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Role of angiotensin AT_1 and AT_2 receptors in mediating the renal effects of angiotensin II in the anaesthetized dog

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1 Experiments were performed using the selective AT_1 receptor antagonist, GR117289, and the selective AT_2 receptor antagonist, PD123177, to assess the relative importance of AT_1 versus AT_2 receptors in mediating the renal effects of angiotensin II (AII) *in vivo*, in salt-replete pentobarbitone-anaesthetized dogs.

2 The AT_1 receptor antagonist, GR117289 ($0.5\,mg\,kg^{-1} + 1\,\mu g\,kg^{-1}\,min^{-1}$, i.v.), caused renal vasodilatation, characterized by a mean increase of $21 \pm 5\%$ in renal blood flow, 45 min post-dose. GR117289 also caused a fall in mean blood pressure ($12 \pm 4\%$), but despite this, sodium and urine excretion were not reduced. Indeed, there was a tendency for urine output and sodium excretion to increase, although the changes were not statistically significant. GR117289 caused a reduction in plasma aldosterone levels ($-35 \pm 16\%$) 45 min post-dose, despite increasing plasma renin activity ($+173 \pm 42\%$). In contrast to GR117289, the AT_2 receptor antagonist, PD123177 ($20\,\mu g\,kg^{-1}\,min^{-1}$ intra-renal artery; i.r.a.) caused no significant change in blood pressure, renal blood flow, or sodium and urine excretion, indicating that the renal effects of endogenous AII in these salt-replete animals are mediated predominantly by AT_1 receptors.

3 Intra-renal artery infusion of AII ($1-300\,ng\,kg^{-1}\,min^{-1}$) caused dose-related renal vasoconstriction, and decreases in urine output, sodium excretion, fractional excretion of sodium, and glomerular filtration rate (GFR). The AT_1 receptor antagonist, GR117289 ($0.5\,mg\,kg^{-1} + 1\,\mu g\,kg^{-1}\,min^{-1}$, i.v.) antagonized these renal effects of AII, causing 15–38 fold rightward displacements of mean dose-response curves for these parameters. In contrast, PD123177 ($20\,\mu g\,kg^{-1}\,min^{-1}$, i.r.a.) failed to antagonize the renal haemodynamic and excretory effects of lower doses of AII ($1-10\,ng\,kg^{-1}\,min^{-1}$, i.r.a.). However, at higher doses of AII ($30-300\,ng\,kg^{-1}\,min^{-1}$, i.r.a.), while PD123177 still failed to antagonize the effects of the peptide on urine output, sodium excretion and GFR, it did cause a small, but significant, degree of inhibition of AII-induced renal vasoconstriction. In addition, at a higher dose ($50\,\mu g\,kg^{-1}\,min^{-1}$, i.r.a.), PD123177 caused a greater degree of antagonism of AII-induced renal vasoconstriction, while renal excretory responses to AII remained unaffected.

4 This study shows that the renal haemodynamic and excretory effects of AII in salt-replete anaesthetized dogs are mainly mediated by angiotensin AT_1 receptors. However, the inhibitory effect of PD123177 on renal vasoconstrictor responses to high doses of AII, raises the possibility that functionally important AT_2 receptors are present in the canine renal vasculature.

Keywords: Angiontensin II; AT_1 receptor antagonist; AT_2 receptor antagonist; GR117289; PD123177; renal function; anaesthetized dog

Introduction

The renin-angiotensin system plays an important role in the physiological control of blood pressure. However, it is now widely thought that overactivity of the system contributes to the pathology of essential hypertension, and angiotensin-converting enzyme (ACE) inhibitors, which prevent the formation of angiotensin II (AII), are effective antihypertensive agents (see Laragh, 1990). An alternative method of inhibiting the activity of the renin-angiotensin system is to block angiotensin receptors and this approach has led to the discovery and development of non-peptide angiotensin receptor antagonists, such as losartan (DuP 753; Chiu *et al.*, 1990) and GR117289 (Robertson *et al.*, 1992). These compounds are being investigated as potential novel treatments for hypertension and heart failure.

