Characterization of angiotensin-II effects on cerebral and ocular circulation by noninvasive methods

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Aims The role of the renin-angiotensin-system (RAS) in the cerebral and ocular circulation is still a matter of controversy. *In vitro* and animal data lead to partially contradicting results. However, direct investigation of locally generated angiotensin II (Ang II) in humans is not possible *in vivo*. Hence, we hypothesised that it might be possible to characterize local effects of Ang II by comparing systemic and local haemodynamic parameters during exogenous Ang II infusion.

Methods In a placebo-controlled, double-blind, two-way cross over study blood flow velocities in the middle cerebral and the ophthalmic artery and ocular fundus pulsations were measured during stepwise increasing doses of Ang II in 10 healthy subjects. Blood flow velocities were assessed by Doppler sonography, fundus pulsation amplitudes (FPA), which estimate local pulsatile ocular blood flow were measured by laser interferometry. Additionally, systemic blood pressure and pulse rate were measured.

Results Ang II dose-dependently decreased resistive index (RI) and increased mean flow velocities (MFV) in both arteries. Fundus pulsation amplitude was dose-dependently decreased by Ang II, whereas mean arterial pressure (MAP) was significantly increased. Pulse pressure amplitude (PPA) was not affected by Ang II administration. There was a high degree of correlation between changes in RIs and the analogously calculated PPA/systolic blood pressure during Ang II infusion, which indicates that the changes in RI after Ang II administration can be attributed to changes in systemic haemodynamics. Calculation of total local ocular blood flow from fundus pulsation amplitudes and changes in flow pulsatility in the ophthalmic artery further argue against significant blood flow changes after Ang II administration.

Conclusions Interpretation of data from Doppler sonography and laser interferometry must be done very carefully when concomitant changes in systemic haemodynamics occur. RI cannot necessarily be taken as an index of distal vascular resistance in these cases and changes in MFV can be caused by changes in vessel diameter or in blood flow. Moreover, FPA cannot be taken as a measure of ocular blood flow if no additional data on flow pulsatility are available. The combination of our systemic and local haemodynamic data indicates that cerebral and ocular circulation are comparably insensitive to changes in local Ang II concentrations. Fundus pulsation and blood flow velocity measurements indicate that neither choroidal nor optic nerve head blood flow are significantly affected by administration of Ang II.

Keywords: Doppler sonography, fundus pulsations, angiotensin II, cerebral blood flow, ocular blood flow

Introduction

Angiotensin II (Ang II), the active peptide of the renin-angiotensin-system (RAS), exerts a broad range of effects on the cardiovascular system including vasoconstriction and stimulation of cell growth [1, 2]. It is well established that Ang II is involved in the regulation of local blood flow in various vascular beds [2]. However, the role of Ang II in cerebral and ocular blood flow is still a matter of discussion.

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Although there is evidence from animal studies that Ang II influences vascular tone of cerebral arteries [3] it has been shown that infusions of intracarotid Ang II do not affect regional cerebral blood flow in man [4]. Moreover, angiotensin converting enzyme (ACE) inhibitors, despite their ability to inhibit the generation of Ang II, do not influence cerebral blood flow [5–7]. On the other hand Ang II decreased blood flow to the choroid plexus in the rat [8], whereas high doses increased cerebral blood flow in the rabbit [9].

Animal data indicate that Ang II is generated locally in ocular tissues [10] and that there exist specific binding sites

for Ang II in retinal vessels [11]. Regulation of local ocular blood flow has been assumed to depend on the RAS in the eye [12, 13], also suggesting a role of the RAS in the pathogenesis of diseases, such as diabetic retinopathy, but *in vitro* studies on the contribution of RAS to the regulation of local ocular blood flow yielded contradictory results [14–16].

