Effects of Topically Instilled Bunazosin, an \(\alpha_1\)-Adrenoceptor Antagonist, on Constrictions Induced by Phenylephrine and ET-1 in Rabbit Retinal Arteries

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PURPOSE. To examine the inhibitory effects of topically instilled bunazosin hydrochloride (bunazosin), a selective \(\alpha_1\)-adrenoceptor antagonist, on the retinal artery constrictions induced by intravitreous phenylephrine hydrochloride (phenylephrine) and endothelin (ET-1) in rabbits.

METHODS. Phenylephrine or ET-1 (20 \(\mu\)L) was injected into the central part of the vitreous in both eyes in pigmented rabbits. Color fundus photographs were taken at 5 minutes before and 60 minutes after the injection. The average diameter of the major retinal arteries at the rim of the optic nerve head (ONH) was normalized with respect to ONH diameter. Bunazosin was instilled into one eye (chosen randomly) and vehicle into the fellow eye at 60 minutes before the intravitreous injection. To examine any interaction between the \(\alpha_1\)-adrenoceptor and ET receptor, phenylephrine and ET-1 were co-injected at individually ineffective doses. In addition, ET-1–induced vasoconstriction was examined after unilateral superior cervical ganglionectomy. The binding affinities of bunazosin for ETA and ET\(_B\) receptors were also evaluated. The series of experiments was performed as masked tests.

RESULTS. Retinal arteries were dose-dependently constricted by both intravitreous phenylephrine and intravitreous ET-1. Topically instilled bunazosin at 0.01% partly inhibited both of these vasoconstrictions on the ipsilateral side, but not on the contralateral side. Bunazosin did not bind to ET receptors. Co-injection of phenylephrine and ET-1 at individually ineffective doses constricted retinal arteries significantly. An adrenergic supersensitivity in retinal arteries was observed after superior cervical ganglionectomy only on the ganglionectomized eye. The ET-1–induced vasoconstriction was significantly weaker in cervical ganglionectomized eyes than in sham-surgery eyes.

CONCLUSIONS. The present findings suggest that topically instilled bunazosin reaches the posterior retina by local penetration at concentrations sufficient to attenuate the phenylephrine- or ET-1–induced constriction of retinal arteries in normal rabbit eyes, and that the inhibitory effect of bunazosin on the ET-1–induced vasoconstriction in this tissue may be partly attributable to an interaction between the \(\alpha_1\)-adrenoceptor and ET receptor. (Invest Ophthalmol Vis Sci. 2004;45:4041–4048) DOI:10.1167/iovs.03-1395

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Moreover, there was an intriguing report that intravitreously injected bunazosin can attenuate the ET-1-induced decrease in ocular blood flow.\(^\text{28}\) The secondary purpose of the present study was therefore to examine whether topically instilled bunazosin might attenuate the intravitreous ET-1-induced constriction of retinal arteries. In addition, we proceeded to investigate the possible mechanism by which bunazosin might attenuate the ET-1-induced vasoconstriction.

**Materials and Methods**

**Experimental Animals**

Male Dutch rabbits (1.5–2.0 kg; Biotech, Shiga, Japan) were used and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. They were housed in an air-conditioned room at approximately 23°C and 55% humidity with a 12-hour light-dark cycle.

**Drugs**

Ophthalmic solutions of bunazosin at 0.0001%, 0.001%, and 0.01%, and the vehicle solution were prepared at Santen Pharmaceutical Co. Ltd (Osaka, Japan).

**Measurement of Retinal Artery Diameter**

The method used was essentially the same as that used by Mizuno et al.\(^\text{3,4}\) In each eye, color fundus photographs were taken at 5 minutes before and 60 minutes after intravitreal injection. For this, we used a fundus camera (RC-XV3; Kowa, Tokyo, Japan; viewing angle covering a fundus area of 50°) and captured the images in an image-filing system (VK-2; Kowa). In a series of studies, we measured two major retinal arteries, which form two major branches of the central retinal artery and then course on the nasal and temporal sides of the optic nerve head (ONH). The average diameter of the major retinal arteries on the rim of the ONH was normalized with respect to ONH diameter, and each experimental value was expressed as a percentage of that obtained at 5 minutes before the intravitreal injection. All measurements were made by a masked observer (YO or YA).