In addition to their potential therapeutic utility, non-peptide angiotensin receptor antagonists are proving valuable in the study of angiotensin receptor pharmacology, and their use in radioligand binding studies has already resulted in the identification of two clear subtypes of angiotensin receptors, termed AT_1 and AT_2 (Bumpus *et al.*, 1991). Angiotensin AT_1 receptors mediate the physiologically important effects of AII such as vasoconstriction and aldosterone release (Wong *et al.*, 1990), and selectively bind non-peptide antagonists such as losartan and GR117289 (Chiu *et al.*, 1989; Robertson *et al.*, 1992). Angiotensin AT_2 receptors selectively bind the non-peptide, PD123177 (Chiu *et al.*, 1989), and the peptide, CGP42112A (Whitebread *et al.*, 1989). In contrast to AT_1 receptors, the functional role of AT_2 receptors remains to be determined, although recently it has been reported that increases in guanosine 3′:5′-cyclic monophosphate (cyclic GMP) occur following stimulation of AT_2 receptors in the neuroblastoma cell line, NIE-115 (Zarahn *et al.*, 1992). The physiological relevance of AT_2 receptors, however, remains unclear (Wong *et al.*, 1990).

AII acts in the kidney to induce arteriolar vasoconstriction, and sodium and water retention. These effects are mediated by receptors at both vascular and proximal tubular sites (Navar *et al.*, 1991; Harris, 1992). In addition, AII stimulates the release of aldosterone which acts on the distal tubule and also stimulates sodium reabsorption. The effects of AII on renal function are pivotal in the role that the

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renin-angiotensin system plays in cardiovascular homeostasis. Autoradiographic studies have recently shown that both angiotensin AT_1 and AT_2 receptors are present in the renal cortex of monkeys (Chang & Lotti, 1991; Gibson *et al.*, 1991) and man (Grone *et al.*, 1992). Studies using the AT_1 receptor antagonist, losartan, indicate an important role for AT_1 receptors in mediating the renal haemodynamic effects of AII in rats and dogs (Batin *et al.*, 1991; Wong *et al.*, 1991a; Clark *et al.*, 1991). However, the importance, if any, of AT_2 receptors in the renal effects of AII remains unclear. Thus, using the selective AT_1 receptor antagonist, GR117289, and the selective AT_2 receptor antagonist, PD123177, the present study aimed to assess the role of AT_1 and AT_2 receptors in mediating the effects of AII on renal haemodynamic and excretory function *in vivo*. A preliminary account of these findings has been published in abstract form (Clark *et al.*, 1992).

Methods

Surgical preparation

Beagle dogs (10–12 kg) of either sex were anaesthetized with sodium pentobarbitone (30–40 mg kg^{-1} i.v.). Anaesthesia was maintained with a constant infusion of sodium pentobarbitone (5–10 mg kg^{-1} h^{-1}, i.v., in a volume of 5–10 ml h^{-1}) administered into the right cephalic vein. Dogs were intubated and artificially respired. Body temperature was maintained at 37 ± 1°C with a thermostatically controlled heating blanket. Dogs subjected to a lengthy period of abdominal surgery tend to develop metabolic acidosis. Consequently, physiological pH was maintained by giving a constant infusion of sodium bicarbonate (0.03 mmol kg^{-1} min^{-1}, in a volume of 0.03 ml kg^{-1} min^{-1}) via a cannula in the left femoral vein. Arterial blood pH, and O_2 and CO_2 tensions, were monitored with a Radiometer ABL30 acid-base analyser. Blood pressure was recorded from a cannula inserted into the abdominal aorta via the right femoral artery, using a Bell and Howell pressure transducer. Heart rate was derived electronically from the blood pressure signal.

A laparotomy was performed and both ureters were cannulated with polythene tubing (Portex, internal diameter = 0.06 mm), for the collection of urine. Urine output was measured gravimetrically. Left renal blood flow was measured with an electromagnetic flow probe (Statham 2.5 mm diameter). Renal vascular conductance was derived from the measurements of renal blood flow and mean blood pressure. A needle (Microlance 26G) connected to polythene tubing (Portex, internal diameter = 0.76 mm) was inserted into the left renal artery, and kept patent with an infusion of saline (0.5 ml min^{-1}). During the surgical period, from the point at which the laparotomy was performed, dogs were saline-loaded (30 ml kg^{-1} at 0.27 ml kg^{-1} min^{-1}) via a cannula in the right femoral vein. The dogs were then maintained in this saline-loaded state by lowering the infusion rate to 0.07 ml kg^{-1} min^{-1}. This saline load was given to ensure a steady state of urine production. Following the surgery, a bolus dose of creatinine (50 mg kg^{-1}. i.v., volume = 5 ml) was administered followed by a continuous infusion (0.75 mg kg^{-1} min^{-1}, i.v.) which was incorporated into the saline maintenance infusion described above.

