There was no statistically significant difference in (1) visual acuity or (2) pupillary size between treatment groups in each treatment day.

No statistically significant differences in the change between pretreatment and post-treatment blood pressure and pulse rate were observed between the two treatment groups during any treatment day, except during weekend 2. During this weekend, the change between pretreatment and post-treatment values of systolic (but not diastolic) blood pressure and pulse rate in the two treatment groups were found to be significantly different (p < 0.05) for the two treatment groups. The timolol group had a drop in systolic blood pressure of 6.67 mm. ± 2.67 and a drop in pulse rate of 3.87 ± 2.25, whereas the placebo group had a rise of systolic blood pressure of 2.71 ± 2.62 and a rise in pulse rate of 4.14 ± 2.90. During this weekend the baseline systolic blood pressure was 5.34 mm. Hg lower and the baseline pulse rate was 5.74 higher in the timolol group, although these differences were not found to be statistically significant.

No adverse effect occurred which could be attributed to the treatment drug. Although some bulbar conjunctiva erythema and an occasional punctate keratitis occurred by five to seven hours in some of the subjects, there was no difference between involvement of treated and untreated eyes. Placebo eyes were equally involved. These changes probably resulted from the Fluress (fluorescein 0.25 per cent and benoxinate HCl 0.4 per cent) eyedrops used to anesthetize the eye in order to measure intraocular pressure.

From the Merck Sharp & Dohme Research Laboratories, West Point, Pa. and the Experimental Therapeutics Section, Tulane University School of Medicine, New Orleans, La. Submitted for publication Nov. 16, 1975. Reprint requests: Dr. Irving M. Katz, Merck Sharp & Dohme Research Laboratories, West Point, Pa. 19486

Key words: beta-blocker, glaucoma, normal volunteers, intraocular pressure, hypotension, pupil, vision.

REFERENCES


Effects of isoproterenol and norepinephrine on regional ocular blood flows. ASRAR B. MALIK, W. A. J. VAN HEUVEN, AND LOWELL F. SATLER.

The effects of norepinephrine and isoproterenol on regional ocular blood flows were studied by the labeled microspheres using the reference sample method in pigs. Total and regional ocular flows increased in response to norepinephrine and isoproterenol infusions. Increase in flows during norepinephrine infusion may be due to increased ocular perfusion pressure induced by the drug, thereby masking its reported direct vasoconstrictor effect in isolated perfused ocular vessels. Increase in flows during isoproterenol infusion occurred despite a decrease in arterial pressure suggesting the existence of vasodilatory beta-adrenergic receptors in the ocular circulation of the pig.

Previous studies have suggested the existence of vasoconstrictor alpha-adrenergic receptors in the ocular circulation.2, 3, 5-7 However, no clear indications have been found for beta-adrenergic receptors.2, 3, 4, 5, 7 The purpose of the present study was to determine the effects of norepinephrine and isoproterenol, alpha- and beta-adrenergic agonists on the regional and total ocular flows as measured in pigs using labeled microspheres.

Methods. Yorkshire pigs (25 kilograms) were anesthetized with 10 mg. per kilogram ketamine intramuscularly, tracheotomized, and connected to a Bird respirator to maintain blood gases and pH at normal levels. A catheter was advanced under fluoroscopic guidance into the left ventricle through a femoral artery. Catheters were also positioned in the other femoral artery and in a brachial artery for the collection of simultaneous timed reference samples. A fourth catheter was advanced into the aorta via the other brachial artery for arterial pressure measurements using Statham PDb23 pressure transducer and monitored on Electronics for Medicine PR-7 recorder. Blood-gas and pH measurements were made using the Radiometer BMS-3 analyzer. After placement of the catheters, sodium pentobarbital (25 mg. per kilogram) was administered to maintain anesthesia.

Labeled carbonized microspheres 15 ± 5 μm in diameter suspended in 2 ml. of 10 per cent dextran with a drop of Tween 80 added to prevent clumping were mixed in a sonicator be-
in six pigs, while two experiments in which the sclera was removed from the extraocular part of the optic nerve were cut flush with the sclera. The total ocular activity was determined by adding each of the activities. The results were analyzed using the paired-t-test. There were no statistical differences between the two control periods and the control flows presented are derived by averaging the two reference samples as well as in the two eyes. The assumption that impaction of spheres does not independently affect ocular flows is comparable to that of the human, and discrete portions are accurately separable. The reference sample method offers certain advantages over other techniques for measuring ocular blood flow. The assumptions of the reference sample method were satisfied. Adequate mixing of spheres was ensured by injecting into the left heart, and was verified by similar activities in the two reference samples as well as in the two eyes. The assumption that impaction of spheres does not independently affect ocular flow was tested by demonstrating that flow values obtained during the initial control period were not significantly different from the control normal values of 87.1 ± 1.1 mm. Hg, 35.6 ± 2.1 mm. Hg, and 7.35 ± 0.021 mm. Hg during the study.

The flows to the retina, iris and ciliary body, and choroid during control period and during isoproterenol and norepinephrine infusions are summarized in Table I. Significant increases (p < 0.01) were observed following infusions of both catecholamines.

