ocular nociceptor sensory nerve terminals.20

Afenac (2-amino-3-benzoylbenzeneacetic acid), a nonselective COX-1 and COX-2 inhibitor21,22 is an NSAID that has been shown to be a potent oral anti-inflammatory and analgesic agent in experimental animals.23,24 Afenac is marketed under the trade name Fenazox (Meiji Seika Kaisha Ltd., Yokohama, Japan) for the treatment of pain and inflammation associated with rheumatoid and osteoarthritis and low back pain, as well as for the treatment of pain and inflammation after surgery, injury, or tooth extraction. Nepafenac is the carboxamide derivative and prodrug form of afenac. Nepafenac applied topically to the eye, has the unique ability to distribute to the vascularized tissues of the eye where it undergoes hydrolytic conversion to afenac. Thus, nepafenac’s anti-inflammatory action is not restricted to the anterior segment, but also extends to the retina, where it is capable of attenuating posterior ocular inflammation and development of retinal edema induced by concanavalin A25 and ischemia-induced retinal neovascularization.26 The superior pharmacodynamics are largely attributable to the conversion of the carboxylic acid to a carboxamide function, thus altering the molecule’s physical, permeability, and pharmacological properties. Although the ocular anti-inflammatory properties of nepafenac have now been well established, its potential ocular analgesic effects have not hitherto been explored in detail.

In the present study, we evaluated the analgesic efficacy of nepafenac measuring the reduction in the nerve impulse activity evoked by repeated chemical noxious stimulation of cor-
neal polymodal nociceptive fibers of anesthetized cats and compared the effects of nepafenac 0.1% (Nevanac; Alcon Laboratories, Inc., Fort Worth, TX) with those of diclofenac 0.1% (Voltaren; Novartis, Basel, Switzerland) and ketorolac 0.4% (Acular LS; Allergan, Irvine, CA), two marketed NSAIDs extensively used for the treatment of postsurgical ocular pain and inflammation. The possibility that analgesic effects of nepafenac were mediated by a reduction of sodium ion channel activity at corneal sensory nerve terminals was also explored by measuring sodium currents in patch-clamped cultured trigeminal ganglion neurons.

**Materials and Methods**

**Experiments In Vivo**

Experiments were performed in 20 cats of both sexes weighing 1.5 to 4.8 kg anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneally). The animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The saphenous vein was cannulated for the administration of balanced saline solution and supplementary doses of pentobarbital sodium (5 mg/kg per hour). The dose of anesthetic was adjusted to maintain the animal in an areflexic state throughout the experiment. The trachea was cannulated and connected to a CO₂ analyzer (model FM1; ADC Ltd., Huddersdon, UK). Mean arterial blood pressure at the femoral artery, end-tidal CO₂, and rectal temperature were continuously monitored and maintained at physiological levels (≥80 mm Hg, 3%-4% CO₂, and 37°C, respectively). At the end of the experiment, the cats were euthanized with an overdose of anesthetic.

**Nerve Fiber Recordings.** The technique previously described by Belmonte et al. was used. Briefly, the cat was placed prone, and the superior and lateral sides of the orbit and the extrinsic muscles of the eye were removed to expose the ciliary nerves. The orbital cavity was then filled with mineral oil, and one of the ciliary nerves was dissected and cut centrally with the aid of a binocular microscope. Filaments were split from the main nerve trunk and placed on an Ag-AgCl electrode for monopolar recording. The presence of corneal units in the filament was confirmed by the multiunit discharge evoked when a paint brush was gently moved over the surface of the cornea. Subsequently, fine nerve strands were split until a single unit was evoked by corneal stimulation. The cornea was kept moist during the experiment by regular application of physiological saline.

