Effects of selective beta\textsubscript{1}- and beta\textsubscript{2}-adrenoreceptor agonists and antagonists on intraocular pressure in the cat

Brenda K. Colasanti and Robert R. Trotter

Ocular tension of cats was measured after topical administration of the selective beta\textsubscript{1}-adrenergic agonist CGP 7760B, the selective beta\textsubscript{1}-adrenergic antagonist atenolol, the selective beta\textsubscript{2}-adrenergic agonist salbutamol, the selective beta\textsubscript{2}-adrenergic antagonist H 35/25, and the mixed beta\textsubscript{1}- and beta\textsubscript{2}-adrenergic antagonist timolol. Although atenolol did not alter intraocular pressure, CGP 7760B produced a modest decrease amounting to 3 to 4 mm Hg. Salbutamol, H 35/25, and timolol each produced a dose-dependent lowering of ocular tension, with maximal reductions amounting to 7, 5, and 5 mm Hg, respectively. Sympathetically denervated cat eyes showed supersensitivity to the pressure-lowering effect of salbutamol. In contrast, sympathectomy markedly reduced the effects of H 35/25 and timolol on ocular tension. Eyes rendered subsensitive to the pressure-lowering effects of cholinomimetics by chronic echothiophate treatment likewise showed diminished responsiveness to H 35/25 and timolol. Pretreatment with timolol (3 hr) completely abolished the pressure-lowering effect of salbutamol, and pretreatment with atenolol likewise completely antagonized the effect of CGP 7760B. These results suggest that beta-adrenergic receptors in the anterior segment of the cat eye are predominantly beta\textsubscript{2}. Although beta-adrenergic antagonists apparently exert their effects on ocular tension by action at beta-adrenergic receptors, a cholinergic mechanism may be involved as well.

Key words: beta\textsubscript{1}-adrenergic agonist, beta\textsubscript{2}-adrenergic agonist, atenolol, salbutamol, beta\textsubscript{2}-adrenergic antagonist, timolol, sympathectomy, cholinergic subsensitivity, intraocular pressure, cat

Lowering of intraocular pressure in response to drugs stimulating beta-adrenergic receptors was first observed after the administration of isoproterenol to glaucomatous eyes.\textsuperscript{1} Since that time, many reports have confirmed the ocular hypotensive effect of isoproterenol in experimental animals\textsuperscript{2-4} and in man.\textsuperscript{5-7} Because this effect was completely blocked by prior administration of the beta-adrenergic antagonist propranolol, isoproterenol was presumed to act specifically at beta-adrenergic receptors.\textsuperscript{3, 7}

Recently, beta-adrenergic agonists have been classified into two groups designated beta\textsubscript{1} (\(\beta_1\)) and beta\textsubscript{2} (\(\beta_2\)) on the basis of relative tissue selectivity of beta stimulation\textsuperscript{8-9}. Although \(\beta_2\)-adrenergic agonists act prefer-
array of other selective β₂-adrenergic agonists have also recently been reported to lower intraocular pressure in the rabbit. Paradoxically, β-adrenergic receptor antagonists cause reduction of intraocular pressure when administered in the absence of an agonist. In early work, the mixed β₁- and β₂-adrenergic antagonist propranolol was reported to exert an ocular hypotensive effect in rabbits and in man. Lowering of human ocular tension in response to the mixed antagonist timolol as well as the selective β₁-adrenergic antagonist atenolol has since been documented. Reports on the effect of timolol on intraocular pressure in normal rabbits, however, have been somewhat conflicting, with some researchers observing a decrease and others little change in ocular tension.

The present studies were undertaken to determine the effects of selective β₁ and β₂-adrenergic agonists and antagonists as well as those of timolol on intraocular pressure in the cat. Because timolol exerted a significant ocular hypotensive effect in this animal species, several additional experiments were conducted to shed further light on the mechanism of action of this antagonist.

Methods

A total of 30 adult cats, mostly female and weighing between 2.2 and 3.1 kg, were utilized in these experiments. In order to facilitate drug administration, nictitating membranes were routinely removed surgically at least 4 weeks prior to tonometry. Intraocular pressure was measured with a pneumatic tonometer (Alcon Laboratories, Fort Worth, Texas) calibrated against eyes of anesthetized cats by open stopcock manometry. The correlation coefficient for pressures up to 30 mm Hg was 0.987. After each cat had been positioned in a commercial restrainer, 1 drop of 0.5% proparacaine (Ophthaine; E. R. Squibb & Sons, Princeton, N. J.) was applied to the corneas. After 10 sec, excess anesthetic was washed away with 5 drops of isotonic saline. The tonometer was then applied tangentially to the cornea for 10 sec. Three successive pressure readings were taken in this manner, and the average value was recorded as intraocular pressure. Ocular tension was measured once hourly for 6 hr. All animals were ex-
Fig. 2. Time courses for the reductions in intracocular pressure occurring after topical application of salbutamol (A) or H 35/25 (B) to cat eyes. Points are the means ± S.E.M. for eight eyes; *p < 0.05; **p < 0.01; ***p < 0.001, significantly different from corresponding predrug baseline value.

