British Journal of Pharmacology (1998) 124, 1467–1474
http://www.stockton-press.co.uk/bjp

Effects of prostaglandins and nitric oxide on the renal effects of angiotensin II in the anaesthetized rat

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1 The potential influences of nitric oxide (NO) and prostaglandins on the renal effects of angiotensin II (Ang II) have been investigated in the captopril-treated anaesthetized rat by examining the effect of indomethacin or the NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), on the renal responses obtained during infusion of Ang II directly into the renal circulation.

2 Intrarenal artery (i.r.a.) infusion of Ang II (1–30 ng kg^{-1} min^{-1}) elicited a dose-dependent decrease in renal vascular conductance (RVC; -38 ± 3% at 30 ng kg^{-1} min^{-1}; $P<0.01$) and increase in filtration fraction (FF; $+49\pm8$%; $P<0.05$) in the absence of any change in carotid mean arterial blood pressure (MBP). Urine output (Uv), absolute (UNaV) and fractional sodium excretion (FENa), and glomerular filtration rate (GFR) were unchanged during infusion of Ang II 1–30 ng kg^{-1} min^{-1} ($+6\pm17$%, $+11\pm17$%, $+22\pm23$%, and -5 ± 9%, respectively, at 30 ng kg^{-1} min^{-1}). At higher doses, Ang II (100 and 300 ng kg^{-1} min^{-1}) induced further decreases in RVC, but with associated increases in MBP, Uv and UNaV.

3 Pretreatment with indomethacin (10 mg kg^{-1} i.v.) had no significant effect on basal renal function, or on the Ang II-induced reduction in RVC (-25 ± 7% vs -38 ± 3% at Ang II 30 ng kg^{-1} min^{-1}). In the presence of indomethacin, Ang II tended to cause a dose-dependent decrease in GFR (-38 ± 10% at 30 ng kg^{-1} min^{-1}); however, this effect was not statistically significant ($P=0.078$) when evaluated over the dose range of 1–30 ng kg^{-1} min^{-1}, and was not accompanied by any significant changes in Uv, UNaV or FENa (-21 ± 12%, -18 ± 16% and $+36\pm38$%, respectively).

4 Pretreatment with L-NAME (10 μg kg^{-1} min^{-1} i.v.) tended to reduce basal RVC (control -11.8 ± 1.4, +L-NAME -7.9 ± 1.8 ml min^{-1} mmHg$^{-1}\times10^{-2}$), and significantly increased basal FF (control $+15.9\pm0.8$, +L-NAME $+31.0\pm3.7$%). In the presence of L-NAME, renal vasoconstrictor responses to Ang II were not significantly modified (-38 ± 3% vs -35 ± 13% at 30 ng kg^{-1} min^{-1}), but Ang II now induced dose-dependent decreases in GFR, Uv and UNaV (-51 ± 11%, -41 ± 14% and -31 ± 17%, respectively, at an infusion rate of Ang II, 30 ng kg^{-1} min^{-1}). When evaluated over the range of 1–30 ng kg^{-1} min^{-1}, the effect of Ang II on GFR and Uv were statistically significant ($P<0.05$), but on UNaV did not quite achieve statistical significance ($P=0.066$). However, there was no associated change in FENa observed, suggesting a non-tubular site of interaction between Ang II and NO.

5 In contrast to its effects after pretreatment with L-NAME alone, Ang II (1–30 ng kg^{-1} min^{-1}) failed to reduce renal vascular conductance in rats pretreated with the combination of L-NAME and the selective angiotensin AT_1 receptor antagonist, GR117289 (1 mg kg^{-1} i.v.). This suggests that the renal vascular effects of Ang II are mediated through AT_1 receptors. Over the same dose range, Ang II also failed to significantly reduce GFR or Uv.

6 In conclusion, the renal haemodynamic effects of Ang II in the rat kidney appear to be modulated by cyclooxygenase-derived prostaglandins and NO. The precise site(s) of such an interaction cannot be determined from the present data, but the data suggest complex interactions at the level of the glomerulus.

Keywords: Renin angiotensin system; angiotensin II; renal vasculature; renal tubules; nitric oxide; prostaglandins

Introduction

The renin angiotensin system (RAS) is a major controller of sodium and water homeostasis, and is hence pivotal in the control of blood pressure (Hall, 1986). The effector octapeptide of the RAS, angiotensin II (Ang II), induces sodium retention through a variety of actions on renal haemodynamic and tubular function, mediated by angiotensin receptors located on the afferent and efferent arterioles, glomerulus, medullary interstitial cells, and tubule epithelium (Ichikawa & Harris, 1991). Recent studies have suggested that receptors for Ang II in the rat kidney are of the losartan-sensitive AT_1 subtype (Zhuo *et al*., 1992). These receptors have been localized to the afferent and efferent arterioles, glomerulus, interstitial cells, and proximal tubule of rat kidney (Edwards & Aiyar, 1993; Zhuo *et al*., 1992, 1994).

