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Dilatation by angiotensin II of the rat femoral arterial bed *in vivo* via pressure/flow-induced release of nitric oxide and prostaglandins

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1 The haemodynamic effects of angiotensin II (AII) and, for comparison, arginine vasopressin (AVP) in the femoral and superior mesenteric artery of urethane-anaesthetized rats were analysed with the ultrasonic transit time shift technique.

2 I.v. bolus injection of AII (0.1–3 nmol kg^{-1}) and AVP (0.03–1 nmol kg^{-1}) increased blood pressure which was accompanied by a decrease in blood flow through the superior mesenteric artery and an increase in femoral blood flow. The femoral hyperaemia was in part due to vasodilatation as indicated by a rise of femoral vascular conductance up to 200% relative to baseline. The femoral vasodilatation caused by AVP, but not AII, was followed by vasoconstriction.

3 Blockade of angiotensin AT_1 receptors by telmisartan (0.2–20 μmol kg^{-1}) prevented all haemodynamic responses to AII.

4 The femoral dilator responses to AII and AVP depended on the increase in vascular perfusion pressure since vasodilatation was reversed to vasoconstriction when blood pressure was maintained constant by means of a gravity reservoir. However, the AII-evoked femoral vasodilatation was not due to an autonomic or neuroendocrine reflex because it was not depressed by hexamethonium (75 μmol kg^{-1}), prazosin (0.25 μmol kg^{-1}) or propranolol (3 μmol kg^{-1}).

5 The AII-induced femoral vasodilatation was suppressed by blockade of nitric oxide (NO) synthesis with N^G-nitro-L-arginine methyl ester (L-NAME, 40 μmol kg^{-1}) and reversed to vasoconstriction when L-NAME was combined with indomethacin (30 μmol kg^{-1}), but was left unaltered by antagonism of endothelin $ET_{A/B}$ receptors with bosentan (37 μmol kg^{-1}).

6 These results demonstrate that the effect of AII to increase systemic blood pressure and the resulting rise of perfusion pressure in the femoral artery stimulates the formation of NO and prostaglandins and thereby dilates the femoral arterial bed. This local vasodilator mechanism is sufficient to mask the direct vasoconstrictor response to AII.

Keywords: Femoral artery; blood flow; nitric oxide; prostaglandins; angiotensin II; angiotensin AT_1 receptor antagonist; arginine vasopressin

Introduction

The cardiovascular system is subject to autonomic regulation by neural and humoral mechanisms, the sympathetic nervous system and the renin-angiotensin-aldosterone axis being the most eminent factors. Blood flow at the organ level is modulated by additional mechanisms in order to meet the local metabolic demands independently of blood pressure and vasoconstrictor tone (Crissinger *et al*., 1988; Remak *et al*., 1990). Thus, blood pressure, blood flow and vascular tone are interrelated with each other, as an increase in perfusion pressure results in myogenic vasoconstriction (Metting *et al*., 1989; Hellebrekers *et al*., 1990; Pohl *et al*., 1991; Kuo *et al*., 1991) whereas alterations in blood flow are amplified by synergistic changes of vascular conductance (Pohl *et al*., 1986; Drexler *et al*., 1989; Sinoway *et al*., 1989; Anderson & Mark, 1989R). *In vitro* studies have, in addition, demonstrated that myogenic vasoconstriction opposes flow-dependent vasodilatation (Griffith *et al*., 1987; Pohl *et al*., 1991; Kuo *et al*., 1991).

These complex interrelationships between blood pressure, blood flow and vascular conductance are likely to regulate the regional haemodynamics in the face of blood pressure alterations, but have been little explored *in vivo*. The aim of the present study, therefore, was to analyse the hypertensive effects of angiotensin II (AII) in its impact on local haemodynamics in the anaesthetized rat. In order to test for regional differences in somatic and visceral vascular beds, the responses to the two vasoconstrictor peptides were examined in the femoral and superior mesenteric artery. Pharmacological experiments were designed to identify the mediators by which drug-induced hypertension causes local vasodilatation.

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Methods

Animal preparation

All experiments of this study were approved by the Federal Ministry of Science, Traffic and Arts of the Republic of Austria. Female Sprague-Dawley rats weighing 180–230 g were fasted for 20 h but allowed free access to water. After the induction of anaesthesia with urethane (1.5 g kg^{-1}, s.c.) the rats were placed on a heated table, to maintain rectal temperature at 37°C, and fitted with a tracheal cannula, to facilitate spontaneous respiration. Blood pressure was measured via a cannula in the right carotid artery and recorded with a pressure transducer (ISOTEC; HSE, March-Hugstetten, Germany). A second cannula was placed in the left jugular vein for the i.v. administration of drugs. In some experiments the left femoral vein was cannulated for i.v. infusion of sodium nitroprusside. For blood flow measurements in the superior mesenteric artery a midline laparotomy was performed. The right femoral artery was visualized through a 1.5 cm incision in the inguinal region. The arteries were isolated over a length of

5 mm and special care was taken to remove any fat tissue adherent to the adventitia.

Haemodynamic measurements

Blood flow was determined by the ultrasonic transit time shift technique with the use of a small animal flowmeter (model T206; Transonic, Ithaca, NY, U.S.A.) and a 1 mm ultrasonic flow probe (model 1RB; Transonic). The flow probe was factory-calibrated and contains two ultrasonic transducers. Wide beams of ultrasound pass back and forth the full width of the vessel alternately interesecting the flowing blood in an upstream and downstream direction. Blood flow, in ml min^{-1}, is calculated from the modification of the beam transit time, which is solely dependent on blood flow but independent from the vessel's diameter and largely insensitive to probe misalignment (Barnes *et al*., 1983; Burton & Gorewit, 1984). The 1 mm flow probe has been developed for measurements in small vessels and repeatedly used and validated in different arteries of the rat (Myers & Hernandez, 1992; Wachter *et al*., 1995; D'Almeida *et al*., 1995).

The blood flow signal from the flowmeter and the amplified blood pressure signal were fed into a personal computer via an analogue-digital converter. Mean arterial pressure (MAP), heart rate and vascular conductance, in μl min^{-1} $(mmHg)^{-1}$ as blood flow divided by MAP, were calculated on-line. Drug-induced changes in blood flow and vascular conductance were expressed as % of baseline values.

Experimental protocol and study groups

After preparation the rats were allowed to equilibrate until haemodynamic parameters became stable (30–40 min). The animals received a pretreatment according to the study group which they were assigned to. After an adequate period to reach full efficacy of the pretreatment, vehicle (1 ml min^{-1}) or increasing doses of AII (0.1–3 nmol kg^{-1}) or arginine vasopressin (AVP; 0.03–1 nmol kg^{-1}) were given as i.v. bolus injections to construct dose-response curves. The intervals between the injections were such that the haemodynamic parameters had returned to baseline at least 10 min before the next injection was made. In each animal only one dose-response curve was recorded.

The first study investigated the effect of the selective angiotensin AT_1 receptor antagonist telmisartan (Wienen *et al*., 1993) on the AII-induced alterations of MAP and blood flow in the superior mesenteric and femoral artery. To this end rats were pretreated with 0.2, 2 or 20 μmol kg^{-1} telmisartan or its vehicle (1 ml kg^{-1}) 20 min before the AII dose-response curves were begun.

