Local Action of the Renin Angiotensin System in the Porcine Ophthalmic Circulation: Effects of ACE-Inhibitors and Angiotensin Receptor Antagonists

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Purpose. The renin angiotensin system and endothelium-derived substances are important regulators of the microcirculation. The authors studied the roles of angiotensins (Ang), angiotensin converting enzyme (ACE)-inhibitors, and Ang II-receptor antagonists in the porcine ophthalmic circulation.

Methods. Isolated porcine ciliary arteries were studied in myographs and the intact porcine eye in a perfusion system at 80 cm H2O perfusion pressure with Krebs-ringer bicarbonate solution (37°C, 95% O2, 5% CO2).

Results. ACE-inhibitors enalaprilat and benazepril (both 10^-5 M) did not change ciliary vascular tone nor flow of perfused porcine eyes. However, enalaprilat or benazepril enhanced the relaxation of ciliary arteries to bradykinin (P<0.02). In the perfused porcine eye, enalaprilat (10^-5 M) augmented vasodilation to bradykinin (P<0.02). The bradykinin antagonist Hoe 140 (3 X 10^-7 M) prevented the relaxation of ciliary arteries to bradykinin (P<0.001), but not to acetylcholine. In perfused eyes, Hoe 140 reduced the vasodilation to bradykinin (P<0.01). Ang II (10^-10 to 10^-6 M) evoked a contraction of ciliary arteries and was more potent than Ang I. Enalaprilat abolished the effect of Ang I. The AT1-receptor antagonist, valsartan (10^-3 to 10^-3 M; 30 minutes) inhibited the response of ciliary arteries to Ang II, whereas the AT2-receptor ligand CGP 42112 B (10^-7 to 10^-8 M) was ineffective. In the perfused porcine eye, valsartan restored the decrease in flow to Ang II.

Conclusions. Angiotensins play an important regulatory role in the porcine ophthalmic microcirculation through AT1-receptors. ACE-inhibitors prevent the effects of Ang I and augment endothelium-dependent relaxation to bradykinin, which releases nitric oxide through B2-receptors. Invest Ophthalmol Vis Sci. 1995; 36:555-562.

The renin angiotensin system is an important regulatory system in the circulation. Recently, circulatory as well as tissue vascular renin-angiotensin systems have been delineated. Angiotensin II binding sites and angiotensin converting enzyme (ACE) activity have recently been detected in human, feline, and bovine retinal vascular tissue. Angiotensin II can exert contractile effects in human and bovine posterior ciliary arteries, but not in bovine retinal arteries. Renin is the enzyme that transforms angiotensinogen into angiotensin I. Endothelial cells play a crucial role in the activation of the biologically inactive angiotensin I into angiotensin II because the ACE is located on the endothelial cell membrane. Depending on the vascular tissue, however, non-ACE-peptidases may also contribute to the conversion of angiotensin I. Angiotensin II is a potent vasoconstrictor and also evokes proliferation and migration of vascular smooth muscle cells by activation of specific angiotensin receptors. Several subtypes of angiotensin receptors recently have been characterized.

The most important subtypes are AT1 and AT2 receptors. Although it appears that in peripheral vessels AT1 receptors are mediating vasoconstrictor responses to angiotensin II, the physiological role, if any, of angiotensin AT2 receptors remains unclear.

In addition to its important role in the activation...
Tissue Harvesting

of angiotensin II, ACE inactivates bradykinin. Bradykinin is released from endothelial cells and is capable of activating specific receptors on the endothelium linked to the formation of prostacyclin and nitric oxide. Indeed, the activation of the L-arginine–nitric oxide pathway by bradykinin accounts in large part for its hemodynamic effects in the peripheral circulation as well as in the ophthalmic circulation. Hence, in certain, but not all, peripheral arteries (depending on whether they express enough ACE), ACE-inhibitors are able to augment the effects of bradykinin through inhibition of the breakdown of this agonist. The latter effects may be important for the vascular protective effects of ACE-inhibitors because nitric oxide is a potent vasodilator and an inhibitor of platelet function. Furthermore, nitric oxide has antiinflammatory and antiproliferative properties. However, these beneficial effects of ACE-inhibitors cannot be demonstrated in all blood vessels. Indeed, although in the human coronary artery and saphenous vein ACE-inhibitors markedly augment the effects of bradykinin, the drugs have no effect in the human internal mammary artery, presumably because this blood vessel expresses very low levels of converting enzyme.

