Reduction by Antiinflammatory Drugs of the Response of Corneal Sensory Nerve Fibers to Chemical Irritation

Xiaojie Chen, Juana Gallar, and Carlos Belmonte

Purpose. Nonsteroidal antiinflammatory drugs (NSAIDs) have been applied topically to reduce ocular pain caused by corneal injury or anterior segment surgery. The authors investigated whether the analgesic effects of the NSAIDs diclofenac, indomethacin, and flurbiprofen and of the calcium channel antagonist diltiazem on corneal pain are mediated by a reduction of nerve activity in corneal polymodal nociceptive fibers.

Methods. Impulse activity of single A-δ and C corneal nerve fibers was recorded from the ciliary nerves of anesthetized cats. Polymodal units were identified by their response to both touching with the Cochet-Bonnet esthesiometer and to acidic stimulation with 30-second pulses of 80% or 98.5% CO₂ or 60 μl of 10 mM acetic acid, applied to the corneal receptive area. Ongoing impulse activity, firing responses to CO₂ or acetic acid, and mechanical threshold of single fibers were recorded before and at various times (5 to 90 minutes) after topical application of 0.1% sodium diclofenac, 0.03% sodium flurbiprofen, 0.1% indomethacin, and 0.045% diltiazem hydrochloride or of their vehicles.

Results. Indomethacin, diclofenac, and flurbiprofen, in decreasing order of potency, gradually reduced the mean frequency of the impulse response of corneal polymodal nerve fibers evoked by CO₂ stimuli. The progressive increase of ongoing activity, observed in vehicle-treated eyes after repeated CO₂ stimulation was also prevented by NSAIDs. Diltiazem also attenuated the response to CO₂ for a shorter period of time and with a faster time course. The mechanical threshold of corneal polymodal fibers was not affected by treatment with any of these drugs.

Conclusions. Indomethacin, diclofenac, and flurbiprofen, as well as the calcium antagonist diltiazem, diminish the responsiveness of corneal polymodal nociceptors to chemical stimuli. This appears to be caused, in part, by a direct effect of these drugs on the excitability of polymodal nerve endings, but also by an inhibition by NSAIDs of the formation of cyclooxygenase products such as prostaglandins, thus reducing the enhanced responsiveness of nociceptors caused by local release of arachidonic acid metabolites from injured cells.

Inj activity arising from corneal injury is the consequence of an activation by noxious stimuli of trigeminal nociceptive neurons innervating the cornea. Excitation of corneal nerve terminals is directly caused by the noxious stimulus but also induced by inflammatory substances released by damaged cells at the site of injury. Inflammatory mediators, which include arachidonic acid metabolites, neuropeptides, biogenic amines, and kinins, contribute to pain by direct activation of nociceptive terminals and also through their sensitization. Sensitization is characterized by a decreased threshold and an increased sensitivity of nociceptive terminals to the stimulus, as well as by the development of ongoing impulse activity. The overall enhancement of neural nociceptive discharges during sensitization contributes to the maintenance of ocular pain and to hyperalgesia after corneal damage.

Prostaglandins (PGs), products of the arachidonic cyclooxygenase pathway, have a well-established role as mediators of inflammatory pain. When applied locally, PGE₂ and PGF₂α sensitize the response of nocicep-
tive endings of various tissues (skin, testis, muscle, joints), thus suggesting that PGs, locally released at the injured area, both excite and sensitize nociceptors.\textsuperscript{6–9} Nonsteroidal antiinflammatory drugs (NSAIDs) exert their antiinflammatory activity through an inhibition of the cyclooxygenase responsible for the conversion of arachidonic acid into biologically active PGs.\textsuperscript{10} In the eye, topical NSAIDs have been claimed to have an analgesic effect on pain produced by corneal surgery.\textsuperscript{11–15} However, it has not been established whether this analgesic action results from a blockade of the local formation of prostanoids by NSAIDs or is caused by a direct effect of the drug on nociceptive nerve terminals.

