Selective Suppression by Bunazosin of Alpha-Adrenergic Agonist Evoked Elevation of Intraocular Pressure in Sympathectomized Rabbit Eyes

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Purpose. To determine whether there is α₁-adrenergic receptor heterogeneity associated with the regulation of intraocular pressure (IOP) and mydriasis in rabbits, the authors tested the hypothesis by characterizing the ability of the selective α₁-adrenergic antagonist, bunazosin, to block the ocular hypertensive and mydriatic responses to α₁-adrenoceptor stimulation by either norepinephrine (NE) or phenylephrine (PE).

Methods. The effects of topical application of bunazosin on IOP and pupillary diameter were measured in unilateral superior cervical ganglionectomized (SCGX) rabbits after exposure to either NE or PE.

Results. Bunazosin (0.1%) alone only lowered the IOP in the normal eye and did not elicit a pupillary response on either side. NE (0.01–1.0%) by itself caused a concentration-dependent rise in IOP on both sides, but mydriasis did not occur on the normal side. In SCGX eyes, the sensitivity of the IOP response to NE increased tenfold over that measured on the normal side. Unlike on the normal side, concentration-dependent mydriatic responses occurred with 0.1 and 1% NE. After pretreatment with bunazosin (0.1%), neither NE (0.1%) nor PE (0.1%) evoked a rise in IOP. However, the mydriatic response to either one of these agonists in the SCGX eyes was less affected. By contrast, pretreatment with the α₂-adrenergic antagonist, 0.5% yohimbine, did not change the IOP increase elicited by 0.1% NE.

Conclusions. These results suggest that the α₁-adrenergic receptors that regulate IOP and pupillary diameter are different from one another in the rabbit. Invest Ophthalmol Vis Sci. 1993;34:1761-1766.

Stimulation of α₁-adrenoceptors by either norepinephrine (NE) or phenylephrine (PE) is known to cause mydriasis1–5 and, initially, a rapid rise in intraocular pressure (IOP).2–6 Bunazosin hydrochloride is a potent and highly selective postsynaptic α₁-antagonist that is used clinically as an antihypertensive drug.7 We previously reported that the topical application of bunazosin reduced IOP, but it had little or no effect on pupillary diameter in rabbits8,9 and humans.10 Such selectivity by bunazosin could mean that each one of these responses is linked to a different α₁-adrenergic subtype. This notion is consistent with accumulating data showing that many tissues exhibit α₁-adrenoceptor heterogeneity.11 A drawback of our earlier studies in characterizing the bunazosin-elicited effects on IOP

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and mydriasis was that sympathetic neuronal input was not eliminated. Such input was a confounding factor in the characterization of adrenergic receptor regulation of these responses.

The current study was undertaken to determine whether or not pretreatment with bunazosin changes the IOP and mydriatic responses to either NE or PE in sympathectomized rabbits. This procedure eliminates any possible endogenous catecholamine effects resulting from their possible release and reuptake at pre-junctional terminals. With this approach, we characterized more directly the hypotensive mode of action of bunazosin on IOP after exposure to exogenous α1-adrenoceptor agonists.

**MATERIALS AND METHODS**

**Animals and Drugs**

This investigation adhered to the principles of the ARVO Resolution on the Use of Animals in Research. Male Japanese albino rabbits weighing 3–4 kg were housed at a temperature of 23 ± 1°C and humidity of 55 ± 10% with a 12 hr:12 hr light/dark cycle. Lights on was defined as 7:00 AM, and lights off occurred at 7:00 PM. The drugs used were as follows: bunazosin HCl (Eisai, Tokyo, Japan), yohimbine HCl (Sigma, St. Louis, MO), D,L-norepinephrine HCl (Aldrich, Milwaukee, WI), and L-phenylephrine HCl (Kasei, Tokyo, Japan). Bunazosin was dissolved in a borate-buffered solution that was adjusted to pH 6. All other drugs were dissolved in saline. The drugs were applied topically (50 μl) around 11:00 AM into both eyes.

**Superior Cervical Ganglionectomy**

The 19 rabbits were anesthetized with a combination of xylazine (1.8 mg/kg) and ketamine (30 mg/kg) intramuscularly. Unilateral superior cervical ganglia were surgically excised. The contralateral superior cervical ganglion remained intact. Two weeks after surgery, the success of the unilateral sympathectomy was determined by comparing the mydriatic responses evoked by the application of 50 μl of 0.1% NE to both eyes.