The present study was performed to investigate the regulatory role of the local renin-angiotensin system, as well as the effects of the ACE-inhibitors and newly developed specific angiotensin II-receptor antagonists in the porcine ophthalmic circulation.

MATERIALS AND METHODS

Tissue Harvesting

Porcine eyes with surrounding tissue were obtained from a slaughterhouse 10 minutes after death and transported in cold (4°C) modified Krebs–Ringer bicarbonate solution containing: NaCl 118.6 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; MgSO₄ 1.2 mM; KH₂PO₄ 1.2 mM; NaHCO₃ 25.7 mM; edetate calcium disodium 0.026 mM, and glucose 11.1 mM. All experiments adhered to the ARVO Statement for the use of Animals in Ophthalmic and Vision Research.

Myograph System for Isolated Extraocular Arteries

Under a microscope, a short segment (7 to 8 mm) of the ciliary arteries (the two main branches of the common ophthalmic artery) was dissected and cut into small rings (2 mm) as described. During the preparation procedure, the tissues were constantly kept in control solution.

After preparation, rings (diameters ranged between 200 to 400 μm) were immediately mounted in a myograph system. Two tungsten wires (diameters 40 μm and 80 μm) were passed through the lumen. One (80 μm) was connected to a force transducer (Showa Sokki LB-5, Rikadenki GmbH, Freiburg, Germany), and the other (40 μm) was fastened to a micromanipulator (Narishige, Tokyo, Japan) for adjustment of muscle length. The mounted rings were immersed in organ chambers filled with 12.5 ml of control solution (37°C; 95% O₂, 5% CO₂, pH = 7.4) and equilibrated for 45 minutes.

The vessels were stretched stepwise at increasing tension, and at each level of tension they were exposed to 100 mM KCl. The optimal passive tension was defined as that tension at which the contraction to 100 mM KCl was maximal; this tension averaged 977 ± 60 mg in ciliary arteries (n = 6). In all subsequent experiments, arterial rings were stretched slowly in steps of 100 mg until optimal tension was reached. The active resting tone was defined as the difference between optimal passive tension and tension after maximal relaxation with bradykinin (10⁻⁴ M) of these blood vessels, and it averaged 174 ± 38 mg (n = 6).

Before the experiment, endothelial function was checked in each ring by adding bradykinin (3 × 10⁻⁷ M) on top of a contraction to serotonin (3 × 10⁻⁷ M). If bradykinin evoked a full relaxation, the endothelium was considered functionally intact. Repeated concentration–response curves to bradykinin were fully reproducible (not significant; n = 6, data shown under reference 36).

Perfusion System of the Intact Porcine Eye

The fatty tissue surrounding the extraocular muscles was carefully removed. During preparation, the ocular globes were constantly kept in cold (4°C), modified Krebs–Ringer bicarbonate solution. The globes were then cannulated under a microscope (Wild & Leitz, Zürich, Switzerland) with a short polyethylene tube (diameter 600 μm) through the common ophthalmic artery and secured with two sutures (80 cm H₂O) and temperature (37°C). After preparation, the ocular globe with the tube inserted in the common ophthalmic artery was immediately connected with a needle (diameter 500 μm) to a Langendorff perfusion system according to Schuler (Hugo Sachs Elektronik KG, Freiburg, Germany) and perfused within 1 hour of death with previously filtered (filter paper circles: middle fine, crystalline; Schleicher & Schuell, Dassel, Germany) and oxygenated (95% O₂, 5% CO₂) Krebs solution containing 0.5% albumin (control solution) at constant pressure (80 cm H₂O) and temperature (37°C).