Mechanical stimulation of the cornea was performed with a Cochet-Bonnet esthesiometer, provided with a no. 12 filament to determine force thresholds and receptive field borders. Chemical sensitivity was tested by applying to the corneal surface an air jet containing 98.5% CO₂ in air for 30 seconds. CO₂ causes the local formation of carbonic acid and acts as an acidic stimulus. The flow of the gas mixture was initially adjusted using only air, to a flow level below that required to activate the fiber mechanically. Conduction velocity of single nerve fibers was estimated from the latency of the response to suprathreshold electric shocks (0.1–0.5 ms, 0.5–3 mA) applied to the receptive field area with a bipolar silver electrode. Conduction distance (3.0–3.4 cm) was measured by placing 0.5–3 mA) applied to the receptive field area with a bipolar silver electrode. Conduction distance (3.0–3.4 cm) was measured by placing a micropipette, in 60-μL drops. In some instances, a piece of tissue paper was placed on the cornea, covering the receptive field area to restrict the effect of the drug to the boundaries of the recorded unit. This allowed recording and testing, whenever possible, more than one unit in the cornea of the same animal. One minute later, the response to chemical stimulation was again tested. The mechanical threshold was measured 1 minute after- ward. Stimulation with CO₂ was repeated seven times per fiber, with a time interval of 15 minutes between stimuli. The mechanical threshold was measured again after the third CO₂ stimulation.

**Experiments In Vitro**

**Culture of Trigeminal Ganglion Neurons.** Trigeminal ganglion neurons from adult mice (postnatal days 25–40) were cultured as described previously. In brief, male Swiss OF1 mice were killed by inhalation of 100% CO₂ followed by decapitation and removal of trigeminal ganglia (TGs). TGs were cut in small pieces and incubated for 1 hour at 37°C in a dissolution solution containing (mM): 155 NaCl, 1.5 K₂HPO₄, 5.6 HEPES, 4.8 NaHEPES, and 5 glucose. The solution also contained 0.07% collagenase type XI (Sigma-Aldrich, St. Louis, MO) and 0.3% dispase (Invitrogen, Carlsbad, CA). After incubation, tissue fragments were gently triturated with a fire-polished glass pipette, and the resultant suspension was centrifuged at 2000 rpm for 8 minutes. The pellet obtained was resuspended and cultured in a medium containing: 88% minimum essential medium (MEM), 10% fetal bovine serum (Invitrogen), supplemented with 1% MEM vitamins (Invitrogen), 100 μg/mL penicillin/streptomycin, nerve growth factor (NGF; mouse 7S, 100 ng/mL; Sigma-Aldrich). Cells were plated on poly-L-lysine–coated glass coverslips and used after 1 to 2 days in culture.

**Whole-Cell Recordings of Sodium Currents.** Voltage-clamp recordings in the whole-cell configuration were performed on small to medium sized neurons with a patch-clamp amplifier (Multi- clamp 700B; Molecular Devices, Sunnyvale, CA). Stimulus delivery and data acquisition were then performed (pClamp 9 software; Molecular Devices). Depolarizing voltage steps (−10 mV) of 15-ms duration were delivered from two holding potentials (−100 and −50 mV) every 10 seconds.

The standard bath solution contained (in mM): 140 NaCl, 5 KCl, 1.3 MgCl₂, 2.4 CaCl₂, 10 HEPES, 10 glucose (297 mOsm/Kg) and had a pH of 7.4 adjusted with NaOH. To isolate sodium currents, we prepared an external solution containing (mM): 35 NaCl, 30 TEACl, 85 choline chloride, 0.1 CaCl₂, 5 MgCl₂, 10 glucose, 10 HEPES (310 mOsm/Kg [pH 7.4] adjusted with TEAOH). Standard patch-pipettes (3–5 M) were fabricated from borosilicate glass capillaries (Harvard Apparatus Ltd., Edenbridge, UK) and contained (in mM): 140 CsF, 10 NaCl, 10 HEPES, 1 EGTA (302 mOsm/Kg [pH 7.2, adjusted with CsOH]). All recordings were performed at room temperature.
Drugs