Uveal flow was calculated as the sum of flows to the iris and ciliary body, and choroid. Uveal flow increased (p < 0.01) during isoproterenol and norepinephrine infusions (Fig. 1).

Total ocular flow was calculated as the sum of flows to the iris and ciliary body, choroid, retina, sclera, and intraocular part of the optic nerve. Flow to cornea and lens was insignificant and is not included in the calculation. Total ocular flow increased (p < 0.01) during isoproterenol and norepinephrine infusions (Fig. 2).

Discussion. The reference sample method using labeled microspheres was used to determine total and regional ocular blood flow in pigs. The pig was selected because its ocular vascular geometry is comparable to that of the human, and discrete portions are accurately separable. The reference sample method offers certain advantages over other techniques for measuring ocular blood flow. The assumptions of the reference sample method were satisfied. Adequate mixing of spheres was ensured by injecting into the left heart, and was verified by similar activities in the two reference samples as well as in the two eyes. The assumption that impaction of spheres does not independently affect ocular flow was tested by demonstrating that flow values obtained during the initial control period were not significantly different from the control period following recovery from the first drug infusion. The final assumption that microspheres are removed by trapping from the ocular circulation beds was not tested; however, previous studies indicate that only 1 to 2 per cent of the 15 μm spheres are not trapped in the first pass through

Table I. Effects of isoproterenol and norepinephrine infusions on flows to retina, iris and ciliary body, and choroid in right (R) and left (L) eyes. Mean values are shown with ± 1 S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Isoproterenol 2 μg/min./Kg.</th>
<th>Norepinephrine 5 μg/min./Kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ml./min./100 Gm.)</td>
<td>(ml./min./100 Gm.)</td>
<td>(ml./min./100 Gm.)</td>
</tr>
<tr>
<td>Retina</td>
<td>R</td>
<td>152.3 ± 43.1</td>
<td>462.9 ± 157.5</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>145.6 ± 58.1</td>
<td>471.5 ± 142.3</td>
</tr>
<tr>
<td>Iris and ciliary body</td>
<td>R</td>
<td>1,351.5 ± 410.5</td>
<td>2,980.6 ± 805.3</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>1,280.2 ± 401.8</td>
<td>2,640.5 ± 780.2</td>
</tr>
<tr>
<td>Choroid</td>
<td>R</td>
<td>16,500.8 ± 2,102.2</td>
<td>33,211.1 ± 5,900.2</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>16,780.2 ± 1,905.0</td>
<td>30,150.0 ± 6,112.2</td>
</tr>
</tbody>
</table>

kilogram). The order of infusion was randomized and the data were not affected by the order. All measurements were taken when the arterial pressure had stabilized. Infusion of the microspheres did not affect the subsequent measurements since the initial control and second control measurements taken after recovery from the first drug infusion were not significantly different. Approximately 6,000,000 microspheres were injected for each measurement over a 40-second period and reference samples were withdrawn from two peripheral arteries using Harvard withdrawal pumps. Successful experiments were carried out in six pigs, while two experiments in which arterial pressures were altered significantly were rejected. These experiments were also rejected because of differences in the ocular flows between the two eyes indicative of poor ventricular mixing.

At the end of the experiment the eyes were enucleated and placed in 10 per cent formalin for 10 to 14 days. This process enabled accurate dissection of the entire iris and ciliary body, sclera, choroid, retina, cornea, and lens. The muscles, nerves, Tenon's capsule, and conjunctiva were removed from the sclera and the extraocular part of the optic nerve were cut flush with the sclera. The total ocular activity was determined by adding each of the activities. The tissues were dried and weighed, and the activities were counted on a Nuclear-Chicago well counter. Blood flow was then computed from the following formula: flow = (reference flow/reference activity) times activity in tissue. All flow data were also normalized per 100 Gm. dry weight of tissue. The results were analyzed using the paired-t-test.

Results. There were no statistical differences between the two control periods and the control flows presented are derived by averaging the two control values. There were also no significant differences between the right and left ocular flows of each pig.

Mean arterial pressure increased from 110 ± 4 mm. Hg to 163 ± 4 mm. Hg during norepinephrine infusion. The pressure fell from 112 ± 4 to 85 ± 4 mm. Hg during isoproterenol infusion. Microsphere injections did not alter the pressures during infusions of the drugs. Arterial oxygen tension, carbon dioxide tension, and pH did not change significantly from control normal values of 87.1 ± 1.1 mm. Hg, 35.6 ± 2.1 mm. Hg, and 7.35 ± 0.021 mm. Hg during the study.
the systemic circulation. Small spheres were used instead of the larger 50 μm spheres because they offer fewer problems with plasma skimming; thus the use of smaller spheres is suggested for studies on ocular blood flow. However, choroidal blood-flow measurements may have been underestimated using the 15 μm spheres since larger vessels are present in the choroidal bed. The number of spheres injected was determined by the relatively small flow to the eye and the need for adequate resolution. Approximately 6,000,000 spheres were injected so that approximately 12,000 were impacted in each eye since each eye receives approximately 0.2 per cent of the cardiac output. Spheres were injected without obvious hemodynamic effects, and yet enabled 200 to 400 sphere impactions necessary for accurate regional flow determinations in a tissue sample.

