Reduction of Capsaicin-Induced Ocular Pain and Neurogenic Inflammation by Calcium Antagonists

Gertrudis G. Gonzalez, Pilar Garcia de la Rubia, Juana Gallar, and Carlos Belmonte

Purpose. To examine whether blockade of chemosensitivity of corneal nociceptors by Ca\textsuperscript{2+} antagonists decreases pain and irritation induced by capsaicin.

Methods. In adult rabbits, the number of lid-squeezing movements and the degree of palpebral opening, miotic response, and conjunctival vasodilation evoked by a bilateral instillation of 30 \mu l of capsaicin (33 mM) were measured at different times (up to 5 hours) after the drug. Irritative responses to capsaicin in eyes pretreated with diltiazem, verapamil, or nifedipine were compared with those that received only the vehicle. Protein content in aqueous humor was also measured at the end of the experiment.

Results. Diltiazem at doses of 1 to 28 mM, administered 15 minutes before the application of capsaicin, significantly decreased scratching movements, conjunctival hyperemia, closure of the eye, and elevated aqueous protein concentration induced by capsaicin; however, it did not significantly reduce miosis. Nifedipine (2.8 and 10 mM) diminished the number of scratching movements but not other inflammatory parameters, whereas verapamil (2.8 and 10 mM) was totally ineffective in attenuating ocular signs of irritation produced by capsaicin.

Conclusions. These results suggest that by lowering capsaicin-induced neural activity in nociceptive terminals, diltiazem decreases pain and neurogenic inflammation and may be useful as both an analgesic and an antiinflammatory agent in the eye. Invest Ophthalmol Vis Sci. 1993;34:3329-3335.

When applied topically to the eye of experimental animals, capsaicin, the pungent substance of the red pepper, evokes immediate pain as evidenced by lid-squeezing and vocalization, as well as by signs of acute ocular irritation (conjunctival edema and hyperemia, miosis, aqueous flare).\textsuperscript{1,2} In the human eye, topical capsaicin at low doses also elicits a sharp sensation of pain.\textsuperscript{3} The algesic and inflammatory effects of capsaicin, which are followed by an insensitivity of the area to chemical irritation, are caused by a selective action of the drug on polymodal nociceptors, that is, sensory fibers responding to injurious mechanical forces, irritant chemicals, and heat.\textsuperscript{4,5}

The cornea, conjunctiva, and anterior uvea are densely innervated by polymodal nociceptive fibers.\textsuperscript{6}

Capsaicin acts on polymodal nerve terminals through the opening of nonselective cationic channels,\textsuperscript{7} presumably the same ionic channels that mediate response of nociceptors to acid and other irritant chemicals,\textsuperscript{8,9} this leads to depolarization and the subsequent discharge of nerve impulses.\textsuperscript{10,11} In addition, Ca\textsuperscript{2+} influx releases neuropeptides contained in nociceptive afferents that contribute to local inflammatory reactions (neurogenic inflammation);\textsuperscript{12,13} toxic accumulation of intracellular Ca\textsuperscript{2+} ultimately determines damage and inactivation of nociceptive terminals.\textsuperscript{14}

Recently, it has been demonstrated that the impulse response of corneal polymodal nociceptors to certain chemicals (H\textsuperscript{+}, inflammatory mediators) can be selectively eliminated by blockade with calcium antagonists without affecting mechanical responsiveness of the same nerve fibers.\textsuperscript{15,16} A suppression of the response of corneal nociceptive terminals to chemicals may prevent their excitation by capsaicin and the subsequent release of neuropeptides, thus reducing neurogenic inflammation. To explore this possibility, we studied in rabbits whether calcium antagonists atten-
uated pain and anterior segment irritation induced by capsaicin administration. A preliminary report of these findings has been published.17

METHODS
Unrestrained albino California adult rabbits weighing 2.5 to 3 kg were used in this study. The animals were treated according to the ARVO Resolution on the Use of Animals in Research.

