The trabecular effect of noradrenalin in the rabbit eye

M. L. Sears and T. E. Sherk

Adrenergic effects upon aqueous outflow facility of the rabbit eye have been investigated with a constant rate infusion technique. Unilateral cervical ganglionectomy was performed one week prior to study, and a supersensitive response to noradrenalin was obtained. Millimicrogram doses could then be used to study the increase in outflow facility after subconjunctival, intracameral, and intravitreous noradrenalin injections. The increase in outflow facility was greatest after intracameral injection, but there were significant facility increases after subconjunctival injection at levels which did not dilate the highly supersensitive iris. The results indicate that the rabbit outflow channels can be made supersensitive to noradrenalin and that the site of action of noradrenalin on outflow facility is probably intrascleral.

Increase in the facility of aqueous outflow following use of adrenergic agents in glaucoma[1-3] has prompted several investigations[4-5] into the mechanism of this effect. The development of supersensitivity of outflow facility following sympathetic denervation[6] has proved a useful technique to study the specificity and locus of this response to adrenergic agents. A direct action of noradrenalin on the trabecular meshwork was postulated, since the dose of intravitreal noradrenalin required to increase outflow facility was fifty times the intracameral dose. The present work provides further evidence for this effect.

From the Department of Surgery, Section of Ophthalmology, School of Medicine, Yale University, New Haven, Conn.

Presented in part at the Glaucoma symposium sponsored by the National Institutes of Health, Del Monte, Calif., Sept. 28, 1962, and at the Current Research in Glaucoma symposium sponsored by the National Society for the Prevention of Blindness, Nov. 2, 1962.

This work was supported by United States Public Health Service Grant No. B3781, the Connecticut Lions Eye Research Foundation, the James Hudson Brown Memorial Fund of Yale University School of Medicine, and by Research to Prevent Blindness, Inc.

Methods

More than 200 New Zealand albino rabbits weighing between 2 and 3 kilograms were subjected to right cervical ganglionectomy under thiopental anesthesia. One week later, the animals received levarterenol bitartrate* (hereinafter referred to as noradrenalin) bilaterally by several routes, and the outflow facility was measured after the following intervals: 60 minutes after subconjunctival injection, 60 to 90 minutes after intracameral injection, and 150 minutes after intravitreal injection. Preliminary experiments indicated that facility-increasing effects were prominent at these intervals. Three comparable groups received isotonic saline. A fourth control group consisted of noninjected animals. Intraocular injection volumes were always 10 μl, and were performed with a Hamilton precision ground syringe and a 30 gauge needle. Subconjunctival injections used volumes of 0.2 ml. Intraocular

*California Biochemical Corp.

pressure and outflow facility were measured in the manner previously described in detail.[7, 8] The rabbits were anesthetized with intravenous urethane (ethyl carbamate),* and the eyes cannulated by means of a spring and trigger device.[9] Intraocular pressures were recorded with a Sanborn electromanometer system. The facilities of aqueous outflow were determined by measuring the steady state increase in intraocular pressure caused by infusion of isotonic saline at 2 μl per minute simultaneously into the anterior chamber of both eyes.

Aqueous humor protein was determined bilaterally in four groups of rabbits: (1) normal rabbits, (2) rabbits which had right cervical ganglionectomy one week earlier, (3) rabbits which had right cervical ganglionectomy one week earlier and had received bilateral intracameral noradrenalin in doses ranging from 0.1 to 5.0 μg, and (4) rabbits which had right cervical ganglionectomy one week earlier and had received bilateral intracameral isotonic saline. In Groups III and IV, aqueous was obtained one hour after anterior chamber injection. Protein content was determined from reference curves made with bovine serum albumin.[10]† Noradrenalin added in increments up to 5.0 μg had no effect on the values obtained.

Dose effect curves relating noradrenalin concentration to outflow facility were plotted semilogarithmically for convenience. Each point on the dose effect curves represents the average of facility determinations made for at least 6 rabbits. Dosage is stated in this article according to weight of bitartrate salt, i.e., roughly twice the weight of noradrenalin.

