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THE ACTION OF SYMPATHOMIMETIC AMINES ON THE OUTFLOW OF AQUEOUS HUMOUR FROM THE EYE

BY

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The actions of adrenaline, noradrenaline and isoprenaline on the resistance to outflow of aqueous humour from the rabbit eye are described. In some experiments, correlations have been made with pupil diameter. Intravitreous injections of adrenaline, noradrenaline and large doses of isoprenaline decreased the resistance to outflow of fluid from the eye and dilated the pupil. Intravitreous injection of phentolamine was without effect on the intraocular pressure and resistance to outflow of aqueous humour, although such injections resulted in miosis. The actions of adrenaline, noradrenaline and large doses of isoprenaline on the outflow resistance were antagonized by phentolamine. Postganglionic sympathetic denervation did not affect the aqueous humour dynamics when the eyes where examined 2 weeks later, but all the denervated eyes exhibited miosis at this time. Much smaller doses of noradrenaline were required to lower the intraocular pressure and decrease the resistance to outflow of aqueous humour in the denervated eyes; in addition, the dose/response curve for the effect of noradrenaline on the outflow resistance was shifted to the left in these experiments. These results support the view that adrenaline α-receptors are associated with the resistance to outflow of aqueous humour from the rabbit eye.

There is now evidence suggesting that adrenaline α-receptors, as originally classified by Ahlquist (1948), are involved in the resistance to outflow of aqueous humour from the angle of the anterior chamber of the mammalian eye (Bárány, 1962a; Eakins, 1963 ; Eakins & Eakins, 1964). Occupation of α-receptors, possibly situated in the trabecular meshwork, is thought to lead to a decrease in the resistance to outflow of fluid from the eye which then results in a lowered steady state intraocular pressure.

The present investigations were designed to determine first, whether the decrease in the resistance to outflow of aqueous humour from the rabbit eye caused by noradrenaline, adrenaline and large doses of isoprenaline, could be inhibited by blockade of α-receptors, and secondly, whether chronic postganglionic sympathetic denervation would increase the sensivity of the outflow structures to noradrenaline. In some experiments, correlations between aqueous humour dynamics and pupillary size were sought.

METHODS

Adult rabbits of the New Zealand white strain weighing between 2.5 and 3.5 kg were anaesthetized with 1 to 2 g/kg of urethane given intravenously into a marginal ear vein as a 25% (w/v) solution in 0.9% saline.

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Determination of intraocular pressure. The intraocular pressures were recorded manometrically using Sanborn pressure transducers (Model 267b) in conjunction with Sanborn 1100 A.S. carrier preamplifiers and a multichannel 150 M rectilinear recorder. A four-way Perspex block was used to interconnect the pressure reservoir, the pressure transducer and the anterior chamber of the eye, as described originally by Langham (1959).

Measurement of the outflow facility of aqueous humour. The steady state intraocular pressure was determined immediately after cannulation of the anterior chamber. The outflow facility of aqueous humour from the eye was determined from an analysis of the pressure decay curve in the following manner. The intraocular pressure was increased to 35 mm Hg by connection of the anterior chamber of the eye to the saline reservoir previously set at 35 mm Hg. After equilibration, the tap to the reservoir was turned off and the spontaneous decay of the intraocular pressure to normal was recorded. Under these conditions, the rate of fall of the pressure depends on the ease with which fluid can leave the eye. Quantitative values of the outflow facility were derived from the pressure-decay curve using the values of the pressure/volume relationship of the living rabbit eye (Eisenlohr & Langham, 1962) in a manner identical to that described previously (Langham & Eisenlohr, 1963; Eakins, 1963).

Administration of the drugs into the eye. The compounds being investigated were dissolved in 0.9% saline and injected through the sclera into the vitreous body of the proptosed rabbit eye. An Agla (Burroughs Wellcome) micrometer syringe was used for the injection with a 30 gauge hypodermic needle. In all these experiments, a constant dose-volume of 10 μl. was used. All drugs were left in contact for 6 hr to allow adequate diffusion within the eye (Eakins, 1963). The contralateral eye was untreated and was used as a control. The lack of effect of very small volumes of saline injected into the vitreous body on aqueous humour dynamics has been reported elsewhere (Eakins, 1963).