To date, *in vivo* data from human subjects are not available, since characterization of the effects of locally generated Ang II in the ophthalmic or cerebral circulation is not possible. We hypothesised, however, that characterization of the sensitivity to Ang II in these vascular beds might be possible by systemic administration of Ang II and assessment of systemic and local haemodynamic parameters. We therefore compared the results of transcranial Doppler ultrasound in the middle cerebral artery [17], transorbital Doppler ultrasound in the ophthalmic artery [18], and topical measurement of fundus pulsations with laser interferometry [19, 20] with systemic blood pressure during stepwise increasing doses of Ang II.

Methods

Subjects

After approval from the Ethics Committee of Vienna University School of Medicine was obtained, 10 healthy male volunteers were studied (age range: 20–32 years; mean $\pm$ s.e. mean: 27.6 $\pm$ 1.2). The nature of the study was explained and all subjects gave written consent to participate. Each subject passed a screening examination that included medical history and physical examination, 12-lead electrocardiogram, and laboratory screening. Subjects were excluded if they were taking any medication or if any abnormality was found as part of the pretreatment screening unless the investigators considered an abnormality to be clinically irrelevant. Furthermore an ophthalmic examination, including slit lamp biomicroscopy and indirect funduscopy was performed. Inclusion criteria were normal ophthalmic findings and ametropia <3 dioptres.

Study design

Subjects were studied in a double-blind, randomized, two way cross over design with a washout period between study days of at least 5 days. Subjects were randomly assigned to stepwise increased doses of Ang II (angiotensin II, Clinalfa, Läufelfingen, Switzerland; doses: 0 (=baseline), 0.65, 1.25, 2.5, 5.0 ng kg^{-1} min^{-1}, infusion period/dose level: 30 min, infusion rate 1 ml min^{-1}) or placebo (to maintain double-blind conditions, five numbered syringes containing physiological saline solution were prepared and infused sequentially). On the second trial day subjects crossed over to the alternate treatment. Haemodynamic measurements were performed during the last 10 min of each infusion step in a predetermined order (sonography of the middle cerebral artery, fundus pulsation measurement, blood pressure and pulse rate, sonography of the ophthalmic artery).

All subjects were asked to refrain from alcohol and caffeine for at least 12 h before trial days. In order to standardize the sodium balance all subjects received 3 g/day of sodium chloride for 3 days prior to the trial days, in addition to the usual salt intake. Studies were performed after an overnight fast in a quiet room with an ambient temperature of 22° C that had complete resuscitation facilities.

Doppler sonography

Mean blood flow velocity (MFV), peak systolic flow velocity (PSV), and end diastolic flow velocity (EDV) were determined in the right middle cerebral artery (MCA) using transcranial ultrasound [17] and in the right ophthalmic artery (OA) using transorbital ultrasound [18]. MFV was measured manually as the time mean of the spectral outline. For the measurements a 2 MHz probe (CFM 750, Vingmed Sound, Horten, Norway) was used. Middle cerebral artery resistive index (RImca) and ophthalmic artery resistive index (RIoa) were calculated as RI = (PSV-EDV)/(PSV). All parameters were determined as mean values over cardiac cycles.

Fundus pulsations

Pulse synchronous pulsations of the eye fundus were assessed by laser interferometry on the subject's right eye. The method is described in detail by Schmetterer *et al.* [19]. Briefly, the eye is illuminated by the beam of a single mode laser diode with a wavelength (λ) of 783 nm. The light is reflected at both the front side of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. Distance changes between cornea and retina lead to a corresponding variation of the interference order ($\triangle N(t)$). This change in interference order can be evaluated by counting the fringes moving inwards and outwards during the cardiac cycle. Changes in optical distance ($\triangle L(t)$), corresponding to the cornea-retina distance changes, can then be calculated by $\triangle L(t) = \triangle N(t).\lambda/2$. The maximum distance change is called fundus pulsation amplitude (FPA) and estimates the local pulsatile blood flow in the selected ocular vessels [20]. The short-term and day-to-day variability of the measurements is small, which allows the detection of even small changes in local pulsatile blood flow following pharmacological stimulation [20, 21]. In contrast to systems recording ocular pressure pulse [22–24], information on the ocular circulation can be obtained with high transversal resolution. Fundus pulsation measurements were performed in the macula, where fundus pulsation amplitude is influenced by choroidal circulation (FPAM), and in the optic disc, where choroidal and retinal blood flow contribute to the signal (FPAO) [25, 26]. The measurements in the optic disc were performed in regions without surface vessels and were located temporally between the outer margin of the pallor and the margin of the optic nerve head.