Specificity of the adrenergic increase in outflow facility was tested in four consecutive experiments in which rabbits were given 20 mg. per kilogram of phenoxybenzamine[11] (Dibenzyline)‡ 6 days after right cervical ganglionectomy and 24 hours prior to intracameral injection of 1.0 μg noradrenalin. Outflow facility measurements were made in the usual manner. Complete blockade (alpha) was confirmed for each animal at the conclusion of each experiment by noting the absence of any pressor effect on femoral arterial blood pressure with 20 μg per kilogram of intravenous noradrenalin.

Results

General appearance. After ganglionectomy, the rabbits were observed for any gross ocular changes. One week later the eyes were free of irritation, and no abnormalities could be detected by slit-lamp examination. Following injection of noradrenalin by subconjunctival, intracameral, or intravitreal route, neither right nor left eyes exhibited hyperemia. On a few occasions, with anterior chamber injection of either saline or noradrenalin, a transient dilation of iris vessels occurred at the 12:00 o'clock margin of the pupil, which subsided within 5 to 10 minutes.

Pupil. All rabbits exhibited a right miosis following ganglionectomy. With gentle handling this pupillary difference persisted until noradrenalin was applied to the eyes. The denervated side then exhibited marked sensitivity. As little as 20 μg of intracameral noradrenalin could produce wide pupillary dilation. There was no consistent pupillary dilation in the left normally innervated irides until a dose of 1.0 μg was injected into the anterior chamber. When pupillary dilation occurred, it generally persisted for the duration of the infusion experiment, about 2 hours.

Protein measurements. The possibility of a change in vascular permeability following anterior chamber injection prompted the measurement of aqueous humor protein. The results are shown in Table I. Control values for eight eyes were 66 ± 8 mg. per cent (Group I). No significant differences were noted between the noninjected denervated and normally innervated sides (Group II). Although there was a two- to threefold increase in aqueous protein bilaterally after intracameral noradrenalin (Group III), the increase did not always parallel the noradrenalin concentration in the dose range studied. Furthermore, there was a similar increase after intracameral saline (Group IV). In this small series there was a tendency for the right denervated eyes to exhibit slightly increased amounts of protein as compared with the left eyes following intracameral noradrenalin (Group III).

Intraocular pressure and outflow facility. Control values are shown in Table II. In

*Merck Sharp & Dohme.
†California Biochemical Corp.
‡Smith Kline & French Laboratories.

the noninjected group, mean intraocular pressure and outflow facility values for denervated right eyes were 18.2 ± 0.5 mm. Hg and 0.15 ± 0.01 μl per minute, mm. Hg, and for normally innervated left eyes 17.7 ± 0.5 mm. Hg and 0.24 ± 0.02 μl per minute, mm. Hg. The right denervated eyes had a significantly lower outflow facility ($p = < 0.01$) than the corresponding left eyes. This change has been correlated with the depletion of catecholamines developing after denervation (ganglionectomy).[12] Rabbits injected with intraocular saline, whatever the route, had equal facility values for right and left eyes. The associated occurrence of slight transient pupillary dilation on the right side after intraocular saline injection suggests that with slight ocular trauma small amounts of catecholamines, derived from the systemic circulation, are permitted to enter the eye so that in the presence of supersensitized receptors, i.e., structures in the right eye, a facility increase occurs yielding a value equal to the left eye under its condition of normal innervation. This effect did not appear to be additive, however, until doses greater than 0.1 μg noradrenalin were injected intracamerally.

Intraocular pressure and outflow facility after intracameral noradrenalin are shown in Table III. The dose-facility curve is illustrated in Fig. 1. There was an increase in outflow facility after intracameral noradrenalin on both denervated and normally innervated sides, but eyes that were normally innervated required at least 3.0 μg before there was any increase in outflow facility. Some of the denervated eyes exhibited an increase in outflow facility at 0.1

Table I. Aqueous humor protein content effect of ganglionectomy and noradrenalin*