Fig. 3. Intraocular pressure over a 6 hr period after topical administration of timolol to cat eyes. Points are the means ± S.E.M. for eight eyes; *p < 0.05; **p < 0.01; ***p < 0.001, significantly different from corresponding predrug baseline value.

posed to this experimental procedure several times before initiation of the studies.

The left superior cervical ganglion together with small portions of the postganglionic fibers was surgically removed from four cats under anesthesia induced by sodium pentobarbital, 40 to 50 mg/kg intraperitoneally. These animals were utilized at periods ranging from 3 to 16 weeks after ganglionectomy.

Four additional cats were treated chronically with echothiophate (Phospholine Iodide; Ayerst Laboratories Inc., New York, N. Y.) on three separate occasions. The drug was applied topically by the microdrop technique of Bárány22 to right eyes twice daily for 15 days. A microliter syringe with polyethylene PE-50 tubing connected to the needle was used for drug delivery. With the eye held open, 15 µl of a 0.25% solution were applied to the cornea by slow placement (1 min) of the microdrops formed by manipulation of the micrometer. In order to permit the solution to dry, the eye was held open for 2 min more. Saline (15 µl) was applied similarly to the contralateral eyes. In order to allow ocular tension to return to predrug levels, drug responses were not determined until 10 days after cessation of the chronic treatment.

Experimental drugs were dissolved in isotonic saline, and the resultant solution was usually applied topically to both right and left eyes with a microliter syringe. A period of at least 1 week intervened between drug administrations to the same animal. Concentrations of each drug were administered at a constant volume of 25 µl. All concentrations were calculated as the percent solution of the available form. The following drugs were used: CGP 7760B [(−)-1-(4-hydroxyphenox)-3-isopropyl-aminopropanol-2-hydrochloride; Ciba-Geigy Corp., Summit, N. J.]; atenolol (Stuart Pharmaceuticals, Wilmington, Del.); the ascorbate salt of salbutamol (Schering Corp., Kenilworth, N. J.); H 35/25 [W542034, 1-(4'-methylphenyl) - 2 - isopropyl - amino - propanol hydrochloride; Astra Pharmaceutical Products, Inc., Worcester, Mass.]; timolol hydrogen ma-
Saline. After topical application of saline to the eyes of cats accustomed to the procedure for measurement, intraocular pressure remained quite constant over a period of 6 hr. Maximal rise in pressure during this period amounted to $0.7 \pm 0.2$ mm Hg (mean $\pm$ S.E.M. for eight eyes) at the 2 hr time point, and the maximal fall in pressure amounted to $0.5 \pm 0.2$ mm Hg at 4 hr.

$\beta_1$-Adrenergic agonist and antagonist. After topical application of the $\beta_1$-adrenergic agonist CGP 7760B, a modest reduction of intraocular pressure from baseline values resulted (Fig. 1, A). Maximal lowering of pressure in response to this agent occurred after application of an 8% solution. After topical administration of the $\beta_1$-adrenergic antagonist atenolol, however, ocular tension did not change over a period of 6 hr (Fig. 1, B).

$\beta_2$-Adrenergic agonist and antagonist. Fig. 2, A, shows the time courses for the fall in intraocular pressure after topical application of two doses of the $\beta_2$-adrenergic agonist salbutamol to cat eyes. Within 1 hr after administration of either dose, maximal reduction of ocular tension from baseline levels had occurred. Time courses for the reduction in intraocular pressure induced by two doses of the $\beta_2$-adrenergic antagonist H 35/25 are depicted in Fig. 2, B. Although intraocular pressure reduction was evident within 1 hr after its administration, the maximal effect was not reached until 4 to 5 hr after the drug.

Timolol. The mixed $\beta_1$- and $\beta_2$-adrenergic antagonist timolol reduced intraocular pressure in the cat. Although the peak effect of a 2% solution was reached by 2 hr after topical application, that of a 4% solution was reached by 3 to 4 hr (Fig. 3). The duration of action of both concentrations extended to 6 hr after delivery to the eye. The dose-response curve obtained for timolol was quite similar to that obtained for the $\beta_2$-adrenergic antagonist H 35/25 (Fig. 4). In the case of both drugs, a maximal fall in pressure amounting to 5 mm Hg occurred in response to a 4% solution.