Although a consequence of interaction with a single class of renal angiotensin receptors, the effects of Ang II may be modulated by other factors, for example prostaglandins and nitric oxide (NO). Ang II stimulates renal prostaglandin production *in vivo* in the rat (Danon *et al*., 1975; Petrulis *et al*., 1981), and from isolated glomeruli (Schlondorff *et al*., 1980). Following blockade of the formation of these predominantly vasodilator prostaglandins, the renal vasoconstrictor effect of Ang II is often augmented (Finn & Arendshorst, 1976). However, there is also evidence for Ang II-induced release of

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the constrictor agent, thromboxane A_2, which then mediates renal vasoconstriction (Wilcox *et al*., 1991).

The question of whether NO and Ang II interact in the control of renal function has been the subject of much investigation. The renal vasoconstriction induced by NO synthase inhibition *in vivo* is attenuated by angiotensin antagonists under conditions of an activated RAS (Sigmon *et al*., 1994), and Ang II stimulates NO release from the afferent arteriole (Ito *et al*., 1991) and mesangial cells (Schultz *et al*., 1990). Whether NO can influence the tubular effects of Ang II has not been elucidated fully, although NO synthase is present in the proximal tubule epithelium (Terada *et al*., 1992), a site associated with the presence of angiotensin receptors (Liu & Cogan, 1987).

Previous studies to investigate interactions of Ang II with prostaglandins or NO may have been complicated by various factors. For example, results obtained using Ang II antagonists are influenced by the extent of endogenous RAS activation. Furthermore, administration of Ang II *via* the intravenous route is associated with complicating haemodynamic changes. Therefore, this study has examined the influence of an NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), and indomethacin on the renal effects of Ang II infused *directly* into the renal circulation. Additionally, by using captopril-pretreated rats, any influence of the extent of background activation of the endogenous RAS should have been removed. In some rats, the influence of a non-peptide angiotensin AT_1 antagonist, GR117289 (Robertson *et al*., 1992) on the effects of Ang II were investigated.

A preliminary account of these findings was made to the British Pharmacological Society (Polley *et al*., 1995).

Methods

Surgical preparation

Male, albino Glaxo Wellcome-bred, Wistar-derived rats (mean body weight 305 ± 9 g) were fasted overnight, anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1} intraperitoneally), and cannulae were placed in the trachea to facilitate respiration, and in the left carotid artery for measurement of mean blood pressure (MBP). Cannulae were also placed in the left and right femoral veins, for infusion of saline (1.5 ml h^{-1}) or saline (1.5 ml h^{-1}) containing Sagatal (20 mg kg^{-1} h^{-1}), respectively. A fine cannula (Portex pp10, stretched at one end to an external diameter of approximately 0.2 mm) was inserted into the left femoral artery, and advanced retrogradely into the abdominal aorta (Aiken & Vane, 1973). This cannula was kept patent by constant infusion of saline (3 ml h^{-1}).

A midline, abdominal incision was made, and the ureters of both kidneys were cannulated (Portex pp10). The left renal artery was carefully cleared of connective tissue, and exposed close to its origin at the aorta. The tip of the fine cannula inserted *via* the femoral artery was then located in the aorta, and positioned close to the origin of the left renal artery. The fine tip of the cannula was then manipulated into the renal artery, allowing a constant intrarenal artery (i.r.a.) infusion of saline directly to the left kidney. An ultrasonic flow probe (Transonic 1RB, Linton Instrumentation, Diss, U.K.) was then placed around the renal artery, proximal to the fine cannula tip, for measurement of renal blood flow (RBF), and subsequent calculation of renal vascular conductance (RVC). Following completion of surgery, a bolus injection of inulin was administered (20 mg i.v.), and the infusion into the right femoral vein (saline + Sagatal, 20 mg kg^{-1} h^{-1} at 1.5 ml h^{-1}) was supplemented with inulin at a concentration of 40 mg ml^{-1}. Captopril (50 μg kg^{-1} min^{-1} i.v.) was introduced into the saline infusion into the left femoral vein 75 min after completion of surgery and maintained for the duration of the experiment. This dose of captopril was demonstrated, in 10 pilot experiments, to produce an $86 \pm 7\%$ and $89 \pm 4\%$ inhibition of the pressor and renal vasoconstrictor responses, respectively, to exogenous angiotensin I (300 ng kg^{-1} i.v.). The preparation was then allowed to equilibrate for 2 h prior to commencing renal clearance studies.

Experimental protocol

Following equilibration, a series of basal 15 min clearance periods was started. For each 15 min period, urine was collected into pre-weighed tubes, and the volume voided measured gravimetrically. An arterial blood sample (0.25 ml) was collected into lithium-heparin coated tubes at the midpoint of each clearance period, and MBP and RBF were measured at the end of each period. Basal stability was considered to have been achieved when there was less than 15% variation in urine volume or cardiovascular measurements over three consecutive clearance periods. For each measured or derived parameter, the average of these three basal periods was taken as a mean basal value. Changes occurring during subsequent infusion of Ang II or vehicle were expressed relative to this basal value.

