Adrenergic Modulation of Choroidal Blood Flow in the Rabbit

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Purpose. To determine whether the choroidal pressure-flow relationship is altered by the α-adrenergic antagonist, phentolamine, or the β-adrenergic antagonist, propranolol.

Methods. In two groups of pentobarbital-anesthetized rabbits, the choroidal pressure-flow relationships were determined by raising the intraocular pressure (IOP) at mean arterial pressure (MAP) of 50, 60, 70, and 80 mm Hg before and after phentolamine (0.5 mg/kg, intravenously, n = 7) and propranolol (0.25 mg/kg, intravenously, n = 7) administration. Hydraulic occluders on the thoracic aorta and inferior vena cava were used to control MAP, which was measured in the central ear artery. The eye was cannulated with two 23-gauge needles, one to manipulate the ocular volume and the other to measure the IOP. Choroidal blood flow was measured by laser Doppler flowmetry with a probe positioned over the posterior retina. The protocol consisted of setting the MAP, then infusing saline into the eye at 30 μl/minute until the IOP increased from baseline to 100 mm Hg.

Results. At the MAP of 70 mm Hg, α-adrenergic blockade caused an upward shift in the choroidal pressure-flow relationship; β-blockade shifted the relationship downward.


Previous studies in this laboratory show evidence of strong autoregulation in the posterior choroid of the rabbit. In the current study, we begin evaluating potential neurohumoral modulators of choroidal blood flow by examining the effect of α- and β-adrenergic blockade on the choroidal pressure-flow relationship.

Pharmacologic and histochemical studies indicate the presence of α- and possibly β-adrenergic receptors on choroidal vessels, and direct electrical stimulation of the ocular sympathetic nerves or local infusions of catecholamines cause choroidal vasoconstriction. However, the physiological role of the choroidal sympathetic nerves and the choroidal response to humoral catecholamines is unclear. Bill found no effect of cervical sympathectomy on choroidal microsphere entrapment when mean arterial pressure (MAP) was reduced by hemorrhage from 90 to 52 mm Hg, suggesting that the choroidal sympathetic nerves are not activated by the arterial baroreflex. However, Bill et al also observed that when MAP was increased by aortic ligation, cervical sympathectomy caused uveal "overperfusion" that could be prevented by direct sympathetic stimulation, suggesting that the choroidal sympathetics may be tonically active or may become activated during acute, mechanically induced arterial hypertension.

Given that the choroidal vasculature is innervated with sympathetic nerves that may be tonically active, this study tests the hypothesis that nonselective α-adrenergic blockade and β-adrenergic blockade cause choroidal vasodilation and vasoconstriction, respectively. To test this hypothesis and to verify that ocular sympathetic nerve activity is not under baroreflex control, the choroidal pressure-flow relationships were determined by varying the intraocular pressure (IOP) at four different MAPs.
METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and it adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animal Preparation

Locally obtained New Zealand white rabbits of both sexes were housed in the institutional animal care facility and given food and water ad libitum for at least 1 day before the experiments. At approximately 8 AM on the day of the experiments, animals were anesthetized with pentobarbital sodium (30 mg/kg intravenously [IV], supplemented as necessary). Gallamine triethiodide (10 mg/kg IV) was administered to eliminate saccadic eye movements and extraocular muscle tone. Animals were intubated through a tracheostomy and respired with room air to maintain the expired PCO$_2$ at 40 to 45 mm Hg. Body temperature was maintained at 37°C to 39°C with a heating pad.

To control MAP, hydraulic occluders were placed around the descending thoracic aorta and inferior vena cava through a right thoracotomy. The aortic occluder was used to redirect the cardiac output to the upper half of the body, thus increasing the MAP at the eye. The caval occluder was used to decrease MAP by impeding venous return to the heart, thus reducing cardiac output. The MAP was monitored with a pressure transducer connected to a cannula inserted into the central artery of the right ear as an index of MAP at the eye. After surgical preparation, each animal was mounted in a stereotaxic head holder. The right eye was cannulated with two 23-gauge needles inserted into the vitreous through the pars plana. One needle was connected to a pressure transducer to monitor IOP; the other was connected to a saline-filled syringe.