Effects of saline alone

Following a post-surgical equilibration period of at least 60 min, a series of 10 min urine collections was made, while saline was continuously infused (0.5 ml min^{-1}) into the left renal artery. At the end of each 10 min period, readings of cardiovascular variables and urine output were made. Basal stability was achieved when urine output and cardiovascular parameters showed less than 10% variation over 3 consecutive collection periods. The intra-renal saline infusion was then continued for 10 consecutive 15 min urine collection periods. At the end of each of these periods, two separate 1 ml arterial blood samples were taken. These blood samples were immediately placed on ice in test tubes containing 0.1 ml EDTA (100 mg ml^{-1}), to prevent blood clotting and further generation of AI. Samples were then centrifuged (15 min, 2000 *g*) at 4°C, after which the plasma was removed and frozen until required for radioimmunoassay to determine plasma renin activity (PRA) or plasma aldosterone concentration. PRA and plasma aldosterone concentration were measured with commercially available radioimmunoassay kits from CIS (UK); SB-REN-1 and SB-ALDO-2, respectively. PRA values are expressed as ng AI formed per ml of plasma per hour at 37°C (ng AI ml^{-1} h^{-1}).

At the midpoint of each 15 min collection period, an arterial blood sample (1.5 ml) was collected into a lithium-heparin coated tube. Following the removal of an aliquot for haematocrit determination, the samples were centrifuged and the plasma removed. Plasma and urine samples were later analysed to assess sodium and potassium concentrations (Beckman electrolyte analyser E2A), and creatinine levels (Beckman creatinine analyser 2). In the dog, creatinine is filtered at the glomerulus, but not secreted or reabsorbed by the renal tubules (Levinsky & Levy, 1973) and hence, creatinine clearance can reliably be used in this species to estimate the glomerular filtration rate (GFR). Filtration fraction was obtained by dividing the GFR by the renal plasma flow (renal blood flow X 1 – haematocrit). For each collection period, sodium excretion (μmol min^{-1}) was calculated by multiplying the urine output (ml min^{-1}) by the urine sodium concentration (μmol ml^{-1}). Fractional sodium excretion (the fraction of filtered sodium which appears in the urine) was then determined by dividing the value obtained for sodium excretion, by the product of the plasma sodium concentration (μmol ml^{-1}) and the GFR (ml min^{-1}). Alterations in fractional sodium excretion provide an index of changes in renal tubular sodium reabsorption.

Effects of angiotensin II

The same protocol was followed as in control experiments with the exception that, after achieving baseline stability, the first dose of AII (1 ng kg^{-1} min^{-1}) was infused (0.5 ml min^{-1}) directly into the left renal artery (i.r.a.). After 15 min, measurements of urine output and cardiovascular parameters were taken and the dose of agonist was increased approximately three fold whilst keeping the infusion rate constant. This procedure was subsequently repeated with several higher doses of AII (3–300 ng kg^{-1} min^{-1}). Mean AII dose-response curves were then plotted.

Effects of AT_1 or AT_2 receptor blockade on basal renal function, and on the renal actions of exogenous angiotensin II

Following the attainment of baseline stability, either the selective angiotensin AT_1 receptor antagonist, GR117289, *or* the AT_2 receptor antagonist, PD123177, was administered. In no experiment was the effect of combined AT_1 and AT_2 receptor antagonism studied.

