Regional Optic Nerve Blood Flow and Its Autoregulation

Joel M. Weinstein,*† R. Bradford Duckrow,‡ Daniel Beard,† and Robert W. Brennan†

An autoradiographic method (14C-iodoantipyrine autoradiography) was used to measure regional optic nerve blood flow (ONBF) under basal conditions in 11 adult female cats. Flow was measured in six regions of the optic nerve: (1) prelaminar, (2) laminar, (3) 1 mm post-laminar, (4) 4 mm post-laminar, (5) 6 mm post-laminar, and (6) intracranial optic nerve and chiasm. A gradient of flow rates was found with relatively high flow in the prelaminar, laminar, and 1-mm post-laminar optic nerve, and significantly lower flow in the 4- and 6-mm post-laminar nerve and in the intracranial optic nerve and chiasm. Blood flow in the 4- and 6-mm post-laminar nerve and in the intracranial nerve and chiasm was comparable to previously reported values for cerebral white matter in the cat. With alteration of mean arterial blood pressure (MABP), optic nerve blood flow demonstrated autoregulatory compensation in all areas. Invest Ophthalmol Vis Sci 24:1559–1565, 1983

The vascular physiology of the optic nerve has been studied extensively by several investigators.1–13 Most prior studies of optic nerve blood flow (ONBF) have focused upon the region of the lamina cribrosa and prelaminar optic nerve, where arterial perfusion pressure has been altered by increasing or decreasing intraocular pressure (IOP).1–10 These studies have sought to define the limits of regional blood flow autoregulation for the distal optic nerve, and are relevant to the pathophysiology of optic nerve damage in human glaucoma. It should be noted, however, that for the prelaminar and anterior laminar optic nerve, perfusion pressure is equal to mean arterial blood pressure (MABP)-intraocular pressure (IOP).3 Regional changes in perfusion pressure also may occur with systemic hyper- or hypotension or in the presence of vascular disease. For this reason, we studied the effect of alterations in MABP upon ONBF in both the distal optic nerve and in its more proximal parts.

Although the vascular physiology of the distal optic nerve has been studied extensively, very few investigators have measured blood flow in the orbital optic nerve (>4 mm retrolaminar), the intracranial optic nerve, or the chiasm. In addition, the response of vascular beds in these regions to changes in arterial perfusion pressure is not well-established. Knowledge of the vascular physiology of these regions may be important for the understanding of optic nerve damage in diverse conditions that may cause ischemia to the retrobulbar optic nerve.14

The present study employed the diffusable indicator, 14C-iodoantipyrine, for regional measurements of ONBF by quantitative autoradiography. This method has advantages over measurement with radioactive microspheres in regions of relatively low flow, such as optic nerve or cerebral white matter.15 ONBF was measured under basal conditions from the prelaminar optic nerve to the chiasm. In addition, the effect of alteration of MABP upon flow in each region was determined.

Materials and Methods

Eleven female mongrel cats weighing from 2.3 to 5.0 kg were used. Each animal was anesthetized with intraperitoneal ketamine hydrochloride (30 mg/kg), and an endotracheal tube was placed. Each animal was paralyzed with pancuronium bromide (1 mg intraperitoneally) and artificially ventilated with a mixture of 70% nitrous oxide and 30% oxygen. Twenty-gauge polyethylene cannulae were placed in one femoral vein and both femoral arteries. One femoral artery catheter was used for continuous monitoring of systemic blood pressure and allowed anaerobic sampling for blood-gas determination. The second arterial catheter, 50 mm in length, was used to collect arterial samples during radioactive tracer infusion. The femoral vein catheter was advanced to the level of the diaphragm and used for injection of 14C-iodoantipyrine.

From the Division of Ophthalmology, Department of Surgery,* and the Division of Neurology, Department of Medicine,† The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania.

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Reprint requests: Joel M. Weinstein, MD, Division of Ophthalmology, The Milton S. Hershey Medical Center, The Pennsylvania State University, P.O. Box 850, Hershey, PA 17033.
A second femoral vein catheter was placed when infusion of metaraminol was performed. Arterial blood-gas tensions and pH were measured frequently using a Radiometer BMS 3 Mk 2 analysis system. Arterial $P_{CO_2}$ was controlled by adjusting the respirator minute volume. Body temperature was monitored by rectal termistor and maintained at 37.5°C by a servocontrolled heat lamp. Heparin (1,000 IU) was given prior to determining blood flow.

