Desensitization to Topical Epinephrine in the Rabbit Eye: Attenuation by Dexamethasone

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Desensitization of ocular adrenergic receptors was examined in vivo in albino rabbit eyes. Repeated topical administration of 2.0% 1-epinephrine bitartrate (equivalent to 1.1% free base) to the rabbit eye resulted in the loss of ocular hypotensive response on the second day of daily or twice-daily treatment. Concurrent topical administration of dexamethasone phosphate (0.1%) prolonged the pressure-lowering effect of 2.0% l-epinephrine for up to 5 days. Loss of pressure-lowering response was accompanied by 100% inhibition of ciliary process beta-adrenergic-sensitive adenylate cyclase activity following 6 days of topical administration of l-epinephrine; inhibition of c-AMP synthesis was attenuated by dexamethasone (27% decrease in activity after 6 days of treatment). The data suggest that modulation of epinephrine-induced desensitization by corticosteroids in the rabbit eye is associated with stabilization of beta-adrenergic-sensitive adenylate cyclase activity. Invest Ophthalmol Vis Sci 27:1737-1740, 1986

Materials and Methods

Animals and Treatment

Male New Zealand white rabbits (2.0–2.5 kg) were housed individually and fed ad libitum. Animals were acclimated to the laboratory environment (12 hr light, 12 hr dark) for 7–10 days, until intraocular pressures and variation in pressure between 10 AM and 1 PM had stabilized. Rabbits used for daily (q.d.) dose experiments and twice-daily (b.i.d.) dose experiments were obtained from different vendors. Consequently, mean baseline intraocular pressures (±S.E.M.) were 20.9 ± 0.5 mm Hg in animals used for the q.d. experiments and 26.1 ± 0.5 mm Hg in the b.i.d. trials. Animals were divided into groups so that the mean baseline pressure and mean change from baseline between 10 AM and 1 PM varied by no more than 2 mm Hg among treatment groups. Experimental groups were pretreated with 50 μl of 0.1% dexamethasone phosphate in 0.9% saline (Merck, Sharp & Dohme, West Point, PA) or vehicle (0.9% saline) applied topically to both eyes. Five minutes after pretreatment, animals received 50 μl of topical 2.0% 1-epinephrine bitartrate (equivalent to 1.1% free base) (Sigma Chemical Co., St. Louis, MO) applied bilaterally. Control animals received vehicle (0.9% saline) in two doses given 5 min apart. Animals were treated with drugs once or twice daily for 6 successive days. Drugs were administered at 10:30 AM in q.d. experiments, and at 10:30 AM and 4:30 PM in b.i.d. experiments. Dexamethasone and epinephrine solutions were prepared immediately before use.
IOP was measured at 10 AM (prior to instillation of drugs) and at 1 PM (2.5 hr following treatment), using a Digilab pneumotonometer model 30 R/T (Digilab, Inc., Cambridge, MA). Corneas were anesthetized with one drop of 0.5% proparacaine hydrochloride (Alcon, Ft. Worth, TX) prior to applanation. Restraint was not required for IOP measurements and topical drug administration. A total of seven animals (14 eyes) per group, in two trials, were used for b.i.d. dosage experiments. On the sixth experimental day, animals were euthanized with intravenous sodium pentobarbital (75 mg/kg), and irides were used for q.d. dosage experiments. On the sixth experimental day, animals were euthanized with intravenous sodium pentobarbital (75 mg/kg), and irides were used for q.d. dosage experiments. On the sixth experimental day, animals were euthanized with intravenous sodium pentobarbital (75 mg/kg), and irides were used for q.d. dosage experiments. 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2, 4, and 6 (P < 0.05). Post-treatment pressures in epinephrine/dexamethasone-treated eyes were significantly lower than pressures in eyes treated with epinephrine alone on days 2-6. Epinephrine-treated eyes exhibited a hypertensive rise on days 2 and 3, although the difference in IOP was significant on day 3 only (P < 0.02). Treatment with dexamethasone in conjunction with epinephrine prevented this rise.

Ciliary process beta-adrenergic-sensitive adenylate cyclase activity was completely inhibited after b.i.d. treatment with 2.0% epinephrine for 6 days (Table 1). The loss of l-isoproterenol-stimulated activity was partially prevented when 0.1% dexamethasone was given in conjunction with epinephrine. Stimulation index (stimulated activity/basal activity) was used to show relative responsiveness to l-isoproterenol in pooled tissues from control and desensitized eyes (Table 1). In this manner, it may be seen that dexamethasone attenuates 63% of the loss of beta-adrenergic-sensitive adenylate cyclase activity. However, basal activity, used in the determination of the stimulation index, was consistently lower (10.38 ± 0.81 pmol c-AMP/min/mg protein) after steroid treatment. Lower basal adenylate cyclase activity following dexamethasone treatment may be attributable to a decreased level of endogenous prostaglandins, since antiinflammatory steroids are known to suppress prostaglandin synthesis by viable, intact cells.12 No difference in basal adenylate cyclase activity was observed between control and epinephrine-desensitized tissues (18.13 ± 2.22 and 20.09 ± 3.39 pmol c-AMP/min/mg protein ± S.E.M., respectively).