In the experiments performed in cats, test solutions (60 µL) were applied to the corneal surface as a drop or in a piece of paper tissue covering the receptive field. No washing was performed afterward. The following drugs were assayed: nepafenac, 0.1% (Nevanac); diclofenac 0.1% (Voltaren; Novartis); and ketorolac 0.4% (Acular LS; Allergan). The vehicle for nepafenac (mannitol, cabomer 974P, sodium chloride, tyloxapol, edetate disodium, benzalkonium chloride 0.005%, sodium hydroxide, and/or hydrochloric acid to adjust pH and purified water, USP) was also tested in separate experiments. Nepafenac, 0.1% was prepared by Alcon Laboratories, Inc. and represents the clinical formulation (Nevanac), diclofenac 0.1% (Voltaren), and ketorolac 0.4% (Acular LS) were obtained from a local pharmacy and represent marketed products. Test articles were transferred under sterile conditions into identical opaque containers and coded as A, B, or C. The samples were then sent to the investigators. The investigators were unaware of the test drug selection, their composition or coding until the complete series of experiments had been finished and the effects of the different drugs had been summarized and tabulated.

In the experiments performed in cultured trigeminal neurons, the following drugs were tested during patch-clamping: nepafenac (Alcon Laboratories Inc.), amfenac (Alcon Laboratories, Inc.), and diclofenac (Sigma-Aldrich). Drugs were prepared as a stock solution of 50 mM in DMSO and stored at −20°C until use. Drugs were prepared as a stock solution of 50 mM in DMSO and stored at −20°C until use. Aliquots of the drug stock solutions were appropriately diluted in external solutions. Drugs were applied by a gravity perfusion system at a rate of 1 mL/min.

Drugs were tested in the following order: nepafenac, amfenac, and diclofenac. Test articles were transferred under sterile conditions into identical opaque containers and coded as A, B, or C. The samples were then sent to the investigators. The investigators were unaware of the test drug selection, their composition or coding until the complete series of experiments had been finished and the effects of the different drugs had been summarized and tabulated.

RESULTS

General Properties of Corneal Sensory Fibers

Neural activity was recorded from 49 corneal fibers sensitive to mechanical and chemical stimulation and thus classified as polymodal nociceptors. Three pure mechanosensory fibers, insensitive to CO2 stimulation, and two cold thermoreceptor fibers were also recorded in separate experiments to test the effect of nepafenac on these functional types of fibers.

Conduction velocity was measured in 33 polymodal fibers and ranged from 3.6 to 16 m/sec (mean. 6.6 ± 0.5). Therefore, all of them belonged to the thin, myelinated A alpha fiber.

The receptive fields of polymodal nociceptor fibers were round or oval, with a mean long diameter of 5.4 ± 0.2 mm (n = 47). In 9 of 47 units, the receptive field was restricted to the cornea, and in 38, it also extended into the sclera. In two units, the receptive area was not mapped.

Effect of Drugs on Neural Activity

Nepafenac. Topical nepafenac was tested in a total of 17 polymodal nociceptors, two cold thermoreceptors, and three pure mechanonociceptor fibers.

Direct Effects. Mean impulse frequency of the spontaneous activity was measured in 15 polymodal nociceptor fibers before and 1 minute immediately after application of nepafenac. Although mean impulse frequency was slightly reduced 1 minute after drug application, differences with the control were not significant (Fig. 1; Table 1).

Response to CO2. As shown in Figure 2 and Table 2, the mean firing frequency of the impulse discharge evoked by 30-second CO2 pulses was significantly reduced immediately after application of nepafenac. This effect persisted 100 minutes afterward (Fig. 2; Table 2). The latency and time to peak values of the impulse discharge were slightly longer than during the control period, but differences were not statistically significant (data not shown).

Diclofenac. Diclofenac 0.1% was tested in 11 polymodal nociceptor fibers.

Direct Effects. Application of diclofenac evoked in most of the fibers (7/11) a short impulse discharge and/or a slight and transient increase in ongoing activity frequency. This effect was reflected in a higher mean impulse frequency during the 1-minute period that followed application of the drug (Fig. 1, Table 1) in comparison with the control. However, differences did not reach significance.