Regional optic nerve blood flow was determined by quantitative autoradiography using $^{14}$C-iodoantipyrine in the manner of Sakurada et al. $^{16}$ $^{14}$C-iodoantipyrine (80 $\mu$Ci/kg, 56 mCi/mM, Amersham) was dissolved in 2 ml of normal saline and injected into each animal at a constant rate. The time course of the arterial concentration of radioactive tracer was determined by continuously collecting blood dropping from the short arterial catheter during the period of tracer injection. Drops were pooled over five-second intervals and 20-$\mu$l aliquots were removed, solubilized, decolorized, and counted in a scintillation counter. After 60 seconds of tracer injection, a bolus of saturated potassium chloride (approximately 3 ml) was injected through the same catheter to stop the heart. The cat was decapitated and the chiasm was treated was removed. The nerve was frozen in liquid Freon-12 at -30°C. Forty-micron longitudinal sections of each nerve were cut in a cryostat (Slee), mounted on glass slides, and dried at 60°C. The chiasm was dissected separately. The intracanalicular portion of each optic nerve was not studied. Each optic nerve was dissected from the globe and the dural nerve sheath was removed. The nerve was frozen in liquid Freon-12 at -30°C. Forty-micron longitudinal sections of each nerve were cut in a cryostat (Slee), mounted on glass slides, and dried at 60°C. The chiasm was treated in a similar fashion. These slides were applied to single emulsion mammography film with a set of $^{14}$C standards. Correction for attenuation of radioactivity by nonradioactive optic nerve sections of various thickness against a $^{14}$C source. Relative penetration was determined by exposing mammography film to this tissue attenuated source.

Regional optic nerve blood flow was determined using the equations presented by Eckman et al. $^{18}$ The arterial concentration curve was fitted by the sum of two exponential terms using a Marquardt-Levenberg algorithm. $^{19}$ Regional $^{14}$C-iodoantipyrine specific activity within the optic nerve was determined by measuring the optical density of the autoradiogram with a single spot densitometer (TCX, Tobias Inc.) with an aperture of 0.3 mm. Localization of each region on the autoradiograph was established by correlation with adjacent stained histologic sections. The immediate prelaminar area generally was located adjacent to the choroid, which appeared on the radiograph as a very black area (Fig. 1). Iodoantipyrine diffusion limitation was assumed to be negligible ($m = 1$), and the partition coefficient ($\lambda$) was assumed to be 0.80. $^{16,19}$ Systemic blood pressure fell rapidly after cardiac arrest induced by intravenous KCl. The end of perfusion was taken as the moment when blood pressure fell to morbid level, determined by extrapolating the slope of the rapid pressure fall to the subsequent morbid steady-state pressure. Arterial sampling of tracer concentration continued during cardiac arrest to document the continued rise in arterial blood radioactivity during this period.

Optic nerve blood flow autoregulation was tested by hypotension induced by exsanguination or hypertensive conditions were maintained for at least 20 minutes before determining blood flow. Optic nerve blood flow determined for animals with mean arterial pressures between 82 and 127 mmHg was considered to reflect the normotensive state.

Intraocular pressure was monitored in seven cats using a pneumotonometer (Digilab) which had been calibrated previously by simultaneous IOP measurements of a directly cannulated anterior chamber. In a separate experiment, the effect of altered MABP upon IOP was determined. The anterior chamber was cannulated with a 25-gauge needle attached by a saline column to a strain gauge pressure transducer. Systemic arterial pressure was increased by metaraminol infusion and decreased by exsanguination to reproduce the range of pressures used in the autoregulation studies. MABP was maintained at each level for 30 minutes.

**Results**

Under conditions of normotension and prior to infusion of $^{14}$C-iodoantipyrine, the mean arterial blood-gas tensions for the 11 animals studied were: $P_{CO_2}$ 26.7 ± 3.2 (SE) mmHg, and $P_{O_2}$ 132 ± 16 mmHg. Mean arterial $pH$ was 7.40 ± 0.05.

The absorption coefficient ($k$) for adult cat optic nerve was 0.007 m$^{-1}$. No variation in $\beta$ particle penetration through optic nerve sections was observed along the length of this structure.