We have previously found that losartan can antagonize the renal effects of AII in the dog over a 2 h period when given at a dose of 5 mg kg^{-1}, i.v., along with a continuous infusion of 20 μg kg^{-1} min^{-1}, i.v. (Clark *et al.*, 1991). GR117289 has approximately 10–30 fold higher affinity than losartan for AT_1 receptors *in vitro* (Chiu *et al.*, 1990; Robertson *et al.*, 1992), and negligible affinity ($pK_i < 6$) for AT_2 sites (Robertson *et al.*, 1992). Thus, in the present study, GR117289 was administered at a dose of 0.5 mg kg^{-1}, i.v., plus a constant intravenous infusion of 1 μg kg^{-1} min^{-1}. In a separate group of dogs, since a limited quantity of PD123177 was available, the drug was infused locally at a dose of 20 μg kg^{-1} min^{-1},

i.r.a., for the duration of the experiment. An approximation of the concentration of PD123177 achieved in the renal circulation was made by dividing the drug infusion rate ($\sim 200\,\mu g\,min^{-1}$) by the average renal blood flow ($\sim 80\,ml\,min^{-1}$) to give a figure of $\sim 2.5\,\mu g\,ml^{-1}$ which equates to 4.5 μM. Since PD123177 has nanomolar affinity for AT_2 receptors (Chiu *et al.*, 1989), the dose given in the present study was anticipated to be high enough to block AT_2 receptors, even if there was high renal clearance of the drug. Conversely, we anticipated that the concentration of PD123177 achieved in the kidney would be unlikely to exceed 5 μM, and possibly become non-selective, unless significant renal concentration of the compound occurred. It has previously been reported that PD123177 does not inhibit functional responses mediated by AT_1 receptors at doses as high as 100 μM (Wong *et al.*, 1990). The effects of PD123177 were also studied in another group of dogs at the slightly higher dose of $50\,\mu g\,kg^{-1}\,min^{-1}$.

Following their administration, the effects of either GR117289, PD123177 or saline on renal haemodynamic and excretory function were monitored over three consecutive 15 min periods. AII ($1-1000\,ng\,kg^{-1}\,min^{-1}$) was then administered as described above. AII mean dose-response curves were plotted and compared with those obtained in dogs which received AII in the absence of antagonist. Where appropriate, the degree of rightward displacement of agonist curves caused by an antagonist was estimated from linear parts of the mean dose-response curves.

Expression of results and statistical analysis

Generally, drug-induced alterations in renal and cardiovascular variables have been expressed as mean percentage change ± s.e.mean from the experiment basal values. Data have been expressed in this manner to facilitate the comparison of results (especially dose-response relationships) obtained from different treatment groups. Changes in renal haemodynamics, excretory function, or glomerular filtration rate, refer, unless otherwise stated, to changes in the *left* kidney only.

Basal values In experiments in which the effects of saline alone, GR117289 or PD123177 were studied, the basal value for all parameters was taken as the mean of the readings obtained at the end of each of the three, 10 min collection periods, immediately prior to infusion of drug or saline.

Where the effects of AII were studied in the presence of GR117289 or PD123177, the basal value was taken as that obtained immediately prior to AII infusion (i.e. 45 min post-antagonist).

Statistical analysis was carried out using the RS1 package for data handling (BBN Software Products Corporation). Statistical significance was assumed when $P<0.05$.

Effects of GR117289 or PD123177 on basal renal function Changes in response to GR117289 or PD123177 were compared with those occurring over the corresponding time period in the saline controls. Unless otherwise stated, data for each individual parameter from the different treatment groups were examined by one way analysis of variance, followed by Dunnett's multiple comparison test. Dunnett's test is appropriate for comparing changes at several time points in two treatment groups (GR117289 and PD123177) with those observed in one control group.

Effects of angiotensin II in the absence and presence of antagonist In the case of AII dose-response relationships, for each parameter to be tested, mean percentage change over a range of AII doses (similar to area under the curve) was compared, by Student's *t* test for unpaired data, to that which occurred over the corresponding time period in the saline controls. Where warranted, the same type of analysis was used to compare differences between the effects of AII in the absence and presence of PD123177.

Drugs used

Drugs used were AII (Human sequence, Sigma); GR117289 (1-[[3-bromo-2-[1H-tetrazol-5-yl)phenyl]-5-benzofuranyl] methyl]-2-butyl-4-chloro-1H-imadazole-5-carboxylic acid), potassium salt (synthesized by Glaxo Group Research); PD123177 (1-[(4-amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imadazo[4,5-c] pyridine-6 carboxylic acid) hydrochloride (synthesized by Glaxo Group Research); pentobarbitone sodium (May & Baker). Drugs were dissolved and diluted in saline. Doses of drugs quoted in the text refer to the parent compound or peptide.