Postganglionic denervation. Surgical procedures were carried out under aseptic conditions on rabbits anaesthetized with a combination of pentobarbitone sodium (20 mg/kg. intravenously) and ethyl chloride (by inhalation). Postganglionic denervation of a rabbit eye was effected by unilateral extirpation of the ipselateral superior cervical ganglion together with a small amount of the postganglionic fibre. The animals were then allowed to recover.

Blood-aqueous barrier. The protein content of aqueous humour was estimated semi-quantitatively by the addition of an equal volume of an 8% solution of trichloracetic acid. Samples of normal aqueous humour taken from rabbit eyes gave a faint turbidity under these conditions (10 to 40 mg of protein/100 ml.). Experiments in which the aqueous humour showed excessive protein content were rejected on the grounds that the increased protein content indicated a breakdown of the blood-aqueous barrier.

The following drugs were used in this study: (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (+)-isoprenaline hydrochloride and phentolamine methanesulphonate (Regitene, Ciba). All doses refer to the salts.

RESULTS

Effect of intravitreous injections of noradrenaline on the resistance to outflow of aqueous humour. The result of a typical experiment is shown in Fig. 1. The steady state intraocular pressure in the untreated left eye was 21.0 mm Hg, whereas in the right eye, treated with 200 μg of noradrenaline, the steady state intraocular pressure was lowered to 16.0 mm Hg. The rate of fall of the intraocular pressure to normal in the test right eye was greater than that observed in the untreated eye. Analysis of the pressure-decay curves showed a marked reduction in the resistance to outflow of aqueous humour in the eye treated with noradrenaline. All eyes treated with noradrenaline exhibited mydriasis.

Effect of locally injected phentolamine on the response of the outflow mechanism to noradrenaline. In this series of experiments, a standard dose of 200 μg of phen-

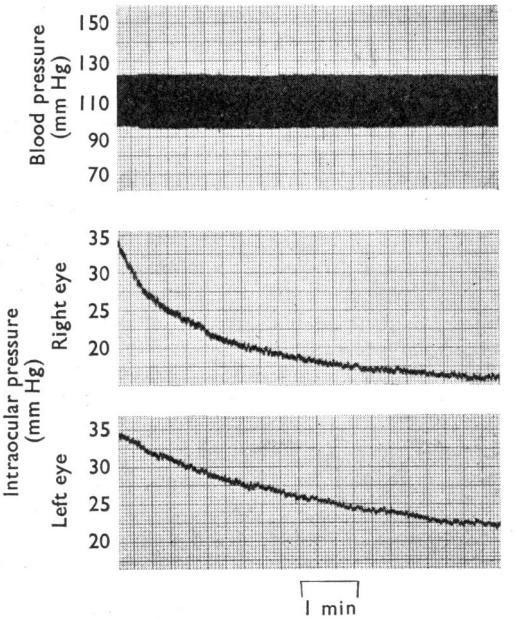

Fig. 1. Rabbit 3·0 kg, anaesthetized with urethane (1 to 2 g/kg). Pressure-decay curves recorded manometrically from the anterior chambers of both eyes. Records, from above downward : mean arterial blood pressure recorded from a femoral artery ; spontaneous decay of the intraocular pressure to normal in the right eye treated with 200 μg of noradrenaline ; as above for the untreated control left eye.