Catheterization of the common ophthalmic arteries assures complete perfusion of the choroidal and retinal vascular systems as well as small extraocular muscular arteries. The perfused ocular globe, hanging with the cornea downward, was immersed in a...
were added after this period. If sudden changes in double-jacketed glass bath (volume: 200 ml) filled with endothelium and ophthalmic microcirculation 557 vessels were incubated with bradykinin-antagonist were tested by adding the drug on top of a contraction in which the eyeglobes were immersed corresponded control solution (37°C). The overflow from the bath in which the eyeglobes were immersed corresponded double-jacketed glass bath (volume: 200 ml) filled with control solution (37°C). The overflow from the bath in which the eyeglobes were immersed corresponded to ophthalmic flow (ml/min) and was collected during 1- to 5-minute periods. Experiments were started after at least 20 minutes of equilibration; all drugs were added after this period. If sudden changes in double-jacketed glass bath (volume: 200 ml) filled with control solution (37°C). The overflow from the bath in which the eyeglobes were immersed corresponded to ophthalmic flow (ml/min) and was collected during 1- to 5-minute periods. Experiments were started after at least 20 minutes of equilibration; all drugs were added after this period. If sudden changes in flow occurred after equilibration, the tissues were discarded. In time control experiments, ophthalmic flow (2.7 ± 0.2 ml/min at 80 cm H2O) remained constant for as long as 120 minutes (n = 6). Hence, all experiments were performed within 120 minutes of starting perfusion.

Protocols

Myograph System. For each series of experiments, one ring of porcine ciliary artery from one eye of one animal was used for study; n, therefore, refers to the number of animal studies in each series of experiments. After confirming endothelial function (see Myograph System for Isolated Extracocular Arteries) in each ring, the effects of increasing concentration (cumulative concentration–response curves) of bradykinin (10^-9 to 10^-6 M) were tested by adding the drug on top of a contraction evoked by serotonin (3 × 10^-7 M). After washout, vessels were incubated with enalaprilat or benacepril (both 10^-9 M; 30 minutes). Rings were again contracted with serotonin (3 × 10^-7 M), and bradykinin (10^-9 to 10^-6 M) was given on top of an equally potent contraction. In another series of experiments, the effects of increasing concentrations (cumulative dose–response curves) of bradykinin (10^-9 to 10^-6 M) or acetylcholine (10^-9 to 10^-5 M) were tested by adding the drug on top of a contraction evoked by serotonin (3 × 10^-7 M). After washout, vessels were incubated with bradykinin–antagonist Hoe 140 (3 × 10^-6 M); rings were again contracted with serotonin, and bradykinin (10^-9 to 10^-6 M) or acetylcholine (10^-9 to 10^-7 M) was given on top of a comparable contraction. Time control experiments demonstrated excellent reproducibility of the response to bradykinin. In other experiments, rings were contracted with angiotensin II (10^-8 M, 10^-7 M, or 10^-6 M; one dose per vessel, because of tachyphylaxis with repeated applications). The effect of angiotensin II (10^-7 M) was studied after preincubation with angiotensin II receptor antagonist subtype AT1, valsartan (10^-9 to 10^-5 M, 30 minutes), or after preincubation with angiotensin II receptor ligand subtype AT2, CGP 42112 (10^-7 M, 30 minutes) compared to time control without any antagonists. In some experiments, rings were contracted with angiotensin I (10^-8 M), and enalaprilat (10^-5 M) was added on top of a contraction evoked by angiotensin I.