Following attainment of baseline stability, one group of rats continued to be infused with saline only into the left renal artery for the duration of the experiment. In other groups, this infusion was replaced with saline containing Ang II (1 ng kg^{-1} min^{-1} i.r.a.). After a 5 min equilibration period, a 15 min clearance period was conducted, during which urine was collected, and a blood sample was obtained at the midpoint. Following completion of this clearance period, the intrarenal infusion of Ang II was replaced with a higher concentration of Ang II (3 ng kg^{-1} min^{-1} i.r.a.), and another clearance period was started 5 min later. This procedure was repeated with increasing concentrations of Ang II, to provide a final dose range of 1 to 300 ng kg^{-1} min^{-1} i.r.a.

Three further groups of rats received additional pretreatment prior to commencing basal clearance periods: (a) indomethacin (INDO; 10 mg kg^{-1} i.v.), administered 90 min prior to commencing basal clearances, (b) L-NAME (10 μg kg^{-1} min^{-1} i.v.), commencing 90 min prior to basal clearances, and (c) L-NAME commencing 90 min prior to basal clearances plus GR117289 (1 mg kg^{-1} i.v.) administered 60 min later (i.e. 30 min prior to basal clearances). Thereafter, the protocol described above was followed, using intrarenal infusion of Ang II 1–300 ng kg^{-1} min^{-1} i.r.a.

Calculation of indices of renal function

A small aliquot of each blood sample was removed for determination of haematocrit. The remaining blood was centrifuged, and plasma and urine samples stored at 4°C until further analysis. Subsequently, plasma and urinary sodium concentration was determined using ion-selective electrodes (Beckman analyser E2A), and inulin concentration was determined by colorimetric assay. Calculations of urine output (Uv), absolute (UNaV) and fractional excretion of sodium

(FENa), and of glomerular filtration rate (GFR) and filtration fraction (FF) were then made using standard formulae.

Materials used

Sodium pentobarbitone was obtained from Sanofi Animal Health. GR117289 (prepared as a sodium salt) was synthesized at Glaxo Wellcome Research & Development Ltd., and was dissolved in saline, after correcting for the quantity of active compound. All other chemicals were purchased from Sigma. INDO was dissolved in 0.1 M NaOH, and subsequently diluted with saline. All other chemicals were dissolved and diluted in saline.

Statistical analysis

Basal values obtained in different groups of rats were compared using Student's *t*-test for unpaired data. Changes in MBP markedly influence renal excretory function, and hence analysis of the effects of drugs on renal function should be made in the absence of changes in renal perfusion pressure. Therefore analysis was focused on the effects of non-pressor doses of Ang II ($1-30$ ng kg^{-1} min^{-1} i.r.a.).

The slopes of the log dose response curves constructed for each cardiovascular and renal parameter in the different treatment groups were determined as a summary statistic for each group. This removes the possible influence on comparisons of different basal values between groups. The calculated slopes were compared by analysis of covariance. The effect of increasing dose on each parameter was also assessed by comparing the mean slope of each treatment group against zero slope. The slopes measured in individual groups were compared by Student's *t*-test for unpaired data. Statistical significance was assumed at $P<0.05$.

Results

Unless stated otherwise, all changes in renal excretory function refer to the left, infused kidney.

Basal values obtained in different groups of rats are shown in Tables 1 and 2. All experiments were conducted in the presence of captopril, which induces a significant fall in MBP and rise in RBF in these rats (unpublished observations). Thus, the measured level of RBF was consistent with previous reports using captopril-pretreated rats (Deng *et al*., 1996). In rats infused with saline, only (i.r.a.), basal values of RBF, RVC and GFR (Table 1) were higher than measured in other groups of rats (Table 2), although measures of renal excretory function were similar. The reason for this difference is unknown. However, the statistical analysis applied (see above) enabled comparisons to be made between groups with different basal values.

Effects of Ang II or vehicle (saline) infusion i.r.a.

In rats infused with saline, only (i.r.a.), there was a tendency for MBP to decline over time, and for RBF and RVC to increase (Table 1). Concomitant with the decline in MBP were small, non-significant decreases in Uv, UNaV, GFR and FF (Table 1).

Infusion of Ang II ($1-30$ ng kg^{-1} min^{-1} i.r.a.) reduced RBF (by -1.1 to -4.8 ml min^{-1}; $P<0.01$) in the absence of any significant change in MBP (Figure 1). Higher doses of Ang II (100 and 300 ng kg^{-1} min^{-1}) reduced RBF further, although dose-related increases in MBP ($+19$ to $+32$ mmHg) were also observed. Thus, RVC decreased significantly ($P<0.01$) in a dose-related fashion over the entire dose range of Ang II (Figure 1), a response significantly different from the effects of the infusion of saline alone ($P<0.01$).

