Angiotensin (1-7) Reduces Intraocular Pressure in the Normotensive Rabbit Eye

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PURPOSE. In the present study the effects of exogenous angiotensin II and its breakdown metabolite angiotensin (1-7) on the intraocular pressure (IOP) and on aqueous humor dynamics in normotensive rabbit eye were evaluated.

METHODS. Male New Zealand White rabbits with normal IOP were used for intravitreous and topical administration of the test compounds. IOP was measured in conscious rabbits by pneumotonometer after topical anesthesia. Outflow measurements were made with a two-level constant pressure method in anesthetized animals.

RESULTS. Angiotensin (1-7) administered intravitreally reduced IOP within 1 to 5 hours (P < 0.05). This effect was abolished by the selective angiotensin (1-7) antagonist A-779, and partially by the selective angiotensin II type 2 receptor antagonist PD123319. When olmesartan, an angiotensin II type 1 receptor blocker, was administered simultaneously with an angiotensin (1-7), no antagonism was seen. Intravitreous administration of CGP42112 A, an angiotensin II type 2 receptor agonist, and angiotensin II did not significantly influence IOP, nor did topical administration of these compounds alter IOP. Angiotensin II significantly reduced outflow facility (P < 0.01) dose dependently, whereas angiotensin (1-7) had no effect.

CONCLUSIONS. Angiotensin (1-7) is a biologically active vasodilatory and antiproliferative heptapeptide, and its vascular effects counteract those of angiotensin II. It reduces intraocular pressure possibly by a selective Mas receptor, without changing aqueous humor outflow facility in the normotensive rabbit eye. (Invest Ophthalmol Vis Sci. 2008;49:2557–2562) DOI: 10.1167/iovs.07-1399

The renin-angiotensin system (RAS) plays an important part in the control of blood pressure and electrolyte homeostasis. Recent studies have demonstrated that angiotensin (Ang) II is not only a potent vasoconstrictor and a stimulant of the release of aldosterone from the adrenal gland, but also a potent vasoconstrictor and a stimulant of the release of aldosterone from the adrenal gland, but also a stimulant of cell proliferation and apoptosis and tissue fibrosis and participates in inflammatory responses in a nonhemodynamic manner.1–5 Recent evidence suggests that besides circulating RAS, tissue or local RAS also exists in the vasculature, adrenal gland, kidney, brain, testis, and ovary.4–6 Many of the known RAS components can be identified in the human4–7,9 and rabbit5,10 eye. RAS expression and secretory function have been shown even in cultured human and rabbit ciliary body nonpigmented epithelium, the tissue responsible for aqueous humor secretion.11,12 There is as yet only limited evidence of RAS in the trabecular meshwork,9 but Ang II can induce cell proliferation in bovine trabecular meshwork cells and increase the synthesis of collagen in vitro.13

Evidence is accumulating to indicate that the nonhemodynamic effects of Ang II are essential in hypertensive end-organ damage.1–3 Tissue RAS is activated in pathophysiological situations, and local synthesis of Ang II appears to contribute to altered tissue function and morphology in the heart.4–6 There has been a debate as to the effects of local RAS in the human eye: Does intracocular angiotensin originate from local production or is it originated from the blood compartment? It has been shown that Ang I, Ang II, and angiotensinogen are not able to pass the blood–brain barrier (BBB).4–15 The blood–retina barrier (BRB) is comparable to the BBB.16 If the BRB is intact, circulating angiotensin cannot reach the vitreous fluid.4 When the BRB is disrupted the vitreous is accessible.17 In any recent case studies have shown that topical application of angiotensin-converting enzyme (ACE) lowers intraocular pressure (IOP) in patients with ocular hypertension and primary open-angle glaucoma18 and in healthy volunteers (Denis P et al. IOVS, 1995;36:ARVO Abstract 3388). CS-088 (olmesartan), an Ang II type 1 receptor antagonist, has been shown to lower IOP in monkey eyes with unilateral laser-induced glaucoma.19 Ang (1-7) has been reported to contribute to the systemic antihypertensive effects of ACE inhibitors,20–22 whereas the effects on the eye have not been studied.