Perfusion System. The effects of bradykinin (10^-9 to 10^-7 M) were tested by adding cumulative dosages of the drug to the next respective perfusate. In some experiments, the effects of bradykinin (10^-9 to 10^-7 M) were tested after the incubation (30 minutes) and continued presence of enalaprilat (10^-5 M). To study the effect of the bradykinin antagonist Hoe 140, bradykinin (10^-9 to 10^-6 M) was applied in different eyes in the presence or absence of an infusion of Hoe 140 (3 × 10^-7 M and 3 × 10^-6 M, started 30 min before the addition of bradykinin). The effect of angiotensin II (10^-9 M) was studied under control conditions and after prior infusion of the antagonist of the AT1 receptor (10^-5 M, 30 minutes).

Drugs. Drugs were obtained from the following sources: Bradykinin, indomethacin, angiotensin I, and angiotensin II from Sigma (St. Louis, MO); bradykinin–antagonist (Hoe 140) from Hoechst Pharmacutica (Frankfurt, Germany); enalaprilat from Merck, Sharp & Dohme (Rahway, NJ); angiotensin II AT1 receptor antagonists valsartan and AT2 ligand CGP 42112 from Ciba Geigy, Basel, Switzerland. All drugs were dissolved in distilled water except indomethacin, which was dissolved in 10^-5 M Na2CO3. All concentrations are expressed as final molar concentrations in the perfusate.

Statistical Analysis

Results are given as mean ± standard error of the mean. N equals the number of animals used for a given experimental protocol, in which only one eye per animal was used. The dose–response curves were fitted with the linear model (least squares method) and compared with analysis of variance for repeated measurements. In some experiments, paired or unpaired Student’s t-tests were used for statistical analysis. A two-tailed P value smaller than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Bradykinin

Effect of ACE-Inhibition. In isolated ciliary arteries with endothelium, bradykinin (10^-9 to 10^-6 M) evoked concentration-dependent relaxations (Fig. 1; n = 6).
The addition of ACE-inhibitors enalaprilat (10^-5 M) or benazepril (10^-5 M) did not change tension of the blood vessels. However, enalaprilat or benazepril enhanced the relaxation of ciliary arteries to bradykinin (Fig. 2; P < 0.02 versus control for enalaprilat and benazepril; n = 6 to 7). Figure 1 shows a representative myograph recording of the effects of benazepril (10^-5 M) on the relaxation evoked by bradykinin (10^-9 to 10^-6 M). The area under the concentration-response curves was decreased after incubation with enalaprilat or benazepril as compared to control (P < 0.02 versus control).

In perfused porcine eyes, bradykinin (10^-9 to 10^-7 M) caused concentration-dependent increases in ophthalmic flow (Fig. 3; P < 0.05, analysis of variance for repeated measurements; n = 5); the absolute increase of flow averaged 0.2 ± 0.05 ml/min for 10^-9 M, 0.21 ± 0.04 ml/min for 10^-8 M, and 0.3 ± 0.06 ml/min for 10^-7 M bradykinin. In contrast, in time-control experiments, flow did not change. After pretreatment of the eyes with ACE, enalaprilat (10^-5 M; 30 min) flow did not change, but the vasodilator effects of bradykinin were augmented (Fig. 3; left panel, P < 0.02 versus control effect of bradykinin, analysis of variance for repeated measurements; n = 5; data not shown). In perfused porcine eyes, the bradykinin antagonist Hoe 140 (3 × 10^-7 M or 3 × 10^-6 M) was infused for 30 minutes. No change in ophthalmic flow occurred during infusion of the antagonist (not significant; n = 5; data not shown). The vasodilator effects of bradykinin, however, were reduced in a concentration-dependent manner in the presence of Hoe 140 (Fig. 3; right panel, n = 5; area under the curve for Hoe 140 3 × 10^-7 M not significant, for Hoe 140 3 × 10^-6 M < 0.01). After incubation with Hoe 140 (3 × 10^-7 M), the flow increase to bradykinin was 0.05 ± 0.02 ml/min for 10^-9 M, 0.1 ± 0.02 ml/min for 10^-8 M, 0.21 ± 0.05 ml/min for 10^-7 M, and 0.22 ± 0.02 ml/min for 10^-6 M (not significant versus control). After incubation with Hoe 140 (3 × 10^-6 M), the changes in flow of bradykinin were 0.08 ± 0.04 ml/min for 10^-9 M, -0.07 ± 0.02 ml/min for 10^-8 M, -0.05 ± 0.05 ml/min for 10^-7 M, and -0.15 ± 0.04 ml/min for 10^-6 M bradykinin (not significant versus control).