Table 1 Cardiovascular and renal parameters in rats infused with saline, only, i.r.a. Data are shown as arithmetic mean±s.e.mean from five animals

		Change from basal at time intervals during infusion of saline i.r.a.		
	Basal	*0.5 h*	*1 h*	*1.5 h*
MBP (mmHg)	106±7	−10±5	−12±4	−14±5
RBF (ml min^{-1})	17.9±1.5	−0.2±0.9	0.5±0.7	1.0±1.1
RVC (ml min^{-1} mmHg$^{-1}\times10^{-2}$)	17.0±1.0	1.6±0.7	2.8±0.5	3.6±0.3
GFR (ml min^{-1})	1.8±0.2	−0.2±0.2	−0.2±0.2	−0.4±0.2
Uv (μl min^{-1})	41.7±6.1	−7.9±1.8	−7.8±3.7	−14.1±5.0
UNaV (μmol min^{-1})	7.0±1.2	−1.0±0.2	−0.2±0.6	−1.1±1.0
FENa (%)	2.6±0.3	0.2±0.5	0.5±0.4	0.4±0.7
FF (%)	17.2±1.0	−2.9±1.6	−3.5±1.3	−5.5±1.7

Table 2 Basal values of cardiovascular and renal parameters prior to infusion of Ang II i.r.a. Data are arithmetic mean±s.e.mean from four to six animals

	Pretreatment			
	Saline (control) ($n=6$)	*INDO* ($n=5$)	*L-NAME* ($n=6$)	*L-NAME+ GR117289* ($n=4$)
MBP (mmHg)	114±9	110±4	116±4	102±9
RBF (ml min^{-1})	13.0±1.1	12.0±2.5	8.9±1.9	9.7±1.2
RVC (ml min^{-1} mmHg$^{-1}\times10^{-2)}$	11.8±1.4	11.0±2.3	7.9±1.8	9.9±1.5
GFR (ml min^{-1})	1.3±0.1	1.3±0.1	1.5±0.2	1.6±0.4
Uv (μl min^{-1})	34.5±6.7	37.7±5.5	47.0±6.6	42.0±12.6
UNaV (μmol min^{-1})	6.8±1.6	6.8±1.6	8.0±1.1	7.4±2.7
FENa (%)	3.4±0.7	4.2±1.3	3.6±0.5	4.2±2.2
FF (%)	15.9±0.8	20.7±3.4	31.0±3.7*	24.6±3.6

*Denotes $P<0.05$ compared with Saline (Control).

Infusion of Ang II, at doses not changing MBP (1–30 ng kg^{-1} min^{-1}), did not influence GFR, Uv or UNaV (Figure 1). Changes in these parameters were similar to the effects of saline alone, despite the marked reductions in RVC. At pressor doses of Ang II (100 and 300 ng kg^{-1} min^{-1}) GFR was maintained at a basal level, but marked increases in Uv and UNaV were observed (Figure 1). Associated with the maintained GFR, but reduced RBF, was a small, but significant ($P<0.05$), dose-related increase in FF (+7.8% at 30 ng kg^{-1} min^{-1}) during infusion of Ang II.

Infusion of Ang II at non-pressor doses (1–30 ng kg^{-1} min^{-1} i.r.a.). into the left kidney had no significant effect on

