The biphasic intraocular pressure response of rabbits to epinephrine

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A study has been made of the pupillary and intraocular pressure responses of conscious rabbits to daily topical applications of submaximal doses of epinephrine. On the first day, epinephrine caused rapid pupil dilation which preceded a prolonged decrease of intraocular pressure. On the second and subsequent days, the application of the same dose of epinephrine increased the duration of the pupillary response and caused a biphasic pressure response in all treated eyes; an initial increase of intraocular pressure lasting two to four hours followed by decrease of intraocular pressure below the initial value which lasted for more than twenty-four hours. The β-receptor antagonist, propranolol, and the α-receptor antagonist, phenoxybenzamine, caused small and large reductions, respectively, in the hypertensive response to epinephrine. Phenoxybenzamine, but not propranolol, also inhibited the pupillary dilation and the hypotensive response to epinephrine. Topical administration of phenoxybenzamine strongly inhibited the hypertensive response to epinephrine but left unaffected the pupillary response.

Key words: epinephrine, biphasic pressure response, pupil, phenoxybenzamine, propranolol, thymoxamine.

It has been reported that adrenergic compounds having β-agonist activity may increase intraocular pressure under certain experimental and clinical conditions. In particular, epinephrine, a mixed α- and β-adrenergic receptor agonist, has been reported to cause transient increments of pressure in normal and glaucomatous human eyes and in the eyes of patients with Horner's syndrome. A hypertensive response to high concentrations of epinephrine in some but not all rabbits was observed by Norton and Viemstein. The slow onset of ocular hypotension with epinephrine observed by Langham, Simjee, and Josephs was reported to be further delayed by a second application of epinephrine. A small hypertensive ocular response, associated with decreased outflow facility, to the topical application of 2 per cent epinephrine in anesthetized vervet monkeys was observed by Bill.

The hypertensive response in rabbits and monkeys to epinephrine has been attributed to stimulation of β-adrenergic receptors and this explanation is consistent with subsequent studies using salbutamol, a highly selective β-adrenergic...
agonist with intense vasodilator activity.\textsuperscript{13-15}

The present paper deals with the time courses of the pupillary and pressure responses to daily applications of submaximal doses of epinephrine. On the second and subsequent days, a transient hypertension developed in all treated eyes, and the nature of the receptors mediating this response has been investigated.

**Methods**

Adult male and female rabbits weighing 2.5 to 3.5 kilograms were used. The pupil diameter was measured visually in uniform artificial illumination with a transparent millimeter rule. Readings taken in the vertical and horizontal meridians and average readings were recorded.

The intraocular pressure was measured by the Langham pneumatic tonometer with the floating tip sensor.\textsuperscript{16-18} Oxygen was supplied to the tonometer at a pressure of 20 pounds per square inch, and the sensor pressure was measured with a Sanborn 207B pressure transducer connected to a Sanborn 296 recorder. The tonometer was standardized each day against a pressurized silastic membrane, and the readings were interpreted from a mean calibration curve for rabbit eyes.\textsuperscript{19}

**Experimental procedure.** Tonometry was made after application of one drop of 0.5 per cent proparacaine (Ophthaine; Squibb and Sons, New York); five to ten seconds after instillation, the excess anesthetic was washed from the conjunctival sac with physiologic saline. This restricts sensory loss to a few minutes. Solutions of the experimental drugs were applied topically using a 25 or 50 µl pipette (Coleman Instruments, Maywood, Ill.). The vehicle used as solvent for the epinephrine was found to have no effect on either the pupil or intraocular pressure in a series of control experiments. This vehicle was not applied to the untreated eyes in the experimental studies.

**Preparation of drugs.** The following drugs were used: 1-epinephrine borate as the commercial preparation Eppy N (Barnes-Hind Laboratories, Sunnyvale, Calif.); phenoxybenzamine (Dibenzyline; Smith, Kline, and French Laboratories, Philadelphia); propranolol (Aldrich Chemical Company, Milwaukee).

All results are expressed statistically as the arithmetic mean ± standard error of the mean, and
Fig. 2. The mean time courses of the pupillary and intraocular pressure responses of the same six conscious rabbits (Fig. 1) on the fourth day of the study. ○○ and ●● represent the untreated and drug-treated eyes, respectively.

Results

The mean time courses of the intraocular pressure and pupillary responses to a single application of one per cent epinephrine are summarized in Fig. 1. An immediate and large pupillary dilation occurred in the treated eyes, with maximum values developed within one to two hours; the pupil size recovered almost completely within three to four hours. The intraocular pressure of the treated eyes showed a small but significant pressure decrease within one hour (p < 0.01, based on comparison of difference in pairs of eyes of individual rabbits before and after treatment). The maximal pressure response occurred within four to six hours in individual animals, and a small response was still present at twenty-four hours.