The second study was designed to determine as to how the local haemodynamic responses to AII and AVP are related to the concomitant hypertensive effects. In order to eliminate the increases in MAP in response to the peptides the rats were injected with heparin (2500 iu kg^{-1}, i.v.) and the carotid cannula was connected via a side-to-end anastomosis to a reservoir filled with saline (Metting *et al*., 1989). To avoid inflow of saline from the reservoir into the cardiovascular space of the rat the height of the reservoir was adjusted such that the intrasystemic pressure measured in the carotid cannula was slightly below the initial MAP value. By this procedure MAP was maintained constant since any drug-induced changes in pressure were buffered by gravity. When the effect of telmisartan (2 μmol kg^{-1}) or its vehicle (1 ml kg^{-1}) on femoral blood flow was determined in blood pressure-controlled rats, the reservoir was set equivalent to an intracarotid pressure of approximately 65 mmHg, which was found to be the average MAP value in the telmisartan-treated rats of study 1. As the gravity reservoir buffered the hypotensive effect of telmisartan it became possible to estimate the haemodynamic consequences of endogenous AII blockade.

In the third study the effects of a continuous i.v. infusion of AII (1 nmol min^{-1} kg^{-1} for 8 min) on femoral haemodynamics were studied under normal conditions and during gravity control of MAP.

In study 4 the haemodynamic responses to bolus injections of AII were recorded after blockade of ganglionic neurotransmission and antagonism of α- or β-adrenoceptors. The rats were pretreated i.v. with vehicle (1 ml kg^{-1}), hexamethonium (75 μmol kg^{-1}), prazosin (0.25 μmol kg^{-1}), **S**-propranolol (3 μmol kg^{-1}) or **R**-propranolol (3 μmol kg^{-1}) 40 min before the recording of AII dose-response curves was begun. These doses of hexamethonium, prazosin and propranolol have previously been shown to block effectively ganglionic transmission and α- and β-adrenoceptor activation, respectively (Celler *et al*., 1981; Timmermans & Van Zwieten, 1980; Hainsworth *et al*., 1974).

Study 5 assessed the modulator role of prostaglandins and nitric oxide (NO) in the systemic and femoral haemodynamic responses to AII. Sixty min before the effects of i.v. bolus injections of AII were tested, the rats were injected i.p. with vehicle (1 ml kg^{-1}) or indomethacin at a dose (30 μmol kg^{-1}) that has been shown to inhibit effectively cyclo-oxygenase activity (Dembinska-Kiec *et al*., 1991). To probe for an involvement of NO the rats were injected i.v. with vehicle (1 ml kg^{-1}), N^G-nitro-L-arginine methyl ester (L-NAME), at a dose (40 μmol kg^{-1}) that is maximally effective in increasing MAP (Moncada *et al*., 1991), or the inactive enantiomer D-NAME (40 μmol kg^{-1}). These pretreatments were made 30 min before the recording of the AII dose-response curves was begun. Since L-NAME markedly altered the haemodynamic parameters at baseline an additional control group was included, in which before the AII test MAP was restored to pre-L-NAME values by continuous i.v. infusion of sodium nitroprusside (20–100 nmol min^{-1} kg^{-1}). In a further study group the effect of combined administration of indomethacin (30 μmol kg^{-1}) and L-NAME (40 μmol kg^{-1}) was examined, with dose-response curves to AII being constructed 60 min later. An additional series of experiments tested the hypothesis that the haemodynamic effects of AII involve the activation of endothelin receptors. Rats were pretreated with vehicle (2 ml kg^{-1}) or the non-selective endothelin $ET_{A/B}$ receptor antagonist, bosentan, at a dose of 37 μmol kg^{-1}, which has been shown to antagonize effectively the cardiovascular effects of endothelins (Clozel *et al*., 1994).

Substances

Urethane (Fluka, Buchs, Switzerland) was dissolved in distilled water at a concentration of 25% (wt/wt). Stock solutions (1 mM) of arginine vasopressin and angiotensin II (Bachem; Bubendorf, Switzerland) were prepared in distilled water. Further dilutions used for injection were made with saline. Sodium nitroprusside (Merck; Darmstadt, Germany), N^G-nitro-L/D-arginine methyl ester (Bachem; Bubendorf, Switzerland), hexamethonium, prazosin, **R**- and **S**-propranolol (Sigma; Vienna, Austria) were dissolved in saline. Indomethacin (Merck, Sharp & Dohme; München, Germany) was dissolved in 2% Na_2CO_3 at a concentration of 90 μmol ml^{-1}.

Bosentan (Ro 47-0203), 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulphonamide (Hoffmann-La Roche; Basel, Switzerland), was dissolved in bidistilled water at a concentration of 17.5 μmol ml^{-1}.

Telmisartan (BIBR 277), 4-[(1,4-dimethyl-2-propyl-[2,6-bi-1H-benzimidazol]-1-yl)methyl]-[1,1-biphenyl]2-carboxylic acid, (Thomae; Biberach, Germany) was dissolved by placing 40 μmol of the drug in a vial and adding in a stepwise manner 800 mg distilled water, 44 mg 1 M NaOH, 900 mg distilled water and 100 mg mannitol, the emerging solution being stirred and heated to 90°C. To obtain a concentration of 20 μmol ml^{-1} telmisartan, the solution was made up to a final volume of 2 ml by adding distilled water, the pH being adjusted to ~9.5 with 1 M HCl. The blank vehicle was prepared in an analogous manner.

Statistics

All data are presented as mean ± s.e.mean. Statistical evaluation of the results was performed with the Mann-Whitney U test or Kruskal-Wallis H test followed by Dunn's test, as appropriate. Probability values of $P<0.05$ were regarded as significant.

Results

General

The baseline values for MAP, mesenteric and femoral haemodynamics are shown in Table 1. Bolus injections of AII dose-dependently increased MAP (Figure 1) and heart rate (data not shown). The positive chronotropic effect of AII was not further evaluated since it did not reach the level of statistical significance in all study groups. The hypertensive reaction to AII was accompanied by a decrease in mesenteric blood flow (data not shown) and mesenteric vascular conductance (MVC; Figure 1), whereas blood flow in the femoral artery increased (Figures 2 and 3). The AII-evoked femoral hyperaemia was in part due to femoral vasodilatation as indicated by a dose-dependent increase in femoral vascular conductance (FVC; Figures 1 and 2). The AII-induced changes in MAP, mesenteric haemodynamics and femoral blood flow were rapid in onset and waned during the following 5–10 min (Figure 2), whereas the increase in FVC was slightly delayed. For example, the time lag between the injection of 1 nmol kg^{-1} AII and the onset of femoral hyperaemia was 0.78 ± 0.28 s whereas FVC did not begin to increase before 10.44 ± 2.43 s ($P<0.0001$, $n=9$) post-injection.