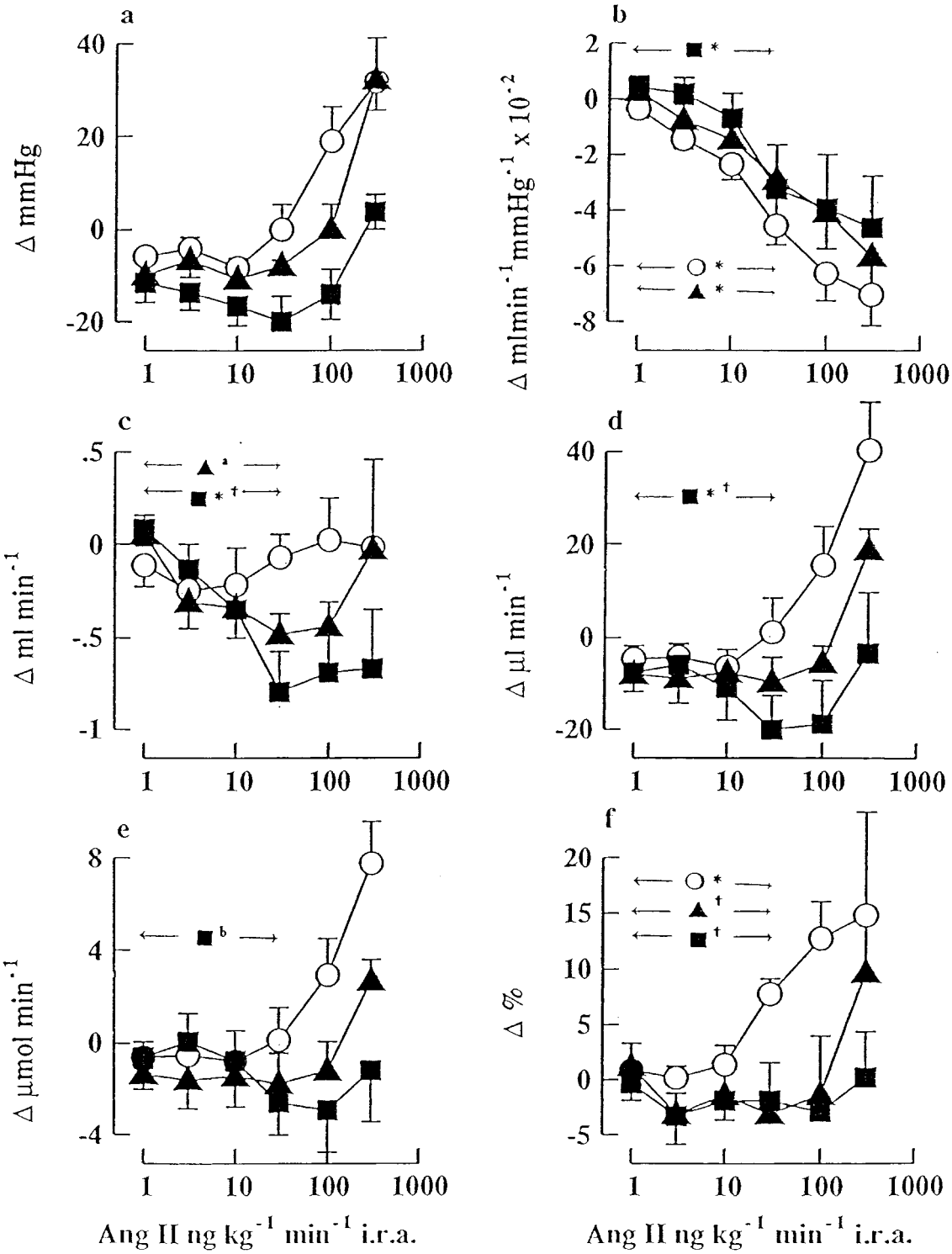

Figure 1 The effect of increasing doses of Ang II on (a) MBP, (b) RVC, (c) GFR, (d) Uv, (e) UNaV and (f) FF, in the absence (○) and presence of INDO (▲) or L-NAME (■). Data are arithmetic mean ± s.e.mean from $n=5-6$ animals. The slopes of the Ang II log dose response curves (1–30 ng kg^{-1} min^{-1}) were subjected to statistical analysis. Significant differences in slopes are depicted for data in the absence (← ○ →) and presence of INDO (← ▲ →) or L-NAME (← ■ →). *$P<0.05$ for change from basal, †$P<0.05$ compared with the effects of Ang II in the absence of pretreatment (○), and [a] and [b] denote $P=0.078$ and $P=0.066$ for change from basal, respectively.

GFR, FENa, Uv or UNaV of the right, non-infused, kidney in any treatment group. As MBP increased, there were concomitant rises in Uv, UNaV and FENa in the right kidney.

Influence of INDO or L-NAME on basal parameters

Basal values measured following saline infusion or after INDO or L-NAME pretreatment, but prior to Ang II infusion, are shown in Table 2. INDO pretreatment did not significantly change any basal value compared with control rats (saline i.v. + Ang II i.r.a.), although FF of INDO-treated rats was increased slightly. Infusion of L-NAME tended to reduce RBF and RVC, although values failed to achieve statistical significance compared with control rats. Although RBF declined, GFR was not changed compared with control rats, and consequently FF was increased significantly ($P<0.05$). Basal cardiovascular and renal parameters measured in rats pretreated with L-NAME and the angiotensin AT_1 receptor antagonist, GR117289, were not significantly different from measurements made in control rats, or in rats treated with L-NAME alone (Table 2).

Influence of INDO on effects of Ang II i.r.a.

In the presence of INDO, Ang II (1–30 ng kg^{-1} min^{-1} i.r.a.) caused dose-related decreases in RBF (−0.9 to −3.7 ml min^{-1}; $P<0.05$) and RVC (0.3 to −2.9 ml min^{-1} $mmHg^{-1} \times 10^{-2}$; $P<0.05$) without increasing MBP (Figure 1). These changes were slightly, but not significantly, smaller than those observed in control rats. At 100 ng kg^{-1} min^{-1} i.r.a., the increase in MBP caused by Ang II (+19 mmHg) appeared attenuated in the presence of INDO (−0.1 mmHg), although this difference failed to achieve statistical significance. At an infusion rate of 300 ng kg^{-1} min^{-1} i.r.a., Ang II caused a pressor response of equal magnitude in both control and INDO-pretreated rats (+32 mmHg).