The present study was aimed at establishing the effects of several NSAIDs on nerve activity evoked by repeated noxious stimuli (mechanical and chemical) in corneal polymodal nociceptive fibers of the cat. Preliminary results have been reported elsewhere.\textsuperscript{14–17}

\section*{METHODS}

\subsection*{General}

Experiments were performed in cats of both sexes weighing 2.0 to 3.5 kg, anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg, ip). The animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The brachial vein was cannulated for administration of balanced saline solution and supplementary doses of diluted anesthetic (5 mg/kg per hour). The animal was kept in an areflexic state throughout the experiment. The trachea was cannulated and connected to a CO\textsubscript{2} analyzer (ADC Ltd., model FM1; Hoddesdon, England). Mean arterial blood pressure at the contralateral carotid artery and end-tidal CO\textsubscript{2} were continuously monitored and maintained at physiological levels (\textgreater;80 mm Hg and approximately 3% to 4%, respectively). Rectal temperature was monitored and maintained at 37°C with a regulated heating blanket. At the end of the experiment, cats were killed with an overdose of anesthetic.

\subsection*{Electrophysiological Recordings}

The technique previously described by Belmonte et al\textsuperscript{18–20} was employed. The cat was placed in a prone position. 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 free 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 fine paint brush was slid over the cornea; then, fine nerve strands were split until a single unit was evoked by corneal stimulation. The cornea was kept moist during the experiment by application of physiological saline with an ultrasonic humidifier.

Mechanical stimulation of the cornea was performed with a Cochet-Bonnet esthesiometer,\textsuperscript{21} provided with No. 12 and No. 8 filaments, to determine force thresholds and receptive-field borders.

Chemical sensitivity was tested by applying a humidified gas jet containing 80% or 98.5% CO\textsubscript{2} in air to the corneal surface for 10 to 30 seconds. CO\textsubscript{2} causes the local formation of carbonic acid and acts as an acidic stimulus.\textsuperscript{22} The flow of gas was initially adjusted with air to a level less than mechanical threshold. In some experiments, sensitivity to acid was examined by topical application of 30 to 60 \textmu{l} of a solution of 10 mM acetic acid in 155 mM NaCl (pH = 3.8) onto the unit’s receptive field.

Conduction velocity of the fiber was estimated from the latency of suprathreshold electric shocks (0.1 to 0.5 msec, 0.5 to 3 mA) applied to the receptive field or limbal area with a bipolar silver electrode. Conduction distance (2.5 to 3.0 cm) was measured by placing an 8.0-gauge thread along the trajectory of the nerve.

Impulse discharges were recorded on an FM magnetic tape and analyzed off-line with a window discriminator, an analog-to-digital converter (CED 1401; Cambridge, England), and a computer. The response to CO\textsubscript{2} was analyzed by measuring the following parameters: (1) latency: delay between onset of the CO\textsubscript{2} pulse and the first impulse given by the unit; (2) time-to-peak: delay between the first impulse and the bin in which the maximal number of impulses was measured; (3) mean discharge rate: number of impulses per second (imp/sec) during a definite time of the CO\textsubscript{2} stimulation period, taking the beginning of the CO\textsubscript{2} pulse as zero time; and (4) peak frequency: maximal firing frequency during one bin. Impulse frequency during interstimulus periods (ongoing activity) was also measured and expressed in imp/sec. Data are presented as mean ± SEM. Both parametric and nonparametric statistical tests were used to compare responses before and after drug and the effects of the different drugs.

\subsection*{Drugs}

Test solutions (30 to 60 \textmu{l}) were applied to the corneal surface with a micropipette. No washing was performed afterwards. The following drugs were assayed: 0.1% sodium diclofenac (Voltaren, CIBA Vision, Duluth, GA), 0.03% sodium flurbiprofen (Ocufen, Allergan, Irvine, CA); and 0.1% indomethacin (Indocol-
lyre, Laboratoire Chauvin, Montpellier, France). Each drug was dissolved in its commercial vehicle. Drugs and their vehicles were generously provided by the manufacturers. Diltiazem hydrochloride (0.045%, Sigma Chemical, St. Louis, MO) was dissolved in 124 mM NaCl, 5 mM KCl, and adjusted to pH 7.5 with 20 mM Hepes.