AVP, injected i.v. as bolus, had a more sustained effect than AII, which at higher doses (0.3–1 nmol kg^{-1}) lasted up to 20 min. Like AII, AVP increased MAP (Figure 4) and constricted the superior mesenteric artery whereas heart rate decreased (data not shown). In contrast to the effect of AII the femoral haemodynamic response to AVP was biphasic. Along with the hypertensive reaction to the peptide, FVC initially increased for 1–2 min but then gradually decreased below the baseline value. The initial dilator and delayed constrictor effects of AVP in the femoral artery were evaluated separately and are shown in Figure 4.

Continuous i.v. infusion of AII (1 nmol min^{-1} kg^{-1}) led to a sustained increase in MAP and femoral blood flow (Figure 5) and decreased mesenteric blood flow and MVC (data not shown). The increase in FVC was less pronounced than observed after bolus injection of the peptide (compare Figure 1 and 5). Femoral vasoconstriction (i.e., a decrease in FVC below baseline) did not occur at any time (Figure 5).

Telmisartan

The effects of the AT_1 receptor antagonist telmisartan (0.2, 2 or 20 μmol kg^{-1}, i.v.) on the haemodynamic parameters under study are summarized in Table 1. Despite a tendency to reduce MAP, 0.2 μmol kg^{-1} telmisartan had no significant effect while the doses of 2 and 20 μmol kg^{-1} reduced MAP and femoral blood flow and increased mesenteric blood flow and MVC to a comparable degree (Table 1). In contrast, FVC remained unchanged by any dose of telmisartan (Table 1).

As shown in Figure 1, the haemodynamic responses to bolus injections of AII were dose-dependently depressed by telmisartan (0.2, 2 or 20 μmol kg^{-1}). The antagonist inhibited the hypertensive, mesenteric vasoconstrictor and femoral vasodilator responses to AII with a comparable degree of potency. The highest dose of telmisartan (20 μmol kg^{-1}) abolished the responses to AII, which indicates an at least 1000 fold shift of the AII dose-response curves to the right.

Gravity-controlled blood pressure

In order to test the hypothesis that the femoral vasodilator effects of AII and AVP are related to the peptides' hypertensive action and not the result of direct smooth muscle relaxation the haemodynamic responses to AII and AVP were investigated under gravity control of MAP. When the gravity reservoir was set at the animal's normotensive level, connecting the rat's circulation with the reservoir did not change mesenteric or femoral blood flow (data not shown). As expected, i.v. bolus injection of AII (Figures 2 and 3) or AVP (Figure 4) and i.v. infusion of AII (1 nmol min^{-1} kg^{-1}) for 8 min (Figure 5) failed to increase MAP under gravity control. The AII-induced femoral vasodilatation was abolished and, in fact, reversed to vasoconstriction as both femoral blood flow and FVC dropped markedly below baseline values after bolus injection (Figures 2 and 3) and during infusion (Figure 5) of AII. In the case of AVP the initial femoral vasodilatation was no longer present under gravity control of MAP whereas the delayed vasoconstrictor response was significantly augmented (Figure 4). The mesenteric vasoconstrictor responses to bolus injection of either peptide were not altered under gravity control of MAP as compared to control experiments (data not shown).

In an additional set of experiments the effect of AT_1 receptor blockade with telmisartan (2 μmol kg^{-1}) on femoral

Table 1 Effects of telmisartan on systemic, mesenteric and femoral haemodynamic parameters

Treatment	*(dose)*	n		*MAP* (mmHg)	*MBF* (ml min^{-1})	*MVC* (μl min^{-1} $mmHg^{-1}$)	*FBF* (ml min^{-1})	*FVC* (μl min^{-1} $mmHg^{-1}$)
Vehicle	(1 ml kg^{-1})	9	pre	92 ± 4	8.4 ± 1.1	92 ± 11	2.5 ± 0.5	26.8 ± 5.2
			post	92 ± 4	8.4 ± 1.0	92 ± 9	2.5 ± 0.6	26.2 ± 5.2
Telmisartan	(0.2 μmol kg^{-1})	5	pre	95 ± 3	9.8 ± 1.1	104 ± 10	3.0 ± 0.6	32.1 ± 6.5
			post	87 ± 3	10.0 ± 1.0	114 ± 12	2.5 ± 0.4	29.2 ± 5.3
Telmisartan	(2 μmol kg^{-1})	5	pre	90 ± 6	8.3 ± 2.4	90 ± 23	2.4 ± 0.7	26.4 ± 6.9
			post	67 ± 6*	10.3 ± 3.1	153 ± 45	1.8 ± 0.5	26.5 ± 6.9
Telmisartan	(20 μmol kg^{-1})	5	pre	90 ± 2	10.8 ± 1.3	120 ± 15	2.6 ± 0.7	28.3 ± 6.8
			post	65 ± 2*	13.0 ± 1.7*	201 ± 30*	2.0 ± 0.7	30.9 ± 10.7
				(ΔmmHg)	(%)	(%)	(%)	(%)
Vehicle	(1ml kg^{-1})	9		−0.2 ± 2.2	102 ± 4	102 ± 5	99 ± 4	100 ± 5
Telmisartan	(0.2 μmol kg^{-1})	5		−7.2 ± 2.0	101 ± 2	110 ± 4	86 ± 6	93 ± 5
Telmisartan	(2 μmol kg^{-1})	5		−22.4 ± 3.0*	123 ± 2*	166 ± 6*	75 ± 4*	101 ± 3
Telmisartan	(20 μmol kg^{-1})	5		−25.2 ± 2.1*	119 ± 5*	166 ± 10*	73 ± 4*	101 ± 8

Mean arterial blood pressure (MAP), blood flow (MBF, FBF), and vascular conductance (MVC, FVC), in the superior mesenteric (MBF, MVC), and femoral artery (FBF, FVC), were measured immediately before (pre) and 20 min after (post) the i.v. administration of vehicle or telmisartan at the doses indicated. The upper part of the table shows the absolute values before and after vehicle/drug administration whereas the lower part of the table depicts relative changes. Data are mean ± s.e.mean, n as indicated. *$P<0.05$ versus vehicle.

haemodynamics was determined under gravity control of MAP. To this end the gravity reservoir was set such that the intracarotid pressure was similar to that measured after telmisartan administration under normal conditions. When by this procedure MAP was reduced to 65 ± 2 mmHg ($n = 10$) both mesenteric and femoral blood flow decreased significantly (data not shown). However, MVC did not significantly fall (105 ± 7 and 92 ± 4 μl min^{-1} mmHg^{-1} before and after gravity-induced hypotension, respectively; $n = 10$), whereas FVC significantly decreased from 24.5 ± 3.8 to 15.9 ± 2.2 μl min^{-1} mmHg^{-1} ($P < 0.001$, $n = 10$). As expected, neither telmisartan (2 μmol kg^{-1}) nor its vehicle (1 ml kg^{-1}) had any effect on gravity-controlled MAP (-0.6 ± 0.2 and $+0.4 \pm 0.2$ mmHg, respectively; $n = 5$). While the vehicle had no effect on MVC (103 ± 4% relative to pre-injection values, $n = 5$), telmisartan dilated the mesenteric artery as indicated by a MVC increase to 162 ± 12% ($P < 0.01$ versus vehicle, $n = 5$), which was of a similar magnitude as that seen under physiological conditions (Table 1). However, under gravity control of MAP, telmisartan also increased FVC, a reaction that was not observed under normal conditions (Table 1). After telmisartan injection FVC rose to a value of 142 ± 18% while vehicle was without effect (99 ± 4%; $P < 0.05$, $n = 5$).