Regional optic nerve blood flow was found to vary along the length of the nerve in all animals studied. A gradient of blood flow was found to exist, with the highest flows in the prelaminar and laminar regions. Blood flow values decreased along the nerve in a gradual fashion from the globe to the optic chiasm. This
gradient was maximal in the first 4–6 mm of optic nerve. The gradient can be seen in a representative autoradiograph of a single 40-micron optic nerve section shown in Figure 1. Of the 11 animals studied, five had mean systemic arterial blood pressures between 82 mmHg and 127 mmHg. MABP for the normotensive group was somewhat less than that previously reported (130–145 mmHg), an effect which we presume is related to the effects of anesthesia, paralysis, and artificial ventilation. The mean optic nerve blood flow for these normotensive cats ranged from 50 ± 2 (SE) ml/100 g/min in the prelaminar region to 13 ± 1 ml/100 g/min for the intracranial optic nerve and chiasm. Flow values under normotensive conditions for other regions of the optic nerve were as follows: laminar, 48 ± 4 ml/100 g/min; 1 mm post-laminar, 40 ± 3 ml/100 g/min; 4 mm post-laminar, 21 ± 1 ml/100 g/min; and 6 mm post-laminar, 18 ± 2 ml/100 g/min. The values for the intracranial optic nerve and chiasm, the 6-mm retrobulbar optic nerve, and the 4-mm retrobulbar optic nerve were each significantly different from the values for the prelaminar, laminar, and 1-mm post-laminar optic nerve (P < 0.01). These figures are displayed in Figure 1. These readings and the appearance of the autoradiograph show a smooth decrease in blood flow along this interval (Fig. 1).

Using exsanguination and metaraminol infusion, mean systemic arterial blood pressures from 41 to 225 mmHg were obtained. Regional ONBF for five optic nerve regions and the optic chiasm are displayed as a
function of mean arterial blood pressure in Figure 2. Autoregulation of optic nerve blood flow is present in each region. In addition, hypertension-induced flow increases are not seen in the retrolaminar areas.

Because distal optic nerve perfusion pressure is related to IOP as well as mean systemic arterial pressure, the effect of alterations of arterial pressure upon anterior chamber pressure was determined. The new relationship between MABP and IOP was established within 5 to 10 minutes and was found to be unchanged when 30 minutes was allowed to elapse at each new level of MABP. The slope of the regression line between these two variables determined in one cat was 0.1. This indicates that the rise in IOP induced by systemic hypertension will be approximately 10% of the systemic blood pressure increment.

**Discussion**

**14C-Iodoantipyrine Autoradiography**

This method has been used extensively in studies of cerebral blood flow and has been found to be particularly accurate in areas of low flow. In the present study, a dose of 80 μCi/kg of 14C-iodoantipyrine injected over 60 seconds resulted in sufficient tissue labeling for determination of ONBF. This method is superior to the radioactive microsphere technique, which may yield inaccurate results due to: (1) insufficient number of microspheres in areas of low flow; (2) axial streaming; and (3) obstruction of arterioles. Noninvasive autoradiographic techniques may also be superior to methods that require insertion of microelectrodes, such as measurement of optic disc P02, H2 washout, or thermal conductivity; however, the effect of insertion of microelectrodes upon optic nerve circulation is unknown.

**Regional ONBF under Basal Conditions**

The vascular anatomy of the optic nerve and retina in cats is somewhat different from that of primates and humans. Despite these differences, the response of the cat optic nerve vasculature to increased intraocular pressure is similar to that of primates. In addition, similar blockade of axoplasmic transport is found in response to increased intraocular pressure. For these reasons, the cat was felt to be a suitable subject for this study.

Our values for regional blood flow for the prelaminar (50 ± 2 ml/100 g/min), laminar (48 ± 4 ml/100 g/min), and 1-mm post-laminar (40 ± 3 ml/100 g/min) optic nerve are somewhat lower than previously published data using other methods. Ernest measured optic disc blood flow at the lamina using an H2 microelectrode (hydrogen clearance method) and reported a flow of 95 ml/100 g/min. Geijer and Bill, using radioactive microspheres, reported a prelaminar flow of 120 ± 30 ml/100 g/min, a laminar flow of approximately 200 ml/100 g/min, and a 1-mm retrolaminar flow of 59 ± 6 ml/100 g/min. Methodologic differences obscure direct comparison of these disparate results, and we have no further explanation for the disparity. Kollarits et al employed a similar method using 14C-antipyrine to determine average flow for the entire orbital optic nerve and obtained results similar to our own. The authors used a nonregional technique to obtain an average value of 29 ml/100 g/min. Although the techniques are not directly comparable, this result is similar to our measurements of between 40 ± 3 and 18 ± 2 ml/100 g/min for the three regions of the retrobulbar optic nerve.