**Experimental Protocol**

A filament of a ciliary nerve containing sensory fibers that responded to mechanical stimulation of the corneal surface with a wet brush was localized. Splitting of the filament was performed until a single corneal unit was identified, based on the size and shape of its action potential. The receptive field border was then mapped and the mechanical threshold determined with the Cochet-Bonnet esthesiometer. Conduction latency was subsequently measured.

A CO2 pulse was applied for 10 to 30 seconds to the unit’s receptive field to establish the chemosensitivity of the unit. In some experiments, 60 µl of 10 mM acetic acid solution was used for this purpose. Five minutes after this control stimulation, 60 µl of the test drug was dropped on the corneal receptive field of the recorded unit. After a 5-minute pause, the CO2 stimulus was repeated. The mechanical threshold was measured immediately afterward. Responsiveness to acidic stimulation was subsequently tested every 15 minutes, for 1.5 to 2 hours. Mechanical threshold was determined again at the end of this period. In some experiments, a second unit was studied after this procedure was completed. In these cases, units having their receptive field in a region of the cornea not previously exposed to the drug were selected.

In a separate set of experiments, three applications of 10 mM acetic acid were made at 5-minute intervals, before and after application of flurbiprofen. Frequency of impulses of the evoked responses and of the ongoing activity during interstimulus periods was determined.

**RESULTS**

**General Properties of Corneal Polymodal Nociceptors**

Neural activity of 103 corneal fibers, sensitive to mechanical and chemical stimulation (polymodal nociceptors) was recorded. Pure mechanosensory fibers, insensitive to CO2 stimulation, and “cold” nociceptors12,20 were not included in this study.

The conduction velocity of polymodal corneal units ranged from 0.6 to 23 m/sec (mean, 5.7 ± 0.6 m/sec; n = 90). Based on their conduction velocity (cv), units were classified as A-δ (cv > 2 m/sec, n = 63) or as C fibers (cv ≤ 2 m/sec, n = 27). In 13 units, conduction velocity was not measured. Receptive fields were round or oval, with a mean long diameter of 5.8 ± 0.3 mm (n = 85). In 83 of 97 units, the receptive field was restricted to the cornea, and in 14 fibers (all of them A-δ), it extended also into the sclera. In 6 units, the receptive area was not mapped. Confirming previous observations,20 significant differences in receptive field size (P < 0.05, Mann-Whitney rank sum test) were found between A-δ and C polymodal fibers.

**Response to Mechanical and Chemical Stimuli.** Approximately 66% of the polymodal nociceptive units included in this study presented a low-frequency impulse activity in the absence of any intentional stimulation. This ongoing activity (mean frequency, 0.10 ± 0.03 imp/sec; n = 57) persisted throughout the experiment (Figs. 1, 2). The percentage of A-δ and C units displaying ongoing activity was similar, although mean firing frequency was significantly greater in C units than in A-δ units (C: 0.18 ± 0.08 imp/sec, n = 15; A-δ: 0.07 ± 0.02, n = 42; P < 0.05; Mann-Whitney rank sum test).

Mechanical stimulation of the corneal receptive field with a suprathreshold value of the Cochet-Bonnet esthesiometer elicited a brief discharge of one to three nerve impulses. When a jet of gas containing 80% or 98.5% CO2 was applied on the receptive area, a continuous discharge of impulses was evoked with a variable latency (2 seconds up to 15 seconds), usually reaching a frequency peak within the initial 5 seconds of the response and then declining slowly (Figs. 1, 3). No differences were found in the firing frequency or the duration of the response evoked by either 80% or 98.5% CO2. Application onto the cornea of a drop of 10 mM acetic acid elicited a discharge of impulses with a similar time course to that of CO2, although the mean firing frequency was significantly lower (CO2: 2.7 ± 0.3 imp/sec; acetic acid: 0.9 ± 0.1 imp/sec; P < 0.001, Mann-Whitney rank sum test).