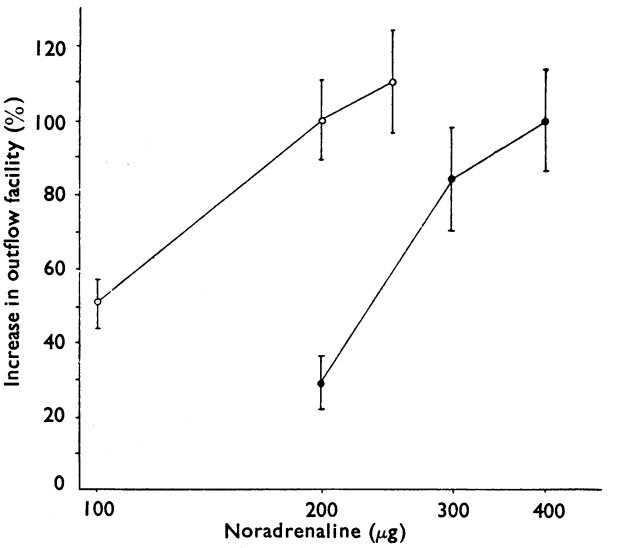

Fig. 2. Effect of phentolamine in blocking the response of the outflow mechanism of the rabbit eye to noradrenaline. Ordinate, percentage increase in outflow facility of aqueous humour determined by analysis of pressure-decay curves ; abscissa, doses of noradrenaline injected into the vitreous body, plotted on a log scale ; ○ —— ○, dose/effect relationship for noradrenaline alone ; ● —— ●, dose/effect relationship for noradrenaline in eyes treated with 200 μg of phentolamine. Each point is the mean of at least four observations. The standard error of each set of observations is represented by a vertical line.

tolamine in 10 μl. of saline was injected immediately before the noradrenaline into one eye. The steady state pressures and pressure-decay curves of the test, and remaining untreated control eyes were compared 6 hr later. In this manner, two dose/response curves were obtained, one for the effect of noradrenaline on the facility of outflow (C), and one for the effect of noradrenaline in the presence of a standard dose of 200 μg of phentolamine. The results are shown in Fig. 2. The simultaneous injection of phentolamine resulted in a shift of the noradrenaline dose/effect curve to the right, indicating a competitive antagonism of the noradrenaline effect.

In a further series of experiments, it was found that the intravitreous injection of 200 μg of phentolamine, alone, was without effect on the aqueous humour dynamics, although all such injections resulted in miosis. In a series of five rabbits, the intraocular pressure and outflow facility (means and standard errors) of the untreated eyes were 19.9 ± 0.75 mm Hg and 0.20 ± 0.03 μl./min/mm Hg respectively: The corresponding values for the phentolamine-treated eyes were 19.0 ± 0.6 mm Hg and 0.18 ± 0.02 μl./min/mm Hg.

It was of interest that qualitative observations of the pupil diameters before cannulation suggested that phentolamine abolished the noradrenaline-induced mydriasis before inhibiting the effect of the drug on aqueous humour dynamics.

Effect of local phentolamine on the response of the outflow structures to intravitreous injections of adrenaline and isoprenaline. Adrenaline is more potent than noradrenaline in its action both on the pupil and on the resistance to outflow of aqueous humour from the rabbit eye (Bennett, Reinke, Alpert, Baum & Vasquez-Leon, 1961; Eakins, 1963). In the present study, doses of adrenaline producing a submaximal increase in the outflow of aqueous humour were injected alone, and in the presence of a standard dose of 200 μg of phentolamine. The results are shown in Table 1. Blockade of ocular adrenaline α-receptors considerably reduced the increase in outflow facility normally observed after the intravitreous injection of adrenaline. In addition, the mydriasis observed in the adrenaline-treated eyes was effectively abolished following treatment with phentolamine.

TABLE 1

INHIBITION BY PHENTOLAMINE OF THE DECREASE IN RESISTANCE TO OUTFLOW OF AQUEOUS HUMOUR FROM THE RABBIT EYE AFTER ADRENALINE AND LARGE DOSES OF ISOPRENALINE

Values are means and standard errors. Numbers of experiments are in parentheses. All drugs were injected into the vitreous body 6 hr before examination of the eye

Drug	Dose (mg)	Facility of outflow of aqueous humour (μl./min/mm Hg)	
		Control eye	Drug-treated eye
Isoprenaline	1·0	0·21±0·02 (5)	0·31±0·04 (5)
Isoprenaline + phentolamine	1·0+ 0·2	0·24±0·02 (4)	0·25±0·03 (4)
Adrenaline	0·05	0·25±0·03 (4)	0·45±0·05 (4)
Adrenaline + phentolamine	0·05+ 0·2	0·25±0·03 (4)	0·32±0·03 (4)

Doses of isoprenaline, much greater than those required to cause a maximum fall in intraocular pressure by depression of the inflow mechanism (Eakins, 1963), dilated the pupil and reduced the resistance to outflow of aqueous humour. Both these latter effects were antagonized by α-receptor blockade induced by the intravitreous injection of phentolamine (see Table 1).