**Effect of Topically Instilled Bunazosin on Retinal Artery Diameter in Normal Rabbits: Experiment 1**

Bunazosin solution at 0.01% or its vehicle was instilled into one randomly chosen eye in normal rabbits. Color fundus photographs were taken at 5 minutes before and 120 minutes after the instillation.

**Phenylephrine- or ET-1–Induced Vasoconstriction: Experiment 2**

This series of experiments was performed as in previous reports.\(^\text{3,4}\) Each pupil was dilated with 1 drop of 0.4% tropicamide (Mydrin M; Santen). Then, 20 μL of a solution of either phenylephrine (100 or 1000 μM) or ET-1 (500, 500, or 1000 nM) was injected into the vitreous in both eyes. Color fundus photographs were taken at 5 minutes before and 60 minutes after the injection. As a separate experiment, intracocular pressure (IOP) in vehicle- and bunazosin-instilled eyes was measured at 60 minutes after an intravitreous injection of phenylephrine at 100 μM or of ET-1 at 300 nM. Measurements were made by masked investigators (YO and YA) using a pneumotonometer (model 30 Classic; Medtronic Ophthalmics, Jacksonville, FL).

**Effect of Intravitreous Injection on the Penetration of Bunazosin into the Vitreous: Experiment 4**

In this series of experiments, phenylephrine and/or ET-1 was injected into the vitreous body after topical instillation of bunazosin. Hence, there was a possibility that the intravitreous injection procedure itself might have induced bunazosin transfer from the conjunctiva, sclera, or choroid into the vitreous. To investigate, we measured by HPLC the concentrations of bunazosin in vitreous bodies that had or had not received an injection. Bunazosin solution (1%; 50 μL) was topically instilled into both eyes. Sixty minutes later, 20 μL of saline was injected into the vitreous body of the right eye in each animal, with the left eye remaining untreated. Because the vitreous concentration of bunazosin after topical instillation of 0.01% or 0.1% solution was too low to detect subtle differences, a 1% solution of bunazosin was instilled in this experiment. Sixty minutes after the injection, the rabbits were anesthetized with pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co. Ltd, Osaka, Japan). Then, both eyes were enucleated, and after the vitreous body was collected, its wet weight was recorded.

The concentrations of bunazosin in the vitreous samples were measured by a masked investigator using the reverse phase HPLC fluorescence-detection method. The vitreous samples were subjected to extraction with acetonitrile containing an internal standard (prazosin hydrochloride; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The dried extracts obtained by evaporation of acetonitrile were dissolved in HPLC solvent (mobile phase, described later). The samples were then injected into an HPLC system (HP1100; Hewlett Packard Japan, Tokyo, Japan), which was used in the reverse-phase mode for this assay. The stationary phase used was a packed column (250 mm length × 4.6 mm internal diameter; TSKgel ODS-80T M, Tosho Inc., Tokyo, Japan). A mixture of 66 mM sodium phosphate (pH 7.5) and acetonitrile (7:3 vol/vol) was used for the mobile phase, with a flow rate of 1.0 mL/min. Retention of the drug was monitored with a spectrophotometric detector (excitation wavelength 350 nm, emission wavelength 405 nm). The concentrations of bunazosin were determined with external standards by using the peak area ratios of bunazosin to the internal standard.

**Binding Affinities of Bunazosin for ET Receptors: Experiment 5**

Using conventional procedures, we evaluated the binding affinities of bunazosin for ET\(_A\) and ET\(_B\) receptors. Briefly, a cell membrane fraction containing the target receptor was initially fixed on the filter membrane, and a radio isotope (RI)-labeled ligand specifically recognizing this receptor was allowed to bind to the membrane. Subsequently, 0.01, 1, or 100 μM bunazosin was added to the membrane, and the replacement rate for bunazosin was calculated by determining the amount of RI-labeled ligand released from the receptor.

**Effect of Intravitreous Co-injection of Phenylephrine and ET-1 on Retinal Arteries: Experiment 6**

One eye was intravitreally injected with 20 μL of one of the following: vehicle (group 1), phenylephrine at 30 μM (group 2), ET-1 at 30 nM (group 3), or a mixed phenylephrine/ET-1 solution (comprising 10 μL of 60 μM phenylephrine and 10 μL of 60 nM ET-1, group 4). Color
fundus photographs were taken at 5 minutes before and 60 minutes after the intravitreal injection. As a separate experiment, IOP was measured at 60 minutes after the injection of phenylephrine, ET-1, or mixed solution by masked investigators (YO and YA) using a pneumotonometer. The effect of topical 0.01% bunazosin on the vasoconstriction induced by the intravitreal mixed phenylephrine/ET-1 solution was studied as just described.