Ongoing Activity. A gradual increase in the frequency of the spontaneous impulses that appeared during the intervals between repeated CO2 pulses was normally observed with vehicle treatment. This increase was prevented by nepafenac, which also significantly reduced the mean frequency of this spontaneous impulse activity (Fig. 1, Table 1).

The spontaneous activity of cold receptors was not modified by nepafenac treatment (data not shown).

Ketorolac. Ketorolac 0.4% was tested in 12 polymodal nociceptor fibers.

Direct Effects. Application of ketorolac evoked in most of the fibers (7/11) a short impulse discharge and/or a slight and transient increase in ongoing activity frequency. This effect was reflected in a higher mean impulse frequency during the 1-minute period that followed application of the drug (Fig. 1, Table 1) in comparison with the control. However, differences did not reach significance.

Mechanical Threshold. The mechanical threshold of corneal polymodal units measured 2 and 17 minutes after application of nepafenac did not change significantly when compared with control thresholds (Table 3).

The impulse response of mechanonociceptors to mechanical stimuli was not affected by nepafenac treatment (data not shown).

Ongoing Activity. Nepafenac did not affect the mean frequency of spontaneous impulse activity of polymodal nociceptor fibers, but prevented the gradual increase in firing frequency of the ongoing activity observed in vehicle-treated eyes after repeated CO2 pulses (Fig. 1, Table 1).

Ketorolac. Ketorolac 0.4% was tested in 12 polymodal nociceptor fibers.

Direct Effects. Application of ketorolac, in five polymodal fibers ongoing activity did not vary: In one fiber, it was slightly reduced, and in six fibers the ongoing activity increased, in two of them producing a burst of impulses ime-
The effect of the different NSAIDs on ongoing activity of polymodal nociceptors before and at different times after drug application is presented in Table 1. 

TABLE 1. Effect of the Different NSAIDs on Ongoing Activity of Polymodal Nociceptors before and at Different Times after Drug Application

<table>
<thead>
<tr>
<th>Drug</th>
<th>Vehicle</th>
<th>Nepafenac 0.1%</th>
<th>Diclofenac 0.1%</th>
<th>Ketorolac 0.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>0.31 ± 0.20</td>
<td>0.96 ± 0.50</td>
<td>0.08 ± 0.02</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>0–1 min</td>
<td>0.31 ± 0.17</td>
<td>0.30 ± 0.11</td>
<td>0.35 ± 0.19</td>
<td>0.19 ± 0.09</td>
</tr>
<tr>
<td>15–16 min</td>
<td>0.57 ± 0.47</td>
<td>0.63 ± 0.35</td>
<td>0.15 ± 0.05</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>30–31 min</td>
<td>1.00 ± 0.95</td>
<td>1.05 ± 0.96</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>45–46 min</td>
<td>1.38 ± 1.36</td>
<td>0.52 ± 0.37</td>
<td>0.13 ± 0.09</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>60–61 min</td>
<td>Not tested</td>
<td>0.55 ± 0.40</td>
<td>0.08 ± 0.05</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>75–76 min</td>
<td>0.53 ± 0.38</td>
<td>0.05 ± 0.02</td>
<td>0.11 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>90–91 min</td>
<td>Not tested</td>
<td>0.17 ± 0.10</td>
<td>0.11 ± 0.04</td>
<td>0.05 ± 0.03</td>
</tr>
</tbody>
</table>

Mean discharge rate (in impulses/s) of ongoing activity was measured during the 1-min period before (control), immediately after (0–1 min), and at different times after drug application. Data are the mean ± SEM. Tested drugs: vehicle (n = 3–9), nepafenac (n = 9–17), diclofenac (n = 5–11), ketorolac (n = 5–12). No significant differences (one-way repeated-measures ANOVA).

Immediately after drug application. On average, the mean impulse frequency immediately after ketorolac was slightly higher than during the previous control period, but differences were not significant (Table 1, Fig. 1).

Response to CO2. The mean firing frequency of the discharge evoked by repeated 30-second CO2 pulses was progressively reduced after application of ketorolac (Fig. 2, Table 2). No changes in the latency or time course of the impulse discharge were observed after drug treatment (data not shown).