Non invasive systemic haemodynamics

Blood pressure, pulse rate, and ECG: systolic, diastolic, and mean blood pressures (SBP, DBP, MAP) were measured on the upper arm by an automated oscillometric device

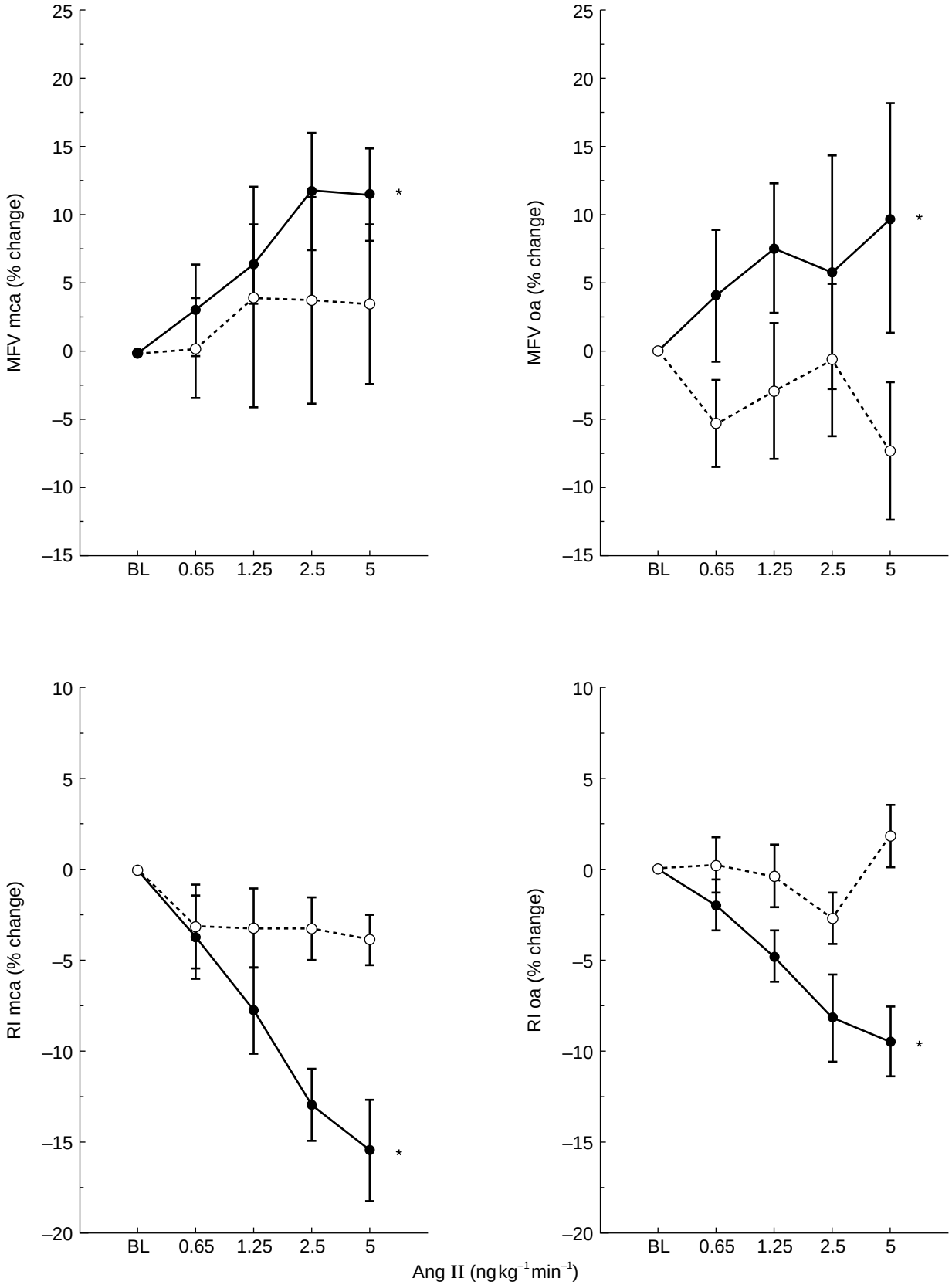

Figure 1 Dose-response relationship for angiotensin II on mean flow velocity and resistive index of the middle cerebral artery (MFV mca, RI mca) and the ophthalmic artery (MFV oa, RI oa). The % changes from baseline (BL) measurements during stepwise infusions of angiotensin II (solid lines with solid symbols) or during infusion of placebo (dotted lines with open symbols) are shown. Results are presented as means $\pm$ s.e. mean ($n = 10$). Asterisks indicate statistically significant differences *vs* placebo as calculated by ANOVA for repeated measurements ($P < 0.05$).