**Effect of Superior Cervical Ganglionectomy on Phenylephrine-Induced Vasoconstriction: Experiment 7**

Superior cervical ganglionectomy (SCGx) was performed as in previous reports. Briefly, the rabbit was anesthetized with a combination of xylazine (1.8 mg/kg; Bayer, Leverkusen, Germany) and ketamine (30 mg/kg; Sankyo Co. Ltd., Tokyo, Japan), injected intramuscularly. The superior cervical ganglion on the right side was then surgically excised (SCGx group). The contralateral superior cervical ganglion remained intact. SCGx rabbits were used as a model in experimental situations.

**Data Analysis**

All data, expressed as the mean ± SE, were analyzed with an unpaired or paired t-test, with or without the Bonferroni correction or by one-way ANOVA followed by the Dunnett multiple-comparison test, as appropriate. *P < 0.05 was considered statistically significant.

**RESULTS**

**Effect of Topically Instilled Bunazosin on Retinal Artery Diameter in Normal Rabbits: Experiment 1**

In normal rabbits, there was no significant difference in the retinal artery diameter between vehicle- and bunazosin-instilled eyes. The relative diameters at 120 minutes after instillation against 5 minutes before instillation were 94.5% ± 3.7% in vehicle-instilled eyes and 98.3% ± 2.4% (mean ± SE, n = 4) in bunazosin-instilled eyes. Neither value was significantly different from 100%.

**Phenylephrine- and ET-1–Induced Constrictions of Retinal Arteries: Experiment 2**

Retinal arteries were constricted dose-dependently by intravitreal injection of phenylephrine or ET-1. The vasoconstriction induced by phenylephrine was significant at doses of 100 and 1000 μM (Fig. 1), whereas ET-1–induced vasoconstriction was significant at doses of 300 to 1000 nM (Fig. 2). In a preliminary examination, the vasoconstrictions induced by phenylephrine at doses of 100 and 1000 μM had almost reached their maximum within 15 minutes after the intravitreal injection (data not shown). In contrast, the ET-1–induced vasoconstriction increased progressively and reached an almost maximum response at 60 minutes after the injection (data not shown). In another preliminary experiment, ET-1 at 300 nM constricted retinal arteries by 53.7% ± 1.9% (mean ± SE, n = 20). In this experimental situation (n = 5), a change greater than 14.7% (percentage of constriction) would be detected with a significance level (α) of 0.05 and a statistical power (1 – β) of 0.8.

**Effect of Topically Instilled Bunazosin on the Vasoconstrictions Induced by Phenylephrine or ET-1: Experiment 3**

Retinal arteries were constricted by intravitreal phenylephrine at 100 μM (Figs. 3A, 3B), and the vasoconstriction was almost completely inhibited by an instillation of 0.01% bunazosin (Figs. 3C, 3D) administered at 60 minutes before the intravitreal injection. Topically instilled bunazosin at 0.001% to 0.01% attenuated the vasoconstriction induced by 100 μM phenylephrine (Fig. 3E), with the effects being significant at 0.01% bunazosin. Bunazosin at 0.01% did not inhibit the vaso-
Effect of Intravitreous Injection Procedure Itself on the Penetration of Bunazosin into the Vitreous: Experiment 4

In saline-injected and noninjected eyes, the bunazosin concentrations in the vitreous after its topical instillation at 1% were 3.17 ± 0.16 and 3.42 ± 0.45 ng/g wet tissue (mean ± SE, n = 6), respectively (no significant difference).

Binding Affinities of Bunazosin for ET Receptors: Experiment 5

Table 1 shows that bunazosin displayed no binding affinity for either ET_A or ET_B receptors, even at a concentration of 100 μM.