Group	*Dose†*	*Right eye*	*Left eye*
I. Normal rabbits		62 ± 10 (4)	70 ± 13 (4)
II. Noninjected‡		70 ± 13 (3)	65 ± 8 (3)
III. Intracameral saline‡	0.9	104 ± 28 (4)	164 ± 31 (3)
IV. Intracameral noradrenalin‡	0.1§	123 ± 24 (3)	89 ± 17 (3)
	0.4	173 ± 26 (3)	113 ± 24 (3)
	1.0	157 ± 28 (4)	101 ± 14 (4)
	5.0	157 ± 15 (2)	147 ± 7 (3)

*Values are in mg. % ± S.E. (n).
†Injection volumes were 10 μl.
‡Right cervical ganglionectomy performed one week preceding aqueous tap.
§Dose in micrograms of bitartrate salt.

Table II. Intraocular pressure and outflow facility one week after right cervical ganglionectomy

Goup	*Right eye*		*Left eye*	
	*P**	*C†*	*P*	*C*
I. Noninjected contols (n = 11)	18.2 ± 0.5	0.15 ± 0.01	17.7 ± 0.5	0.24 ± 0.02
II. Subconjunctival saline (n = 4)	17.4 ± 0.4	0.27 ± 0.06	17.9 ± 0.8	0.29 ± 0.06
III. Intravitreal saline (n = 4)	15.4 ± 0.5	0.20 ± 0.04	16.2 ± 1.2	0.22 ± 0.03
IV. Intracameral saline (n = 4)	16.8 ± 0.5	0.24 ± 0.12	17.3 ± 0.4	0.19 ± 0.09

*mm. Hg ± S.E.
†μl/min., mm. Hg ± S.E.

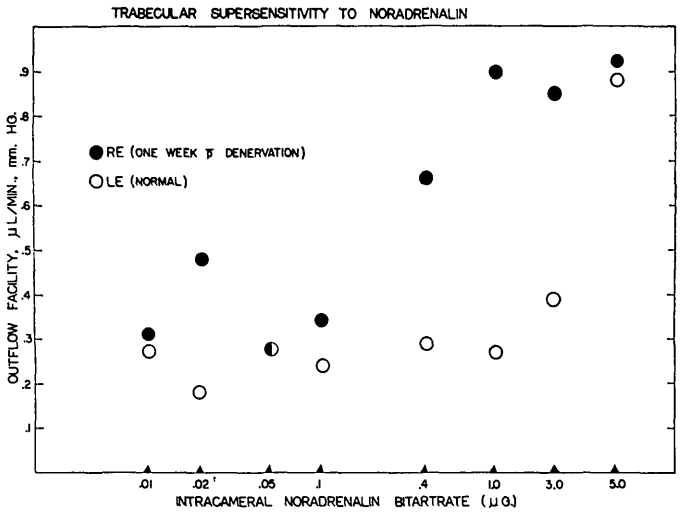

Fig. 1. At the dose level 0.02 µg, 6 rabbits were subjected to bilateral cervical ganglionectomy and received right intracameral noradrenalin bitartrate, 0.020 µg in 10 µl isotonic saline, and left intracameral 10 µl isotonic saline. Average facilities: right eye, 0.45 ± 0.10; left eye, 0.15 ± 0.01.

µg of noradrenalin. Statistically significant differences in response between normally innervated and denervated eyes appeared at a dose level of 0.4 µg. The supersensitivity phenomenon could be augmented considerably by bilateral ganglionectomy, presumably because of partial crossed sympathetic innervation to the rabbit eye. In 6 rabbits subjected to bilateral cervical ganglionectomy, 20 µg of noradrenalin injected into the anterior chamber (Fig. 1) produced a mean "c" value of 0.45 ± 0.1 (n = 6). An approximately equal facility value of 0.38 ± 0.07 (n = 11) was elicited with 3.0 µg in normally innervated eyes. This difference corresponds to at least a one hundred and fifty-fold increase in sensitivity.