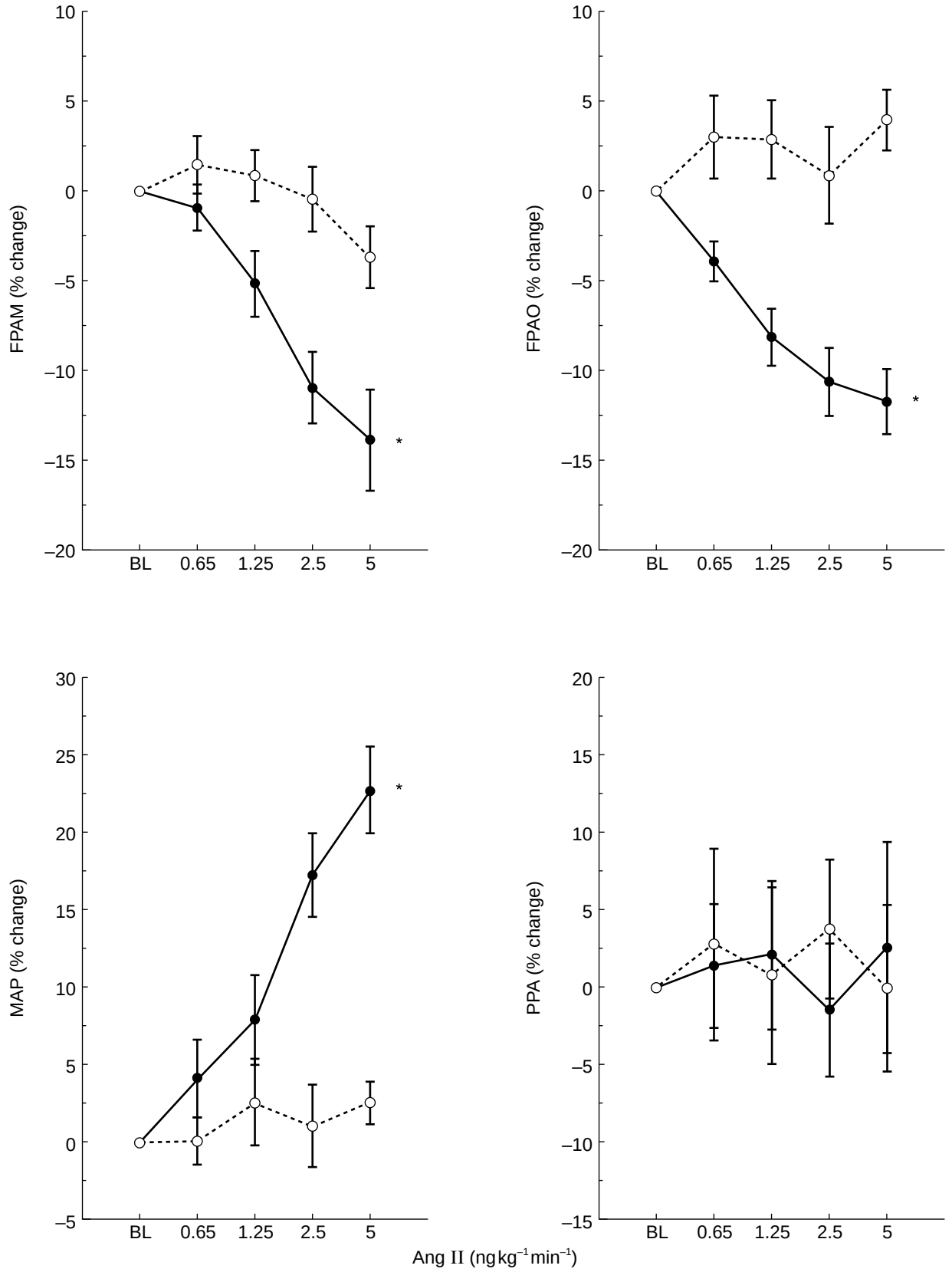

Figure 2 Dose-response relationship for angiotensin II on fundus pulsation amplitudes in the macula (FPAM) and the optic disc (FPAO) and on mean arterial pressure (MAP) and pulse pressure amplitude (PPA). The % changes from baseline (BL) measurements during stepwise infusions of angiotensin II (solid lines with solid symbols) or during infusion of placebo (dotted lines with open symbols) are shown. Results are presented as means $\pm$s.e. mean ($n=10$). Asterisks indicate statistically significant differences *vs* placebo as calculated by ANOVA for repeated measurements ($P<0.05$).

(HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA, USA). Pulse pressure amplitude was measured as PPA = SBP-DBP. Pulse rate was automatically recorded from a finger pulse-oxymetric device. ECG was monitored using a standard 4 lead device (HP-CMS patient monitor).

Data analysis

In analogy to the RI, which is calculated by (PSV-EDV)/PSV, (SBP-DBP)/SBP was calculated. The association between drug induced changes in (SBP-DBP)/SBP and RI in the MCA and the OA was calculated by linear regression. The pulsatile fraction of blood flow in the ophthalmic artery was calculated by (MFV-EDV)/MFV. It was assumed that flow pulsatility in the choroid after administration of Ang II changed approximately the same way as it did in the ophthalmic artery. As FPAM and FPAO are indirect measures of pulsatile blood flow, (FPAM*MFV)/(MFV-EDV) and (FPAO*MFV)/(MFV-EDV) were taken as relative estimates of choroidal and optic disc blood flow (CHBF, ODBF), respectively.

All statistical analyses were done using the Statistica® software package (Release 4.5, StatSoft Inc., Tulsa, OK, USA). For analysis of drug effects the measurements at each consecutive Ang II (or placebo) infusion step were expressed as %-change from baseline. The statistical significance versus placebo and baseline was calculated by ANOVA for repeated measurements. *Post hoc* comparisons were done with paired *t*-test at individual time points. A two-tailed $P<0.05$ was considered the level of significance.

Results

No significant differences between baseline values of the two study days were observed (Table 1).

As shown in Figure 1, RI in the MCA was decreased dose dependently by Ang II ($P<0.001$ *vs* baseline and placebo); this effect was paralleled by a smaller but significant increase in MFV, amounting to $11.8\pm3.4\%$ at the highest dose level ($P<0.05$ *vs* baseline and placebo).