Synergistic Effect of Phenylephrine and ET-1: Experiment 6

Next, we examined whether stimulation of α_1-adrenoceptors modulates the vasocostrictor effect of ET-1. As shown in Figure 5, single intravitreous injections of phenylephrine at 30 μM or ET-1 at 30 nM failed to constrict retinal arteries (groups 2 and 3). However, when these two agents were co-injected into the vitreous body at those doses, retinal arteries were constricted to a degree that was significantly greater than

constriction induced by 1000 μM phenylephrine. These topical instillations of bunazosin had no significant effects on the responses to phenylephrine in the fellow control eyes (Fig. 3E). In vehicle- and 0.01% bunazosin-instilled eyes, the IOPs at 60 minutes after intravitreous injection of phenylephrine at 100 μM were 19.9 ± 0.6 and 20.5 ± 0.7 mm Hg (mean ± SE, n = 4), respectively (no significant difference).

Vasoconstriction was induced by intravitreous ET-1 at 300 nM (Figs. 4A, 4B), and this effect was inhibited by an instillation of bunazosin at 0.01% (Figs. 4C, 4D) administered at 60 minutes before the intravitreous injection. Bunazosin at 0.0001% and 0.001% did not inhibit the vasoconstriction induced by ET-1 at 300 nM. Topical instillation of bunazosin at 0.01% had no significant effect on the response to ET-1 in the fellow control eye (Fig. 4E). At 500 and 1000 nM of ET-1, there was no significant difference between the two eyes (Fig. 4E). In vehicle- and 0.01% bunazosin-instilled eyes, the IOPs at 60 minutes after an intravitreous injection of ET-1 at 500 nM were 21.3 ± 0.8 and 20.0 ± 1.8 mm Hg (mean ± SE, n = 4), respectively (no significant difference).
Table 1. Binding Affinities of Bunazosin for ET Receptors

<table>
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<tr>
<th>Receptors</th>
<th>Tissue</th>
<th>Replacement Rate by Bunazosin (%)</th>
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<tbody>
<tr>
<td>ET_A</td>
<td>Human neuroblastoma cells</td>
<td>0.01 1 100 (µM)</td>
</tr>
<tr>
<td>ET_B</td>
<td>Human astrocytoma cells</td>
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Data are replacement rate by bunazosin (%) and were calculated by determining the amount of radio isotope-labeled ligand released from the receptor (n = 2). The final concentration of [125I]-ET-1 was 0.053 nM for the ET_A receptor and 0.025 nM for the ET_B receptor. The incubation conditions under which reactions were performed were as follows: ET_A receptor, in 50 mM Tris-HCl (pH 7.4) containing 0.5 mM CaCl_2 and 0.1% BSA at 37°C for 90 minutes; ET_B receptor, in 50 mM Tris-HCl (pH 7.4) containing 0.5 mM CaCl_2 and 0.1% bacitracin at room temperature for 4 hours. Specific binding assays were performed for the ET_A and ET_B receptors under these experimental conditions.

either of the constrictions induced by the individual drugs (group 4). In groups 1, 2, 3, and 4, the IOPs at 60 minutes after the intravitreous injection were 22.1 ± 0.9, 22.7 ± 1.4, 20.0 ± 1.3, and 21.0 ± 1.4 mm Hg (mean ± SE, n = 4), respectively. There were no significant differences in IOP among these four groups. Topical instillation of 0.01% bunazosin almost completely inhibited the constriction induced by co-injection of phenylephrine and ET-1 (group 5).

Effect of SCGx on Phenylephrine-Induced Vasoconstriction: Experiment 7

Although rabbits subjected to SCGx have been used as a model for the elimination of sympathetic adrenoceptor stimulation, the effect of SCGx on the retinal arteries has not been reported. To confirm the supersensitivity of α_1-adrenoceptors in the retinal arteries, the effect of phenylephrine at a normally ineffective dose (30 µM) was examined in SCGx rabbits (Fig. 6). The relative values obtained for retinal artery diameter (after versus before surgery) were 92.7% ± 4.8% (mean ± SE, n = 5) in the sham-surgery eyes and 100.6% ± 4.9% (n = 6) in the ganglionectomized eyes (no significant difference), suggesting that SCGx itself did not affect the diameters of retinal arteries. Neither sham-surgery eyes nor nonsurgical eyes showed constriction of the retinal arteries in response to 30 µM phenylephrine. In contrast, in the ganglionectomized eyes, retinal arteries were constricted by intravitreous injection of phenylephrine at 30 µM to a degree that was significantly greater than the responses in the contralateral eyes (P = 0.015), the nonsurgical eyes (P = 0.022) or the sham-surgery eyes (P = 0.035). This indicates that a supersensitivity to this α_1-adrenoceptor agonist in the retinal arteries was present only in ganglionectomized eyes, or at least that the response was much more evident.