In contrast to its effect in control rats, Ang II 1–30 ng kg^{-1} min^{-1} i.r.a. appeared to cause a dose-related

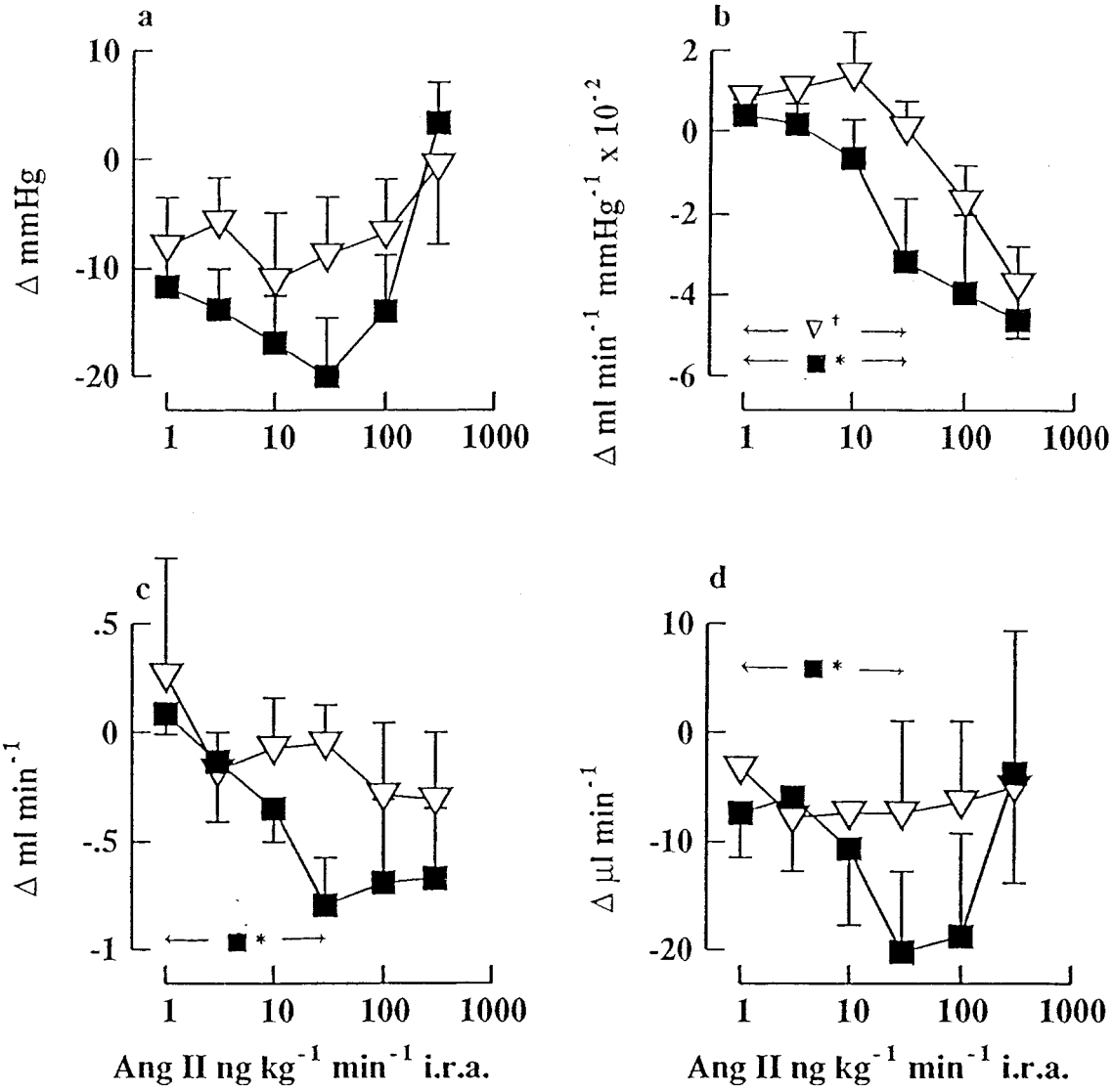

Figure 2 The effect of increasing doses of Ang II on (a) MBP, (b) RVC, (c) GFR and (d) Uv in rats pretreated with L-NAME in the absence (■) and presence (▽) of GR117289. Data are arithmetic mean ± s.e.mean from $n=4-6$ animals. The slopes of the Ang II log dose response curves (1–30 ng kg^{-1} min^{-1}) were subjected to statistical analysis. Significant differences in slopes are depicted for data in the absence (← ■ →) and presence of GR117289 (← ▽ →). *$P<0.05$ for change from basal, and †$P<0.05$ compared with the effects of Ang II in the absence of GR117289.

reduction in GFR in INDO-pretreated rats (Figure 1), although this effect failed to achieve statistical significance ($P=0.078$). FF was not changed significantly during infusion of Ang II, thereby being significantly different ($P<0.05$) from the effect of Ang II in control, saline-infused rats. There was no change in Uv, UNaV or FENa (Figure 1) during infusion of non-pressor doses of Ang II in INDO-treated rats. At pressor doses of Ang II, GFR, FF, Uv, UNaV and FENa increased above basal levels.

Influence of L-NAME on effects of Ang II i.r.a.

In the presence of L-NAME, Ang II caused dose-related, significant reductions in RBF and RVC (Figure 1). This renal vasoconstrictor effect of Ang II appeared attenuated compared with control rats, although this difference was not significant. A fall in MBP occurred (−18 mmHg) during infusion of Ang II ($1-30$ ng kg^{-1} min^{-1}), although similar falls in MBP occurred in rats infused with saline alone without significant reduction in any other parameter (Figure 1).