RI in the OA also decreased dose dependently in response to Ang II ($P<0.001$ *vs* baseline and placebo, Figure 1). MFV was slightly increased by Ang II, amounting to $9.8\pm8.5\%$ at the highest dose level ($P<0.05$ *vs* baseline, NS *vs* placebo).

The changes in fundus pulsation amplitudes in response to stepwise increased doses of Ang II are depicted in Figure 2. Ang II dose dependently decreased FPAM ($P<0.001$ *vs* baseline and placebo) and FPAO ($P<0.001$ *vs* baseline and placebo).

Stepwise increased doses of Ang II caused a dose dependent increase in MAP ($P<0.001$ *vs* baseline and placebo; Figure 2), and a small decrease in pulse rate ($P<0.05$ *vs* baseline, NS *vs* placebo, data not shown). In contrast PPA was not affected by administration of Ang II (Figure 2).

There was a high degree of correlation between Ang II induced changes in RIs in the MCA and the OA and PPA/SBP (Figure 3). The correlation coefficient between RI in the MCA and PPA/SBP was $r=0.997$ ($P<0.001$). The correlation coefficient between RI in the OA and PPA/SBP was only slightly lower ($r=0.994$, $P<0.001$).

Figure 4 depicts the calculated values of CHBF and ODBF in response to the different doses of Ang II. Neither CHBF nor ODBF were significantly affected by Ang II, although there was a slight decrease in CHBF with increasing doses of Ang II. Hence Ang II decreased pulsatile blood flow (Figure 2 FPAM and FPAO) component in the choroid

Table 1 Mean baseline values (±s.e. mean) on the 2 study days ($n=10$).

	Day 1	*Day 2*
Resistive index middle cerebral artery	0.56±0.01	0.57±0.01
Mean flow velocity middle cerebral artery (cm s^{-1})	59.3±6.1	60.1±4.6
Resistive index ophthalmic artery	0.72±0.02	0.73±0.02
Mean flow velocity ophthalmic artery (cm s^{-1})	19.3±1.9	19.0±2.1
Fundus pulsation amplitude in the macula (μm)	3.5±0.3	3.3±0.4
Fundus pulsation amplitude in the optic disc (μm)	7.6±0.9	7.8±0.6
Systolic blood pressure (mm Hg)	118±2	120±4
Diastolic blood pressure (mm Hg)	61±3	59±3
Mean arterial blood pressure (mm Hg)	77±2	83±3
Pulse rate (beats min^{-1})	62±3	70±2