Effect of Superior Cervical Ganglionectomy on ET-1–Induced Vasoconstriction: Experiment 8

To examine whether adrenoceptors are involved in the ET-1–induced constriction of retinal arteries, this vasoconstriction was examined in SCGx rabbits (Fig. 7). In the sham-surgery group, the ET-1–induced constriction was similar to that in the nonsurgical control group. In contrast, in the SCGx group, the ET-1–induced vasoconstriction was significantly smaller than in either the nonsurgical group (P = 0.015 with the Bonferroni correction) or the sham-surgery group (P = 0.012 with the Bonferroni correction) and significantly greater than zero (P = 0.02). In the sham-surgery and SCGx groups, the IOPs obtained at 60 minutes after intravitreous injection were 24.5 ± 0.2 and 22.5 ± 0.8 mm Hg (mean ± SE, n = 6), respectively (no significant difference).

Discussion

In the present study, intravitreous injection of phenylephrine constricted rabbit retinal arteries in a dose-dependent manner, and topical instillation 0.01% bunazosin, a selective α_1-adrenoceptor antagonist, inhibited the vasoconstriction only in the ipsilateral eye. Although it has been reported that both bunazosin and phenylephrine affect IOP in normal rabbit eyes,
there was no significant difference in IOP between vehicle- and bunazosin-instilled eyes at 60 minutes after intravitreous injection of phenylephrine (the time point at which we measured vessel diameter). The lack of an IOP change in the present study may be attributable to the fact that we administered phenylephrine by intravitreous injection. At least, we can say that the effect of topically instilled bunazosin on the intravitreous phenylephrine-induced retinal artery constriction seemed to be independent of any IOP reduction. Topical bunazosin might be thought to decrease blood pressure. However, instillation of 0.1% bunazosin, a concentration 10 times higher than the highest one (exception: experiment 4) used in this study, did not change blood pressure in rabbits (Santén's unpublished data). In the present series of studies, phenylephrine was injected into the vitrous. Because the concentration of phenylephrine would be highest at the retinal surface, it is difficult to attribute the vasodilative effect of bunazosin to the action on retrobulbar vessels. In systemic arteries, the predominant control of arterial tone is by sympathetic innervation. Several pieces of evidence support the involvement of adrenoceptors in the control of vascular tone in the retina. Adrenoceptors, including the $\alpha_1$-adrenoceptor, are expressed in bovine retinal vessels and in rabbit retina, and a series of enzymes involved in catecholamine synthesis is localized on the retina in rat, cat, and monkey. However, the central retinal arteries have been reported to be devoid of adrenergic innervation in monkey and human eyes. After confirming that intravitreous injection of an $\alpha_1$-adrenoceptor agonist increased vascular tone in rabbit retinal arteries, we found that topically instilled 0.01% bunazosin inhibited the phenylephrine-induced vasoconstriction only in the bunazosin-treated eye, not in the contralateral eye. The possibility that the intravitreous injection procedure itself might have induced bunazosin transfer from the conjunctiva, sclera, or choroid into the vitreous was excluded by the results of experiment 4. These findings support the idea that in rabbits, topically instilled bunazosin can reach the posterior retina by local penetration at a concentration high enough to antagonize the action of phenylephrine.

A previous report stated that the IC$_{40}$ of bunazosin on the binding of [3H]-prazosin to the $\alpha_1$-adrenoceptor in human prostate membranes was approximately 1 nM. The bunazosin concentration in 0.01% bunazosin eye drops is 244 $\mu$M. Therefore, if only 1/250,000 of the concentration of topically instilled bunazosin reaches the retinal arteries, there is the potential for bunazosin to exert pharmacologic effects at that location. Recently, it has been reported that topically instilled iganidine, a potent Ca$^{2+}$-channel blocker, reaches the posterior retina at a concentration sufficient to inhibit vasoconstriction induced by intravitreous ET-1 and also that topically instilled nipradilol reaches the posterior retina at pharmacologically active concentrations. These findings confirm that at least some instilled drugs can reach the posterior retina at pharmacologically active levels. The route by which instilled bunazosin reaches the posterior retina is not thought to be through the vitreous, because the result of experiment 4 suggests that the bunazosin concentration in the vitreous after topical instillation of 0.01% bunazosin was only 0.1 nM or so. Further experiments are needed to clarify the precise route.