In marked contrast to its effect in control rats, Ang II infusion in rats pretreated with L-NAME resulted in a dose-related, significant ($P<0.01$) decrease in GFR but no change in FF (Figure 1). These responses were significantly different ($P<0.05$) from the effects of Ang II on GFR and FF in saline-treated rats. Furthermore, in the presence of L-NAME, Ang II caused a small, but significant ($P=0.03$), dose-related reduction in Uv (−19.8 μl min^{-1} at 30 ng kg^{-1} min^{-1}) which was significantly different from the effect of Ang II in saline-treated rats ($P<0.05$). UNaV also tended to decline (−2.6 μmol min^{-1}) although this failed to achieve statistical significance ($P=0.066$). The fall in UNaV was not associated with changes in FENa, which was unchanged during Ang II infusion in both control and L-NAME-treated rats (+0.1% and +1.3%, respectively).

In the presence of L-NAME and GR117289, Ang II i.r.a. failed to significantly change MBP, even at high infusion rates (Figure 2). Ang II-induced renal vasoconstriction was also attenuated in the presence of GR117289, as indicated by a rightward displacement of the Ang II dose-response curve on RVC in GR117289-treated rats (Figure 2). Thus, changes in RVC were significantly different ($P<0.05$) compared with the effects of Ang II in rats pretreated with L-NAME only. Moreover, intrarenal infusion of Ang II in the presence of L-NAME and GR117289 did not significantly change GFR and Uv (Figure 2), or FF, UNaV or FENa, in contrast to the significant effects of Ang II on GFR and Uv in the absence of GR117289.

Discussion

Intrarenal artery infusion of Ang II ($1-30$ ng kg^{-1} min^{-1}) induced marked, dose-related falls in RVC in the absence of any change in MBP. Despite this marked reduction in RVC, GFR was maintained at basal levels throughout the dose range of Ang II, while there was a progressive decrease in RBF. Thus, there was a dose-related rise in FF during Ang II infusion. Although caution should be exercised in deducing arteriolar resistance changes on the basis of FF data alone (Carmines *et al*., 1987), the rise in FF during Ang II infusion is consistent with preferential efferent arteriolar vasoconstriction (Edwards, 1983), and perhaps afferent arteriolar vasoconstriction (Steiner & Blantz, 1979) at higher doses which would contribute to the fall in total RBF.

Despite a marked reduction in RVC, intrarenal infusion of Ang II failed to influence Uv, UNaV or FENa. Furthermore, infusion of Ang II at doses devoid of haemodynamic effects ($0.003-1$ ng kg^{-1} min^{-1} i.r.a.), or the use of short (5 min) clearance periods failed to reveal Ang II-induced changes in these parameters (unpublished observations). Thus, even allowing for a biphasic effect of Ang II (Harris & Young, 1977) or for the potential of long clearance periods to obscure a subtle effect (Barraclough *et al*., 1967), a direct action of Ang II at the renal tubules was not evident under these conditions. The reason for the difference between this and previous studies (Barraclough *et al*., 1967; Malvin & Vander, 1967) showing Ang II-induced reductions in GFR and sodium excretion is unclear, but may be related to the use of anaesthetized compared with conscious animal models. Furthermore, intrarenal levels of Ang II can far exceed circulating plasma levels (Seikaly *et al*., 1990). Therefore, the dose of captopril used may not have completely removed the influence of endogenous Ang II at the renal tubules.

In the presence of INDO, Ang II infusion tended to cause a reduction in GFR, although the effect did not achieve statistical significance ($P=0.078$). The mechanism underlying this trend cannot be elucidated from the present data, but could be speculated to involve the loss of prostaglandin-mediated efferent arteriolar constriction, for example through reduced Ang II-induced generation of thromboxane A_2 (Wilcox *et al*., 1991). Furthermore, reduced post-glomerular vasoconstriction in the presence of INDO would also account for the failure of Ang II to increase FF as it did in control animals, and also explain, in part, the apparent attenuation of Ang II-induced decreases in RBF. Alternatively, the loss of cyclooxygenase-derived dilator agents in the pre-glomerular vasculature, or increased generation of lipoxygenase-derived constrictor leukotrienes (Stern *et al*., 1989), could contribute to Ang II-induced afferent arteriolar vasoconstriction and a decline in GFR.

L-NAME pretreatment caused a transient rise in MBP, and a sustained decrease in RVC without change in GFR. These effects of an NO synthase inhibitor have been described extensively in the rat (Tolins *et al*., 1990), and have been suggested to indicate a preferential increase in post-glomerular resistance (Zatz & DeNucci, 1991), consistent with the significant rise in FF observed in the present study. Despite the reduction, induced by L-NAME, in basal mean RBF and RVC, Ang II caused further, significant dose-related decreases in RBF and RVC; in addition, Ang II significantly reduced GFR in L-NAME pretreated rats, but FF was not further affected. The Ang II-evoked decrease in GFR could be explained by a preferential pre- rather than post-glomerular vasoconstriction in L-NAME pretreated animals. The difference in the balance between the pre- and post-glomerular effects of Ang II in L-NAME pretreated animals, compared with those in which NO synthase was intact, might be attributed to two factors. Firstly, an increase in post-glomerular resistance caused by L-NAME, itself, might diminish the scope for any further effect of Ang II. Secondly, inhibition of NO synthesis might allow unopposed Ang II-induced pre-glomerular vasoconstriction. In this respect, Ang II has been shown to induce NO release from the afferent arteriole of the rabbit (Ito *et al*., 1991) and NO dilates this vessel in the rat (Imig & Roman, 1992; DeNicola *et al*., 1992). Taken together, the different balance of pre- and post-glomerular effects of Ang II could also explain why FF was not increased and GFR was reduced, yet absolute decreases in RBF and RVC were similar in L-NAME pretreated and control rats.

