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

The development of adrenergic subsensitivity upon prolonged or repeated exposure to catecholamines and agonist drugs is a frequent pharmacologic observation. Repeated treatment with topical epinephrine results in diminished hypotensive response in rabbits. Epinephrine-induced desensitization of rabbit eyes has been linked to decreased beta-adrenergic-sensitive adenylate cyclase activity in iris-ciliary body and cornea. Desensitization of ocular adenylate-cyclase-coupled beta-adrenergic receptors may partially explain the loss of pressure-lowering response, since beta-adrenergic receptors, as well as alpha-adrenergic receptors, are involved in the effect of epinephrine on the rabbit eye.

Corticosteroids have been shown to attenuate beta-adrenergic desensitization in circulating cells, and this effect has been linked to stabilization of beta-adrenergic agonist-receptor binding, which is otherwise compromised in desensitized cells. The present study investigates the effect of corticosteroids on the response of intraocular pressure (IOP) and ciliary process beta-adrenergic-sensitive adenylate cyclase activity to repeated topical doses of epinephrine in rabbit eyes.

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% l-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.

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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 q.d. dosage experiments. On the sixth experimental day, animals were euthanized with intravenous sodium pentobarbital (75 mg/kg), and iridociliary bodies were excised.

The treatment of experimental animals in this study was in compliance with the ARVO Resolution on the Use of Animals in Research.

**Adenylate Cyclase Activity**

Ciliary processes were dissected from the iris-ciliary body, pooled by group, and homogenized in 50 mM Tris-HCl buffer (pH 7.4) using a Brinkman polytron (Brinkman Instruments, Westbury, NY) for 15 sec at 65% power. Adenylate cyclase activity was measured in vitro using a modification of the method described by Nathanson.\(^8\) Ciliary process homogenates were incubated at 37°C for 4 min in the presence of 50 mM tris (pH 7.4 at 37°C), 10 mM theophylline, 0.5 mM ethylene glycol-bis-(B-amino-ethyl ether) N,N'-tetra-acetic acid (EGTA), 8 mM MgCl\(_2\), 2 mM adenosine tri-phosphate (ATP), 50 \(\mu\)M guanine tri-phosphate (GTP), and 10 \(\mu\)M l-isoproterenol. All reagents except nucleotides were obtained from Sigma Chemical Company (St. Louis, MO). ATP and GTP were microbiologically derived grades, obtained from ICN (Irvine, CA). Reactions were terminated by heating at 90°C for 2 min. Samples were centrifuged at 1500 \(\times\) g (2700 rpm, Beckman TH-4 rotor) for 15 min to remove insoluble material prior to measurement of adenosine 3',5' cyclic monophosphate (c-AMP).

Cyclic AMP was measured using a modification of the protein binding method of Brown, Elkins, and Albano.\(^9\) Protein was measured by the micro Bio-Rad dye-binding method (Bio-Rad Laboratories, Richmond, CA), using bovine serum albumin as standard.

**Statistical Analysis**

Statistical analysis of data was performed using BMDP-statistical software for basic t-testing.\(^10\) Average values for right and left eyes were used for analysis. In order to adjust for differences in mean baseline pressure among the three q.d. trials, mean control pressure was subtracted from each experimental pressure prior to statistical analysis.

**Results**

Preliminary studies in this laboratory have shown that maximal hypotensive response to 2.0% 1-epinephrine bitartrate and 2.0% 1-epinephrine bitartrate plus 0.1% dexamethasone phosphate occurs 2.0–2.5 hr after topical application to albino rabbit eyes.\(^11\) In the present study, intraocular pressures, measured 2.5 hr after treatment with a single dose of 2.0% epinephrine bitartrate, alone or in combination with 0.1% dexamethasone phosphate, were 6–9 mm Hg lower than control values in groups of animals with mean initial pressures (±S.E.M.) of 20.9 ± 0.5 mm Hg, and 11–13 mm Hg lower than controls when initial IOP was 26.1 ± 0.5 mm Hg. The hypotensive response with epinephrine on day 1 was not significantly different \((P < 0.05)\) from that observed with epinephrine/dexamethasone.

Figure 1 shows the effect of successive daily doses of 2.0% epinephrine bitartrate, with and without 0.1% dexamethasone phosphate, on IOP. On the second day, epinephrine failed to lower IOP significantly from control values \((P < 0.05)\); however, loss of pressure-lowering response was attenuated by administration of dexamethasone in conjunction with epinephrine. IOP, 2.5 hr after treatment with epinephrine/dexamethasone, was significantly lower than controls on days 1–5 \((P < 0.05)\) with q.d. administration. Post-treatment pressures in epinephrine/dexamethasone groups were significantly lower than epinephrine treated eyes on days 2–5 \((P < 0.05)\). Treatment with topical dexamethasone alone (up to 0.2% dexamethasone phosphate b.i.d.) results in no change in IOP after 1 week (data not shown).

When 2.0% epinephrine bitartrate was administered twice daily, the hypotensive effect of epinephrine alone was observed only on day 1 (Fig. 2). IOP, measured 2.5 hr after treatment with epinephrine/dexamethasone, was significantly lower than control on days 1,
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.5,13 Diminished hypotensive 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 hypotensive action of topically applied epinephrine in the rabbit eye.16 In the present study, loss of hypotensive 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 desensitization.

| Table 1. In vivo desensitization—rabbit ciliary processes l-isoproterenol-stimulated adenylate cyclase activity (pmol c-AMP/Minute/Milligram Protein)* |
|-----------------|-----------------|-----------------|
|                 | Control         | Epinephrine     |
| Basal           | 18.13 ± 2.22    | 20.09 ± 3.39    |
| Stimulation     | 65.41 ± 3.98    | 17.97 ± 2.56    |
| index†          | 3.61 ± 0.25     | 0.91 ± 0.06     |
| Epinephrine/    | 10.38 ± 0.81    | 23.58 ± 0.83    |
| Dexamethasone   |                 |                 |

* 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 adenylyl 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 adenylyl 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-receptor occupancy, 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 (beta2-agonist), and is 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: adenylyl cyclase, dexamethasone, desensitization, epinephrine, intraocular pressure

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References