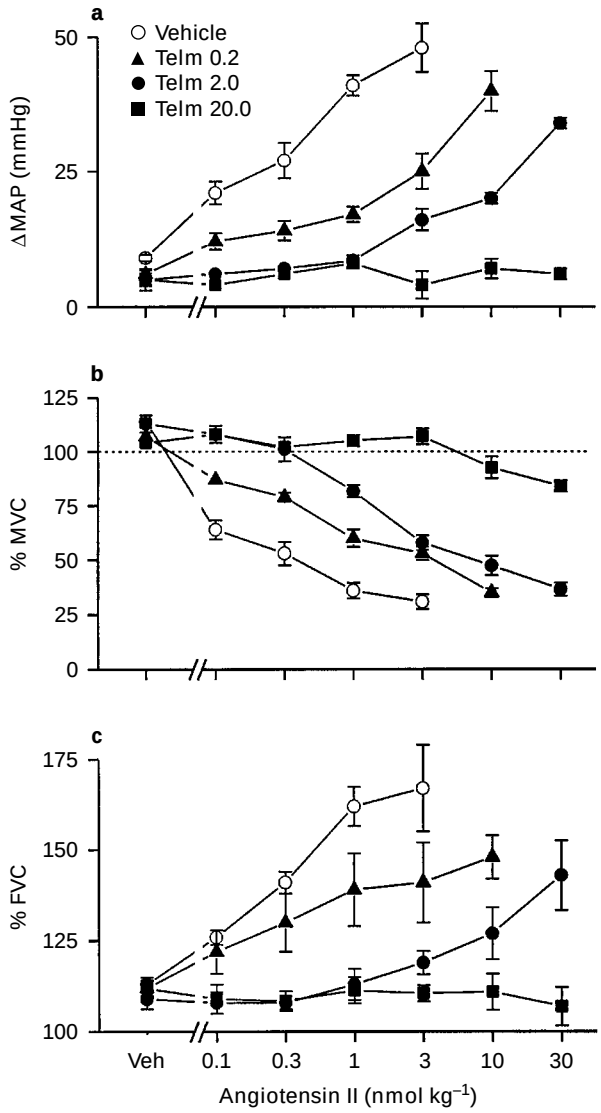

Figure 1 Effects of i.v. bolus injections of angiotensin II on (a) mean arterial blood pressure (MAP), and vascular conductance in (b) the superior mesenteric artery (MVC) and (c) femoral artery (FVC) in the absence and presence of the angiotensin AT_1 receptor antagonist telmisartan. Vehicle (1 ml kg^{-1}) or telmisartan (Telm) at the doses 0.2 μmol kg^{-1}, 2 μmol kg^{-1} or 20 μmol kg^{-1} were injected i.v. 20 min before the angiotensin II dose-response curves were recorded. Data are mean and vertical lines show s.e.mean, $n = 5-9$.

Hexamethonium, prazosin and propranolol

The possibility that the femoral vasodilator response to AII involves autonomic or neuroendocrine reflexes was tested by pretreating rats with hexamethonium (75 μmol kg^{-1}), prazosin (0.25 μmol kg^{-1}) or **S**-propranolol (3 μmol kg^{-1}). Vehicle (1 ml kg^{-1}) and **R**-propranolol (3 μmol kg^{-1}) served as controls. The haemodynamic parameters recorded after these pretreatments are given in Table 2. MAP was significantly reduced by hexamethonium and prazosin whereas FVC was not affected by hexamethonium but was markedly increased by prazosin (Table 2). The efficacy of β-adrenoceptor blockade was indicated by a significant reduction of heart rate after **S**-propranolol while **R**-propranolol and vehicle were inactive (280 ± 10, 356 ± 13 and 329 ± 9 beats min^{-1} after pretreatment with **S**-propranolol, **R**-propranolol and vehicle, respectively; $P < 0.05$ **S**-propranolol versus **R**-propranolol and vehicle, $n = 6$). MAP was left unchanged by **S**-propranolol, whereas femoral blood flow and FVC were significantly decreased as compared with **R**-propranolol (Table 2). Pretreatment with hexamethonium or **S**-propranolol, but not prazosin, significantly augmented the femoral vasodilator responses to bolus injections of AII (0.3 – 3 nmol kg^{-1}; data are shown in Table 3 for 1 nmol kg^{-1} of AII only). The pressor responses to AII were enhanced by hexamethonium but were not significantly altered by prazosin or **S**-propranolol (Table 3).

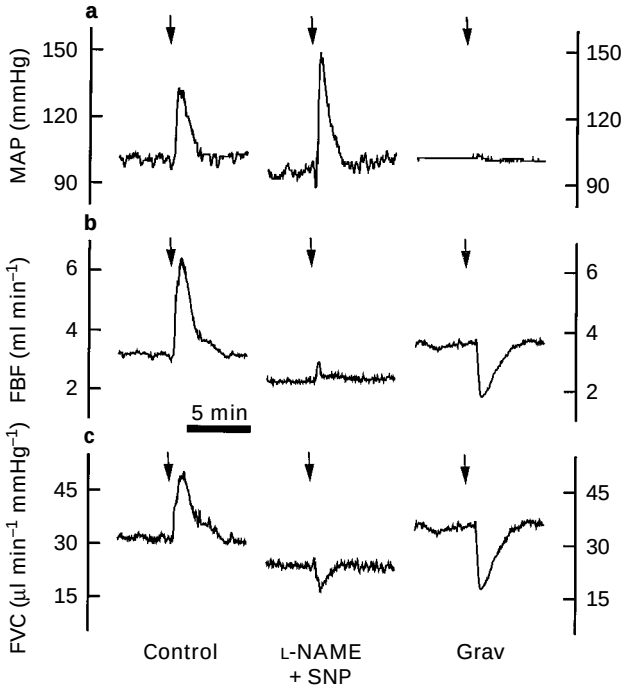

Figure 2 Tracings of the effects of angiotensin II (1 nmol kg^{-1}), injected i.v. as bolus (↓), on (a) mean arterial blood pressure (MAP), (b) femoral arterial blood flow (FBF) and (c) femoral vascular conductance (FVC). Under control conditions the hypertensive effect of angiotensin II was accompanied by an increase in FBF and FVC. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME, 40 μmol kg^{-1}) inhibited the effect of angiotensin II to increase FBF and reversed the rise in FVC to moderate fall of FVC. In these experiments the hypertensive effect of L-NAME was counteracted by i.v. infusion of sodium nitroprusside (SNP, 20 – 100 nmol min^{-1} kg^{-1}). In rats whose MAP was controlled by a gravity reservoir (Grav) angiotensin II failed to alter MAP but markedly reduced both FBF and FVC.