Angiotensinogen is an obligatory component for the eventual production of Ang II. Plasma angiotensinogen is derived primarily from the liver. Ang I and II are generated within the circulation by sequential cleavage of liver-derived angiotensinogen. Renin, synthesized in the kidney, cleaves this substrate to form Ang I. ACE converts Ang I to Ang II. Ang (1-7) is formed from Ang I or from Ang II by carboxypeptidases or endopeptidases.23–25 It is one of the several alternative products of the RAS.25 It is a biologically active vasodilatory and antiproliferative heptapeptide, and its vascular effects counteract those of Ang II.26–28 Ang II regulates hemodynamics at least through Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors.29 The main effects are mediated via AT1 receptors. In rodents, AT1 receptors are further divided into two subtypes: AT1A and AT1B.30 Ang II receptor gene polymorphisms have been found in humans and may be associated with the risk of glaucoma.31 The AT2 receptor appears to be counterregulatory to the action of Ang II at the AT1 receptor and to mediate at least some of the beneficial effects of AT1 receptor blockade via AT2 receptor-mediated generation of bradykinin, nitric oxide, and cyclic guanosine-3,5-monophosphate (cGMP). Emerging evidence suggests the existence of an additional RAS receptor, the G-protein-coupled receptor Mas, whose endogenous ligand is Ang (1-7),26,27 but Mas is not a receptor for Ang II.28

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Supported by grants from The Eye Foundation, Helsinki, Finland; The Glaucoma Research Foundation (Glaukoomatukisaatio) Lux, Helsinki, Finland; and The Paiviikki and Sakari Sohlberg Foundation, Helsinki, Finland.

Submitted for publication October 29, 2007; revised December 10, 2007, and January 11, 2008; accepted March 21, 2008.

Disclosure: A. Vaajanen, None; H. Vapaatalo, None; H. Kautiainen, None; O. Oksala, Santen Oy (E, F)

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In the present study, we investigated the effects of exogenous Ang II and Ang (1-7) on IOP and aqueous humor dynamics in the normotensive rabbit eye.

**MATERIALS AND METHODS**

**Animals**

Male New Zealand White (NZW) rabbits (weighing 2.9–3.9 kg, n = 38; Harlan, Horst, The Netherlands) were used in the study. They were housed in individual cages in a 12-hour light–dark cycle (lights on, 7 AM–7 PM) and had free access to a standard laboratory diet (Stanrath; Special Diet Services, Witham, UK) and water.

**Test Compounds**

Human Ang II acetate was obtained from Sigma-Aldrich (Schnelldorf, Germany); human (Sar\(^1\)Ile\(^8\)) Ang II, a nonspecific Ang II receptor ligand, from NeoMPS (Strasbourg, France), and olmesartan, an AT1 receptor antagonist, from Daiichi Sankyo Co. Ltd., Tokyo, Japan). CGP 42112A, an AT2 receptor agonist; PD123519, an AT2 receptor antagonist, and Ang (1-7), a Mas receptor agonist, were from NeoMPS; and A-779, a Mas receptor antagonist was from (GenScript Corp., Piscataway, NJ).

The compounds were dissolved in either distilled water or isotonic saline. At the beginning of the study, different concentrations were tested to establish relevant levels. The contralateral eye was treated with saline and served as the control in all experiments.

**IOP Measurements**

IOP was measured with a pneumotonometer (Modular One Tonometer; Mentor, Norwell, MA) after topical anesthesia with 0.4% oxybuprocaine (Oftan Obucain; Santen Oy, Tampere, Finland). The pneumotonometer was calibrated for the eye of the rabbit. The animals were accommodated to the IOP measurements before the actual experiments. A washout period of 4 weeks was used between the administrations of different test compounds. Measurements were performed at the same time of day (baseline IOP measurements at 8 and 9 AM) by the same person using the same instrument.