Subconjunctival noradrenalin injections produced increases in outflow facility which were less than the increases in the intracameral group. At 0.4 µg noradrenalin, a significant difference in outflow facilities between right and left eyes appeared: right eye = 0.45 ± 0.07 and left eye = 0.25 ± 0.03 µl per minute, mm. Hg (n = 8), p = < 0.05. At this dose level, however, and also in the rabbits which exhibited facility increases at lower dose levels, e.g., 0.2 µg, pupillary dilation was conspicuously absent. This contrasts sharply with the effects noted with intracameral injection in which group at a dose level of 0.4 µg, larger increases in outflow facility occurred (Table III) with uniformly marked pupillary dilation.

Large facility increases did not become apparent after intravitreous injections of noradrenalin until doses greater than 1.0 µg on the denervated side and greater than 25.0 µg on the normally innervated side were employed. A corresponding decrease in intraocular pressure did not occur, however, indicating that an increase in aqueous inflow may have taken place.

Adrenergic blockade. In four consecutive experiments with intravenous phenoxybenzamine and intracameral noradrenalin (see Methods), outflow facilities for right and left eyes were equalized and decreased to levels found for noninjected denervated eyes one week after ganglionectomy (Table II). Average facility values for right and left eyes were 0.19 and 0.17 µl per minute, mm. Hg, right and left eyes, respectively.

Discussion

There are at least three modes by which noradrenalin could increase outflow facility: mechanical, chemical, or vascular.

A mechanical increase in outflow facility could result from deepening of the anterior chamber[13-15] after intracameral injections. Since increase in outflow facility was apparent only after noradrenalin but not after saline injections, this possibility may be excluded. The mechanical action of the ciliary musculature on outflow facility must also be considered. Van Alphen[16] has shown that strips of rabbit ciliary body will yield only a weak contractile response to adrenalin. Furthermore, the rabbit ciliary muscle has few fibers.[17] These studies suggest that the ciliary muscle plays a minor role in the physiology of outflow resistance in the rabbit eye and are supported by the finding that vitreous injections, given close to the ciliary muscle, do not match the facility-increasing effect of an anterior chamber injection. The discrepancy in concentrations required between anterior chamber and intravitreous injections could be explained by a loss of neurohumor through diffusion, nonspecific protein binding, and degradation in the vitreous cavity prior to receptor binding, but also suggests that the ciliary muscle is not involved in the facility increase.

Nonspecific chemical effects induced in the aqueous could alter the rate of aqueous exit through Schlemm's canal. The finding that phenoxybenzamine blocks the noradrenalin effect points to action on adrenergic receptors. This effect does not bear on the chemical changes at receptor sites induced by combination with the mediator, but does tend to exclude nonspecific effects induced in the aqueous by noradrenalin as accounting for the facility increase. In addition, the fact that closely related compounds like dopamine and normetanephrine in doses as great as 100 μg did not affect the outflow facility also implies a specific receptor mechanism.

Vascular responses at several places might increase outflow facility after noradrenalin. First, diffuse effects mediated by vasoconstriction of posterior segment vessels would be an unlikely explanation in view of the marked discrepancy between anterior chamber and vitreous dose of noradrenalin required to produce an equal facility increase.

A second possible vascular locus for increased outflow facility could be the iris, if changes in permeability of iris vessels cause increased reabsorption of aqueous. A small number of aqueous protein determinations was made to check vascular permeability. The assumption is that protein will enter aqueous via iris vasculature under the conditions of intracameral injection. Control values obtained were comparable to the results of Kuhlman and Kaufman,[18] who used the same method and to those of Anjou and Krakau,[19] who used

Table III. Intraocular pressure and outflow facility after intracameral levarterenol bitartrate

	Right eye		*Left eye*	
Dose (μg)	*P**	*C†*	*P*	*C*
0.01 (5)‡	15.1 ± 0.4	0.31 ± 0.07	15.3 ± 1.2	0.27 ± 0.07
0.05 (5)	16.2 ± 0.9	0.26 ± 0.10	13.6 ± 0.3	0.26 ± 0.07
0.1 (7)	16.2 ± 0.9	0.33 ± 0.07	13.9 ± 0.9	0.23 ± 0.04
0.4 (6)	15.7 ± 0.5	0.66 ± 0.09	15.6 ± 0.6	0.29 ± 0.05
1.0 (6)	14.7 ± 0.9	0.90 ± 0.28	14.1 ± 1.7	0.27 ± 0.04
3.0 (11)	16.3 ± 1.2	0.55 ± 0.07	15.7 ± 0.7	0.38 ± 0.06
5.0 (6)	17.0 ± 1.3	0.89 ± 0.29	16.7 ± 0.3	1.01 ± 0.23