Table 1 Basal values prior to infusion of saline, GR117289, or PD123177

Parameter	*Experimental group*		
	Saline alone ($n = 4$)	*GR117289* ($n = 6$)	*PD123177* ($n = 5$)
Mean blood pressure (mmHg)	113.1 ± 7.4	109.4 ± 4.4	110.0 ± 1.4
Renal vascular conductance ($ml\,min^{-1}\,mmHg^{-1} \times 10^{-3}$)	791 ± 31	792 ± 79	837 ± 59
Renal blood flow ($ml\,min^{-1}$)	88.8 ± 3.3	86.8 ± 9.6	92.0 ± 6.0
Urine output ($ml\ 10\,min^{-1}$)	5.1 ± 0.5	4.5 ± 0.7	4.5 ± 0.8
Sodium excretion ($\mu mol\,min^{-1}$)	118.4 ± 9.6	104.6 ± 13.7	80.4 ± 8.5
Fractional sodium excretion (%)	3.0 ± 0.2	3.1 ± 0.4	2.3 ± 0.3
GFR ($ml\,min^{-1}$)	27.2 ± 1.0	23.7 ± 2.4	20.4 ± 2.6
Filtration fraction (%)	46.7 ± 1.6	45.9 ± 5.0	34.1 ± 3.2
Plasma aldosterone ($pg\,ml^{-1}$)	27.8 ± 7.8	101.0 ± 50.7	64.7 ± 5.4
Plasma renin activity ($ng\,AI\,ml^{-1}\,h^{-1}$)	1.5 ± 0.3	4.2 ± 1.1	5.1 ± 1.8

Values are arithmetic mean ± s.e.mean.

Results

Effects of AT_1 or AT_2 receptor blockade on basal renal function

The effects of the AT_1 receptor antagonist, GR117289 (0.5 mg kg^{-1} + 1 μg kg^{-1} min^{-1}, i.v.), or the AT_2 receptor antagonist, PD123177 (20 μg kg^{-1} min^{-1}, i.r.a.) were examined in separate groups of dogs. For ease of comparison, as explained above, drug effects on renal function have generally been expressed as % change from the experiment basal value. Basal values prior to administration of GR117289, PD123177, or saline are shown in Table 1. There was no significant difference between the antagonist-treated animals and the saline controls for any of the basal parameters shown in Table 1 (one way analysis of variance followed by Dunnett's multiple comparisons test).

Administration of the AT_1 receptor antagonist, GR117289, resulted in a fall in mean blood pressure which was maintained over the 45 min post-dose period (Figure 1a). In addition, GR117289 caused marked renal vasodilatation, characterized by sustained increases in renal vascular conductance and renal blood flow (Figure 1b and c). In contrast, Figure 1 shows that the AT_2 receptor antagonist, PD123177, caused no significant change in blood pressure or renal haemodynamics.

Despite the reduction in blood pressure, there was no decrease in sodium and urine excretion following GR117289; instead there was a tendency for urine output, sodium excretion (Figure 2a and b), and fractional sodium excretion (data not shown) to increase, although the changes failed to achieve statistical significance (analysis of variance/Dunnett's test). GR117289 caused no significant change in GFR (Figure 2c), and since renal blood flow was increased, there was a tendency for filtration fraction to decrease (mean fall of 15 ± 3%, 45 min post-dose) although this was not statistically significant. The AT_2 receptor antagonist, PD123177, caused no significant change in urine output, sodium excretion, fractional sodium excretion (data not shown), or GFR (Figure 2). There was a tendency for sodium excretion to increase, 45 min post-PD123177 (Figure 2b), but this was also not statistically significant. In addition, in four dogs where a slightly higher dose (50 μg kg^{-1} min^{-1}, i.r.a.) was used, PD123177 still failed to cause any significant change in blood pressure, renal haemodynamics, or sodium and urine excretion (data not shown).