One hour before test compound application, the basal IOP was measured in both eyes. Test compounds were administered intravitreally in a volume of 50 \(\mu\)L by 27-gauge needle or topically. Olmesartan (AT1 receptor antagonist) was given simultaneously with Ang (1-7), and the other receptor antagonists, A-779 (Mas receptor antagonist) and PD123519 (AT2 receptor antagonist), were administered 1 hour before Ang (1-7). After administration, IOP was measured at 1, 2, 3, 4, 5, and 6 hours, if not otherwise indicated.

**Aqueous Humor Outflow Measurements**

The test compounds and saline were injected (50 \(\mu\)L) into the vitreous 3 to 24 hours before the outflow studies. When the compounds were administered intracamerally, outflow was registered 30 minutes after administration of the compounds.

For outflow measurements anesthesia was initiated and maintained by intramuscular injection of a combination of ketamine (Ketalar 50 mg/mL; Parke-Davis Warner Lambert Nordic AB, Solna, Sweden) and xylazine (Rompun Vet 20 mg/mL; Bayer AG, Leverkusen, Germany). The femoral artery was cannulated and connected to a pressure transducer (PE-50; Gould/Statham, Bilsboven, The Netherlands) for blood pressure monitoring (model 79-D polygraph; Grass-Telefactor, Quincy, MA). An intravenous infusion of isotonic saline was used in two rabbits, to sustain blood pressure. All animals were pretreated with indomethacin (Confortid 10 mg/kg body weight; Dumex, Copenhagen, Denmark) administered intravenously 30 minutes before the cannulation of the eye. Local anesthesia with a topical drop of 0.4% oxybuprocaine on the cornea was induced before cannulation.

The aqueous humor outflow facility was determined by the two-level constant-pressure perfusion method of Bárány,\(^{32}\) described in detail elsewhere.\(^{33–36}\) At the end of the study, the animals were euthanized with intravenous pentobarbital injection (100 mg/kg Mebunat; Orion Ltd., Espoo, Finland).

**Ethics**

All procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the Guide for the Care and Use of Laboratory Animals, and the methods were approved by the local Animal Experimentation Committee.

**Statistical Analysis**

The IOP results are expressed as the mean ± SEM with 95% CI. The area under the curve (AUC) was calculated by adding the areas between each pair of consecutive time observation, according to the trapezium rule, and dividing by the total observation time. Baseline value was subtracted to get the average change in time (AUC minus baseline). Statistical comparison was made by paired permutation test. The probabilities refer to the difference between the test and control eyes (self-pairing eye-based comparison). The outflow facility results are expressed as the mean ± SEM, and statistical analysis was made with Student's \(t\)-test for paired data.

**RESULTS**

**IOP Measurements**

Ang (1-7) (1 mM), a Mas receptor agonist, significantly lowered IOP after intravitreous injection of a volume of 50 \(\mu\)L (\(P = 0.008\); Fig. 1A). Olmesartan (5 mM), an AT1 receptor antagonist, did not antagonize the Ang (1-7)-lowering effect (\(P = 0.031\); Fig. 1B). PD123519 (1 mM), an AT2 receptor antagonist, had a marginal inhibitory effect on IOP reduction by Ang (1-7) (\(P = 0.09\); Fig. 1C), whereas A-779 (1 mM), a Mas receptor antagonist, abolished the Ang (1-7) effect (\(P = 0.5\); Fig. 1D). When administered alone, these compounds had no effect on IOP.