N^G-nitro-L-arginine methyl ester (L-NAME), indomethacin and bosentan

Pretreatment with L-NAME (40 μmol kg^{-1}) significantly increased MAP and reduced FVC but left femoral blood flow unaltered (Table 2). D-NAME (40 μmol kg^{-1}) had no effect (Table 2). Intravenous infusion of sodium nitroprusside at doses of 20–100 nmol min^{-1} kg^{-1} reversed the effect of L-NAME on MAP and attenuated that on FVC (Table 2). Indomethacin (30 μmol kg^{-1}) had no significant effect on baseline haemodynamic parameters as compared with its vehicle (Table 2) and failed to influence the cardiovascular effects of L-NAME (40 μmol kg^{-1}; Table 2). Similarly, bosentan (37 μmol kg^{-1}) pretreatment was devoid of any haemodynamic effects.

D-NAME (40 μmol kg^{-1}) did not alter the haemodynamic responses to AII (0.1–3 nmol kg^{-1}) as compared with vehicle (data not shown, $n=6$). L-NAME reversed the femoral vaso-

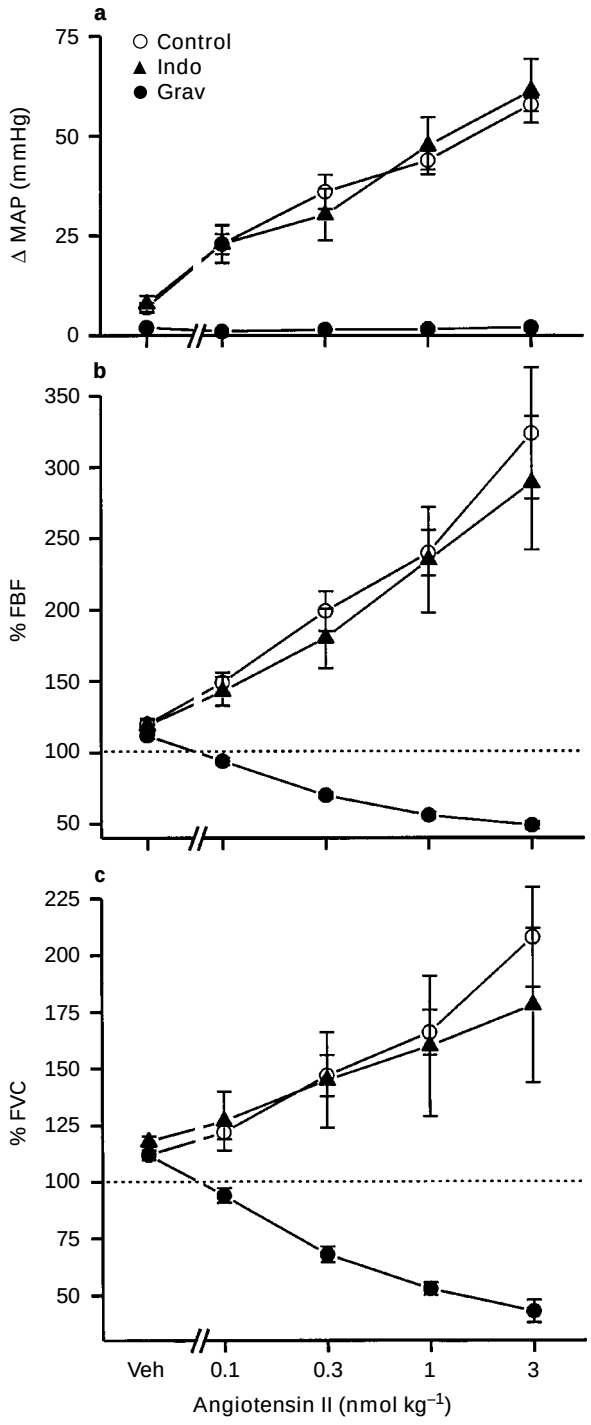

Figure 3 Effects of i.v. bolus injections of angiotensin II on (a) mean arterial blood pressure (MAP), (b) blood flow (FBF) and (c) vascular conductance (FVC) in the femoral artery under control conditions, in the presence of the cyclo-oxygenase inhibitor indomethacin (Indo) and under gravity control of MAP (Grav). Vehicle (1 ml kg^{-1}) or indomethacin (30 μmol kg^{-1}) was injected i.p. 60 min before the angiotensin II dose-response curves were recorded. Data are mean and vertical lines show s.e.mean, $n=6-7$.

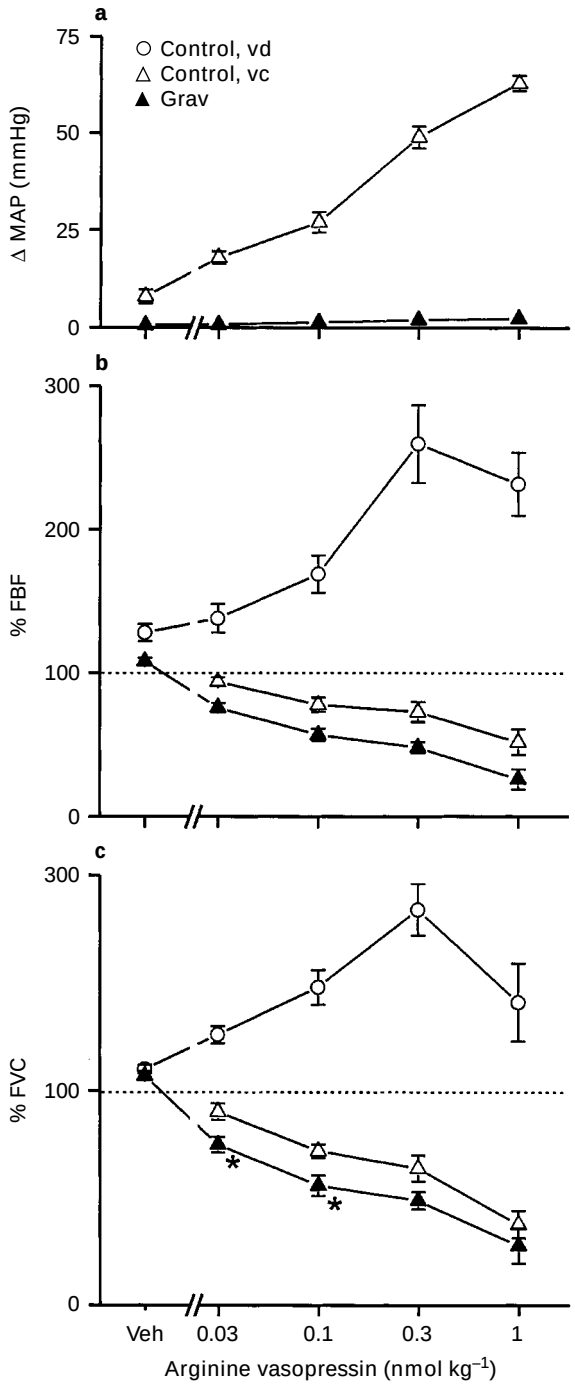

Figure 4 Effects of i.v. bolus injections of arginine vasopressin on (a) mean arterial blood pressure (MAP), (b) blood flow (FBF) and (c) vascular conductance (FVC) in the femoral artery under control conditions and under gravity control of MAP (Grav). Under control conditions the femoral haemodynamic responses to arginine vasopressin were biphasic, vasodilatation (Control, vd) being followed by vasoconstriction (Control, vc) while during gravity control of MAP only vasoconstriction occurred. Data are mean and vertical lines show s.e.mean, $n=6$. *$P<0.05$, FVC decreases under gravity control of MAP versus FVC decreases under control conditions.