Ongoing Activity. The gradual increase in firing frequency of the ongoing activity caused by repetition of CO2 pulses observed with the vehicle, was prevented by application of ketorolac (Fig. 1, Table 1).

Mechanical Threshold. The mechanical threshold of corneal polymodal units did not change significantly 2 or 17 minutes after the application of ketorolac (Table 5).

Effects of NSAIDs on Voltage-Gated Sodium Currents in Cultured TG Neurons

Reduction of CO2-evoked impulse activity in corneal polymodal nociceptor fibers by NSAIDs may be secondary to a nonspecific blockade of voltage-gated sodium channels (i.e., to a local anesthetic effect of the drug). Therefore, we tested the effects of nepafenac, amfenac, and diclofenac at equimolar concentrations (50 μM) on voltage-gated sodium currents evoked by depolarizing pulses in cultured mice trigeminal sensory neurons.

As shown in Figure 3A, the prodrug nepafenac produced only a modest inhibition of sodium current amplitude, averaging 11.5% ± 2.1% (n = 8), that recovered on wash. In contrast, in the same neuron, diclofenac produced a robust inhibition (65.7% ± 7.2%, n = 12) that recovered rapidly after removal of the drug (Figs. 3A, 3B). The effects of amfenac were also subtle but more complex. In five of six cells, there was an initial slight increase that reverted to a slow decline. This slow inhibition averaged 5.9% ± 3.0% (n = 6) and was always much smaller than the effect of diclofenac in the same cells (Figs. 3C, 3D). Mean inhibition of peak sodium current by diclofenac was larger than with the other two NSAIDs tested (P < 0.001, unpaired t-test).

DISCUSSION

The present results show that nepafenac, the carboxamide derivative, and prodrug form of the NSAID amfenac, when applied topically to the cornea, exhibits a rapid onset of action and is very effective in reducing the spontaneous and stimulus-evoked activity of corneal polymodal nociceptors elicited by repeated noxious acidic stimulation. Notably, the spontaneous activity of cold receptors or the response of mechanonociceptors to mechanical stimuli remained largely unaffected after treatment with nepafenac. This result suggests that the effect is quite selective on polymodal nociceptors, the sensory fibers primarily involved in signaling pain associated with tissue inflammation. A similar effect was observed with topical administration of the comparator NSAIDs, diclofenac and ketorolac although with these drugs the action had a slower onset and was on average less pronounced.

Analgesic Effect of NSAIDs

An attenuating effect of diclofenac, indomethacin, and flurbiprofen on impulse activity of polymodal corneal nociceptors of the cat was reported previously by Chen et al. using single fiber recordings of the ciliary nerves in the cat in vivo. Likewise, topical diclofenac reduced corneal sensitivity to mechanical, thermal, and chemical stimulation in healthy human subjects. A similar reduction of spontaneous and acid-evoked impulse activity of polymodal nociceptor fibers of the cat was observed in the present experiments after a masked evaluation of diclofenac, thus confirming the reliability of this experimen-
nal preparation to evaluate and compare the action of analgesic drugs on peripheral nociceptive input from the eye. As expected, ketorolac, another NSAID that has been used topically to relieve ocular pain caused by injury, local inflammatory processes, or surgery,30–32 was also effective in decreasing spontaneous and stimulus-evoked activity of corneal polymodal fibers. Therefore, the data obtained after masked testing shows that nepafenac also decreased polymodal nociceptor fiber spontaneous and stimulus-evoked activity of corneal polymodal fibers,40 an effect that appears to be associated with the chemical structure of diclofenac, because it is not produced by other NSAIDs such as acetylsalicylic acid, antipyrin, and indomethacin. Blockade of voltage-dependent sodium channels will decrease the excitability of all functional types of sensory nerve terminals thus producing local anesthesia. Also, it has been shown recently that diclofenac acts on proton-gated ion channels (ASICs) present in nociceptor neurons, directly inhibiting ASIC currents on these nociceptor neurons,41 both effects explain the reduction by diclofenac of the excitability of all functional types of sensory nerve terminals thus producing local anesthesia. Also, it has been shown recently that diclofenac acts on proton-gated ion channels (ASICs) present in nociceptor neurons, directly inhibiting ASIC currents on these nociceptor neurons.41 Both effects explain the reduction by diclofenac of the response of polymodal nociceptor fibers to acidic stimuli observed by Chen et al.20 and confirmed in the present work. We also confirmed in mice trigeminal ganglion nociceptive neurons the significant blockade of sodium currents by diclofenac. Part of these neurons is the origin of polymodal nociceptive fibers traveling to the cornea. Thus, direct reduction of the excitability of nociceptor terminals by sodium channel blockade appears to be one of the mechanisms used by diclofenac to attenuate the impulse discharge of polymodal nociceptor fibers induced by noxious stimuli.