The measured total and regional ocular blood flows increased during both isoproterenol and norepinephrine infusions. Increases in choroidal blood flow comparable to the present findings were observed during norepinephrine infusion using the Krypton-85 desaturation technique in intact cats. Regional ocular vasoconstriction was demonstrated in isolated segments of the ocular vessels or perfusing small concentrations of norepinephrine directly into the ocular circulation. In contrast, changes in retinal oxygen tension as an indicator of retinal flow indicates that close arterial injections of norepinephrine had no effect on retinal flow. In the present study in the intact pig, we did not observe ocular vasoconstriction in response to norepinephrine. The increase in ocular flow in response to norepinephrine in intact animals may have been due to the norepinephrine-induced increase in arterial pressure suggested by Chandra and Friedman; thereby masking ocular vasoconstrictor effect of norepinephrine in isolated ocular vessels. The increase in ocular flow during norepinephrine infusions were not likely due to decrease in intraocular pressure with the resultant increase in ocular perfusion pressure, since studies have indicated that intraocular pressure increases during intravenous catecholamines infusions.

Isoproterenol resulted in increases in flow to ciliary body and iris, retina, and choroid. The increases may have occurred due to vasodilation since there was a decrease in arterial pressure during isoproterenol infusions, and thus likely a decrease in ocular perfusion pressure. Although the intraocular pressure was not measured, it is unlikely that the increase in flow was due to a decrease in intraocular pressure since isoproterenol has been reported to increase the intraocular pressure. These findings suggest the existence of vasodilator beta-adrenergic receptors in ocular vessels. The results are consistent with the observations that beta-adrenergic receptors mediate dilation in other vascular beds. However, the results are in contrast with previous studies on ocular vessels in which there were no clear indications of beta-adrenergic receptors; although suggestions have been made that there may be dilator ocular beta-adrenergic receptors. The discrepancy may be related to the more invasive nature of previous techniques affecting reactivity of ocular vessels to vasoactive substances. The use of large pigs also permitted a more accurate
dissection of eyes allowing regional flows to be compartmentalized more accurately in the present study. In addition, precautions were taken in the present study to maintain blood-gases and pH in the normal range, since variations in these can independently alter ocular blood flow. The discrepancy may be due to the use of the pig in the present study in contrast to previous studies, suggesting a species specific nature of the ocular vascular response to catecholamines. Finally, there is the possibility that vasodilation induced by isoproterenol is secondary to stimulation of ocular metabolic rate rather than vasodilation due directly to stimulation of beta-adrenergic receptors. Studies using specific antagonists of isoproterenol are needed to confirm the existence of beta-adrenergic receptors in the ocular vasculature.

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Key words: microspheres, reference sample method, pigs, regional ocular flows, isoproterenol, norepinephrine.

REFERENCES

The effect of interruption of the short posterior ciliary arteries on slow axoplasmic transport and histology within the optic nerve of the rhesus monkey.

NORMAN S. LEVY.

Tritiated leucine was injected into the cisterns of rhesus monkey eyes to make it available for protein synthesis by the ganglion cells. The short posterior ciliary arteries were cut three hours later or several weeks prior to the leucine injection. A reduction of labeled protein within the retro-laminar optic nerve was seen in all eyes so treated. Autoradiography revealed a diffuse reduction of axoplasmic transport into these optic nerve heads. There was consistent evidence of focal obstruction of labeled protein at the interface between the lamina scleralis and retro-laminar optic nerve. Vacuoles appeared in the most severely affected areas. These histologic changes were followed by gliosis in the areas of ischemic damage. Glaucomatous cupping of the optic nerve head was not seen within six weeks following the induced ischemia.

Recent studies suggest that the obstruction in axoplasmic transport seen following intraocular pressure elevation in both rhesus and owl monkeys is most likely ischemic in origin. Ischemia of the optic nerve head can be produced without intraocular pressure elevation by compromise of the posterior ciliary arterial supply. Under these conditions, the quantity of slow axoplasmic transport into the optic nerve is also reduced. The purpose of this study is to characterize the effects on slow axoplasmic transport of surgical interruption of the arterial blood supply to the nerve head over periods of varying duration.

Methods and materials. These studies were performed in twelve optic nerves of six rhesus monkeys (Macaca mulatta), weighing 3 to 4 kilograms. Each animal had an ocular examination including tonometry, biomicroscopy, and funduscopy. Photographic documentation of the fundus was obtained before and during the study. Electroretinography and visually evoked responses were obtained in some animals as previously described.

The posterior ciliary arteries were exposed by lateral orbitotomy under ketamine and barbiturate anesthesia. The area at which the optic nerve leaves the eye was observed under the microscope and the short posterior ciliary vessels surrounding the optic nerve cut at that location. In the opposite eye of each animal, a similar ex-