**Effect of Drugs on Neural Activity**

**Sodium Diclofenac. Direct effects.** Impulse activity evoked by an application (60 µl) of 0.1% sodium diclofenac was tested in 11 fibers. Seven units (64%) fired briefly (5 to 60 seconds) immediately after diclofenac application (Fig. 1A). In the remaining fibers, ongoing activity was either unchanged (n = 3) or slightly decreased (n = 1) immediately after administration of diclofenac. On the average, mean impulse frequency immediately after diclofenac was significantly higher than during control (P < 0.05, Table 1). In 10 separate units, the effect of the sodium diclofenac vehicle was assayed. Seven fibers either did not respond if they were silent or did not change their ongoing discharge after administration of the vehicle. Two units fired briefly immediately after vehicle administration. In
pretreatment with diclofenac but not with the vehicle (Table 1, Fig. 2).

Mechanical threshold. Mechanical threshold of corneal polymodal units 10 minutes after diclofenac remained unchanged in six fibers, decreased in one fiber, and increased in the remaining three units (Table 3). Similar results were obtained after topical application of the vehicle. Mechanical threshold was also determined in four corneal units 80 minutes after diclofenac. At this time, mechanical threshold was increased in only one of the tested units (Table 3).

Indomethacin. Direct effects. Impulse activity evoked by an application of 0.1% indomethacin (30 μl) was tested in 25 polymodal units. Ten fibers started to fire immediately after application of the drug (Fig. 1B) with a discharge that lasted for 10 to 60 seconds. The remaining 15 fibers stayed silent or decreased their impulse activity after indomethacin administration. The mean frequency immediately after indomethacin application was significantly higher than during the

one fiber, the background activity ceased for a short period of time (10 seconds) when the vehicle was applied (data not shown). Mean impulse frequency immediately after vehicle application was not significantly different from control.

Response to CO2. The mean firing frequency of the discharge evoked by 30-second CO2 pulses was slightly reduced 5 minutes after diclofenac, and was significantly lower than in control pulses 35 minutes after the drug (Table 2 and Figs. 1A, 3A, 4). No changes in the latency or time course of the impulse discharge were observed after diclofenac (data not shown). Application of the vehicle solution in 10 experiments did not significantly change the firing frequency of the response to CO2 (data not shown).

Ongoing activity. Firing frequency of the ongoing activity, measured before the application of successive CO2 pulses, increased gradually with repeated CO2 applications (Fig. 2A). This effect was prevented by

FIGURE 1. Impulse frequency histograms of the neural response to CO2 (small arrows) before and after application of antiinflammatory drugs at the time indicated by the large arrow. (A) Sodium diclofenac, 0.1%. (B) Indomethacin, 0.1%. (C) Sodium flurbiprofen, 0.03%. (D) Diltiazem hydrochloride, 0.045%.

FIGURE 2. Ongoing activity of corneal polymodal nociceptors after repeated CO2 stimulation after application of (A) commercial vehicle of sodium diclofenac and (B) antiinflammatory drugs. Ongoing activity is expressed as mean discharge rate (imp/sec) during the 3- to 5-minute period preceding CO2 stimulations. Data are mean ± SEM (* P < 0.05, paired t-test.)

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TABLE 1. Effects of Topical Antiinflammatory Drugs on the Ongoing Activity (Impulses/s) of Corneal Polymodal Nociceptors Before (Control) and at Different Times After Drug Application

<table>
<thead>
<tr>
<th></th>
<th>Diclofenac</th>
<th>Indomethacin</th>
<th>Flurbiprofen</th>
<th>Diltiazem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.04 ± 0.02</td>
<td>0.09 ± 0.04</td>
<td>0.06 ± 0.03</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1 min</td>
<td>0.14 ± 0.04*</td>
<td>0.26 ± 0.13†</td>
<td>0.52 ± 0.20*</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>5 min</td>
<td>0.08 ± 0.03</td>
<td>0.07 ± 0.03</td>
<td>0.09 ± 0.03</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>20–25 min</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.04</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>35–40 min</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.01*</td>
<td>0.05 ± 0.02</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td>50–55 min</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>65–70 min</td>
<td>0.06 ± 0.04</td>
<td>0.03 ± 0.01*</td>
<td>0.03 ± 0.02</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td>80–90 min</td>
<td>0.15 ± 0.07</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.14 ± 0.09</td>
</tr>
</tbody>
</table>