**Measurement of IOP and Pupillary Diameter**

IOP was measured in unrestrained conscious rabbits with a calibrated pneumatic tonometer (Alcon, Tokyo, Japan) after topical application of 0.4% oxybuprocaine HCl for corneal anesthesia. Pupillary diameter was measured with a Haab’s pupillometer in 250 lux of light. Pretreatment with bunazosin was done 30 min before the instillation of NE or PE. All experiments were done at intervals separated by 2 weeks to wash out the previous drug.

**Data Analysis**

All data are expressed as the mean ± the standard error of the mean. Statistical significance was determined with Student’s t test for either paired or unpaired variates (P < 0.05).

**RESULTS**

**Basal IOP and Pupillary Diameter**

In Figure 1, we show the diurnal variation in IOP and pupillary diameter measured every 2 hr between 9:00 AM and 9:00 PM in both superior cervical ganglionectomized (SCGX) and the contralateral normal eyes 4 weeks after surgery. With normal eyes, there was a significant decrease in IOP during the day; the SCGX eyes did not show this change. Therefore, the IOP values between 11:00 AM and 3:00 PM were significantly higher in the SCGX eye than in the normal eye. Furthermore, the pupillary diameter was significantly smaller in SCGX eyes than in the normal eyes throughout this period.
Change in IOP and Pupillary Diameter After Topical Application of NE

Concentration–response relationships of the effects of bilateral instillation of NE (0.01%, 0.1%, and 1.0%) on IOP and pupillary diameter are shown in Figure 2. In the normal eyes, NE caused a time-dependent rise in IOP at concentrations of 0.1% and 1.0%, but it caused no remarkable mydriasis even at 1.0%. However, in SCGX eyes, an IOP rise was observed even at a concentration of 0.01% NE; this reached a maximum at 0.1%. By contrast with the lack of a response in normal eyes, there was mydriasis that was apparent at concentrations of 0.1% and 1.0%. PE (0.01%–1.0%) like NE elicited mydriasis and elevated IOP (data not shown).

Effects of Bunazosin on IOP and Pupillary Diameter

As shown in Figure 3 (top panel), bunazosin (0.1%), when applied on either side, caused no reduction in IOP in SCGX eyes, although it reduced the IOP on the contralateral normal side. By contrast, it did not change the pupillary diameter on either side (bottom panel).

Effects of Bunazosin on IOP and Pupillary Responses Evoked by NE or PE

The effects of a pretreatment with 0.1% bunazosin on IOP and pupillary responses evoked by either 0.1% NE or 0.1% PE were determined in SCGX eyes (Fig. 4). The IOP responses to NE alone were biphasic (Fig. 4A), ie, an initial rise in IOP followed by a decrease in IOP below the initial basal level. Bunazosin eliminated both the initial rise in IOP and the subsequent fall in IOP evoked by NE (Fig. 4A), whereas saline instillation did not affect the IOP. Similarly, bunazosin eliminated these changes after exposure to PE (Fig. 4B). However, the mydriatic response evoked by NE was not affected by pretreatment with bunazosin (Fig. 4C). The mydriatic response evoked by PE was significantly inhibited during the early phase at 0.5–1 hr but, never-
FIGURE 4. Effects of pretreatment with 0.1% bunazosin (BZ) on IOP (a, b) and pupillary (c, d) responses to topical application of 0.1% NE (a, c) and 0.1% PE (b, d) in SCGX eyes. Each point represents the mean ± standard error of eight to ten eyes. *P < 0.05 versus 0.1% NE or 0.1% PE. PD = pupillary diameter.

We found that exogenous NE elicited a rise in the IOP and mydriasis in SCGX eyes at similar concentrations; in normal eyes, NE selectively increased only the IOP. These additional effects of NE in SCGX eyes appear to be caused by the induction of postjunctional receptor supersensitivity to NE and PE after the removal of prejunctional uptake mechanisms. The contribution by endogenous catecholamines in the normal eyes is also indicated by the fact that bunazosin did not affect the IOP in SCGX eyes, but it had a lowering effect in the normal eyes.

DISCUSSION

Our previous studies in rabbits and humans demonstrated that topical bunazosin reduced IOP in a dose-dependent manner with a maximum effect at 0.1%. However, bunazosin had little or no effect on pupillary diameter. An explanation for these disparate effects was not apparent because, in the intact eye, endogenous catecholamines are released and taken up across the prejunctional neuronal membranes. This recycling process prevents a definitive understanding of α2-adrenoceptor regulation of IOP and pupillary diameter. Therefore, we studied the effects of two α2-adrenergic agonists on these responses in SCGX rabbits to eliminate these confounding factors.