In L-NAME pretreated animals, Ang II not only reduced GFR, but also caused a small, but significant, decrease in Uv. UNaV was also reduced, albeit not significantly ($P=0.066$). In contrast, Ang II did not significantly reduce Uv or UNaV in indomethacin pretreated rats probably because, despite a tendency for Ang II to reduce GFR, the effect was less pronounced than in L-NAME pretreated animals, and did not achieve statistical significance ($P=0.078$). Furthermore, since Ang II did not change tubular sodium reabsorption (FENa) in indomethacin or L-NAME treated animals, the small changes it did induce in Uv and UNaV may reflect a decrease in filtered load, suggesting that the effects of Ang II were restricted to the glomerulus.

Ang II induces mesangial cell contraction (Ausiello *et al*., 1980) and a reduction in glomerular ultrafiltration coefficient, K_f (Blantz *et al*., 1976), effects which are buffered by prostaglandins (Scharschmidt *et al*., 1983) and NO (Schultz *et al*., 1990). Although it cannot be determined in the present study, pretreatment with INDO or L-NAME may enable unopposed mesangial cell contraction in response to Ang II, leading to reduced glomerular surface area and a decrease in GFR. Indeed, experiments in INDO-treated dogs have shown that Ang II induces falls in GFR *via* an effect at the glomerulus (Bugge & Stokke, 1994). Whether such an interaction between Ang II and NO in this respect could explain the decreases in Uv and UNaV that were observed requires further investigation.

A further possibility which cannot be addressed in these studies, is whether there is alteration of tubuloglomerular feedback (TGF) mechanisms, since Ang II may modulate the sensitivity of this system (Schnermann & Briggs, 1986). NO synthase is present in the macula densa, and a role for NO as a mediator of the TGF signal has been proposed (Wilcox *et al*., 1992). It is therefore possible that when NO synthase is inhibited, the TGF response becomes blunted, and Ang II-induced reductions in GFR and filtered load could continue unchecked, leading to the observed reductions in GFR concomitantly with Uv and UNaV.

Although the precise vascular mechanism(s) by which Ang II reduced RVC, GFR and Uv in the presence of L-NAME is unclear, these effects of Ang II were prevented by co-administration of L-NAME with GR117289. Thus, these data suggest that Ang II mediated its effects via an action at AT_1 receptors, and that in agreement with other literature reports (see Edwards & Aiyar, 1993; Zhuo *et al*., 1992), the angiotensin receptors mediating functional responses at the glomerulus, renal vasculature, and renal tubules of the rat are of the AT_1 subtype.

In summary, no evidence was found in this study for an effect of Ang II at the renal tubules. There are at least two possible explanations for this. Firstly, as has already been alluded to, it is possible that the dose of captopril used was insufficient to abolish the influence of endogenous Ang II at the renal tubules, making it difficult to superimpose any additional effect with exogenous Ang II. Secondly, it is possible, as pointed out by Parekh (1995) that small quantities of Ang II infused continuously into the renal artery do not mix properly with renal arterial blood, and are not distributed evenly throughout the kidney. Under these conditions, the renal vascular, rather than tubular, effects of Ang II might predominate. However, it is interesting to note that the extent to which Ang II infusions (3 and 10 ng kg^{-1} min^{-1}) reduced renal blood flow (15 and 26%, respectively) in the present experiments, bracketed that reported by Parekh (1995) for a single dose of 5 pmol kg^{-1} min^{-1} ($\sim$5 ng kg^{-1} min^{-1}; renal blood flow reduced by 24%), administered in such a way as to ensure adequate mixing. Thus, although we can not discount the possibility, it seems unlikely that inadequate mixing of exogenous Ang II with renal arterial blood in our experiments can be held responsible for our failure to observe a tubular action of Ang II. Nevertheless, this study has demonstrated that, in the absence of MBP changes and following inhibition of angiotensin converting enzyme activity, the renal vascular effects of exogenous Ang II in the rat are opposed by the synthesis and release of cyclooxygenase-derived prostaglandins and NO. This model cannot elucidate the exact mechanism(s) by which this occurs, but suggests complex interactions between Ang II and prostaglandins or NO at the level of the glomerulus.

The authors would like to acknowledge the invaluable assistance provided by Kath Shawley in the statistical analysis of the data.

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(Received April 15, 1997
Revised December 1, 1997
Accepted May 25, 1998)