Studies on the chronically-denervated rabbit eye. Unilateral extirpation of a superior cervical ganglion 2 weeks before examination was without effect on the aqueous humour dynamics of the ipselateral eye; in contrast, the pupil of the denervated eye exhibited marked miosis. In five such experiments, the intraocular pressure and outflow facility (means and standard errors) of the normal control eyes were 20.2 ± 0.65 mm Hg and 0.22 ± 0.03 μl./min/mm Hg; for the denervated eyes, the respective values were 20.8 ± 0.7 mm Hg and 0.20 ± 0.03 μl./min/mm Hg.

Much smaller doses of noradrenaline lowered the intraocular pressure and decreased the outflow resistance in the denervated eyes. The results are shown in Fig. 3. The normal dose/response curve for the effect of noradrenaline on the resistance to outflow of aqueous humour from the rabbit eye was shifted significantly to the left, indicating an increase in sensitivity of the outflow mechanisms to noradrenaline of the order of 100-times. The results obtained by analysis of pressure-decay curves in these experiments were confirmed by pressure infusion studies. Saline (0.9%) was infused into the anterior chamber of the eye at known rates, and the new equilibrium intraocular pressures attained during the infusion rates were

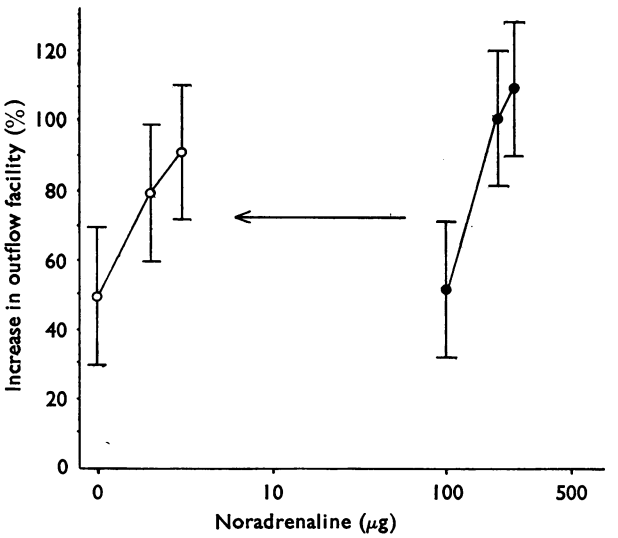

Fig. 3. Effect of chronic postganglionic sympathetic denervation on the response of the outflow structures of the rabbit eye to noradrenaline. Ordinate, percentage increase in outflow facility of aqueous humour determined by analysis of pressure-decay curves; abscissa, doses of noradrenaline injected into the vitreous body, plotted on a log scale. ● —— ●, dose/effect relationship for noradrenaline in normal eyes; ○ —— ○, dose/effect relationship for noradrenaline in chronically denervated eyes. Each point is the mean of at least four observations. The standard errors of the means are represented by the vertical lines.