Mean discharge rate (in impulses/s) of ongoing activity was measured during 3 to 5 minute periods before (control), immediately after drug application (0 to 1 minute), and at different times afterwards. Tested drugs: 0.1% sodium diclofenac (n = 5 to 11), 0.1% indomethacin (n = 12 to 25), 0.03% sodium flurbiprofen (n = 7 to 11), and 0.045% diltiazem hydrochloride (n = 7 to 21). Data are mean ± SEM.

* P < 0.05, † P < 0.01, difference from control, paired t-test or Wilcoxon signed rank test.

Response to CO\(_2\). Figures 1B and 3B show two separate examples of the effect of 0.1% indomethacin on the firing of polymodal units in response to a CO\(_2\) pulse. The mean frequency of the impulse response to CO\(_2\) before indomethacin treatment was 3.0 ± 0.4 imp/sec (n = 25). Five minutes after drug treatment, mean firing frequency was significantly less (Table 2). For 12 units in which CO\(_2\) stimuli were repeated at 15-minute intervals, the firing rate of the response remained significantly reduced (Table 2, Fig. 4). In contrast, the frequency of the impulse discharge evoked by CO\(_2\) was unaffected by treatment with the indomethacin vehicle (data not shown).

Ongoing activity. The mean frequency of the ongoing activity was slightly reduced 5 minutes after indomethacin. This reduction persisted for 65 minutes after the drug (Table 1, Fig. 4B). Ongoing activity was not modified by the vehicle (data not shown).

Mechanical threshold. Fifteen minutes after indomethacin treatment, the mechanical threshold remained unchanged in 17 of 21 fibers and was slightly greater than control in 4 units (Table 3). Seventy-five minutes later, 1 of the 12 explored units was unresponsive to mechanical stimulation. In the remaining 11 fibers, mechanical threshold was unchanged (Table 2).

TABLE 2. Effects of Topical Antiinflammatory Drugs on the Response to CO\(_2\) of Corneal Polymodal Nociceptors Before (Control) and at Different Times After Drug Application

<table>
<thead>
<tr>
<th></th>
<th>Diclofenac</th>
<th>Indomethacin</th>
<th>Flurbiprofen</th>
<th>Diltiazem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.99 ± 0.34</td>
<td>3.01 ± 0.41</td>
<td>2.41 ± 0.37</td>
<td>3.31 ± 0.53</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>1.70 ± 0.32</td>
<td>2.32 ± 0.36†</td>
<td>2.17 ± 0.42</td>
<td>2.3 ± 0.60†</td>
</tr>
<tr>
<td>20–25 min</td>
<td>1.38 ± 0.39</td>
<td>2.18 ± 0.45§</td>
<td>1.83 ± 0.39</td>
<td>1.61 ± 0.49§</td>
</tr>
<tr>
<td>35–40 min</td>
<td>1.35 ± 0.44†</td>
<td>2.03 ± 0.36†</td>
<td>1.57 ± 0.33†</td>
<td>2.28 ± 0.57†</td>
</tr>
<tr>
<td>50–55 min</td>
<td>1.24 ± 0.48</td>
<td>1.94 ± 0.42</td>
<td>1.50 ± 0.28†</td>
<td>2.53 ± 0.28‡</td>
</tr>
<tr>
<td>65–70 min</td>
<td>1.70 ± 0.41†</td>
<td>1.78 ± 0.38§</td>
<td>1.45 ± 0.28†</td>
<td>2.24 ± 0.64‡</td>
</tr>
<tr>
<td>80–90 min</td>
<td>1.40 ± 0.38‡</td>
<td>1.62 ± 0.33‡</td>
<td>1.35 ± 0.40‡</td>
<td>2.11 ± 0.69‡</td>
</tr>
</tbody>
</table>

Response to 80 or 98.5% CO\(_2\) is expressed as the mean discharge rate (impulses/s) during a 30 second CO\(_2\) pulse. Impulse frequency of CO\(_2\) pulses applied before (control) and at different times following drug application (5 to 90 min). Data are mean ± SEM.