Table 2. Effect of NSAIDs on the Response to CO2 of Polymodal Nociceptor Fibers before and at Different Times after Drug Application

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Nepafenac 0.1%</th>
<th>Diclofenac 0.1%</th>
<th>Ketorolac 0.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>1.77 ± 0.27</td>
<td>4.43 ± 1.07</td>
<td>2.65 ± 0.42</td>
<td>2.38 ± 0.38</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>1.49 ± 0.23</td>
<td>2.91 ± 0.97</td>
<td>2.38 ± 0.50</td>
<td>1.94 ± 0.42</td>
</tr>
<tr>
<td>16 min</td>
<td>1.10 ± 0.10</td>
<td>2.82 ± 0.88*</td>
<td>2.18 ± 0.59*</td>
<td>1.89 ± 0.44*</td>
</tr>
<tr>
<td>31 min</td>
<td>1.70 ± 0.20</td>
<td>3.14 ± 1.22</td>
<td>1.77 ± 0.52*</td>
<td>1.78 ± 0.38*</td>
</tr>
<tr>
<td>46 min</td>
<td>1.70 ± 0.70</td>
<td>1.83 ± 0.55*</td>
<td>1.48 ± 0.42*</td>
<td>2.11 ± 0.42*</td>
</tr>
<tr>
<td>61 min</td>
<td>Not tested</td>
<td>2.17 ± 0.77*</td>
<td>1.79 ± 0.69*</td>
<td>1.44 ± 0.52*</td>
</tr>
<tr>
<td>76 min</td>
<td>Not tested</td>
<td>3.38 ± 1.18</td>
<td>1.32 ± 0.38*</td>
<td>1.68 ± 0.53*</td>
</tr>
<tr>
<td>91 min</td>
<td>Not tested</td>
<td>2.00 ± 0.81*</td>
<td>1.04 ± 0.21*</td>
<td>1.68 ± 0.48*</td>
</tr>
</tbody>
</table>

Response to 98.5% CO2 is expressed as the mean discharge rate during 30-s CO2 pulses applied before (control) and at different times after drug application (1–91 min). Data are the mean impulses per second ± SEM. Tested drugs: vehicle (n = 3–9), nepafenac (n = 9–17), diclofenac (n = 5–11), ketorolac (n = 5–12).

*P < 0.05, one-way repeated-measures ANOVA, Holm-Sidak test.