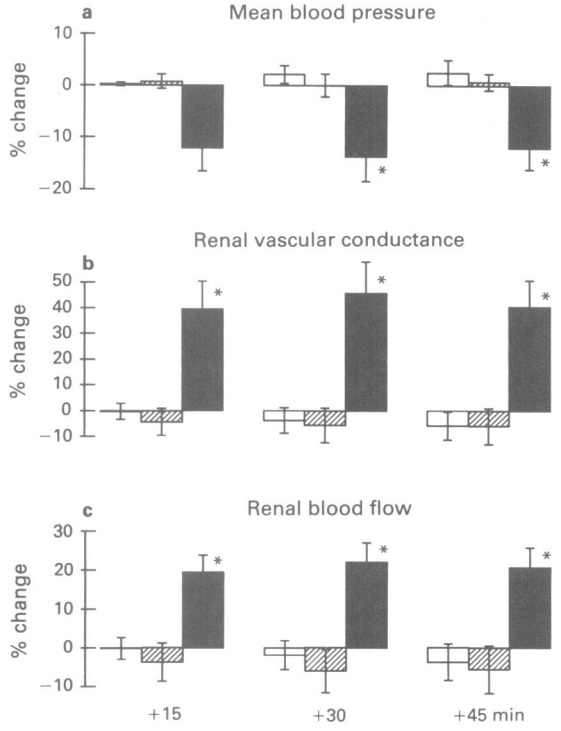

Figure 1 Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR117289, 0.5 mg kg^{-1} + 1 μg kg^{-1} min^{-1}, i.v. (solid columns, $n = 6$), or the AT_2 receptor antagonist, PD123177, 20 μg kg^{-1} min^{-1}, i.r.a. (hatched columns, $n = 5$) on (a) mean blood pressure, (b) renal vascular conductance, and (c) renal blood flow. Columns represent mean % change ± s.e.mean from basal values (see Table 1). *$P < 0.05$, Dunnett's test, drug treated groups vs saline controls.

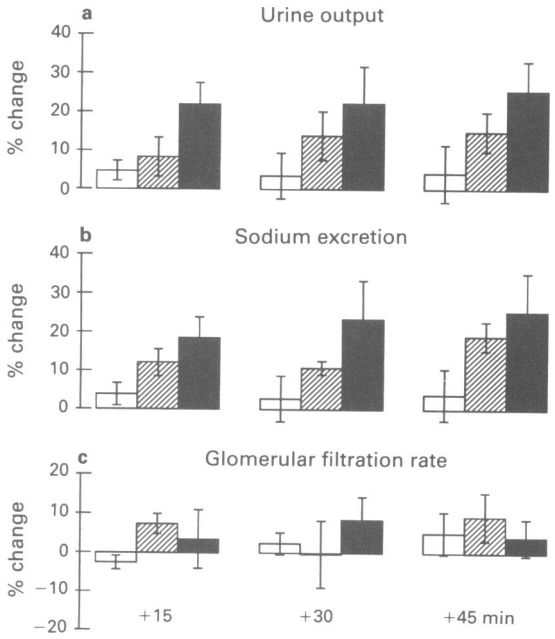

Figure 2 Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR117289, 0.5 mg kg^{-1} + 1 μg kg^{-1} min^{-1}, i.v. (solid columns, $n = 6$), or the AT_2 receptor antagonist, PD123177, 20 μg kg^{-1} min^{-1}, i.r.a. (hatched columns, $n = 5$) on (a) urine output, (b) sodium excretion, and (c) glomerular filtration rate. Columns represent mean % change ± s.e.mean from basal values (see Table 1).

The AT_1 receptor antagonist, GR117289, increased PRA 15, 30, and 45 min post-dose period (Figure 3a). Despite this increase in PRA, a significant reduction in plasma aldosterone concentration was observed 15 and 45 min post-dose (Figure 3b). PD123177 caused no significant change in PRA or plasma aldosterone levels at any time point. (Figure 3).

Effects of AT_1 or AT_2 receptor blockade on the renal actions of exogenous angiotensin II

Table 2 shows basal values measured prior to AII infusion in the different groups of dogs, and demonstrates that for most parameters basal values were not markedly different in the absence or presence of antagonist. Basal sodium excretion was significantly higher in dogs treated with GR117289 compared with antagonist-free dogs. This difference in basal sodium excretion should be borne in mind when comparing the effects of AII on this parameter between the two groups.