In a few experiments timolol was topically applied unilaterally. The decreases in intraocular pressure in response to 2% or 4% solutions ($2.5 \pm 0.8$ mm Hg, $n = 4$, at the 2% concentration; $4.5 \pm 0.4$ mm Hg, $n = 4$, at 4%) were of the same magnitude as those occurring after bilateral application (Fig. 4). No significant reduction of ocular tension oc-
Table I. Intraocular pressure reduction in response to timolol and H 35/25 after sympathetic denervation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>Control (unoperated)</th>
<th>Ganglion-resected</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol</td>
<td>2%</td>
<td>2.75 ± 0.59(8)</td>
<td>1.50 ± 0.88(4)</td>
<td>2.50 ± 0.67(4)</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>4.80 ± 0.17(8)</td>
<td>0 ± 0.71(4)c, d</td>
<td>1.50 ± 0.76(4)c</td>
</tr>
<tr>
<td>H 35/25</td>
<td>4%</td>
<td>4.75 ± 0.45(8)</td>
<td>0.25 ± 0.48(4)c, d</td>
<td>2.0 ± 1.22(4)</td>
</tr>
</tbody>
</table>

No. of eyes in parentheses.

*4 (timolol) or 5 (H 35/25) hr after drug administration.

*b Superior cervical ganglion unilaterally removed 3 to 16 weeks earlier.

*p < 0.05 vs. contralateral eyes.

*p < 0.001 vs. unoperated control.

*p < 0.01 vs. unoperated control.

*p < 0.05 vs. unoperated control.

Table II. Intraocular pressure reduction in response to 3.2% carbachol 10 days after chronic treatment of cat eyes with echothiophate

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Change in intraocular pressure* (mm Hg ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (untreated)</td>
</tr>
<tr>
<td>10</td>
<td>5.0 ± 0.4(4)</td>
</tr>
<tr>
<td>30</td>
<td>6.1 ± 0.5(5)</td>
</tr>
</tbody>
</table>

No. of eyes in parentheses.

*2 hr after carbachol administration.

*Echothiophate (0.25%; 15 μl) administered by microdrop twice daily for 14 days.

*p < 0.01 vs. contralateral eyes.

*p < 0.001 vs. untreated control.

*p < 0.05 vs. untreated control.

Table III. Intraocular pressure reduction in response to timolol and H 35/25 after chronic echothiophate treatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>Control (unoperated)</th>
<th>Chronic echothiophate</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol</td>
<td>2%</td>
<td>2.75 ± 0.59(8)</td>
<td>1.00 ± 0.71(4)</td>
<td>1.25 ± 0.85(4)</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>4.80 ± 0.17(8)</td>
<td>1.75 ± 0.95(4)c</td>
<td>0.50 ± 0.90(4)c</td>
</tr>
<tr>
<td>H 35/25</td>
<td>4%</td>
<td>4.75 ± 0.45(8)</td>
<td>2.67 ± 0.33(4)c</td>
<td>3.00 ± 0.95(4)</td>
</tr>
</tbody>
</table>

No. of eyes in parentheses.

*4 hr after drug administration.

*Echothiophate (0.35%; 15 μl) administered by microdrop twice daily for 14 days.

*p < 0.01 vs. untreated controls.

*p < 0.001 vs. untreated controls.

*p < 0.05 vs. untreated controls.

curred in the contralateral eyes (2%: maximal decrease of 1.0 ± 0.5 mm Hg, n = 4; 4%: 0.5 ± 0.8 mm Hg, n = 4).

Sympathetic denervation. Unilateral sympathetic denervation enhanced the intraocular pressure-lowering effect of 4% salbutamol [3.7 ± 0.2 mm Hg decrease in control (contralateral) vs. 5.9 ± 0.6 mm Hg in denervated; p < 0.05] and 1% salbutamol (1.7 ± 0.4 mm Hg decrease in control, vs. 4.0 ± 0.5 mm Hg in denervated; p < 0.05). In contrast, the effects of H 35/25 and timolol...
were markedly reduced by sympathetic denervation (Table I). Responses of the denervated eyes were considerably less than those of the contralateral control eyes. Responses of the contralateral eyes, moreover, were also reduced in comparison with those of eyes of animals not operated on.

Cholinergic desensitization. Ten days after termination of chronic echothiophate treatment, the intraocular pressure-lowering effect of carbachol was reduced by 50% (Table II). By 30 days responsiveness of these eyes to carbachol was still somewhat attenuated. After topical application of either H 35/25 or timolol to cat eyes at similar time periods after termination of chronic echothiophate treatment, intraocular pressure reduction was also markedly attenuated in comparison with responses of untreated animals (Table III). Responses of contralateral eyes showed much variability, and the effect of 4% timolol was markedly reduced.