The influence of a second topical application of one per cent epinephrine at twenty-four hours to the treated eyes is summarized in Fig. 1. Again, there was rapid pupil dilation to values similar to those seen the previous day. However, the duration of pupillary response increased, the dilation remaining approximately maximal for two to three hours. Prior to the second application of one per cent epinephrine, the intraocular pressure of the treated eyes was 2 to 3 mm Hg lower than in the control eyes. Following application of the epinephrine, the intraocular pressure increased in the treated eyes, and at one hour exceeded that in the control eyes by a mean of 4.2 ± 0.5 (6) mm Hg (p < 0.001). The intraocular pressure then slowly decreased, and at five hours the values in pairs of eyes were
Fig. 3. The influence of intravenous propranolol (a) and intravenous phenoxybenzamine (b) on the biphasic pressure response to epinephrine. Experiments with the antagonists were made after two days of treatment with 1 per cent epinephrine, three times daily. Propranolol (5 mg. per kilogram) was injected intravenously on the third day at the time of the topical application of 50 μl of 1 per cent epinephrine and repeated at T = 1 and 2 hours. Phenoxybenzamine (5 mg. per kilogram was injected intravenously on the third day 90 minutes prior to the topical application of 50 μl of 1 per cent epinephrine at T = 0 hours. ○—○ and ●—● represent the untreated and drug-treated eyes, respectively.

approximately equal. The intraocular pressure in the treated eyes continued to decrease, and at T = 6 hours the mean pressure was 4.0 ± 0.3 (6) mm. Hg less than in the control eyes. Next day (T = 48 hours) the pupil diameters in pairs of eyes were equal, but the pressure in the treated eyes remained below normal (mean pressure difference was 2.1 ± 0.3 (6) mm. Hg).

Repetition of the same experiment on the third, fourth, and fifth days showed qualitative responses similar to those recorded on day 2. However, there was an increase in the hypotensive response recorded twenty-four hours after the previous application of epinephrine. On the fourth and fifth days, the initial mean pressure decrements compared to the pressures in the control eyes were 5.4 ± 0.3 (6) and 4.5 ± 0.4 (6) mm. Hg, respectively. In spite of this significant decrement, the addition of a further drop of epinephrine caused an initial pressure increase in all treated eyes.

Because of the mixed α- and β-adrenergic agonist properties of epinephrine, both α- and β-adrenergic receptor antagonists were used to analyze the specificity of the hypertensive response. Groups of four rabbits were treated with epinephrine, and the inhibitor studies made on either the second or third day of treatment. The pupillary and pressure responses on the first day of treatment of these animals were very similar to those recorded in
Fig. 4. The influence of topically applied phenoxybenzamine (a) and thymoxamine (b) on the biphasic pressure response to one per cent epinephrine. Experiments with the two receptor antagonists were made after two days treatment with one per cent epinephrine, three times daily. Phenoxybenzamine as a one per cent suspension was applied on the third day at the time of application of 50 μl of one per cent epinephrine (T = 0 hours). Thymoxamine (one per cent solution) was applied on the third day at the time of application of 50 μl of one per cent epinephrine (T = 0 hours). ○ and ● represent the untreated and treated eyes, respectively.

Fig. 1. At T = 24 hours, four rabbits were given an intravenous injection of the β-receptor antagonist, propranolol (5 mg. per kilogram), followed by the topical application of one per cent epinephrine. The pupillary and pressure responses to epinephrine were qualitatively and quantitatively similar to those summarized in Fig. 1, B, and appeared largely unaffected by the propranolol. The influence of the propranolol on the ocular response to epinephrine was examined again in the same group of rabbits the following day using a higher intravenous dosage of propranolol (5 mg. per kilogram per hour). Again, the pupil gave a prolonged dilation, and the intraocular pressure of the treated eye increased above that of the control eye within the first hour (Fig. 3). By T = 6 hours, the pressures in the treated eyes were again less than in the untreated eyes (mean pressure differences, 3.8 ± 0.2 mm. Hg). The dose of propranolol used in these experiments has been shown to inhibit the ocular response to the β-agonist, salbutamol.