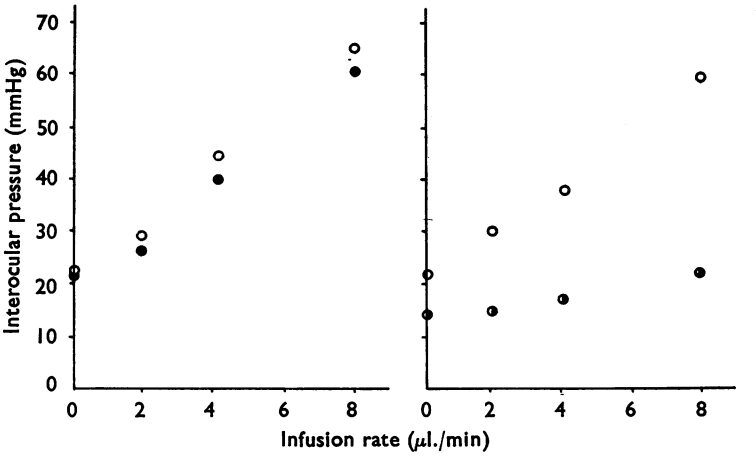

Fig. 4. Effect of noradrenaline on the resistance to outflow of aqueous humour from the rabbit denervated eye as determined from the relationship between steady state intraocular pressure and infusion rates. On the left : ○ —— ○, normal control left eye, and ● —— ●, chronically denervated right eye of the same rabbit. On the right ; ○—— ○, control right eye and ◑——◑, chronically denervated left eye treated with 3 μg of noradrenaline.

determined as described previously by Langham (1959); the results were plotted graphically (Fig. 4). The curves relating the equilibrium intraocular pressures and infusion rates in the normal and untreated denervated eyes were similar. In contrast, the curve for the denervated eye previously treated with 3 μg of noradrenaline showed a distinct difference. The initial intraocular pressure was lower and the slope of the pressure infusion curve was significantly less than those obtained previously. Since the slope of the curve relating steady state intraocular pressure to the rate of infusion depends mainly on the resistance to outflow of fluid from the eye (Langham, 1959), the shallower curve for the noradrenaline-treated eye indicates a decrease in the resistance to outflow of fluid in this eye.

DISCUSSION

It has been reported previously that injections of noradrenaline made directly into the vitreous body of the rabbit eye lower the intraocular pressure by reducing the resistance to outflow of fluid from the anterior chamber of the eye (Eakins, 1963). In the same study, it was observed that the order of potencies of the sympathomimetic amines used, namely adrenaline>noradrenaline>isoprenaline, agreed with that suggested by Ahlquist (1948), for the action of these drugs on adrenaline α-receptors. In the present experiments, these observations have been confirmed and extended. Comparison of dose/response curves for noradrenaline in normal eyes, and in eyes previously treated with phentolamine, indicated that blockade of α-receptors within the rabbit eye effectively antagonized the response of the outflow system to noradrenaline.

It was previously reported (Sears & Bárány, 1960) that local ocular blockade of α-receptors with dibenamine resulted in an increase in the resistance to outflow of

aqueous humour; in the same study, these authors observed that dichloroisoprenaline, a compound known to block β-receptors, decreased the resistance to outflow. On the basis of these results, Sears & Bárány (1960) suggested that in the normal rabbit eye, activation of β-receptors increased, and activation of α-receptors decreased, the resistance to outflow of aqueous humour. However, the presence of β-receptors associated with outflow resistance seems unlikely, since Bárány (1962a) observed that the decrease in resistance to outflow seen after treatment of the animals with dichloroisoprenaline was abolished after α-receptor blockade induced by dibenamine. In addition, Eakins (1963) observed that doses of isoprenaline which caused a submaximal fall in intraocular pressure were without effect on the resistance to outflow. The results reported in the present paper on the effect of α-receptor blockade on aqueous humour dynamics failed to confirm the observations of Sears & Bárány (1960), in that no increase in the resistance to outflow of aqueous humour could be detected following treatment of rabbit eyes with phentolamine. In fact, such doses of phentolamine were without effect on aqueous humour dynamics, although the intense miosis observed in the phentolamine-treated eyes indicated a high degree of α-receptor blockade.

Both adrenaline and large doses of isoprenaline caused a reduction in the resistance to outflow of aqueous humour which was antagonized by phentolamine. The results obtained with isoprenaline are of interest, since the actions of this drug are normally associated with β-receptors. In addition to an effect on outflow resistance, the large doses of isoprenaline used in this study were repeatedly found to dilate the pupil, a response thought to be due to occupation of α-receptors in the dilator pupillae muscle of the iris. The mydriatic effect of isoprenaline has been previously reported for the rabbit eye (Bennett *et al.*, 1961), and for the rat isolated eye (Beaver & Riker, 1962). These observations on the effect of isoprenaline on the resistance to outflow of aqueous humour and on the iris support the suggestion made by Butterworth (1963) that large doses of isoprenaline are capable of exciting α-receptors.