Our flow value for intracranial optic nerve, and chiasm (13 ± 1 ml/100 g/min) is also similar to previously reported results. Weinstein et al measured
intracranial ONBF in sheep using radioactive microspheres and obtained a value of 14 ± 1 mg/100 g/min. These values are similar to previously reported measurements by Sakurada et al of 22 ± 1 ml/100 g/min for cerebral white matter in the cat. 16 Kollarits et al11 using 14C-antipyrine in Rhesus monkeys, obtained a flow of 30 ± 2 ml/100 g/min for the chiasm. This disparity may be caused by the fact that Kollarits et al used a method that is not sensitive to inhomogeneities within a region of the optic nerve, ie, the authors dissolved the entire chiasm in scintillation fluid and measured its radioactivity. As may be noted in our autoradiographs of the chiasm (Fig. 1), a peripheral area of increased radioactivity is present. Our measurements were made from the central homogeneous area of the chiasm and intracranial optic nerve. Inclusion of the more peripheral area, as in the method of Kollarits et al, would result in a higher calculated flow. The significance of the increase in radioactivity at the periphery of the chiasm is unclear.

Of particular interest is the gradient of blood flow (under basal conditions) from the prelaminar optic nerve to the chiasm, with a particularly sharp decline across the lamina cribrosa. Although Geiger and Bill2 also noted a drop in flow across the lamina under basal conditions, no explanation for this phenomenon was offered. It is possible that our measurements for the distal optic nerve were somewhat lower than the true blood flow caused by leakage of tracer from the distal optic nerve into the vitreous. If this is true, the gradient might be even greater than indicated by our measurements. We considered the possibility that the measured flow values for distal OMBF were elevated artifactualily by leakage of tracer from the choroid. If this phenomenon occurred, we would expect blurring of the border between choroid and optic nerve with gradually decreasing labeling from the periphery of the nerve to the center. As can be seen from our autoradiograph (Fig. 1), this phenomenon does not occur and the borders of the choroid are quite sharp and very distinct from the optic nerve.

We considered the possibility that the gradient of flow might reflect a gradient of metabolic requirements along the course of the optic nerve. We are aware of the decreased proportion of metabolically active axoplasm (as opposed to metabolically inactive myelin) in the myelinated retrobulbar optic nerve. This modest decrease in the relative proportion of metabolically active tissue would hardly account for the two- to threefold difference in flow, however. We are unaware of other comparative histologic evidence, such as capillary or mitochondrial counts, which would suggest a gradient of metabolic activity along the nerve. Although mitochondria are in higher concentration in the deeper optic nerve head25 and accumulate at the lamina under conditions of axoplasmic flow stasis,24 we are unaware of studies of the mitochondrial population of the orbital or intracranial optic nerve. At this point, we have no satisfactory explanation for the gradient of blood flow along the optic nerve.

The Effect of Alteration of MABP upon ONBF

ONBF is plotted as a function of MABP in Figure 2. Although perfusion pressure for the prelaminar and anterior laminar optic nerve would be more accurately reflected by MABP-IOP, IOP changed little in absolute terms over the range of arterial blood pressure in the experiment. The shape of the autoregulatory curve, therefore, would have been similar for ONBF as a function either of MABP or [MABP-IOP], although the curve would have been shifted slightly to the left. ONBF, therefore, was plotted as a function of MABP in each region. Figure 2 illustrates autoregulation of ONBF in each region. It is possible that the stability of ONBF with increased MABP is partly due to vasoconstriction of the optic nerve vessels mediated by metaraminol rather than by true autoregulation, as has been suggested by Sossi and Anderson. Although we cannot exclude this possibility, it would seem unlikely in view of the failure of alpha-adrenergic agents to change cerebral blood flow following direct intracarotid injection.26