Tested drugs: 0.1% sodium diclofenac (n = 5 to 11), 0.1% indomethacin (n = 12 to 25), 0.03% sodium flurbiprofen (n = 7 to 11), and 0.045% diltiazem hydrochloride (n = 7 to 21).

† P < 0.05, †‡ P < 0.01, †§ P < 0.005, †¶ P < 0.001, difference from control, paired t-test or Wilcoxon signed rank test.
Antiinflammatory Drugs and Corneal Pain

A Diclofenac

B Indomethacin

FIGURE 3. Sample recording of the impulse discharge evoked by a CO₂ pulse (arrows) in an A-δ polymodal nociceptive fiber before (control) and at different times (5 and 60 minutes) after topical application (60 μl) of 0.1% sodium diclofenac (A) and 0.1% indomethacin (B).

3). No significant differences in mechanical sensitivity were observed after administration of the vehicle (data not shown).

Flurbiprofen. Direct effects. Thirty-two fibers were treated with 0.03% flurbiprofen. Immediately after application of the drug 19 fibers produced an impulse discharge that lasted for approximately 50 seconds (Fig. 1C), eliciting a significant increase in mean frequency (Table 1). A similar, short-lasting frequency increase was also observed in four of seven fibers tested for the effect of flurbiprofen vehicle.

Response to CO₂. Figure 1C shows an example of the effect of flurbiprofen on the firing response of a polymodal unit to CO₂ pulses. Five minutes after the drug, the mean frequency of the impulse discharge evoked by CO₂ was slightly lower than in control (Table 2, Fig. 4). Thereafter, the firing frequency dropped gradually to significantly lower values (Table 2, Fig. 4).

Response to acetic acid. The effect of flurbiprofen on the impulse response to 60 μl topical acetic acid (10 mM) was assayed in a separate group of 21 polymodal units. Ten minutes after flurbiprofen, the average frequency of the impulse discharge evoked by acetic acid in the overall population of units was not statistically different from control. However, when the responses of the A-δ (n = 12) and C (n = 9) polymodal fibers were analyzed separately, it became apparent that after treatment with the drug, the average response of A-δ fibers to acetic acid was not reduced but that of the C fibers was significantly decreased (Table 4; Fig. 5). No differences in the response to acetic acid stimulation were observed after vehicle treatment (data not shown).

Ongoing activity. Mean frequency of the ongoing activity of 11 fibers in which topical flurbiprofen was applied did not increase significantly after repeated CO₂ stimulation in comparison with control values (Table 1, Fig. 2B).

Mechanical sensitivity. Mechanical threshold was determined in 11 fibers before and 10, 30, and 85 minutes after flurbiprofen. Ten minutes after the
TABLE 3. Mechanical Threshold of Polymodal Nociceptors Before and After Topical Application of Antiinflammatory Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time After the Drug</th>
<th>5 to 15 min</th>
<th>30 to 60 min</th>
<th>80 to 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1.19 ± 0.09 (10)</td>
<td>1.20 ± 0.44 (21)</td>
<td>1.05 ± 0.08 (15)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diltiazem</td>
<td></td>
<td></td>
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</tbody>
</table>

Mechanical threshold was measured with a Cochet-Bonnet esthesiometer, before (control) and after topical application of the antiinflammatory drugs at the concentrations described in methods. Data are mean ± SEM; the number of explored units is shown in parentheses.

...drug, mechanical threshold remained unchanged in 7 of 11 fibers, increased in 2 units, and decreased in the remaining 2 units (Table 3). Thirty minutes after flurbiprofen, mechanical threshold remained unchanged in 7 of 9 fibers and increased in 2 units. In 10 fibers, mechanical threshold was also measured 85 minutes after flurbiprofen. No differences were found at this time (Table 3).