No consistent differences in mydriatic (alpha-mediated) response were noted in experiments where pupil diameter was measured prior to treatment and 2.5 hr following administration of drugs on days 1 and 2 (data not shown).

### Discussion

Decreased responsiveness of the rabbit eye to ocular hypotensive agents has been reported following repeated topical application of prostaglandins,13 cholinesterase inhibitors,14 and catecholamines.5,15 Diminished hypertensive response in epinephrine-treated eyes may be related to desensitization of the beta-adrenergic-sensitive adenylate cyclase system. Indeed, elevation of c-AMP has been implicated as a mediator of the hypertensive action of topically applied epinephrine in the rabbit eye.16 In the present study, loss of hypertensive effect was accompanied by complete inhibition of ciliary process adenylate cyclase activity in response to l-isoproterenol after 6 days of treatment with l-epinephrine. Similarly, a single topical dose of 2.0% epinephrine has been shown to inhibit 40% of the beta-adrenergic-sensitive adenylate cyclase activity in rabbit iris-ciliary body,3 however, repeated treatment for 7 days resulted in no further decrease in activity.3 A possible explanation for the difference between this previous finding and the data in this report is that the loss of responsiveness may not have been maximal in the earlier study. Higher concentrations of epinephrine may have been achieved in the anterior chamber in the present studies, since drugs were applied to anesthetized corneas, a condition that facilitates corneal penetration.

Several mechanisms may account for adrenergic desensitization in ocular tissues. A decrease in the number of beta-receptors following prolonged exposure to adrenergic agonists has been demonstrated in several tissues.5,17,18 Beta-adrenergic-sensitive adenylate cyclase activity following topical epinephrine was diminished proportionately to the decreased beta-receptor density in corneal epithelium.4 Since loss of responsiveness following repeated topical exposure persists for up to 72 hr,3 it is likely that this mechanism accounts for some portion of the diminished beta-adrenergic-sensitive adenylate cyclase activity during long-term de-

### Table 1. In vivo desensitization—rabbit ciliary processes l-isoproterenol-stimulated adenylate cyclase activity

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Epinephrine</th>
<th>Epinephrine/Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>18.13 ± 2.22</td>
<td>20.09 ± 3.39</td>
<td>10.38 ± 0.81</td>
</tr>
<tr>
<td>Stimulated activity†</td>
<td>65.41 ± 3.98</td>
<td>17.97 ± 2.56</td>
<td>23.58 ± 0.83</td>
</tr>
<tr>
<td>Stimulation index‡</td>
<td>3.61 ± 0.25</td>
<td>0.91 ± 0.06</td>
<td>2.27 ± 0.08</td>
</tr>
</tbody>
</table>

* Values are the mean ± the S.E.M. of duplicate determinations in pooled tissues from two separate experiments.
† Stimulated activity = c-AMP production in the presence of 10 μM l-isoproterenol.
‡ Stimulation index = stimulated activity/basal activity for each treatment group.
sensitization. It has also been suggested that a decrease in high affinity (cyclase coupled) agonist binding is the mechanism by which rapid desensitization occurs. A functional uncoupling of the beta-adrenergic receptor and nucleotide-regulatory subunit of adenylate cyclase has been demonstrated in the rabbit iris-ciliary body following three successive doses of topical epinephrine administered within a 32-hr period.

Pharmacologic doses of dexamethasone partially reversed the loss of pressure-lowering response and attenuated inhibition of ciliary process beta-adrenergic-sensitive adenylate cyclase activity induced by chronic topical administration of 1-epinephrine. A plausible explanation for this effect is that corticosteroids act by stabilization of high-affinity beta-epinephrine, thereby maintaining receptor-mediated cyclase activation. Certainly, this mechanism could account for suppression of the short-term loss of ocular responsiveness upon rechallenge with epinephrine. The failure of dexamethasone to protect against the loss of hypotensive effectiveness for more than 4–5 days may, therefore, represent decreased beta-receptor density following long-term exposure to catecholamine.

It is interesting to note that dexamethasone prevented an epinephrine-induced rise in intraocular pressure that occurred on the third day of topical administration. Although epinephrine-induced hypertension is considered to be a complex process, possibly involving both alpha- and beta-adrenergic effects, a transient rise has previously been demonstrated on the second and third day of administration of 2.0% (active component) 1-isoproterenol (nonselective beta-agonist) and reproterol (beta-2-agonist), and partially blocked by timolol (nonspecific beta-antagonist). This suggests that desensitization of beta-adrenergic receptors may be occurring at high dose levels, since no elevation in pressure occurs with partial agonists, terbutaline and salbutamol, or in contralateral (untreated) eyes.

Key words: adenylate cyclase, dexamethasone, desensitization, epinephrine, intraocular pressure

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References