Similar experiments were undertaken in which the receptor antagonist, phenoxybenzamine, was given intravenously to a group of four rabbits on the second day of treatment with epinephrine. The intravenous dose of phenoxybenzamine caused a decreased intraocular pressure, and application of the one per cent epinephrine was made ninety minutes later at which time the intraocular pressures were stable (Fig. 3). The blockade of the α-receptors was apparent in the complete inhibition of the pupillary dilation to epinephrine. The ocular hypertensive response was de-
creased significantly but not completely inhibited (Fig. 3). The influence of the α- and β-receptor antagonists applied topically on the hypertensive response to epinephrine was determined in a further series of experiments. A one per cent suspension of phenoxybenzamine applied topically to one eye of three conscious rabbits had no effect on either pupil dilation or intraocular pressure over a period of five hours. However, in rabbits pretreated with epinephrine for two days the topical application of phenoxybenzamine strongly decreased the hypertensive response to epinephrine (Fig. 4). Under these conditions, pupil dilation and the hypotensive response still took place in all individual rabbits.

Similar studies were made with the water-soluble α-adrenergic receptor antagonist, thymoxamine. In previous studies on conscious rabbits, it had been found that thymoxamine applied topically (one per cent solution) and given intravenously (5 mg. per kilogram) failed to block the pupillary dilation and pressure decrement induced by epinephrine applied topically. However, topical application of thymoxamine has been found to inhibit the pupillary dilation to phenylephrine in man. Four rabbits were given one drop of 1.0 per cent solution of thymoxamine topically on the third day of treatment with epinephrine. The hypertensive response to epinephrine was decreased significantly, but the pupil dilation and the pressure decrease occurred in all treated rabbits (Fig. 4).

Finally, experiments were made to determine whether topical application of the β-receptor antagonist, propranolol, would influence the hypertensive response to epinephrine. Norton and Viernstein had reported that in rabbits in which a hypertensive response to high concentrations of epinephrine occurred, the topical application of propranolol would inhibit this effect. Six rabbits were treated for two days with epinephrine, and in all animals a hypertensive response was recorded on the second day of treatment. On the third day, one drop of 0.5 per cent solution of propranolol was applied prior to the application of epinephrine. At this time, the mean intraocular pressure in the treated eyes was $2.4 \pm 0.3 \ (6) \ mm \ Hg$ less than in the control eyes, and the corresponding mean pupil diameter difference was $0.0 \pm 0.1 \ (6) \ mm \ Hg$. One hour after the application of the propranolol and the epinephrine the mean intraocular pressure in the treated eyes was $0.3 \pm 0.3 \ (6) \ mm \ Hg$ less than in the control eyes, and the corresponding pupil diameter difference was $5.5 \pm 0.2 \ (6) \ mm \ Hg$. At six hours, the mean pressure in the treated eyes was $4.6 \pm 0.3 \ (6) \ mm \ Hg$ less than in the control eyes, and there was almost complete recovery of the pupil diameter.

In three untreated normal conscious rabbits, the application of one drop of 0.5 per cent propranolol gave no significant pressure decrease over a period of five hours.

Discussion

In our previous study of the ocular response to epinephrine, the experimental observations were limited to the first twenty-four hours. Under these conditions, a submaximal dose of epinephrine caused an immediate pupillary response which preceded a decrease of intraocular pressure. The pupillary response was prevented, and the pressure response was partially inhibited by an intravenous injection of the α-adrenergic receptor antagonist, phenoxybenzamine. Under the same experimental conditions, the pupillary response to epinephrine was unaffected and the pressure response partially inhibited by the β-receptor antagonist, propranolol. It was concluded that the pupillary response was dependent solely on stimulation of α-adrenergic receptors, and that the pressure response was dependent on stimulation of both α- and β-receptors. Furthermore, there was a difference in the time courses of the pressure responses mediated by the α- and β-receptors in that the
Initial pressure response was determined largely by stimulation of β-receptors, whereas the subsequent prolonged pressure decrease was due largely to stimulation of α-receptors: The α- and β-responses could be differentiated by dosage as well as by use of the appropriate receptor antagonists.

The results of this study have further elucidated the ocular response to epinephrine by the finding and analysis of the biphasic pressure response to multiple applications of the drug. The importance of these new findings lies not only in the understanding of the physiologic mechanisms causing these changes, but also in the therapeutic implications of the use of the drug in favorably influencing the intraocular pressure of the human eye. In this respect, the authors have established that a similar biphasic pressure response to epinephrine may occur in both normal and glaucomatous eyes. In the human studies, the hypertensive phase was found to be associated with a decrease of the outflow facility and the subsequent prolonged, hypotensive phase to be associated with an increase in the outflow facility.