Ang II did not influence IOP in the various concentrations tested: 5 \(\mu\)M, 0.5 mM, and 5 mM. For the results of a concentration of 5 mM Ang II (\(P = 0.88\)), see Figure 2A. (Sar\(^1\)Ile\(^8\)) Angiotensin (5 mM), a nonspecific Ang II antagonist, tended to lower IOP between 2 and 4 hours compared with the effect in the control eye (\(P = 0.12\); Fig. 2B). CGP42112A, an AT2 receptor agonist, had no significant effect on IOP in the various concentrations tested: 30 \(\mu\)M, 0.3 mM, 1 mM, and 1.9 mM. For the concentration of 1.9 mM (\(P = 0.25\)), see Figure 2C. Olmesartan (5 mM) reduced IOP in both eyes. There was no difference between the test and control eyes (\(P = 0.32\); Fig. 2D). To study the effects of intravitreous injection per se, we administered isotonic saline 50 \(\mu\)L to eight rabbits in both eyes and noted no significant changes in IOP (Fig. 2E).

Topical administration of the test compounds did not alter IOP during the 6-hour follow-up.

**Aqueous Humor Outflow Measurements**

An intracameral injection of 0.5 mM Ang II significantly (\(P < 0.05\)) reduced the aqueous humor outflow facility (Fig. 3A). An injection of 5 \(\mu\)M and 5 mM Ang II likewise diminished outflow facility, but the latter concentration (5 \(\mu\)L of 5 mM Ang II) administered intracameral also caused systemic cardiovascular effects in the animals (arrhythmia, increased blood pressure). Overall intracameral administration Ang II caused a brief (5- to 15-minute) increase in blood pressure approximately 5 to 10 minutes after injection. During the next 10 to 15 minutes, blood pressure normalized to that usually recorded in deep anesthesia and was at normal levels when the outflow studies commenced. Intramuscular injections of anesthetics had no direct effect on blood pressure.
Intracameral Ang (1-7) (1 mM) had no effect on outflow facility (Fig. 3B). Outflow measurements were also made after intravitreous administration of Ang (1-7), but it did not influence outflow facility at 3 or 24 hours. IOP was conspicuously lower precisely 3 hours after intravitreous injection of the same concentration of Ang (1-7) (Fig. 1A).

**DISCUSSION**

In the present study, the role of RAS in the regulation of IOP in normotensive rabbits was evaluated. Ang (1-7) significantly reduced IOP after intravitreous injection, and the effect was blocked by a selective Mas receptor antagonist, A-779. Olmesartan similarly lowered IOP, possible via AT1 receptor blockade. The compounds studied also caused a clear contralateral effect in the control eye treated with saline. To verify that intravitreous injections, per se, do not cause any marked decrease in IOP, saline was injected bilaterally, and no significant change in IOP was observed. In addition, in experiments conducted with AT2 and Mas receptor antagonists alone, no significant change in IOP was observed in the experimental or control eyes. According to the literature, Ang (1-7) promotes release of prostanoids from endothelial and smooth muscle cells, release of nitric oxide, vasorelaxation, inhibition of vascular cell growth and less frequently, vasoconstriction. The experiments with, for example, indomethacin-pretreated animals will be the subject of our future studies. Topical administration of these compounds did not lower IOP in normotensive eyes. Presumably, local RAS is more strongly activated in pathophysiological situations such as glaucoma, when the lowering of IOP is more efficient. Ocular hypotensive effects of topical administered ACE inhibitors and AT1 receptor antagonists (olmesartan) have been demonstrated in acute and chronic models of glaucoma in rabbits. Topical application of ACE inhibitors lower IOP in ocular hypertension and primary open-angle glaucoma. Furthermore, orally administered AT1 receptor antagonist, losartan, lowers IOP both in normotensive and glaucomatous human subjects. Preliminary data indicate that also in rabbits with congenitally elevated IOP, the oculohypotensive effect of Ang (1-7) is more pronounced than in normotensive animals (Vaaahanen et al., unpublished data, 2007).