To our surprise, topically instilled bunazosin significantly inhibited the ET-1-induced constriction in retinal arteries without changing the IOP. However, this is consistent with a previous report about intravitreously injected bunazosin. In that report, as in the present study, bunazosin did not reduce IOP in the ET-1-injected eyes. Because ET-1 is known to affect IOP, the lack of an IOP change in the present experiment may be attributable to the use of the intravitreous route for the injection of ET-1. Our experiment with phenylephrine indicated that topically instilled bunazosin can reach the posterior retina in rabbits by local penetration at a concentration high enough to antagonize the action of phenylephrine, which suggests that the effect of bunazosin on the ET-1-induced constriction in retinal arteries may also be due to bunazosin’s reaching the posterior retina by local penetration.

The question we must now consider is how bunazosin inhibits ET-1-induced vasoconstriction. Our receptor-binding assay suggested that bunazosin does not bind to either ET$_A$ or ET$_B$ receptors. In addition, intravitreous co-injection of phenylephrine and ET-1 (each at an individually ineffective dose) constricted retinal arteries significantly, with no change in IOP. This combined effect of phenylephrine and ET-1 was almost completely attenuated by topical instillation of 0.01% bunazosin. Furthermore, the ET-1–induced vasoconstriction was partly inhibited in SCGx eyes. SCGx leads to a depletion of endogenous norepinephrine and thereby to tissue supersensitivity. In the present study, supersensitivity was confirmed by the increased mydriatic response to a low dose of norepinephrine (see the Materials and Methods section) and by the reactivity of retinal arteries to a low dose of intravitreous phenylephrine. In such a situation, where the number of adrenoceptors is increased, there is the possibility of vasoconstriction being induced by $\alpha$-adrenoceptor agonists carried by the blood. However, we found no significant difference in the diameter of retinal arteries between pre- and post-SCGx eyes. Taken together, these findings suggest that a lack of sympathetic $\alpha_1$ stimulation after bunazosin instillation was at least partly responsible for the observed attenuation of the vasoconstrictor effect of ET-1 in rabbit retinal arteries. In fact, some recent reports have suggested an interaction between $\alpha_1$-adrenoceptor stimulation and ET-1 receptor stimulation in several organs and tissues. Moreover, ET-1 induces norepinephrine and epinephrine production in the adrenal gland and also potentiates adrenergic vasoconstrictor responses in rabbit pulmonary and mesenteric arteries in vitro. The present findings suggest that a close interaction between the $\alpha_1$-adrenoceptor and the ET-1 receptor also exists in retinal arteries or...
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retinal tissue, although the details of the mechanism remain unclear. Recent studies have suggested that abnormal ET-1 production in the eye may be involved in a variety of ocular diseases, such as glaucoma, retinitis pigmentosa, and diabetic retinopathy. Exogenous ET-1 causes constriction of retinal vessels and capillaries in ONH, and a misregulation of anterograde axonal transport, as well as axon loss and demyelination of surviving axons, excessive glial proliferation in the optic nerve head, and increased excavation of the optic disc. It is noteworthy that vascular permeability in the retina is increased in such ocular diseases as retinal vascular disease and diabetic retinopathy. This raises the possibility that vasoconstrictor compounds such as ET-1 and/or α-adrenoceptor agonists may leak abnormally through the blood–retinal barrier in these diseases and may be partly responsible for the types of damage mentioned herein.

The present study suggests that topically instilled bunazosin reaches the posterior retina by local penetration at a concentration sufficient to inhibit both the phenylephrine- and ET-1-induced constrictions of retinal arteries, at least in the normal rabbit eye. Topical bunazosin is used at a concentration of 0.01% to reduce IOP in human eyes. However, there are differences in the dimensions of the eyeball and orbit between rabbits and humans, and therefore whether the present results can be extrapolated to human eyes is a matter for further investigation.

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