Angiotensins

Effect of ACE Inhibition. In quiescent ciliary arteries with endothelium, angiotensin I (10^-6 M) evoked a stable vasoconstriction (Fig. 5; P < 0.01 versus control; n = 5). The same concentration of angiotensin II (10^-6 M) induced a greater contraction compared to angiotensin I (P < 0.01). After precontraction with angiotensin I, enalaprilat (10^-5 M) evoked a maximal relaxation of
Ang II, Ang I, and Ang II with enalaprilat (10^-5 M) induced a greater contraction of porcine ciliary arteries (+/- & P < 0.01). Enalaprilat (10^-5 M) abolished the effect of angiotensin I (*P < 0.01).

Angiotensin II Receptor Antagonist. In quiescent ciliary arteries with endothelium, due to the occurrence of tachyphylaxis with repeated exposures to angiotensin II, the effects of each concentration of the vasoactive peptide was studied in separate parallel experiments. Angiotensin II (10^-8 to 10^-6 M) evoked concentration-dependent contractions (Fig. 6; P < 0.01 versus control; analysis of variance for repeated measurements).

After incubation of the vessels with the AT1-receptor antagonist valsartan (10^-9 M to 10^-3 M; 30 minutes), the response to angiotensin II (10^-7 M) was markedly reduced (Fig. 7; P < 0.001 versus control). However, after incubation of vessels with the AT2-receptor ligand CGP 42112 (10^-7 M; 30 minutes), the contractions to angiotensin II were not inhibited (Fig. 7; not significant; n = 5). In perfused porcine eyes, the addition of angiotensin II (10^-6 M) to the perfusion solution evoked a marked reduction in ophthalmic flow (decrease 0.6 ± 0.1 ml/min) compared to control eyes (perfusion solution without drugs) (Fig. 8; P < 0.0001 versus control, n = 6). If AT1-receptor antagonist valsartan was infused for 30 minutes, flow did not change during infusion of the antagonist. The reduction in flow (−0.2 ± 0.1 ml/min) induced by angiotensin II, however, was markedly reversed in the presence of valsartan (Fig. 8; P < 0.003 versus angiotensin II alone).

**DISCUSSION**

In the present study, the local regulatory role of angiotensins in the ophthalmic circulation was delineated using the myograph system for extraocular arteries and a perfusion system for the intact porcine eye. The results show that ACE plays an important regulatory role for the effects of bradykinin, an endothelium-dependent vasodilator that activates the L-arginine nitric oxide pathway,12,37 as well as for the local activation of angiotensin II.2 The receptors involved in the
which is identical to the angiotensin converting enzyme. Bradykinin is inactivated by kininase II, vascular beds in the human, ACE-inhibitors such as sodilator effects of bradykinin were markedly augmented in isolated blood vessels and increases in ophthalmic flow in the isolated perfused eye: The decrease in ophthalmic flow to angiotensin II \(10^{-6} \) M was markedly reduced in the presence of valsartan \(\ast P < 0.002 \) versus control; \(\dagger P < 0.005 \) versus effect of angiotensin II; \(\ast\& P = \) not significant versus control).