Red blood cell flux, an index of tissue blood flow, was measured by laser Doppler flowmetry. The flowmeter (PF-2b; Perimed, Stockholm, Sweden) in this study uses a 2 mW He-Ne laser light source and a probe (PF-303) consisting of three fiber-optic light guides ending in a 10-cm slender, stainless steel sleeve. The flowmeter was calibrated according to factory instructions to provide readings in standardized perfusion units and set at a frequency cut-off of 24 kHz, time constant 0.2 second, and a gain of 1 during the experiments. To measure choroidal blood flow, the probe was advanced through a pars plana incision into the vitreous with a micromanipulator until the tip was positioned near the retinal surface at the posterior pole. Because the probe and eye were isolated from potential sources of motion artifacts and because this region of the rabbit retina is devoid of retinal blood vessels, the flux measurements were caused solely by blood movement within the choroid. During all experiments, the total backscatter was maintained from 2 to 3 V, the range for which the flux is independent of the distance between the probe and the tissue.

Experimental Protocol

Figure 1 shows representative tracings from single animals in the phentolamine and propranolol groups to illustrate the experimental protocol. After initially setting the MAP, saline was infused into the vitreous at 30 μL/minute until the IOP reached 100 mm Hg. The basic protocol was performed at MAPs of 50, 60, 70, and 80 mm Hg before and starting 5 minutes after administration of the α-blocker phentolamine (0.5 mg/kg IV, n = 7) or the β-blocker propranolol (0.25 mg/kg IV, n = 7). The sequence of MAPs before and after the drugs was randomized.

Data Analysis

All measured variables were recorded with a MacLab (World Precision Instruments, Sarasota, FL) data acquisition system connected to a Macintosh SE30 computer (Apple, Cupertino, CA). Digital values for MAP, IOP, and flux were later averaged in 4-second bins and then reaveraged in 5-mm Hg IOP bins. Unless otherwise specified, the results are expressed as the mean ± the standard error of the mean. Drug effects were analyzed by a paired t-test (StatView; Abacus Concepts, Berkeley, CA) and a repeated measures analysis of variance with two within factors (treatment and IOP) followed by paired contrasts using the Huynh-Feldt adjustment (SuperANOVA; Abacus Concepts, Berkeley, CA).

RESULTS

Immediately on cannulation of the eye, the MAP and IOP were 64.3 ± 2 and 15.9 ± 0.7 mm Hg in the phentolamine group and 63.1 ± 2.4 and 16.2 ± 1.3 mm Hg in the propranolol group. Table 1 shows the baseline values for the measured variables and the calculated resistances immediately before and after drug administration in the two groups.

Figure 2 shows the choroidal flux plotted as a function of IOP for the phentolamine group at the four MAPs. At the MAPs of 53.7 ± 0.2 mm Hg, 65 ± 0.2 mm Hg, and 81.2 ± 0.2 mm Hg, phentolamine did not alter significantly the pressure-flow curves. However, at the MAP of 71.4 ± 0.1 mm Hg, the phentolamine curve was shifted upward at all IOPs between 15 and 45 mm Hg.

Figure 3 shows the choroidal flux plotted as a function of IOP for the propranolol group at the four MAPs. At the MAPs of 51.9 ± 0.2 mm Hg, 60.9 ± 0.2 mm Hg, and 80.9 ± 0.1 mm Hg, propranolol did not
significantly alter the pressure-flow curves. However, at the MAP of 71.3 ± 0.1 mm Hg, the propranolol curve was shifted downward at all IOPs between 20 and 60 mm Hg.