Mechanism of Action of NSAIDs

Inhibition of the cyclooxygenase pathway by NSAIDs reduces local production of arachidonic acid metabolites and sensitization of nociceptors caused by PGs released in inflamed tissues,4 thus decreasing inflammatory pain. This has been proposed to be the main mechanism of the analgesic action of NSAIDs.9,35 However, there is experimental and clinical evidence that several NSAIDs have an additional, direct action on peripheral nociceptors that contributes to the analgesic effects of these drugs.17,19,20,30–39 Topical diclofenac rapidly reduced the responsiveness to noxious stimuli of all functional subtypes of peripheral sensory fibers innervating the cat’s cornea,29 suggesting that this drug also has a direct, local anesthetic effect on such nerve fibers. In agreement with this hypothesis, it has been shown that diclofenac reduces sodium currents in dorsal root ganglion primary sensory neurons,40 an effect that appears to be associated with the chemical structure of diclofenac, because it is not produced by other NSAIDs such as acetylsalicylic acid, antipyrin, and indomethacin. Blockade of voltage-dependent sodium channels will decrease the excitability of all functional types of sensory nerve terminals thus producing local anesthesia. Also, it has been shown recently that diclofenac acts on proton-gated ion channels (ASICs) present in nociceptor neurons, directly inhibiting ASIC currents on these neurons.31 Both effects explain the reduction by diclofenac of the response of polymodal nociceptor fibers to acidic stimuli observed by Chen et al.20 and confirmed in the present work. We also confirmed in mice trigeminal ganglion nociceptive neurons the significant blockade of sodium currents by diclofenac. Part of these neurons is the origin of polymodal nociceptive fibers traveling to the cornea. Thus, direct reduction of the excitability of nociceptor terminals by sodium channel blockade appears to be one of the mechanisms used by diclofenac to attenuate the impulse discharge of polymodal nociceptor fibers induced by noxious stimuli.

In contrast to the robust effects of diclofenac on sodium currents, we found that both the prodrug nepafenac and its

Table 3. Effect of NSAIDs on the Mechanical Threshold of Polymodal Nociceptor Fibers before and at Different Times after Drug Application

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Nepafenac 0.1%</th>
<th>Diclofenac 0.1%</th>
<th>Ketorolac 0.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2 min</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.3 ± 0.1</td>
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<tr>
<td>17 min</td>
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<td>1.4 ± 0.2</td>
<td>1.6 ± 0.1</td>
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</table>

The mechanical threshold was measured with a Cochet-Bonnet esthesiometer with a no. 12 filament, before (control) and after the application of the drugs at the concentrations described in the Methods section. Data are expressed as the mean mechanical threshold (mN) ± SEM. Tested drugs: vehicle (n = 3–4), nepafenac (n = 13), diclofenac (n = 10), ketorolac (n = 11–12). No significant differences were found between thresholds before and after the treatments.
active metabolite amfenac exhibited only a minor blocking action on the sodium channel of the soma membrane of TG sensory neurons. This suggests that the rapid action of these drugs on sensory activity of polymodal fibers is not attributable to a local anesthetic effect of the drug on the peripheral nociceptor terminal, which also agrees with the unaltered responsiveness of mecano- and cold-sensitive fibers of the cornea after application of the drug. The lack of a major blocking effect of nepafenac and amfenac on sodium currents is similar to the profile exhibited by other NSAIDs. We did not test the effect of nepafenac or amfenac on H\textsuperscript{+}-induced currents and thus the possibility that their analgesic effects are partly mediated by blockade of ASIC channels on polymodal nociceptor fibers, as has been suggested for aspirin, diclofenac, and flurbiprofen\textsuperscript{41} was not excluded in our experiments. Nonetheless, the analgesic action of nepafenac and amfenac appears to be due mainly to its inhibition of cyclooxygenase and of the subsequent production of PGs.

One of the unique properties of nepafenac is its ability to penetrate ocular tissue rapidly.\textsuperscript{22} This property is largely attributable to the modification of the arylacetic acid function of amfenac to the carboxamide function of nepafenac. This structural change not only eliminates the compound’s anionic character but also simultaneously enhances its partition coefficient with respect to the modification of the arylacetic acid function of nepafenac (thin black trace), and 50 μM diclofenac (gray trace) during a test pulse to −10 mV. (C) Time course of whole-cell peak sodium current and effects of amfenac (50 μM) and diclofenac (50 μM). (D) Whole-cell sodium current with control (thick black trace), 50 μM amfenac (thin black trace), and 50 μM diclofenac (gray trace) during a test pulse to −10 mV. (E) Summary histogram of blockade of sodium current produced by diclofenac, nepafenac, and amfenac. Data are the mean ± SEM of the percentage of reduction of sodium current value from control. ** P < 0.001, t-test.

References