Diltiazem. Direct effects. The effects of 0.045% diltiazem HCl were explored in 21 units. Four of them responded to the application of the drug with a short (less than 20 seconds) discharge. The remaining 17 fibers either remained silent (n = 15) or decreased their ongoing impulse activity (n = 2).

Response to CO₂. An example of the effect of diltiazem on the firing discharge evoked by CO₂ is shown in Figure 1D. The mean discharge rate of the response to CO₂ was significantly reduced by topical application of diltiazem at all the test periods (Table 2, Fig. 4).

Ongoing activity. The ongoing activity of the recorded fibers was unaffected in 1 unit, slightly increased in 10 fibers, and decreased in another 10. Typically, the frequency increase caused by repeated CO₂ stimulation in control experiments was prevented in the first hour after diltiazem treatment and reappeared afterward (Fig. 2B).

Mechanical threshold. Mechanical threshold was determined in five units before and 30 and 60 minutes after diltiazem. At these times, mechanical threshold was slightly increased in two units and reduced in another three units (Table 3).

DISCUSSION

Topical application to the cornea of sodium diclofenac, indomethacin, and flurbiprofen, three NSAIDs

TABLE 4. Response of Polymodal Nociceptors to Repetitive Application of 10 mM Acetic Acid Before and After Flurbiprofen

<table>
<thead>
<tr>
<th>Application</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-6 fibers</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1st</td>
<td>1.2 ± 0.7 (9)</td>
<td>1.1 ± 0.7 (9)</td>
</tr>
<tr>
<td>2nd</td>
<td>2.4 ± 2.3 (5)</td>
<td>1.8 ± 1.0 (5)</td>
</tr>
<tr>
<td>3rd</td>
<td>3.0 ± 3.0 (7)</td>
<td>1.8 ± 1.2 (5)</td>
</tr>
<tr>
<td>C-fibers</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1st</td>
<td>1.0 ± 0.3 (7)</td>
<td>0.6 ± 0.3 (5)</td>
</tr>
<tr>
<td>2nd</td>
<td>1.1 ± 0.3 (7)</td>
<td>0.6 ± 0.3 (5)</td>
</tr>
<tr>
<td>3rd</td>
<td>1.0 ± 0.4 (5)</td>
<td>0.2 ± 0.1* (5)</td>
</tr>
</tbody>
</table>

Three consecutive applications of 10 mM acetic acid (60 μl) were made at 5 minute intervals, before and after topical application of 0.03% flurbiprofen (60 μl). Data are mean ± SEM; the number of explored units is shown in parentheses. * P < 0.05, before vs after, paired t-test.
belonging to diverse chemical groups, reduced the ongoing activity of corneal polymodal nociceptors and attenuated their response to acidic stimulation with CO₂, although mechanical sensitivity remained largely unaffected. A similar effect was obtained with the calcium channel blocker diltiazem, confirming previous reports.²⁵

NSAIDs tested in this work have been used topically to relieve ocular pain caused by injury, local inflammatory processes, or surgery.²⁵-²⁷ Our results show that these compounds, at the same doses and formulations used in patients, effectively reduced impulse activity of polymodal nociceptors evoked by chemical irritation of the cornea. In order of efficiency, indomethacin appeared to be slightly more potent than diclofenac and flurbiprofen. Diltiazem showed a stronger and more rapid initial effect than the NSAIDs although its action on the ongoing activity was less durable. All these substances produced a brief impulse discharge at the moment of application, which may account for the burning sensation reported by many patients immediately after ocular instillation of NSAIDs.¹⁵ The short-lasting impulse discharge evoked in some fibers by topical application of the vehicles may also contribute to the initial sensation of irritation often reported by patients receiving the commercial formulations of these substances.