**DISCUSSION**

Autoregulation is the intrinsic ability of a tissue to control its blood flow during changes in perfusion pressure. Three types of autoregulation are recognized generally, based on the underlying local mechanisms regulating vascular resistance: flow-dependent autoregulation in which the resistance is adjusted to meet parenchymal metabolic demand or to maintain endothelial shear stress, and pressure-dependent (i.e., myogenic) autoregulation in which the resistance is regulated to maintain the vascular wall tension. Relative contributions to vascular resistance by these mechanisms vary from one tissue to another and with the functional status of the tissue, and superimposed on these local determinants of vascular resistance are the neurohumoral mechanisms regulating systemic blood pressure. In some cases, local and neurohumoral control mechanisms exert competing effects on vascular

**TABLE 1. Baseline Values for Mean Arterial Pressure, Intraocular Pressure, Choroidal Blood Flow, and Resistance Immediately Before and 5 Minutes After Administration of Phentolamine and Propranolol**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Phentolamine</th>
<th>P Value</th>
<th>Control</th>
<th>Propranolol</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>66 ± 1</td>
<td>58 ± 3</td>
<td>0.028</td>
<td>60 ± 2</td>
<td>57 ± 2</td>
<td>0.035</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>17 ± 1</td>
<td>14 ± 1</td>
<td>0.002</td>
<td>17 ± 1</td>
<td>15 ± 1</td>
<td>0.002</td>
</tr>
<tr>
<td>Flux (PU)</td>
<td>830 ± 35</td>
<td>835 ± 50</td>
<td>0.931</td>
<td>795 ± 58</td>
<td>775 ± 56</td>
<td>0.142</td>
</tr>
<tr>
<td>Resistance (PU)</td>
<td>0.060 ± 0.002</td>
<td>0.052 ± 0.003</td>
<td>0.044</td>
<td>0.055 ± 0.004</td>
<td>0.056 ± 0.004</td>
<td>0.601</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; IOP = intraocular pressure; Flux = choroidal blood flow; PU = perfusion units.
resistance, such as in the autoregulatory escape from sympathetic nerve stimulation. Conversely, local and neurohumoral control mechanisms can have facilitatory effects, such as in the augmented myogenic response during sympathetic nerve stimulation.

Previous studies from this laboratory found choroidal pressure-flow responses similar to the myogenic autoregulation observed in other tissues. In the current study, we have extended our previous findings by examining the effect of α- and β-adrenergic blockade on the choroidal pressure-flow relationship. Results summarized in Figures 2 and 3 show that, at an MAP of 70 mm Hg, α-adrenergic blockade caused an upward shift in the choroidal pressure-flow relationship and β-adrenergic blockade caused the relationship to shift downward. These results are consistent with known adrenergic vascular pharmacology—i.e., in the presence of an adrenergic stimulus, withdrawal of α-adrenergic constrictor tone causes vasodilation, and withdrawal of β-adrenergic dilator tone causes vasoconstriction.

The fact that adrenergic blockade altered the choroidal pressure-flow relationship indicates the presence of endogenous catecholamines, either at the choroidal resistance vessels or in the extraocular arteries feeding the choroid. The experimental design cannot resolve the site definitively; however, if the site of action were extraocular, one would expect markedly different pressure-intercepts in Figures 2 and 3. If, for example, propranolol constricted the ciliary arteries, the reduced choroidal arterial pressure would cause flow to cease at a lower IOP and shift the pressure-intercept to the left. Conversely, a phentolamine-mediated dilation would raise choroidal arterial pressure and shift the pressure-intercept to the right. Because the pressure-intercepts were not altered by either drug, the primary site of action was most likely intraocular.

A curious aspect of these results is that adrenergic blockade had a significant effect only at the MAP of 70 mm Hg; there was no statistically significant effect at the lower and higher MAPs. This finding is interesting because it suggests that either the choroidal catecholamine exposure or the catecholamine responsiveness varied with MAP.