FIGURE 8. Effect of angiotensin II and AT \(_r\)-receptor antagonist valsartan on ophthalmic flow in the perfused porcine eye: The lack of an effect of these drugs on vascular tone in the ophthalmic circulation, bradykinin is a potent activator of this pathway, and it causes pronounced endothelium-dependent relaxations in isolated blood vessels (however, in vivo its stimulation of the L-arginine-nitric oxide pathway nor the capacitive relaxation to acetylcholine remains unaffected by the antagonist demonstrates that the drug exhibits specific effects and does not generally impair the activity of the L-arginine-nitric oxide pathway nor the capacity of vascular smooth muscle to relax.

The lack of an effect of these drugs on vascular tone or ophthalmic flow indicates that under the current experimental conditions, little bradykinin is locally produced or released even though endothelial cells are a source of bradykinin. Under in vivo conditions, this may be different. As judged from peripheral vascular beds in the human, ACE-inhibitors such as enalapril do not increase local blood flow. However, the potentiation of the vasodilator response to bradykinin induced by angiotensin converting enzyme inhibitors may be important for their beneficial effects in various parts of the circulation. Indeed, stimulation of the L-arginine–nitric oxide pathway by bradykinin not only leads to vasodilation (and, hence, to better organ perfusion) but also to the inhibition of platelet adhesion, monocyte invasion, and migration and proliferation of vascular smooth muscle cells. The bradykinin receptor present on endothelial cells responsible for mediating this response must be of the \(B_2\)-subtype. Indeed, Hoe 140, a selective antagonist of the \(B_2\) bradykinin receptor, prevented the relaxation to bradykinin in isolated arteries as well as the increase in ophthalmic flow in the perfused porcine eye. The fact that endothelium-dependent relaxations to acetylcholine remained unaffected by the antagonist demonstrates that the drug exhibits specific effects and does not generally impair the activity of the L-arginine–nitric oxide pathway nor the capacity of vascular smooth muscle to relax.

On the other hand, ACE also is able to transform angiotensin I into angiotensin II. The vasoconstrictor effects of angiotensin I were fully inhibited by the ACE-inhibitor enalaprilat, demonstrating that in this tissue non-ACE peptidases or a direct effect of angiotensin I does not contribute to the contraction. This suggests that all the effects of angiotensin I in ophthalmic blood vessels are related to its conversion into angiotensin II. The fact that angiotensin II was markedly more potent on a molar basis suggests that only part of the angiotensin I present at the blood vessel wall is transformed into angiotensin II. Again, the fact that enalaprilat or benazepril by itself did not change vascular tone nor ophthalmic flow indicates that there is no continuous formation of angiotensin II from angiotensin I in the ophthalmic circulation under our experimental conditions.

As do other vasoconstrictor agonists, angiotensin II exerts its effects through specific receptors on vascular smooth muscle cells (however, in vivo its stimulatory effects on the release of norepinephrine may also contribute). Two angiotensin receptors have been cloned—the AT \(_r\) and the AT \(_2\)-receptors. Recently, specific antagonists of those receptors have become available for pharmacologic and clinical studies. In isolated ciliary arteries, the AT \(_2\)-receptor antagonist valsartan, in a concentration-dependent manner, inhibited the effects of angiotensin II. The concentration range of its activity resembles its affinity to the AT \(_2\)-receptor, suggesting that valsartan would be highly effective in ophthalmic blood vessels after intravenous or oral administration. In contrast, the AT \(_r\)-receptor ligand CGP 42112 did not reduce the response to angiotensin II. Hence, these results demonstrate that the vascular effects of angiotensin II
in the ophthalmic circulation are mediated by AT\(_1\)-rather than AT\(_2\)-receptors. At the clinical level, AT\(_1\)-receptor antagonists may be effective tools aiming at inhibiting the effects of angiotensin II on the ophthalmic circulation.

**Key Words**
benazepril, enalapril, AT\(_1\)-receptor, bradykinin, valsartan

**Acknowledgments**
The authors thank Dr. H. P. Gurdan, F. Litzer, and F. Germain for technical help at the slaughterhouse (Basel, Switzerland), and they thank Sabine Bohnert for her secretarial help.

**References**