Right cervical ganglion removed in all animals one week prior to measurements.
*mm. Hg ± S.E.
†μl/min., mm. Hg ± S.E.
‡Number of animals.

a new photographic technique. In both saline and noradrenalin injected animals the protein content was in the same range. Furthermore, protein concentrations in a range from 100 to 300 mg. per cent were found in perfused rabbit eyes 2 to 3 hours after cannulation when outflow facilities were normal.[20] These observations tend to exclude a vascular leak as accounting for the increased facility noted after noradrenalin injection.

Third, noradrenalin could produce arteriolar constriction which would reduce episcleral venous pressure. In a few experiments, removal of the episcleral and conjunctival tissues of heparinized rabbits, however, did not yield a significant increase in outflow facility (0.30 ± 0.08 versus 0.25 ± 0.06 [$n = 8$]). Therefore, changes in episcleral venous pressure could not account for the large effects noted in this work. Further, the studies of Bárány[21] and Grant,[22] indicating that the main site of resistance is interior to the episcleral veins, strengthen the idea that only a small effect is exerted by these structures. A direct action of noradrenalin on episcleral vessels might be expected to cause vasoconstriction, an effect in the opposite direction. Finally, subconjunctival noradrenalin in high doses gave high facilities, but low doses, in the range in which anterior chamber injections gave high facilities, elicited less prominent increases. At levels where increases in outflow facility began to appear, i.e., between 0.2 and 0.4 μg of subconjunctival noradrenalin, no changes in pupillary diameter were noted. In other words, facility was affected although little if any noradrenalin entered the anterior chamber. These findings suggest that the locus of adrenergic action on outflow facility is not episcleral but rather intrascleral.

The most likely possibility would be the direct action of noradrenalin on the intrascleral trabecular canals. This term would appear to be more suitable for the rabbit eye than "Schlemm's canal."[23] Two hypotheses concerning the canal mechanism may be considered. One of these stems from Friedenwald's[24] suggestion that Schlemm's canal has afferent arteriolar connections which could easily provide sufficient plasma protein to sustain an osmotic gradient for reabsorption of aqueous. (These afferent connections were not found in the injection studies of Ashton,[25] but, recently, Ruskell[23] may have resolved these discrepant findings for the rabbit eye.) It is barely possible that noradrenalin could influence such a gradient by action on these afferent connections.

The neuroanatomy of the trabeculum suggests a second mechanism. Holland and associates[26] have shown extensive innervation of the trabecular meshwork in rabbits. Holland's work showed nerve endings beneath the endothelium of Schlemm's canal and within the trabecular meshwork that forms the inner wall of this structure. Degeneration studies indicated a sympathetic component.[27] These anatomic studies, taken together with the present perfusion studies, point to the endothelium of the intrascleral canals as the locus of action for noradrenalin.

Dibenzyline was supplied by Mr. J. S. Bird of Smith Kline & French Laboratories.

REFERENCES

1. Garner, L. L., Johnstone, W. W., Ballintine, E. J., and Carrol, M. E.: Effects of 2 per cent levo-rotatory epinephrine on the intraocular pressure of the glaucomatous eye, A. M. A. Arch. Ophth. **62**: 230, 1959.
2. Ballintine, E. J.: The anticholinesterases in glaucoma, *In* Newell, F. W., editor: Transactions of the Fifth Conference, New York, Josiah Macy, Jr. Foundation, 1960, p. 248.
3. Becker, B., Pettit, T. H., and Gay, A. J.: Topical epinephrine therapy of open-angle glaucoma, A. M. A. Arch. Ophth. **66**: 219, 1961.
4. Eakins, K.: The effect of intravitreous injections of norepinephrine, epinephrine, and isoproterenol on the intraocular pressure and aqueous humor dynamics of rabbit eyes, J. Pharmacol. & Exper. Therap. **140**: 79, 1963.
5. Bárány, E. H.: Transient increase in outflow facility after superior cervical ganglionectomy in rabbits, A. M. A. Arch. Ophth. **67**: 303, 1962.