There are only a few reports on the effects of Ang II on IOP. In anesthetized rats intracameral infusion did not increase IOP compared to vehicle infusion. In the present study, exogeneous Ang II had no effects on IOP when administered intravitreously or topically. It did, however, significantly reduce outflow facility after intracameral injection in a dose-dependent manner. This effect was probably not due to an increase in systemic blood pressure, because the pressure had returned to normal at the time of outflow registration. These findings are in accordance with results obtained from monkey studies. Ang II has been reported to have effects on uveoscleral outflow in ocular normotensive rabbits. The experiments with, for example, indomethacin-pretreated animals will be the subject of our future studies. Topical administration of these compounds did not lower IOP in normotensive eyes. Presumably, local RAS is more strongly activated in pathophysiological situations such as glaucoma, when the lowering of IOP is more efficient. Ocular hypotensive effects of topically administered ACE inhibitors and AT1 receptor antagonists (olmesartan) have been demonstrated in acute and chronic models of glaucoma in rabbits. Topical application of ACE inhibitors lower IOP in ocular hypertension and primary open-angle glaucoma. Furthermore, orally administered AT1 receptor antagonist, losartan, lowers IOP both in normotensive and glaucomatous human subjects. Preliminary data indicate that also in rabbits with congenitally elevated IOP, the oculohypotensive effect of Ang (1-7) is more pronounced than in normotensive animals (Vaaahanen et al., unpublished data, 2007).

**FIGURE 1.** IOP in conscious rabbits after intravitreous administration (50 μL) of testcompounds acting on the Ang system in different time points (mean ± SEM). Graphs on the right in each panel describe the change in IOP during 6 hours, presented as the mean and 95% CI. n = 8 (A, B) and n = 6 (C, D). P is the difference between the test and control eyes.
pounds used. The endogenous Ang II concentration in the aqueous humor have been reported to range from 5 to 16 fmol/mg protein in the rabbit and is 0.5 pM in normal human subjects. In the present study, the test compounds were administered into the vitreous space as a single injection in a volume of 50 μL. Thus, the test compounds were injected into

**FIGURE 2.** IOP in conscious rabbits after intra vitreous administration (50 μL) of test compounds acting on the Ang system at different time points (mean ± SEM). Graphs on the right in each panel describe the change in IOP during 6 hours, presented as the mean and 95% CI. n = 6 (A, C), n = 8 (D, E).

**FIGURE 3.** The effect of intracameral injection of test compounds (5 μL) on outflow facility of aqueous humor in anesthetized rabbits. C1 indicates outflow facility at the lower pressure level and C2 at the higher pressure level. Mean ± SEM, n = 6, Ang II; n = 4, Ang (1-7). **P < 0.01 vs. control.
a nonvascularized compartment, from which they had to diffuse into the anterior part of the eye. In the case of intracamer injections when the compounds were administered directly to the point of action, the doses were lower, and the outflow registrations were performed as early as 30 minutes after injection. The half-life of Ang (1-7) in the vitreous space is not known. According to the literature, Ang (1-7) degrades after systemic administration in 30 minutes in canine lung, which is known to have very high ACE activity. However, the ACE activity in the vitreous is known to be much lower which speaks for a longer half-life in the eye. On the other hand, the effect of a compound can be much longer than the half-life of it.

In conclusion, the local RAS is potentially involved in the regulation of IOP. Presumably, this system is more markedly activated in pathophysiological situations such as glaucoma. Exogenous Ang II reduced the outflow facility but had no direct influence on IOP. The breakdown product of Ang I and II, heptapeptide Ang (1-7), reduced IOP, possibly via a newly identified Mas receptor type, without inducing changes in aqueous humor outflow facility in the normotensive rabbit eye. The possibility of decreased aqueous humor formation remains to be clarified.

Acknowledgments
The authors thank Marja Mali and Jaana Tuure for skillful technical assistance and Yuki Asai, MSci, for valuable help in preparation of the manuscript.

References

Reduction of IOP by Angiotensin (1-7)