The presence of receptors associated with the resistance to outflow of aqueous humour raises the possibility of a sympathetic component in the control of the outflow of fluid from the anterior chamber of the eye. It is already well established that 24 hr after removal of a superior cervical ganglion from the rabbit, the intraocular pressure on the operated side is reduced, owing to a decrease in the resistance to outflow of aqueous humour from the eye (Langham & Taylor, 1960a, b; Sears & Bárány, 1960). This phenomenon is thought to be due to the release of noradrenaline from the iris and ciliary body into the aqueous humour (Bárány, 1962a; Eakins & Eakins, 1964). Three days after the operation, the intraocular pressure returns to normal (Langham & Taylor, 1960a), and the ocular tissues are virtually depleted of catechol amines (Eakins & Eakins, 1964). In the present experiments, no difference could be detected between the aqueous humour dynamics of normal eyes and of eyes that had been denervated 2 weeks before examination. However, in contrast to normal eyes, a marked miosis was always observed in the denervated eyes. Thus, the chronically-denervated eyes resembled the eyes treated with phentolamine. At first sight, these results would seem to preclude any sympathetic innervation of the

chamber angle which could affect the resistance to outflow. However, the shift of the noradrenaline dose/response curve to the left in the chronically denervated eyes requires explanation. The increase in sensitivity of the outflow structures to injected noradrenaline under these conditions was of the order of 100-times, an increase that correlates well with that found in other sympathetic effector organs following chronic sympathetic denervation, for example the nictitating membrane of the cat (Innes & Kosterlitz, 1954 ; Kirpekar, Cervoni & Furchgott, 1962 ; Trendelenburg & Weiner, 1962). There is some histological evidence for the presence of nerve fibres in the trabecular meshwork, the structure through which aqueous humour must percolate in order to leave the anterior chamber of the eye. Vrabec (1954) described the innervation of the human trabeculum, while Holland, Sallmann & Collins (1957) observed nonmyelinated nerve fibres and nerve endings within the trabecular meshwork of the rabbit eye, some of which have recently been identified as adrenergic (Laties, personal communication). It has been suggested that the trabecular meshwork can influence the outflow of fluid from the eye (Bárány, 1962a). Thus, it may well be that the increase in sensitivity of the outflow structures to noradrenaline observed after chronic sympathetic denervation in these experiments is a reflection of true denervation supersensitivity, rather than the result of a nonspecific change in the permeability of the denervated ocular cells to noradrenaline; however, at this stage, the latter phenomenon cannot be completely excluded. The fact that much smaller doses of noradrenaline were needed to lower the intraocular pressure in the chronically-denervated eye raises the possibility of a new approach to the treatment of long-term ocular hypertension.

Both chronic postganglionic sympathetic denervation and local blockade of ocular adrenaline α-receptors did not alter the resistance to outflow ; both procedures, however, resulted in miosis, which can be attributed to inhibition of the normal sympathetic influence on the radial muscle fibres of the iris, allowing the parasympathetic tone of the sphincter muscle to constrict the pupil. Thus, pupil size is determined by two opposing autonomic forces, and sympathomimetic agents dilate while parasympathomimetic agents constrict the pupil. It would seem that this situation does not exist in the ocular outflow structures, since there is no evidence that parasympathomimetic agents increase the resistance to outflow of aqueous humour; on the contrary, cholinergic drugs are used in the treatment of glaucoma to lower the intraocular pressure and improve the drainage of fluid from the eye (Becker & Shaffer, 1961). In the primate eye, it has been found that pilocarpine reduces the resistance to outflow of aqueous humour, an action that is slowly antagonized by atropine (Bárány, 1962b).

In conclusion, the evidence reported in this study points to the presence of adrenaline α-receptors associated with the resistance to outflow of aqueous humour from the angle of the anterior chamber of the rabbit eye. The possibility that these receptors are normally stimulated by endogenous noradrenaline released from postganglionic sympathetic nerve endings cannot be excluded at present, although Langham & Rosenthal (unpublished) were not able to detect any alterations in the resistance to outflow of aqueous humour from the rabbit eye following prolonged stimulation of the preganglionic cervical sympathetic nerve.

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