The most likely cause of an MAP-induced change in endogenous catecholamines is the arterial baroreflex. In the rabbit, the MAP range of 50 to 80 mm...
Hg brackets the majority of the baroreflex response for renal sympathetic nerve activity and heart rate, with the maximum baroreflex gain occurring at an MAP of 70 to 75 mm Hg. Thus, it is possible that baroreflex withdrawal could account for the diminished effect of adrenergic blockade at the MAP of 80 mm Hg. However, if the baroreflex were involved, the adrenergic blockade effects should have been augmented at the lower MAPs because of increased baroreflex activation. Because this did not occur, it seems unlikely that the baroreflex was involved. Bill also concluded that the choroidal sympathetic nerves are not under baroreflex control when he found no difference in the choroidal blood flow responses in intact and sympathectomized rabbit eyes when the MAP was reduced by hemorrhage. Moreover, he also found that intact choroidal sympathetic innervation helped to prevent choroidal overperfusion when MAP was increased acutely by aortic ligation. This is a result that would not have occurred in a bed under baroreflex control because raising the MAP would inhibit sympathetic outflow.

Assuming a constant choroidal catecholamine exposure, the other possible explanation for the adrenergic blockade effects at the different MAPs is that the autoregulatory response altered the choroidal responsiveness to catecholamines. Figures 2 and 3 show that the choroidal autoregulatory reserve is largely exhausted at an MAP of 50 mm Hg and marginal at an MAP of 60 mm Hg. Under these conditions, the resistance vessels should be dilated to the fullest extent achievable by local control, possibly overriding the adrenergic influence and accounting for the lack of an adrenergic blockade effect. Conversely, at an MAP of 80 mm Hg, the pronounced autoregulation requires the resistance vessels to start in a constricted state and to relax as the perfusion pressure decreases. If the local control-mediated vasoconstriction were sufficiently strong, it could overwhelm the adrenergic influence and minimize the effect of adrenergic blockade. Following this line of reasoning, if the expression of the adrenergic influence occurs only when local control is not fully activated, it would explain the occurrence of the adrenergic blockade effect only at an MAP of 70 mm Hg.

It should be noted that the evidence for choroidal \( \beta \)-receptors is conflicting. For example, Bill found no change in cat or rabbit uveal vascular resistance in...
response to the β-agonist isopropylnoradrenalin or to epinephrine after α-blockade. Similarly, Koss also saw no effect of β-blockade with propranolol on the anterior choroidal constriction response to epinephrine or sympathetic nerve stimulation in cats. By contrast, Chiou and Chen found an initial decrease in choroidal blood flow 30 minutes after β-blockade with topical L-timolol in rabbits, and Grajewski et al observed a decrease in the IOP pulse amplitude in humans in response to systemic timolol that they attributed to choroidal vasoconstriction. Grajewski et al also found significant binding of a radiolabeled β-blocker in rabbit choroidal vessels. Specific β-receptor binding was found by Elena et al in rabbit choroid and by Bruininck et al in cat choroid. Although the reasons for the discrepant findings in the literature are unclear, current results provide additional evidence of β-receptors in the choroid.

Although the paired design of this study was successful in revealing an effect of α- and β-blockade, the wider range of autoregulation in the propranolol group than in the phentolamine group was unexpected. We do not have a definitive explanation for this finding; however, in three of the phentolamine rabbits, Nembutal (Abbott, Chicago, IL) was used instead of our usual veterinary pentobarbital formulation that had been discontinued by our previous supplier. These animals had blunted autoregulatory responses (see Fig. 4) that contributed to the intergroup variability, but we chose to include their data because they still exhibited a response to phentolamine.

In summary, at the MAP of 70 mm Hg, α- and β-blockade caused choroidal vasodilation and vasoconstriction, respectively. Based on these findings, we conclude that α- and β-receptors are present on choroidal resistance vessels and that the sympathetic nerves innervating these vessels are tonically active and not under baroreflex control.

Key Words
adrenergic, eye, peripheral circulation, phentolamine, propranolol

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References
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