dilatation caused by bolus injection of AII to moderate vasoconstriction. Similar observations were made in rats whose haemodynamic parameters were restored to normal by i.v. infusion of sodium nitroprusside (20–100 nmol min^{-1} kg^{-1}) following L-NAME pretreatment. The mean values were similar to those recorded after pretreatment with L-NAME alone (data not shown, $n=9$). Indomethacin (30 μmol kg^{-1}) alone had no effect on the femoral vasodilator response to AII (Figure 3) whereas combined pretreatment with indomethacin (30 μmol kg^{-1}) and L-NAME (40 μmol kg^{-1}) abolished the femoral hyperaemic response to a bolus injection of AII and gave way to a marked vasoconstrictor effect of AII (Figure 6) which was of a similar magnitude to that observed in blood pressure-controlled rats (Figure 3). After indomethacin/L-NAME pretreatment the AII-induced femoral vasoconstriction was on average significantly more intense than after pretreatment with L-NAME alone (Figure 6). Moreover, the hypertensive effect of AII (1 and 3 nmol kg^{-1}) was significantly enhanced by indomethacin/L-NAME alone (Figure 6). Pretreatment with bosentan (37 μmol kg^{-1}) slightly reduced the hypertensive response to AII but had no effect on the accompanying femoral vasodilatation (Table 3).

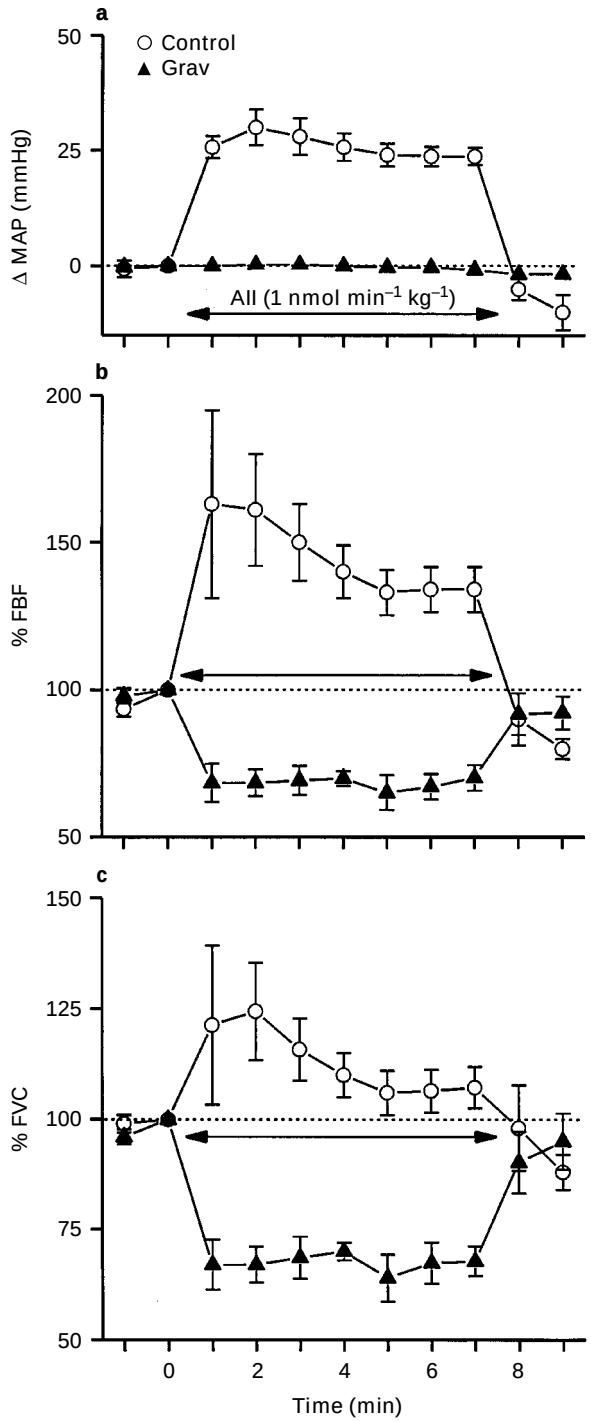

Figure 5 Effects of i.v. infusion of angiotensin II (AII; 1 nmol min^{-1} kg^{-1}) on (a) mean arterial blood pressure (MAP), (b) blood flow (FBF) and (c) vascular conductance (FVC) in the femoral artery under control conditions and under gravity control of MAP (Grav). Data are mean and vertical lines show s.e.mean, $n=7$.

Discussion

The present data demonstrate that AII, injected as i.v. bolus, constricts the superior mesenteric arterial bed but dilates the femoral artery of the rat. This finding is consistent with previous observations that i.v. AII causes femoral hyperaemia and/or vasodilatation (Corder *et al*., 1986; Li & Zimmerman, 1990) but is at variance with data showing that close arterial injection of the peptide constricts the femoral artery *in vivo* (Caldicott *et al*., 1977; Gasic *et al*., 1990). In addition, our results do not accord with the fact that AII is a well-characterized constrictor of the femoral artery *in vitro* (Juul *et al*., 1987; Purdy & Weber, 1988). Since telmisartan (Wienen *et al*., 1993) dose-dependently antagonized AII it is evident that the cardiovascular actions of the peptide observed here are mediated by angiotensin AT_1 receptors. The hypotensive and mesenteric vasodilator effect of telmisartan attests to the importance of endogenous AII in maintaining MAP (Johnson & Davis, 1973) and mesenteric vascular tone, while the inability of the AT_1 receptor antagonist to dilate the femoral artery is in keeping with the lack of effect of angiotensin-converting enzyme inhibitors on femoral arterial resistance (Li & Zimmerman, 1990; Ikeo *et al*., 1992).

The haemodynamic actions of AVP, which were tested here for comparison only, were similar to those of AII except that the AVP-induced dilatation of the femoral artery was followed by constriction. While the vasoconstrictor effect of AVP in the femoral artery has been observed both *in vivo* (Monos *et al*., 1978) and *in vitro* (Katusic *et al*., 1984; Chiba & Tsukada, 1992) AVP-evoked femoral vasodilatation has not yet been described. Certain other vessels, though, are dilated by AVP (Suzuki *et al*., 1989; Walker *et al*., 1989; Cosentino *et al*., 1993; Martinez *et al*., 1994; Rudichenko & Beierwaltes, 1995) or AII (Haberl *et al*., 1991; Maktabi *et al*., 1995; Sai *et al*., 1995). As some of these studies were performed *in vitro* (Walker *et al*., 1989; Cosentino *et al*., 1993; Martinez *et al*., 1994; Sai *et al*., 1995) it appears reasonable to hypothesize that the smooth muscle of some vascular beds is directly relaxed by the peptides. However, the femoral vasodilatation evoked by AII and AVP in the pesent study is not due to direct peptide-mediated vasorelaxation but depends on the marked hypertensive effects of the peptides. This relationship was proved by gravity control of MAP (Metting *et al*., 1989) which prevented any peptide-induced change in MAP and reversed the AII/AVP-evoked femoral vasodilatation to vasoconstriction.