All animals were administered 30 μl of a 1% capsaicin solution (33 mM) applied topically to each eye at a 3-minute interval. In 76 rabbits, 30 μl of a calcium antagonist solution were instilled in one eye 15 minutes before capsaicin, whereas the contralateral eye was pretreated with the vehicle. A separate, control group of nine rabbits received the vehicle in both eyes 15 minutes before capsaicin administration.

The number of scratching movements directed to the eye that received the capsaicin solution was counted during 1 minute after instillation of the drug. Palpebral opening in the center of the eye and pupil diameter were also measured with a transparent ruler under constant illumination. Palpebral closure was expressed as percent of the distance between lids measured at the beginning of the experiment. Miotic responses were evaluated afterward and expressed as reduction of initial pupillary diameter (in mm) by gently pulling the upper eyelid to visualize the pupil and the conjunctiva, when necessary. The severity of conjunctival hyperemia was assessed by biomicroscopy and quantified by a score from 0 (absence) to 4 (maximal vasodilation) in eight steps. Measurements were performed at established times during a 5-hour period by an observer who ignored whether or not eyes had been pretreated with a Ca²⁺ antagonist or with the vehicle.

At the end of the experiment, animals received a lethal dose of intravenous sodium pentobarbital, and the anterior chamber was punctured to collect aqueous humor. Protein concentration was determined by Lowry’s method.

Capsaicin (Sigma, St. Louis, MO) was dissolved in 15% ethanol and 8.5% Tween-80 and then diluted with saline to the final concentration (33 mM). Solutions of the calcium antagonists diltiazem (0.1 to 28 mM), verapamil (2.8 and 10 mM), and nifedipine (2.8 and 10 mM) (Sigma) were prepared in isotonic saline (124 mM NaCl, 5 mM KCl, at pH = 7.5 adjusted with 20 mM HEPES).

When appropriate, data were expressed as the mean ±SEM of the difference between values in the eye treated with the Ca²⁺ antagonist and in the contralateral, vehicle-treated eye. Comparisons between groups were made with the unpaired Student’s t-test. Significance was set at P < 0.05. Comparisons between eyes of the same rabbit were made using parametric (paired Student’s t-tests) and nonparametric tests (Wilcoxon’s signed rank test).

RESULTS
Irritative Response to Capsaicin
Capsaicin (33 mM) applied bilaterally to the eyes of nine rabbits elicited a variable number (3 to 16; mean,
Neurogenic Inflammation and Calcium Antagonists

Effects of Calcium Antagonist Diltiazem

Direct Effects. In 41 rabbits, unilateral administration of diltiazem at doses of 0.1 mM (n = 9), 1 mM (n = 9), 2.8 mM (n = 9), 10 mM (n = 9), or 28 mM (n = 5) did not evoke scratching movements or other immediate signs of ocular discomfort. An absence of reaction was also observed in the contralateral eye, where the vehicle was applied. When concentrations of diltiazem above 10 mM were used, a slight conjunctival vasodilation lasting for 5 to 10 minutes was noticed in test eyes. Mechanical threshold for blinking reflex was not modified after 10 mM diltiazem (control: 4.9 ± 0.3 mg; after diltiazem: 5.2 ± 0.4 mg; n = 6, not significant).

Effects on the Irritative Response to Capsaicin. In capsaicin-treated animals, topical diltiazem administered 15 minutes before capsaicin reduced the signs of pain and the inflammatory response evoked by the toxin. This effect is illustrated in Figure 2, where the time course of pain and irritation parameters are represented for four separate groups of rabbits treated with increasing concentrations of diltiazem (1 to 28 mM). Although the number of scratching movements, the conjunctival hyperemia, and the closure of the eye were markedly diminished by diltiazem at all doses, prevention of the miotic response to capsaicin was less pronounced. Figure 3 depicts the dose-response...
FIGURE 3. Dose-dependent inhibitory effect of diltiazem on irritatory reaction to capsaicin 15 minutes after administration of the drug. Each point is the mean ± SEM of 9 to 18 eyes. Comparisons between eyes of control rabbits (vehicle) and diltiazem-treated rabbits were made with the unpaired Student's t-test. *P < 0.05, **P < 0.01, *P < 0.005, and ++P < 0.001.