A close relationship has been reported to exist between the firing of corneal polymodal nociceptors in response to CO₂ in the cat and intensity of ocular pain in humans.²² Our results provide direct evidence that NSAIDs decrease sensory inflow from corneal polymodal nociceptor fibers to the central nervous system when they are applied topically to the eye at doses used for therapeutic purposes. Thus, the reduction of nerve impulse activity elicited by sodium diclofenac, indomethacin, and flurbiprofen in the cat appears to be the neurophysiological correlate of the ocular analgesia reported in humans after topical treatment with these drugs.²⁶

Recently, an attenuation by sodium diclofenac of neural activity after photorefractive keratectomy has been reported in rabbits.²⁸ These authors recorded multiunit nerve activity of the wounded cornea and used hypertonic NaCl as a nonspecific irritant stimulus. Therefore, these data are not directly comparable to those reported here. However, they confirm that sodium diclofenac, one of the NSAIDs tested in the present work, decreases impulse activity elicited by chemical stimulation of corneal nerves.

It is generally assumed that the analgesic effects of NSAIDs result from their effect on the local synthesis of PGs.⁵,¹⁰,²⁹ Prostaglandins do not act as direct pain mediators but cause hyperalgesia by sensitizing nociceptors to other endogenous algesic substances.³ Inhibition of the cyclooxygenase pathway by NSAIDs is believed to reduce sensitization of nociceptors in inflamed tissues, thus decreasing inflammatory pain. However, there is experimental and clinical evidence that several NSAIDs, including diclofenac, indomethacin, and flurbiprofen, also have a direct action on peripheral nociceptors and separate analgesic and antiinflammatory effects.⁵⁰-⁵³

In our experiments, CO₂ and in some cases acetic acid were used as a chemical stimulus for corneal nociceptors. Local accumulation of CO₂ leads to carbonic acid formation in the tissue and a decrease of corneal pH,³⁴ which excites corneal polymodal nociceptors.¹⁹,²⁰,²² Protons appear to act directly on nonselective cationic channels of nociceptor terminal membranes, causing their depolarization.³⁵,³⁶ Therefore, the decrease of the impulse response to CO₂ caused by NSAIDs or by diltiazem may be attributable to a direct effect of these compounds on nociceptor excitability. A local anesthetic effect on corneal nociceptors is dubious because mechanical sensitivity was not clearly affected by treatment, although this possibility cannot be completely excluded. Local anesthetics block sodium channels of sensory nerve fibers more prominently when they fire repetitively, as happens during CO₂ pulses, compared with when only a few impulses are produced, as occurs with mechanical stimulation.³⁷,³⁸ Nevertheless, the NSAIDs acetylsalicylic acid and salicylic acid reduced in dose-dependent manner pH-induced pain in humans and nociceptor excitation in the isolated rat skin–saphenous nerve preparation, without apparent anesthetic effects.³⁹ Thus, attenuation of the responses to CO₂ by the drugs tested in this study may be caused by a direct blockade of ionic channels, as has been suggested for diltiazem,²³ or by other mechanisms interfering with the excitability of the polymodal nerve ending. For example, it has been speculated that diclofenac, but not indomethacin, may downregulate sensitized nociceptors through the stimulation of the nitric oxide–cyclic guanosine monophosphate pathway, which will counteract the effect of cyclic adenosine monophosphate formed during excitation by endogenous mediators.⁴⁰

In addition to a direct effect, inhibition of PG synthesis must also be considered as an explanation for the attenuating action of NSAIDs on nociceptor activity. Small amounts of PGE₂ and PGF₂α are synthesized under basal conditions by the cornea;¹¹ repeated CO₂ applications and the ensuing tissue acidosis presumably stimulate local production of PGs.³⁹ It is known that PGs enhance the excitability of nociceptors in several tissues, including the cornea, and increase their response to acid.⁴²

An increase in local PG levels after repeated noxious stimulation could be the origin of the slow buildup of ongoing activity and its prevention by NSAIDs. Conversely, the gradual decrease of corneal
PG formation induced by NSAIDs may decrease the response to CO₂. The slower time course for the effects of NSAIDs, in comparison with diltiazem, favors this interpretation. Thus, inhibition of PG synthesis by NSAIDs appears to contribute in an unknown but possibly significant degree to a reduced response of corneal polymodal nociceptors to noxious chemical stimuli.

**Key Words**
calcium channel antagonists, corneal pain, inflammation, nonsteroidal anti-inflammatory drugs, prostaglandins

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**References**