The observation that the mesenteric vasoconstrictor responses to bolus injections of AII and AVP were left unchanged by gravity control of MAP suggests that the short-term constrictor effects of the peptides are independent of pressure-dependent regulatory mechanisms. However, under steady state conditions, pressure-induced myogenic vasoconstriction (Metting *et al*., 1989; Hellebrekers *et al*., 1990) may come into play as deduced from the gradual decline of femoral vasodilatation during continuous infusion of AII (Figure 5). The rundown of vasodilatation, which occurred despite maintained hypertension, is likely to result from a delayed pressure-induced vasoconstriction (Griffith *et al*., 1987; Pohl *et al*., 1991; Kuo *et al*., 1991) which counteracted the pressure-dependent vasodilatation.

Table 2 Effects of hexamethonium, prazosin, bosentan, L-NAME and indomethacin, and of the combinations of L-NAME with sodium nitroprusside or indomethacin, on systemic and femoral haemodynamic parameters

Treatment	n	*MAP* (mmHg)	*FBF* (ml min^{-1})	*FVC* (μl min^{-1} $mmHg^{-1}$)
Vehicle	8	86±4	2.4±0.3	27.9±4.2
Hexamethonium	8	54±4*	1.4±0.2*	25.3±2.8
Prazosin	5	59±5*	2.6±0.3	44.5±5.0*
R-propranolol	6	86±5	2.4±0.3	27.0±3.0
S-propranolol	6	81±2	1.5±0.1*	18.7±1.6*
Vehicle	6	92±4	2.7±0.2	30.2±2.8
Bosentan	8	85±5	2.9±0.4	34.5±4.6
Vehicle	9	90±4	2.7±0.4	30.5±3.6
D-NAME	6	98±6	2.6±0.4	26.8±3.3
L-NAME	13	127±6*	2.3±0.3	17.7±2.2*
L-NAME+SNP	9	75±6	1.5±0.2*	20.3±1.9
Vehicle	7	87±5	1.6±0.2	18.7±2.7
Indomethacin	7	77±6	2.1±0.3	24.8±4.2
Indomethacin+L-NAME	12	138±4*	2.2±0.3	16.2±2.3*

Mean arterial blood pressure (MAP), blood flow (FBF) and vascular conductance (FVC) in the femoral artery were meausred 40 min (hexamethonium, prazosin and propranolol), 30 min (N^G-nitro-L-arginine methyl ester (L-NAME)) or 60 min (indomethacin) after the respective pretreatment. Vehicle, hexamethonium (75 μmol kg^{-1}), prazosin (0.25 μmol kg^{-1}), **S**-propranolol (3 μmol kg^{-1}), **R**-propranolol (3μmol kg^{-1}), bosentan (37 μmol kg^{-1}), L-NAME (40 μmol kg^{-1}) and N^G-nitro-D-arginine methyl ester (D-NAME, 40 μmol kg^{-1}) were injected i.v. L-NAME+SNP: the i.v. infusion of sodium nitroprusside (SNP, 20–100 nmol min^{-1} kg^{-1}) was started 10 min after pretreatment with L-NAME. Indomethacin (30 μmol kg^{-1}) and its vehicle (1 ml kg^{-1}) were administered i.p. Indomethacin+L-NAME: the i.p. injection of indomethacin (30 μmol kg^{-1}) was followed by i.v. administration of L-NAME (40 μmol kg^{-1}) 30 min later, baseline haemodynamics being measured after another 30 min period. Data are mean±s.e.mean, *n* as indicated. $*P<0.05$ versus the respective control.

Table 3 Effects of hexamethonium, prazosin, propranolol and bosentan on the systemic and femoral haemodynamic changes evoked by angiotensin II (1 nmol kg^{-1})

Treatment	n	*MAP* (ΔmmHg)	*FBF* (%)	*FVC* (%)
Vehicle	8	44±2	250±17	177±11
Hexamethonium	8	56±4*	387±41*	242±22*
Prazosin	5	41±4	220±25	170±17
R-propranolol	6	44±3	206±11	150±6
S-propranolol	6	48±5	298±28*	211±16*
Vehicle	6	49±2	225±13	170±12
Bosentan	8	42±2*	210±23	166±12

Effects of 1 nmol kg^{-1} of angiotensin II, injected i.v. as bolus, on mean arterial blood pressure (MAP) and blood flow (FBF) and vascular conductance (FVC) in the femoral artery after pretreatment with hexamethonium, prazosin, propranolol or bosentan. Vehicle, hexamethonium (75 μmol kg^{-1}), prazosin (0.25 μmol kg^{-1}), **S**-propranolol (3 μmol kg^{-1}), **R**-propranolol (3 μmol kg^{-1}) and bosentan (37 μmol kg^{-1}) were injected i.v. 30 min before the haemodynamic effects of angiotensin II were recorded. Data are mean±s.e.mean, *n* as indicated. $*P<0.05$ versus the respective control.

Another type of interaction between vasodilatation and vasoconstriction is exemplified by the haemodynamic responses to telmisartan. Antagonism of endogenous AII by this drug resulted in femoral vasodilatation only when the hypotensive effect of the antagonist was prevented by gravity control of MAP. Since gravity-controlled hypotension *per se* caused femoral vasoconstriction, it would seem that the lack of effect of telmisartan on FVC under control conditions results from the functional balance between femoral vasodilatation by AT_1 receptor blockade and vasoconstriction by systemic hypotension.

From the present observations it is inferred that both endogenous and exogenous AII constrict the femoral arterial bed but that this effect is overridden by a dilator mechanism which is stimulated through the simultaneous hypertensive action of the peptide. The same mechanism counteracts the femoral vasoconstrictor effect of AVP, causing initial vasodilatation and attenuating the subsequent vasoconstriction. Since the increase in MAP and femoral blood flow preceded the rise of FVC, and both hypertension and the rise in femoral blood flow were prevented by gravity control of MAP, it would appear that the femoral vasodilatation is a consequence of the increase in vascular perfusion pressure and the associated increase in blood flow. Flow-dependent vasodilatation is an important component in the local control of vascular tone (Holtz *et al*., 1984; Pohl *et al*., 1986; Griffith *et al*., 1987; Drexler *et al*., 1989; Sinoway *et al*., 1989; Anderson & Mark, 1989), and the present study indicates that this mechanism is also operated by the AII/AVP-evoked increase in perfusion pressure.