It has been suggested that at least for short-term changes in MABP, compensatory changes in IOP occur that tend to minimize changes in perfusion pressure for the distal optic nerve. These changes in IOP may contribute to some extent to stabilization of blood flow in the distal optic nerve (pseudo-autoregulation, as may occur with increased intracranial pressure due to arterial hypertension). We do not know whether this mechanism is effective when changes in MABP are maintained for longer intervals. We are uncertain to what extent this response represents changes in choroidal vascular volume, facility of outflow, aqueous production, or a combination of these factors. In absolute terms, this phenomenon probably contributes very little. At the low end of the curve, decreasing MABP from 100 to 50 would result in a true decrease in perfusion pressure of 45 mmHg instead of 50 mmHg. Further posteriorly in the nerve, compensatory changes in IOP would have comparatively little effect upon perfusion pressure.

Prior studies of distal ONBF autoregulation (with alteration of IOP) have yielded conflicting results. Alm and Bill1 and Geiger and Bill2 studied autoregulation of the distal optic nerve using the microsphere technique. The first study found impaired autoregulation. The second study concluded that autoregulation was intact. As noted by the authors of both studies, however, their specimens contained a relatively small
number of microspheres, a methodologic problem known to introduce a high degree of variability. The small number of microspheres was related to the relatively low blood flow in the optic nerve. Ernest and Potts studied autoregulation of optic disc blood flow using temperature as an indirect index of regional blood flow. These authors concluded that autoregulation did not occur with increased IOP. In a later experiment, Ernest found autoregulation of oxygen tension (not blood flow) when optic disc perfusion pressure was lowered by increasing IOP. In another study, Ernest measured blood flow in the lamina using a micro-electrode hydrogen clearance method. The author found that autoregulation was present with changes in IOP. Sossi and Anderson found good autoregulation of the distal optic nerve in cats with changes in IOP using 125I-iodoantipyrine, a method similar to our own. Their lower limit for autoregulation was about 30 mmHg. Although we cannot precisely define the break point for autoregulation in our prelaminar and laminar optic nerves, blood flow is significantly different from mean values in the prelaminar and laminar optic nerve at MABPs of 52 mmHg and 63 mmHg, respectively. If IOP was approximately 15 mmHg, this would correspond to perfusion pressures of between 37 mmHg and 48 mmHg, a value only slightly higher than that of Sossi and Anderson.

Inspection of the autoregulatory curves shows that blood flow is reduced to 50–60% of the mean at MABP below 52 mmHg in each region. Although we cannot precisely define the limits of autoregulation for each region, inspection of the curve suggests similar autoregulatory capacity throughout the nerve. For the limited number of animals studied, autoregulation appears to be as efficient for the prelaminar and laminar optic nerve as for the postlaminar segment. Our data suggest that the selective vulnerability of the distal optic nerve noted clinically under conditions of arterial hypotension ("shock-induced optic neuropathy") is not caused by less efficient autoregulation in this region. The gradient of flow that we have described suggests the possibility of increased metabolic activity in the distal optic nerve, a hypothesis which may be tested by autoradiographic studies employing metabolic tracers such as 14C-glucose. If this is the case, the distal optic nerve may be more susceptible to small decrements in blood flow because of increased metabolic requirements. The relationship of these observations to arteriosclerotic ischemic optic neuropathy remains unclear.

We know of no prior studies of ONBF autoregulation of the orbital (>4 mm retrobulbar) optic nerve. Although Kollarits measured ONBF in the retrobulbar optic nerve, autoregulation was not studied. Intact autoregulation of the intracranial optic nerve has been reported previously by Weinstein et al using the microsphere technique in sheep.

Our results neither support nor exclude an ischemic mechanism as the primary cause for optic nerve damage in human glaucoma. It is possible that a decrease in arterial perfusion pressure for the entire optic nerve (resulting from systemic arterial hypotension) may result in a pattern of blood flow that is quite different from that which occurs when perfusion pressure of the distal optic nerve is selectively decreased by increasing IOP; eg, shunting of blood away from the optic nerve head may occur in the latter situation as proposed originally by Gafner and Goldmann, and later suggested by Geijer and Bill. Further studies are needed to clarify what role, if any, is played by ischemia in human glaucoma.

Key words: optic nerve, blood flow, glaucoma, anterior ischemic optic neuropathy

References


14. Miller NR: Retrobulbar ischemic optic neuropathies. In Walsh