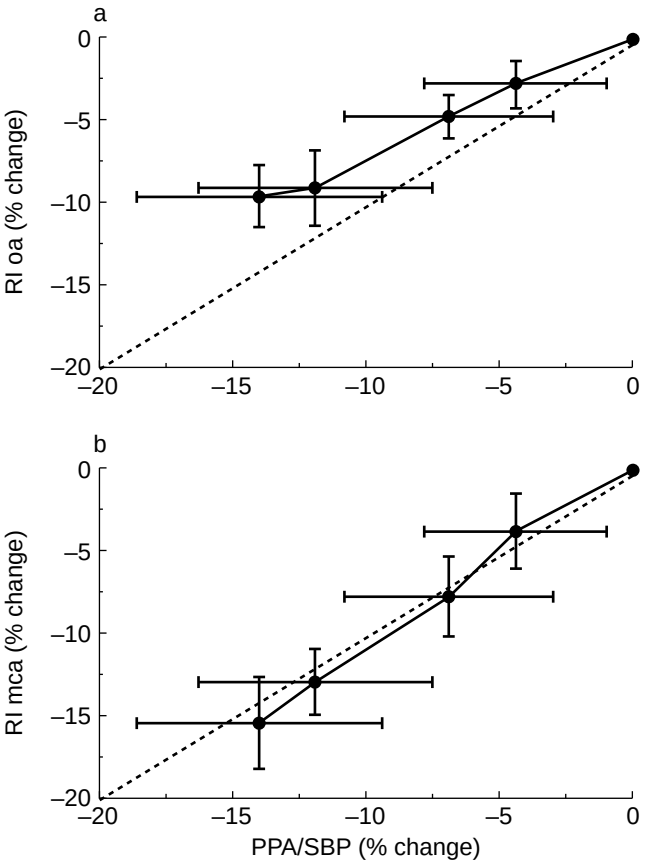

Figure 3 Association between Ang II induced changes in PPA/SBP and RI oa (a) and PPA/SBP and RI mca (b). The dotted lines represent the 45 degree correlation line between the variables. The solid line connects dose levels of Ang II (increasing dose levels from right to left).

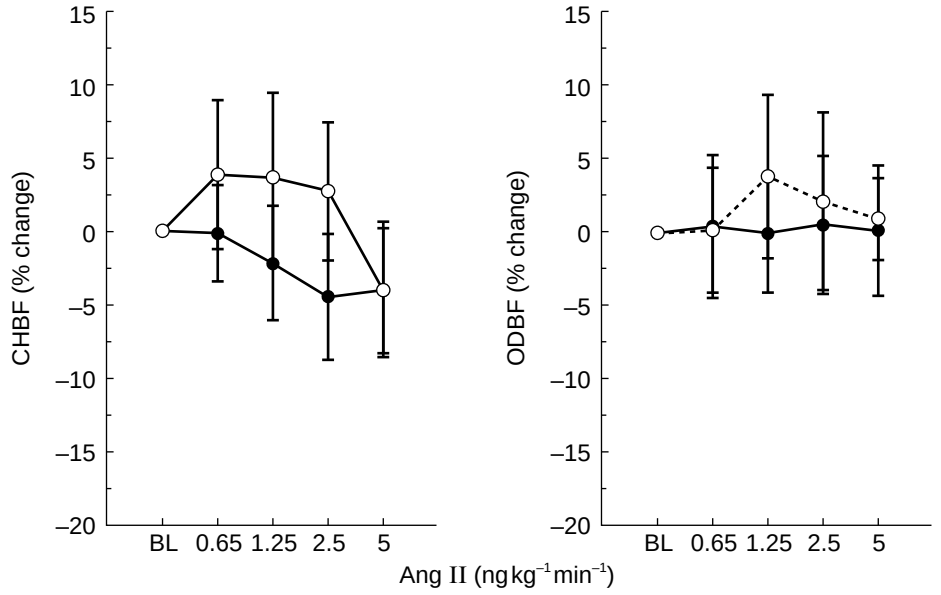

Figure 4 Dose-response relationship for angiotensin II on calculated choroidal and optic disc blood flow (CHBF, ODBF). The % changes from baseline (BL) measurements during stepwise infusions of angiotensin II (solid lines with solid symbols) or during infusion of placebo (dotted lines with open symbols) are shown. Results are presented as means ± s.e. mean ($n = 10$).

and the optic disc, but most likely increased the steady blood flow (as evidenced from the decrease in RIoa, Figure 1) component and therefore did not alter total choroidal or optic disc blood flow (Figure 4).

Discussion

Ang II decreased resistive index in the middle cerebral and ophthalmic artery as determined by transcranial Doppler sonography; the effect was dose-dependent and significant *vs* placebo (Figure 1). Resistive index was introduced as a measure of vascular resistance distal to the vessel under study [27], and a decrease in resistive index is often interpreted as a vasodilation of these resistance vessels. However, it has been pointed out that an increase in proximal vascular resistance has the same effect on RI as a decrease in distal vascular resistance [28]. In our experiments the observed decrease in RI was strongly correlated to the analogously calculated changes in PPA/SBP-ratio during administration of Ang II (Figure 3) and the angle of the regression line is close to 45 degrees. Hence, the decrease in RI after administration of Ang II is likely attributable to the propagation of the pressure wave through the vascular system rather than to dilation of peripheral resistance arteries in the brain and eye. This lack of effect on resistance vessels in our experiments might be partially a consequence of myogenic autoregulative mechanisms due to the increase in perfusion pressure [29]. Nevertheless our results indicate that the effect of exogenous Ang II on changes in vascular resistance distal to the measurement site is probably small and that the sensitivity to Ang II in these vascular beds is comparatively low.