β-Adrenergic agonist-antagonist interactions. Administration of 8% atenolol to cat eyes 3 hr after application of the β1-adrenergic agonist CGP 7760B, 8%, (i.e., immediately prior to the time-to-peak effect of CGP 7760B) resulted in complete antagonism of the pressure-lowering response to this drug, with a return of intraocular pressure to baseline values (Fig. 5) within 1 hr. This reversal had a short duration. By 2 hr after administration of the antagonist, ocular tension had again fallen.

Administration of 4% timolol 30 min prior to 4% salbutamol (i.e., immediately prior to the time-to-peak effect of salbutamol) resulted in partial antagonism of the effect of salbutamol as well as partial reduction of the effect of timolol (Fig. 6, A). Administration of 4% timolol 3 hr prior to 4% salbutamol (i.e., salbutamol given immediately prior to the time-to-peak effect of timolol), moreover, resulted in complete abolition of the pressure-lowering response normally occurring after administration of either agent, with a return of ocular tension to baseline values (Fig. 6, B).

Discussion

The results of this study have demonstrated that the selective β2-adrenergic agonist salbutamol produces an ocular hypotensive effect of rapid onset in the cat similar to that previously reported in the rabbit, 7, 12 primate,7 and human.7, 12 In addition, a selective β1-adrenergic agonist, CGP 7760B,23 has been found to produce a modest reduction in ocular tension. Although the selective β2-adrenergic antagonist H 35/2524 produced a more pronounced ocular hypotensive effect of longer duration, the selective β1-adrenergic antagonist atenolol did not alter intraocular pressure. Because the latter antagonist reversed the pressure-lowering effect of CGP 7760B, adequate ocular penetration probably occurred. The relative activities of these selective β-adrenergic stimulants and blockers may thus indicate that β-adrenergic receptors mediating pressure changes in the anterior segment of the cat eye are predominantly β2.

The mixed β1- and β2-adrenergic antagonist timolol proved to be an effective ocular hypotensive agent in the cat. As reported by others,51 this antagonist did not alter intraocular pressure in normal rabbits (unpublished observations). The doses required to lower ocular tension in the cat are quite high in comparison with those effective in man.16, 17 Reported doses of timolol for man, however,
are expressed in terms of the free base rather than the salt, and the volume in 1 drop is at least 1.4 times greater than the 25 μl used in the present study. After consideration of the anterior chamber volume of the cat eye as well, which is about four times that of man, doses of timolol effective in the cat are quite comparable to those effective in humans.

Sympathetically denervated cat eyes showed supersensitivity to the ocular hypotensive effects of salbutamol. These results are in agreement with those previously reported for the nonselective β-adrenergic agonist isoproterenol. In contrast, the effect of timolol and that of the β2-adrenergic antagonist H 35/25 were almost completely abolished by denervation. Intact adrenergic innervation is accordingly required for mediation of the actions of these drugs.

Because the ocular hypotensive responses to both timolol and salbutamol were completely abolished by administration of the antagonist 3 hr prior to the agonist, the effect of timolol may be mediated in part by action at a β-adrenergic receptor. The profound loss of responsiveness to timolol and the β2-adrenergic antagonist H 35/25 of eyes rendered subsensitive to cholinomimetics by chronic echothiophate treatment additionally suggests that ocular cholinergic input may modulate the effects of these antagonists.

The lack of a contralateral effect of timolol after unilateral topical application indicates that systemic effects do not contribute appreciably to the lowering of ocular tension. The marked reduction of effectiveness of timolol observed in eyes contralateral to those treated chronically with echothiophate, on the other hand, is probably due to systemic absorption of the cholinesterase inhibitor. Responses to timolol of eyes contralateral to the ganglionectomized side of sympathetically denervated cats likewise were significantly less than those of unoperated controls. This latter phenomenon was previously observed in a study on prostaglandin and catecholamine effects in rabbits and is most likely due to the existence of crossed sympathetic innervation.

The cat appears to be a suitable experimental animal for the study of β-adrenergic drug effects on ocular tension.

Fig. 6. Intraocular pressure reduction in eyes treated topically with timolol either 30 min (A) or 3 hr (B) prior to salbutamol. Although the 30 min pretreatment (salbutamol given at the zero time point) resulted in partial antagonism of the pressure-lowering effect of either drug, the 3 hr pretreatment (salbutamol given at the 3 hr time point) resulted in complete antagonism, with a return of ocular tension to baseline values. Each point represents the mean ± S.E.M. for eight eyes; *p < 0.05, **p < 0.01, ***p < 0.001, significantly different from values for eyes given timolol alone at corresponding time period. A, *p < 0.05, significantly different from values for eyes given salbutamol alone at corresponding time period. B, *p < 0.05 and **p < 0.01, significantly different from values for eyes given salbutamol alone at the 1 and 2 hr time points, respectively.

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REFERENCES