Nevertheless, was not eliminated by pretreatment with bunazosin (Fig. 4D). In other experiments, if the α2-adrenoceptor antagonist, yohimbine (0.5%) was used instead of bunazosin during the preexposure period, the subsequent responses of mydriasis and IOP to NE were unaltered (Fig. 5).

Based on the results of studies in a host of other tissues, there is evidence that the agonist effects on IOP and pupillary responses are elicited by two different α1-adrenoceptor subtypes. For example, in blood vessels, there are different α1-adrenoceptor subtypes based on resolvable differences in affinity for prazosin. Recently, evidence was reviewed suggesting that α1-adrenoceptors can be further subdivided into pharmacologically distinct subtypes that are coupled to specific second-messenger pathways. Others also proposed a similar subclassification based...
Ocular Hypotensive Mechanism of Bunazosin

Bunazosin appears to be a selective $\alpha_1$-adrenoceptor antagonist because pretreatment with a selective $\alpha_2$-adrenoceptor antagonist, yohimbine, instead of bunazosin, affected neither the NE-induced IOP increase nor mydriasis. The selective suppressive effect of bunazosin on a NE-induced elevation of IOP further suggests that bunazosin interacts with a specific $\alpha_1$-adrenoceptor subtype because NE elevates IOP and induces mydriasis only by stimulating $\alpha_1$-adrenoceptors. Other evidence for distinct subtypes includes the report that the $pA_2$ values of the quinazolines, such as prazosin and bunazosin, were approximately tenfold lower in the rabbit iris dilator muscles than in the rabbit aortic strips.

The previous indication of the selectivity of bunazosin as an $\alpha_1$-adrenoceptor antagonist is strengthened by our observation that pretreatment with the $\alpha_2$-antagonist, yohimbine, affected neither the NE-induced rise in IOP nor mydriasis in SCGX eyes, whereas bunazosin selectively suppressed the NE-evoked IOP increase in these eyes without affecting mydriasis. This apparent selectivity by bunazosin supports the hypothesis of $\alpha_1$-adrenoceptor heterogeneity linked to these two responses because both of these effects result from $\alpha_1$-adrenoceptor occupancy by NE. Even though bunazosin has an action similar to that reported by another $\alpha_1$-adrenoceptor antagonist, prazosin, the effects of these two drugs on IOP were not identical. Only bunazosin did not change the IOP of SCGX eyes, whereas prazosin decreased the IOP in both normal and SCGX eyes. This difference in effects could mean that their selectivities for $\alpha_1$-adrenoceptors are not the same or that prazosin has actions in addition to its effects on $\alpha_1$-adrenoceptors.

Our evidence, which we obtained in support of $\alpha_1$-adrenoceptor heterogeneity, stems from the dose-response relationships provided in Figure 2. We found that, in SCGX eyes, a greater than tenfold higher concentration of NE was required to elicit maximal mydriasis than to elevate IOP maximally. In these eyes, NE (0.01%) elicited an increase in IOP that was 60% of the maximum increase obtained with 0.1% NE. However, in SCGX eyes, the pupillary response to 0.01% NE was much less because it only increased the pupillary diameter by a value that was 10% of the mydriatic response elicited by 0.1% NE. Nevertheless, there is some indication that bunazosin also interacts with the $\alpha_1$-adrenoceptor linked to mydriasis because bunazosin partially suppressed the pupillary response to PE.

There is other earlier evidence for $\alpha_1$-adrenoceptor heterogeneity based on studies of the pupillary response. The mydriatic response to $\alpha_1$-adrenoceptor agonists was not inhibited by either prazosin or yohimbine in rabbits. Moreover, in cats, the pupillary response was not inhibited by $\alpha_2$-antagonists, such as prazosin and WB4101, which led the authors to suggest that this response was linked to adrenoceptors that differed from the classic $\alpha_1$ and $\alpha_2$ subtypes.

In conclusion, we found that there may be two distinct subtypes of $\alpha_1$-adrenoceptors that regulate IOP and pupillary diameter. The ocular hypotensive effect of bunazosin is probably the result of the blockade of one specific $\alpha_1$-adrenoceptor subtype that is different from the one linked to the regulation of pupillary diameter.

**Key Words**
- bunazosin, intraocular pressure, pupillary diameter, rabbit, $\alpha_1$-adrenoceptor

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