The Response of Retinal Vasculature to Angiotensin

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A retinal arterial constriction was produced in anesthetized cats with a continuous transvitreal infusion of angiotensin I or antitensin II (Ile-5). Constriction of vessels near the infusion cannula tip occurred over a range of angiotensin II concentrations from $10^{-9}$ to $10^{-4}$ molar, and was reversibly blocked by a ten-fold excess of the competitive antagonist saralasin. Constriction did not occur in response to angiotensin I if angiotensin-converting enzyme was blocked with Captopril. Control infusions of saline did not elicit a contraction of the retinal arteries. Severe axonal and inner retinal damage and necrosis occurred when angiotensin II produced a prolonged vasospasm, but not after infusion with control solutions, or when constriction caused by angiotensin was brief. Invest Ophthalmol Vis Sci 28:676-682, 1987

While studying the effects of elevated intraocular pressure on the physiology of the optic nerve head, Sossi and Anderson1 found that the blockage of axonal transport by elevated intraocular pressure was augmented when angiotensin was used to elevate the systemic arterial blood pressure. These findings suggested that angiotensin is capable of constricting the vessels of the optic nerve head, or at least capable of preventing autoregulatory dilation of the vessels when the adequacy of blood flow is challenged by elevation of intraocular pressure. There are interesting potential clinical correlations and implications if such a pathophysiologic mechanism participates in the optic nerve damage of glaucoma.2

Constriction of vessels in the central nervous system (CNS), including the retina and optic nerve,3,4 might be expected as an autoregulatory response to elevated blood pressure. Such an indirect autoregulatory response (not due to angiotensin acting on receptors of the vessels) should not incapacitate the autoregulatory system, or produce constriction that is excessive to the point of interfering with neuronal physiology. Thus the interference with axonal transport suggests that angiotensin may be exerting direct vasoconstrictive action on the vessels of the optic nerve head, with the muscular tone preventing their redilation as an autoregulatory response to tissue ischemia.

Such a direct effect on CNS vessels (including the retina and optic nerve) is unexpected, because the tight junctions of the endothelium should preclude access of circulating octapeptide angiotensin II (MW: 1067 Daltons) to the muscular coat of the vessels. Thus, the vessels should not have a direct response to angiotensin, unless either there are angiotensin receptors on the endothelium, which in turn transmit a message to the muscular coat, or else there is access to the muscular coat through a breach in the blood–brain barrier. We are attracted to the latter explanation for our experimental result, because the axonal transport abnormalities were localized at the optic nerve head, where there exists a breach in the blood–brain barrier by virtue of unimpeded diffusion of substances from the choroid.5

Although our experiments indicated that these vessels may constrict in response to extravasated angiotensin, there is reason to doubt that the exterior wall of CNS vessels would have receptors for a circulating hormone to which they are not exposed under normal circumstances. Therefore, experiments were conducted to determine whether or not CNS vessels (specifically those of the retina and optic nerve head) exhibit a specific direct response to angiotensin when it bathes the exterior surface of the vessel.

Materials and Methods

All applicable federal and ARVO guidelines for care and use of animals for experimentation were followed. Cats of either sex (weight 1.5–5.0 Kg) were anesthetized with intraperitoneal nembutal (30 mg/kg) and atropine (0.035 mg/kg). Animals with signs of ocular trauma, inflammation, synechiae, or retinal scarring were not used. Some animals were given intravenous indo-
methacin (20 mg/kg) dissolved in normal saline with pH+ adjusted to 8.0–8.5 by 1.0 normal NaOH to enable more satisfactory solubilization. In these animals, Indomethacin was used to block the generation of prosstaglandins in the eyes during experimentation. Atropine 1% solution was used topically to dilate the pupil.

The cats were placed in a lateral recumbent position with the head stabilized. Respirations were spontaneous in room air, and the animal was wrapped in a blanket to reduce the loss of body heat. No artificial ventilation was used. In many animals, arterial pressure monitoring was performed by cannulation of the left femoral artery. A lateral canthotomy was performed, and episcleral traction sutures were placed. Through a conjunctival incision, a sclerotomy was made with a 20-gauge needle 4–5 mm posterior to the limbus. A 30-gauge cannula was then passed through the sclerotomy and positioned over a major retinal artery or the optic disc. A preloaded tuberculin syringe was mounted on a continuous infusion pump, and connected to the cannula with flexible tubing. Final positioning of the cannula was performed under direct visualization with a posterior pole lens and an operating microscope. In cases where the retina was injured by cannula placement, the experiment was terminated.