With the doses we used our results concerning the cerebral circulation are in good agreement with the findings of Olesen [4] in man, although other authors presented experimental evidence of a slightly decreased cerebral blood flow after administration of Ang II [30]. With much higher doses an increase of cerebral blood flow has been observed after intracarotid administration of Ang II in rabbits [9]. This phenomenon was explained by the fact that Ang II relaxes cerebral arteries preconstricted with prostaglandin $F_{2\alpha}$ *in vitro* [31], even though Ang II contracts non-preconstricted cerebral arteries [3]. For the ocular circulation it has been assumed from data on the *in vitro* effect of Ang II, that the RAS may only play a minor role in blood flow regulation [14, 15]. On the other hand data from ACE inhibitors and angiotensin II receptor antagonists in isolated porcine arteries argue in favour of an important role of the RAS in ophthalmic microcirculation [16].

In our study we observed a small increase in blood flow velocities in the middle cerebral artery and the ophthalmic artery. However, increased blood flow velocity in large cerebral arteries may be due to vasoconstriction in these vessels or in case of constant vessel diameter, can be indicative of an increase in cerebral blood flow [32]. As we have not measured vessel diameter in the present study we cannot decide which of the two effects was responsible for the increased blood flow velocities. It has already been discussed that the data concerning the effect of Ang II on cerebral and ocular arteries are contradictory and it cannot be entirely decided whether vasoconstriction occurs in the middle cerebral and ophthalmic artery *in vivo*.

Additionally, we performed laser interferometric measurement of fundus pulsations, which yields an indirect measure of pulsatile blood flow in the choroid and the optic nerve head. Due to its high reproducibility [20, 33], this method can be considered a valuable tool for determination of drug effects on pulsatile ocular blood flow. Ang II decreased dose-dependently FPAO and FPAM reflecting a reduced pulsatile blood flow in the choroid. As these measurements only estimate local pulsatile ocular blood flow, they cannot necessarily be used to quantify total ocular blood flow. In fact the observed decrease in the resistive index in the ophthalmic artery indicates a shift from pulsatile to non-pulsatile blood flow. Hence the decreased FPAs most likely do not reflect a decreased ocular blood flow, but might be

attributed to a shift from pulsatile to non-pulsatile blood flow after administration of Ang II as evidenced from the calculated relative blood flow in the choroid and the optic disc. However, it is obvious that our method of calculating total blood flow yields only an indirect measure of blood flow in these vascular beds. Especially it has to be emphasised that ocular blood flow is only a small portion of ophthalmic blood flow. However, our method for calculating changes in relative ocular blood flow does not require that baseline pulsatility in ophthalmic and ocular vessels is similar, but only that Ang II induced %-changes in pulsatility in these vascular beds are comparable. As recent experiments indicate that choroidal blood flow is at least partially autoregulated [34–35], it might well be that autoregulation also contributes to the results observed in the present study.

Our study shows that results from Doppler sonographic measurements and measurements of pulsatile blood flow have to be analysed very carefully when concomitant changes in systemic haemodynamics occur after pharmacologic stimulation. Especially interpretation of Doppler sonographic data alone could lead to incorrect conclusions. Combining information from laser interferometry and Doppler ultrasound, however, indicates that neither choroidal nor optic nerve head blood flow are affected by administration of Ang II. Moreover, our results indicate that the reactivity of cerebral and ocular resistance vessels to changes in Ang II concentrations is comparably small.

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