DISCUSSION

As in previous reports,\(^1\,^2\) capsaicin, applied topically to the rabbit eye, evoked immediate signs of pain and inflammation in the anterior uvea, cornea, and conjunctiva, that subsided gradually within 5 to 6 hours. These signs of ocular irritation induced by capsaicin were reduced in a dose-dependent manner by the Ca\(^{2+}\) antagonist diltiazem, whereas nifedipine and verapamil were not effective. The attenuation by diltiazem of irritative effects of capsaicin was more pronounced on corneal pain, conjunctivai hyperemia, and blood-aqueous barrier breakdown than on miosis. This could be explained in part by a reduced access of the drug to intraocular structures but also by the different mechanisms postulated for pupillary and vascular responses to ocular injury that involve substance P and calcitonin gene-related peptide, respectively.\(^1^8\) In
Diltiazem is a calcium channel blocker of the group of benzothiazepines, while nifedipine and verapamil belong respectively to the families of dihydropyridines and phenylalkylamines. All of them act at micromolar concentrations on L-type Ca^{2+} channels. However, diltiazem at higher concentrations (0.5 to 1 mM), also has a blocking action on ionic channels involved in transduction by sensory cells. For instance, diltiazem reduced the proton-induced sodium current mediating responses of cultured dorsal root sensory neurons to acid, it also antagonized ionic currents associated to light transduction in photoreceptors and responses to odorants of olfactory cells. Moreover, activation by acid of polymodal nociceptive terminals of the cornea was markedly reduced by diltiazem, suggesting that chemosensitive ionic channels in nociceptors were affected by the drug.

Capsaicin increases membrane conductance to cations and raises intracellular Ca^{2+} levels, leading to depolarization and release of neuropeptides by nociceptive terminals. Whether this action of capsaicin on nociceptors is mediated by the transducing mechanism used by H_{4} or other chemical irritants is open to discussion; however, it is conceivable that the same ionic channels are finally gated by the various chemical stimuli for depolarization of polymodal nociceptors.

### TABLE 1. Protein Concentration in Aqueous Humor (mg/ml) 30 Minutes and 5 Hours After Capsaicin in Rabbits Pretreated With a Ca^{2+} Antagonist in One Eye and With the Vehicle in the Contralateral Eye

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mM)</th>
<th>Treated Eye</th>
<th>Contralateral Eye</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem</td>
<td>0.1</td>
<td>1.9 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.4 ± 1.4</td>
<td>3.7 ± 0.9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>1.9 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.5 ± 0.5</td>
<td>2.7 ± 0.6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.6 ± 0.5</td>
<td>1.5 ± 0.2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10*</td>
<td>3.7 ± 0.7†</td>
<td>6.2 ± 0.8</td>
<td>5</td>
</tr>
<tr>
<td>Verapamil</td>
<td>2.8</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.4 ± 0.6</td>
<td>1.5 ± 0.2</td>
<td>9</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2.8</td>
<td>2.1 ± 0.9</td>
<td>2.0 ± 0.4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *30 minutes after capsaicin. †P < 0.01, paired t-test.

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nerve endings. Our observation that diltiazem, which blocks nociceptive excitation by acid, also reduced behavioral signs of pain evoked by capsaicin, suggests that this is the case.

The decreased responsiveness to chemicals of nociceptive nerve endings after diltiazem may also explain the attenuating effect of this drug on capsaicin-induced neurogenic inflammation. It is well established that inflammation evoked by various types of ocular injury (chemical irritation, argon laser, or infrared radiation of the anterior segment) is largely dependent on intact sensory nerves that release neuropeptides. However, unlike local anesthetic drugs that block completely nerve excitation or impulse conduction, diltiazem maintains mechanosensitivity of the same nerve terminals made unresponsive to chemicals. Therefore, diltiazem may be a potentially useful drug to reduce ocular pain and neurogenic inflammation while it preserves corneal sensitivity to mechanical stimuli.

Key Words

cornea, conjunctiva, iris, nociceptors, diltiazem

Acknowledgments

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References