6. Sears, M. L., and Sherk, T. E.: Supersensitivity of aqueous outflow in rabbits after sympathetic denervation, Nature **197**: 387, 1963.
7. Sears, M. L.: Outflow resistance of the rabbit eye: Technique and effects of acetazolamide, A. M. A. Arch. Ophth. **64**: 823, 1960.
8. Sears, M. L., and Bárány, E. H.: Outflow resistance and adrenergic mechanisms, A. M. A. Arch. Ophth. **64**: 839, 1960.
9. Sears, M. L.: Miosis and intraocular pressure changes during manometry, A. M. A. Arch. Ophth. **63**: 707, 1960.
10. Lowry, O. H., Rosebrough, N. H., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent, J. Biol. Chem. **193**: 265, 1951.
11. Nickerson, M., and Nomaguchi, G.: Adrenergic blocking action of phenoxyethyl analogues of Dibenamine, J. Pharmacol. & Exper. Therap. **101**: 379, 1951.
12. Sears, M. L., Mizuno, K., and Sherk, T. E.: Analysis of ganglionectomy effect on outflow resistance in the rabbit eye, INVEST. OPHTH. Submitted for publication.
13. Grant, W. M.: Tonography: Glaucoma, *In* Newell, F. W., editor: Transactions of the Third Conference, New York, 1958, Josiah Macy, Jr. Foundation, p. 60.
14. Francois, J., Rabaey, M., and Neetens, A.: Perfusion studies on the outflow of aqueous humor in human eyes, A. M. A. Arch. Ophth. **55**: 193, 1956.
15. Nihard, P.: Influence of the depth of the anterior chamber on the resistance to flow of aqueous humor, Exper. Eye Res. **1**: 229, 1962.
16. Van Alphen, G. W., Robinette, S. L., and Macri, F.: Drug effects on ciliary muscle and choroid preparations in vitro, A. M. A. Arch. Ophth. **68**: 81, 1962.
17. Sheppard, L. B.: The anatomy and histology of the normal rabbit eye with special reference to the ciliary zone, A. M. A. Arch. Ophth. **67**: 117, 1962.
18. Kuhlman, R. E., and Kaufman, H. E.: A microchemical study of the aqueous humor enzyme-protein interrelations, A. M. A. Arch. Ophth. **63**: 41, 1960.
19. Anjou, I., and Krakau, C. E. T.: Aqueous flare and protein content in the anterior chamber of normal rabbit eyes, Acta ophth. **39**: 95, 1961.
20. Sears, M. L.: Unpublished observations.
21. Bárány, E. H.: Resistance to aqueous outflow: Glaucoma, *In* Newell, F. W., editor: Transactions of the First Conference, New York, 1960, Josiah Macy, Jr. Foundation, p. 140.
22. Grant, W. M.: Further studies on facility of flow through trabecular meshwork, A. M. A. Arch. Ophth. **60**: 523, 1958.
23. Ruskell, Gordon L.: Aqueous drainage paths in the rabbit. A Neoprene latex cast study, A. M. A. Arch. Ophth. **66**: 119, 1961.
24. Friedenwald, J. S.: Circulation of the aqueous. V. Mechanism of Schlemm's canal, A. M. A. Arch. Ophth. **16**: 65, 1936.
25. Ashton, N., and Smith, R.: Anatomical study of Schlemm's canal and aqueous veins by means of Neoprene casts: III. Arterial relations of Schlemm's canal, Brit. J. Ophth. **37**: 577, 1953.
26. Holland, M. G., von Sallmann, L., and Collins, E. M.: A study of the innervation of the chamber angle, Am. J. Ophth. **42**: 148 (part 2), 1956.
27. Holland, M. G., von Sallmann, L., and Collins, E. M.: A study of the innervation of the chamber angle: II. The origin of trabecular axons revealed by degeneration experiments, Am. J. Ophth. **44**: 206, (part 2), 1957.