The experimental evidence that epinephrine can increase intraocular pressure through stimulation of adrenergic receptors was not unexpected in view of recent observations of Norton and Vierstein using isoproterenol and epinephrine, and those of Langham and Diggs using salbutamol and epinephrine. Norton and Vierstein found that the intraocular pressure declined monotonically in all rabbits given a topical application of 2 per cent epinephrine, whereas some but not all rabbits responded with a transitory increase of intraocular pressure when given 4 and 8 per cent solutions of epinephrine. They found that the hypertensive and hypotensive responses were associated with a decreased and an increased outflow facility, respectively. The former response but not the latter was inhibited by the β-receptor antagonist, propranolol. In studies of the ocular response to the highly selective β-adrenergic agonist, salbutamol, Langham and Diggs found that the initial application caused a monotonic decrease of intraocular pressure, whereas on the second day salbutamol caused an initial hypertensive response followed by a hypotensive response. In this case, both responses were blocked by propranolol.

The hypertensive response to epinephrine differed from that induced by salbutamol and isoproterenol in that it involved stimulation of both α- and β-adrenergic receptors. Propranolol did not completely block the hypertensive response, but phenoxybenzamine did have a large inhibitory effect. The finding that the β-responses were quantitatively less than the α-responses is consistent with our previous studies that the initial ocular responses to epinephrine in rabbits are due mainly to stimulation of α-receptors. By way of comparison, the ocular responses to the mixed α, β-agonist, isoproterenol, is determined principally by stimulation of β-receptors.

In his response to epinephrine, man differs from the rabbit in that the pressure response is more readily elicited to stimulation of β- than α-receptors and is earlier in onset. In this respect it has been a general finding in both animal and human eyes that the pressure decrease mediated by β-agonist is rapid, a significant response occurring within one hour. The conclusion that stimulation of α-adrenergic receptors could induce an increase of intraocular pressure was unexpected but has been confirmed in subsequent studies with norepinephrine. The ocular responses to a single application of norepinephrine include pupil dilation which precedes a prolonged decrease of intraocular pressure. These responses are completely blocked by the α-antagonist, phenoxybenzamine, but uninfluenced by the β-receptor antagonist, propranolol. In extending these studies, it has been shown that multiple applications of norepinephrine caused a biphasic pressure response.
and that both the pressure increase and the pressure decrease were blocked by the α-adrenergic receptor antagonist, phenoxybenzamine, but uninfluenced by intravenous injection of the β-adrenergic receptor antagonist, propranolol.23

The conclusion that pressure increments and decrements may be mediated by stimulation of α-receptors extends and complements previous findings that biphasic pressure responses may also be mediated by stimulation of β-adrenergic receptors in the eye. These apparently paradoxical findings are consistent with recent theoretical analysis supported by much experimental work on the role of the intraocular vascular circulation in the regulation of intraocular pressure and aqueous humor dynamics.24-26 The basic tenets of the concept contrast with the more traditional viewpoint that aqueous humor secretion and the outflow facility are little influenced by blood flow. It is postulated that the secretion of the aqueous humor is proportionate to the vascular perfusion of the ciliary processes and the outflow resistance to vary with the extent of the vascular perfusion of the intrascleral plexus, where blood and aqueous humor draining from the eye compete for the same vascular vessels. The theory further postulates that the neural activity of the adrenergic nerves to the smooth muscles of the vessels supplying blood to these two critical areas determines whether corresponding changes occur in the rate of aqueous humor formation and in the resistance to outflow of the aqueous humor.

The flow resistance to the outflow of aqueous humor in the living rabbit is believed to be determined by two anatomic systems, the trabecular meshwork and the intra- and episcleral systems where blood and aqueous mix. There have been numerous attempts to determine which site is the major contribution to the total outflow resistance without entirely satisfactory results.27-32

Vasodilation associated with increased blood filling of the intrascleral plexus would decrease the number of channels available for the drainage of the aqueous humor; this would be a mechanism causing decreased outflow facility. Parri passu, a vasoconstriction of the blood supply to the intrascleral plexus would increase the availability of channels for the outflow of aqueous humor; this would be a mechanism causing an increased outflow facility. Vasomotor changes in the episcleral vessels could further modify the drainage of aqueous humor and intrascleral blood. The episcleral vasoconstriction induced by epinephrine would cause a high flow resistance where normally it is very low. Under these conditions increased vascular filling of the intrascleral system would occur and decrease the pathways for aqueous outflow; this would be a further mechanism for decreasing the outflow facility.

REFERENCES
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