In analysing the pathways and mediators of the pressure-induced femoral vasodilatation, the possibility that cardiovascular reflexes via the sympathetic nervous system or the sympatho-adrenal axis are involved was ruled out because hexamethonium, prazosin and propranolol failed to depress the femoral vasodilator response to AII. The efficacy of hexamethonium at inhibiting ganglionic neurotransmission and prazosin at blocking α-adrenoceptors was proven by the hypotensive effects of these drugs, while the effectiveness of β-adrenoceptor blockade by propranolol was evident from an enantiomer-selective fall of baseline FVC and bradycardia. It is hence realistic to hypothesize that the pressure-dependent vasodilatation which AII evokes in the femoral artery arises from a local pathway within the vascular bed under study. This surmise is consistent with the observation that flow-dependent vasodilatation requires the presence of the endothelium (Holtz *et al*., 1984; Pohl *et al*., 1986; Lamping & Dole, 1988) and involves NO and prostaglandins (Lamontagne *et al*., 1992; Hecker *et al*., 1993; Koller *et al*., 1993; 1994; Joannides *et al*., 1995). An important mediator role of NO in the action of AII to dilate the femoral artery has been proved with the NO synthase inhibitor L-NAME which suppressed the AII-induced vasodilatation in an enantiomer-selective manner. This effect of L-NAME was independent of any baseline alterations, because counteracting the hypertensive effect of L-NAME with sodium nitroprusside did not restore the AII-evoked femoral vasodilatation.

Although the cyclo-oxygenase inhibitor indomethacin did not *per se* alter the femoral vasodilator response to AII, it

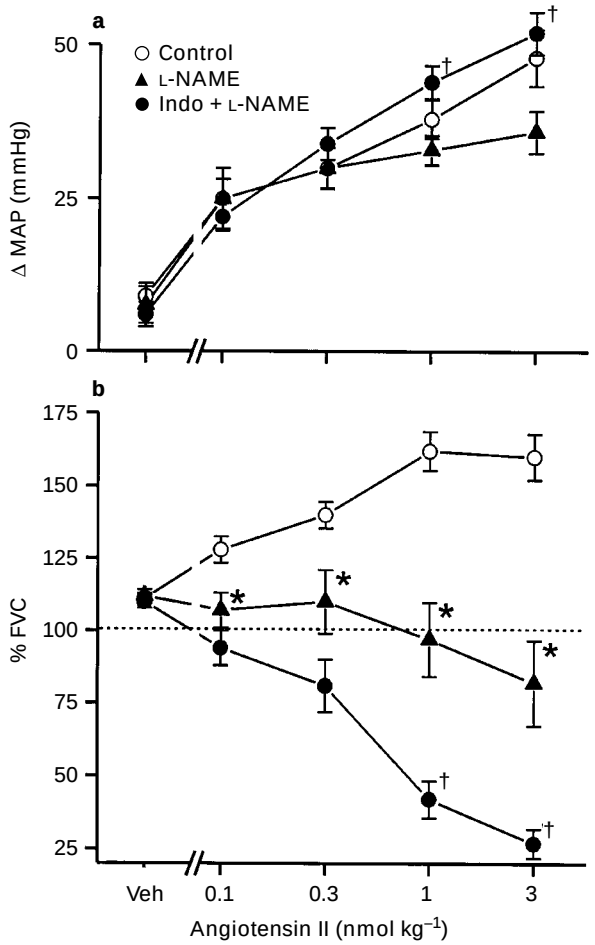

Figure 6 Effects of i.v. bolus injections of angiotensin II on (a) mean arterial blood pressure (MAP) and (b) vascular conductance in the femoral artery (FVC) in the absence (control) and presence of N^G-nitro-L-arginine methyl ester (L-NAME) or indomethacin plus L-NAME (Indo + L-NAME). L-NAME (40 μmol kg^{-1}) or its vehicle (1 ml kg^{-1}) was injected i.v. 30 min, and indomethacin (30 μmol kg^{-1}) was administered i.p. 60 min, before the angiotensin II dose response-curves were recorded. Data are mean and vertical lines show s.e.mean, $n=9-13$. *$P<0.05$ L-NAME versus vehicle; †$P<0.05$ indomethacin plus L-NAME versus L-NAME.

enhanced the ability of L-NAME to reverse the AII-evoked femoral vasodilatation to vasoconstriction. As a consequence, combined administration of indomethacin and L-NAME augmented the hypertensive effect of AII and caused AII to constrict the fermoral artery to the same extent as was seen under gravity control of MAP. It follows that not only NO but also prostaglandins contribute to the femoral dilator effect of AII. The interaction between NO and prostaglandins is unlikely to be serial or additive but appears to arise from reciprocal inhibition of their synthesis. It is known that NO can inhibit the release of prostacyclin (Doni *et al*., 1988; Barker *et al*., 1996) and that prostacyclin blunts the formation of NO (Barker *et al*., 1996). As a consequence, full inhibition of an effect mediated by both NO and prostacyclin may be observed only when the generation of both mediators is suppressed simultaneously. The observation that L-NAME *per se* inhibited the AII-induced femoral vasodilatation whereas indomethacin alone was inactive, suggests that either NO is a quantitatively more important mediator than prostacyclin or that cyclooxygenase blockade disinhibits the NO pathway to a larger degree than NO elimination enhances the release of prostaglandins. Support for the latter conjecture comes from the ability of indomethacin to augment the hypertensive effect of L-NAME in autonomically blocked rats (Ingles *et al*., 1995).

Recently, endothelins have been implicated in the hypertensive action of AII (Balakrishnan *et al*., 1996). Since endothelin-1 can dilate the hindquarter arterial bed via the release of NO (Fozard & Part, 1992), we tested the hypothesis that the femoral vasodilator effect of AII may involve the activation of endothelin receptors. A dose (37 μmol kg^{-1}) of bosentan, which has been shown to antagonize effectively the vasodilator response to endothelin-1 in the hindquarter (Gardiner *et al*., 1994), did not alter the AII-induced femoral vasodilatation, arguing against a significant role of endothelins in this AII effect.

In other respects, the data of this study emphasize that the femoral and mesenteric arterial beds are differentially regulated by changes in MAP (i.e., perfusion pressure). Unlike the corresponding FVC responses, the mesenteric vasoconstrictor responses to AII and AVP and the mesenteric vasodilator effect of telmisartan remained unchanged under gravity control of MAP, which suggests that the mesenteric arterial bed is less reactive to short term changes in perfusion pressure/blood flow than the femoral artery. However, gravity control of MAP revealed that AII and AVP were about three fold more potent in constricting the mesenteric artery (data not shown) than the femoral artery. These observations might, at least in part, explain why the mesenteric artery does not respond with dilatation to systemic hypertension evoked by AII and AVP.

In summary, the present study shows that the femoral vasodilatation caused by exogenous AII is a consequence of the hypertensive action of the peptide which, by increasing the vascular perfusion pressure and probably shear stress, stimulates the formation of the vasodilator autacoids, NO and prostaglandins.

Abbreviations

AII, angiotensin II; AVP, arginine vasopressin; FBF, femoral blood flow; FVC, femoral vascular conductance; L-NAME, N^G-nitro-L-arginine methyl ester; MAP, mean arterial blood pressure; NO, nitric oxide; MBF, mesenteric blood flow; MVC, mesenteric vascular conductance

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