Fresh solutions of angiotensin I, angiotensin II (Sigma, St. Louis, MO) and saralasin (courtesy of Norwich-Eaton Pharmaceuticals Inc., Norwich, NY) or Captopril (courtesy of E.R. Squibb & Sons, Inc., Princeton, NJ) were prepared immediately prior to use in Intraocular Balanced Salt Plus (BSS⁺) Solution (Alcon Laboratories, Fort Worth, TX) with parts I and II reconstituted as directed. In order to avoid adsorption of the low concentrations of angiotensin and saralasin, glass containers were not used. Infusions were initiated 1 hr after indomethacin treatment when indomethacin was used. Duration of infusions was from 1–4 hr at a rate of 300 µl/hr. Control infusions were either the reconstituted BSS⁺ solution alone, or a solution of angiotensin II with a ten-fold excess of the competitive antagonist saralasin (sar₁-al₈ angiotensin II).

Fundus photography was performed with a Nikon MD-12 camera with Ektachrome ASA-100 film (Eastman Kodak, Rochester, NY). When tissues were to be examined microscopically, the abdominal aorta was cannulated, and a 2–3 min retrograde perfusion of normal saline was used to rinse out the vascular tree, followed by a perfusion of 5% glutaraldehyde in 0.1 m phosphate buffer (pH 7.3). Specimens for electron microscopy were postfixed in osmium tetroxide (2%). Sections for electron microscopy were stained with uranylacetate and lead nitrate. Histopathological results were used only in cases where excellent fixation of tissues occurred, and where the fellow eye had not been treated and could serve for comparison to the angiotensin-treated fellow eye.

### Results

Infusions of angiotensin I or II through a transvitreal cannula placed near the retinal surface or optic nerve resulted in observable constrictions of the retinal arteries over a wide range of concentrations. Pretreatment with intravenous indomethacin did not noticeably affect the subsequent response of cat retinal arteries to angiotensin II (Table 1). The constrictive response began as a focal vasospasm in the region of the retinal artery nearest the infusion cannula tip. This usually began 3–15 min after initiation of the infusion, and spread both proximally toward the optic nerve and distally (Figs. 1–4). Many animals demonstrated tachyphylaxis, so the response only lasted 7–15 minutes, with the first area to constrict also being the first to relax. Histopathologic and ultrastructural examination of these retinas failed to demonstrate any evidence of ischemic damage. Many other animals developed a prolonged arterial constriction that persisted for the duration of the experiment (3 or 4 hr). Retinal haziness developed in these cases (Fig. 4). Histopathologic examination of these eyes demonstrated moderate to severe inner retinal (but not outer retinal) damage after periods of infusion of angiotensin from 2–4 hr (Figs. 5–6). In the angiotensin-treated eyes that demonstrated inner retinal damage, the muscular arterioles had a configuration that suggested active constriction at the time of fixation (Figs. 7, 8). Electron micrographs confirmed cellular swelling and disintegration in the inner retina typical of ischemia (Figs. 9, 10).

Control infusions (Intraocular BSS⁺) failed to elicit a constriction of retinal arteries (N = 13), and did not result in any discernable retinal damage. Infusion of the competitive inhibitor saralasin failed to elicit a constriction of the retinal arteries. A 1-hr,
Fig. 1. (top, left) Fundus of the left eye of a cat prior to angiotensin II infusion. The 30-gauge infusion cannula is in position, near to a major retinal artery (arrow) and vein.

Fig. 2. (top, right) Same fundus after ten minutes of continuous angiotensin infusion ($9 \times 10^{-5}$ molar). Note mild constriction of branch retinal artery (arrow). The retinal vein (larger caliber vessel) is diffusely narrowed.

Fig. 3. (bottom, left) Twenty minutes after start of infusion. Note marked arterial constriction (arrow) in proximity to the tip of the infusion cannula.

Fig. 4. (bottom, right) Severe attenuation of retinal arterial vessels (arrow) 1 hr after start of infusion. Note that the constriction has spread distally from the previous site of cannula placement to involve almost the entire arterial tree within view of the photograph. There is extensive retinal haziness.
pre-infusion of saralasin failed to block the constrictive effect of subsequently infused angiotensin II (three of four trials). However, when the subsequently infused antidiotensin II solution also contained saralasin in a ratio of 10:1 ([Ang II] = 8 \times 10^{-6} \text{ M}, [saralasin] = 8 \times 10^{-5} \text{ M}), the constriction was blocked (N = 5).

Similarly, captopril, an angiotensin converting enzyme inhibitor, blocked the response of retinal arteries to angiotensin I (N = 6).

Systemic mean arterial pressure did not change during intraocular angiotensin infusions.

**Discussion**

This study demonstrated that, over a wide range of concentrations, the potent vasoactive octapeptide, angiotensin II, has a specific vasoconstrictive effect on the cat retinal arteries when administered into the vitreous. When sustained, the response resulted in retinal and optic nerve infarction from severe retinal arterial constriction. These effects are not seen with a control solution (Intraocular BSS-plus), they occur at the lowest concentrations of angiotensin known to be physiologically active, and they are blocked by the competitive antagonist saralasin. Also, the response to angiotensin I, the decapeptide precursor of angiotensin II, is prevented by Captopril, a converting enzyme inhibitor. The localized nature of the vasospastic response and the lack of a significant elevation in mean arterial pressure show that the constrictive effect is a direct effect, and not an indirect autoregulatory response to increased arterial systemic blood pressure.

Although measurements of actual blood flow were not made in this investigation, the axonal and inner retinal damage achieved is evidence that a significant decrease in blood flow did occur with angiotensin II. Certainly there was sufficient ischemia to produce the chemical messengers (hypoxia, hypercapnia, acidosis) that call for autoregulatory vasodilation, but evidently angiotensin can overwhelm the autoregulatory mechanisms of the retina sufficiently to lead to necrosis. The
Fig. 7. Retina treated with angiotensin II (1.5 x 10^{-7} molar) for 2 hr. Note the tightly constricted conformation of the retinal artery cut in cross-section. The vessel wall thickness is increased relative to the vascular lumen diameter, and endothelial cell nuclei protrude toward the vessel lumen (715X). Compare to Figure 9.

ability to limit or to overcome the autoregulatory response would be an important characteristic of circulating vasoconstrictors, if it is to be speculated that they help determine the level of intraocular pressure that will damage the optic nerve in glaucoma.2

We cannot explain why, in some eyes, the vascular constriction persists and, in others, it is short-lived, despite continued infusion of the angiotensin. Tachyphylaxis to the angiotensins is an established phenomenon both in vivo and in vitro,7-10 and persistence of tachyphylaxis may range from 1-1½ hr after cessation of angiotensin administration.7,11 Tachyphylaxis is a dose-related phenomenon,7,12 with a greater degree of tachyphylaxis at the higher doses or concentration of angiotensin used. However, in our experiments, both the unsustained and the sustained vasoconstrictive responses occurred at both the nonphysiologic high doses of angiotensin II, and the more physiologic doses. The occurrence or nonoccurrence of tachyphylaxis was unpredictable, and we were unsuccessful in preventing tachyphylaxis by adjusting the angiotensin concentration or by varying the pH+ of the infusate. It should be noted that the concentration of angiotensin infused is not the concentration to which the retinal artery is exposed. Diffusion gradients and tissue proteases would reduce the effective concentration delivered to the retinal arteries.

We also could not prevent tachyphylaxis by administering indomethacin. Prostaglandins are known to decrease the effectiveness of angiotensin,13 and as a result may cause tachyphylaxis. Therefore, indomethacin pretreatment was used to block the generation of prostaglandins, but both transient and prolonged responses occurred in each of the indomethacin-pretreated and the non-pretreated groups of animals.

Finally, we could not correlate the dose of anesthetic...
agent or the depth of anesthesia with the occurrence of transient or sustained responses to angiotensin. However, in principle, the anesthetic agent could affect responsiveness in our system (but could not explain the in vitro tachyphylaxis demonstrated by others). For example, barbiturates depress respiratory activity,\(^{14}\) and resultant elevations of pCO\(_2\) could have an effect on autoregulatory capacity. Further, barbiturate anesthesia diminishes metabolic demand of the central nervous system,\(^{13}\) and presumably also of the retina. Barbiturate-induced diminution of O\(_2\) demand could indirectly influence autoregulatory requirements by varying the amount of tissue ischemia generated by a reduced blood flow. Edvinsson and McCulloch\(^{15}\) found that pentobarbitone diminished the response of isolated feline middle cerebral arteries to potassium, norepinephrine and prostaglandin F\(_2\). The question of whether or not barbiturate anesthesia also has its own direct effect on cerebral vasculature is not clear. Chin et al\(^{16}\) demonstrated that pentobarbitone in the live dog decreased cerebral venous outflow in proportion to decreased O\(_2\) demand, suggesting no direct effect on the cerebral vasculature. It must be remembered that in vivo responsiveness in an awake individual may not be the same as is shown in an anesthetized experimental animal.

In 14 of the total seventy angiotensin II treated eyes, there was no notable constriction. It is possible that there was no reaction to the angiotensin II, suggesting an absence of angiotensin receptors in these animals. An alternative explanation is that we failed to measure small changes in vascular caliber that were within the range of error in our photographic measurement technique. The latter possibility would suggest a difference in autoregulatory capabilities between these different eyes, and could also be a manifestation of different depths of anesthesia.

Further investigation will include attempts to identify and localize retinal and optic nerve vascular re-

\(\text{Fig. 9.} \) Electron micrograph of an area of severe disruption in the nerve fiber layer (7100X), near the optic disc of eye treated with angiotensin II (9 \times 10^{-3} \text{ mol})

\(\text{Fig. 10.} \) Normal nerve fiber layer in control eye (7200X).
ceptrons for angiotensin II, and possibly other vasoactive substances.

Key words: angiotensin, optic nerve blood flow physiology, glaucomatous cupping, autoregulation of blood flow, retinal arteries

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References