Renal haemodynamics

In antagonist-free animals, intra-renal artery infusion of AII (1–300 ng kg^{-1} min^{-1}) resulted in dose-related renal vasoconstriction, as indicated by decreases in renal vascular conductance and renal blood flow (Figure 4a and b). These effects of AII occurred in the absence of any change of blood pressure except at the highest dose (300 ng kg^{-1} min^{-1}), where a small pressor response was observed (Figure 4c). The AT_1 receptor antagonist, GR117289, antagonized the AII-induced renal vasoconstriction, causing approximately 12 and

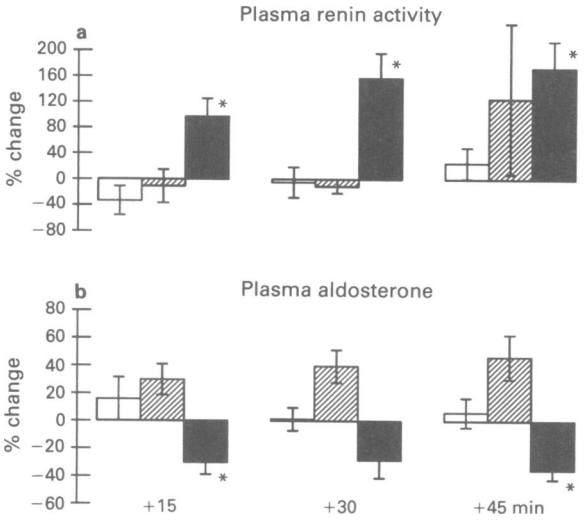

Figure 3 Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR117289, 0.5 mg kg^{-1} + 1 μg kg^{-1} min^{-1}, i.v. (solid columns, $n = 6$), or the AT_2 receptor antagonist, PD123177, 20 μg kg^{-1} min^{-1}, i.r.a. (hatched columns, $n = 5$) on (a) plasma renin activity, and (b) plasma aldosterone levels. Columns represent mean % change ± s.e.mean from basal values (see Table 1).
*$P < 0.05$, Dunnett's test, drug treated groups vs saline controls.

16 fold rightward displacements of the mean AII dose-response curves for renal vascular conductance (Figure 4a) and renal blood flow (Figure 4b), respectively. The AT_2 receptor antagonist, PD123177 (20 μg kg^{-1} min^{-1}), did not inhibit the renal vasoconstrictor effects of AII over the dose-range 1–10 ng kg^{-1} min^{-1}. Interestingly, however, PD123177 did cause inhibition of the vasoconstrictor responses to higher doses of AII (30–300 ng kg^{-1} min^{-1}), causing a small (~4 fold) rightward displacement of the AII curve for renal vascular conductance (Figure 4a), and decreasing the ability of AII to reduce renal blood flow (Figure 4b). Comparing mean % change over a range of agonist doses in the absence and presence of antagonist, AII (30–300 ng kg^{-1} min^{-1})-induced reductions in renal vascular conductance and renal blood flow were significantly inhibited by PD123177 (unpaired t test).

Renal excretory function

Changes in renal perfusion pressure can result in alterations in renal tubular function, and consequently, sodium and urine output. To avoid this complicating factor, the following results describe the effects of non-pressor doses of AII on renal function, in the absence or presence of antagonists.

AII (1–100 ng kg^{-1} min^{-1}) caused marked dose-related reductions in urine output and sodium excretion (Figure 5a and b) which occurred concomitantly with falls in fractional sodium excretion and GFR (Figure 5c and d). The AT_1 receptor antagonist, GR117289, antagonized the effects of AII on these parameters to a similar extent as it inhibited the renal vascular effects of AII. Mean AII dose-response curves for urine output, sodium excretion, fractional sodium excretion, and GFR (Figure 5) were displaced approximately 16, 20, 15, and 38 fold, respectively, to the right of those observed in the absence of antagonist. The AT_2 receptor antagonist, PD123177, caused no significant inhibition of the effects of AII on urine output, sodium excretion, or fractional sodium excretion (Figure 5). From Figure 5c, it could be deduced that PD123177 antagonized the ability of high doses of AII (30 and 100 ng kg^{-1} min^{-1}) to reduce GFR. However, this apparent effect of PD123177 was heavily influenced by the data from one animal in the group, and was not statistically significant.

Thus, the data indicate that the AT_2 receptor blocker, PD123177 (20 μg kg^{-1} min^{-1}, i.r.a.), did not antagonize the effects of AII on renal excretory function, but did cause a small, but significant, inhibition of the renal vasoconstrictor effects of high doses (30–300 ng kg^{-1} min^{-1}) of AII. The dose-dependency of this phenomenon was examined in a further group of four dogs where PD123177 was administered at the slightly higher dose of 50 μg kg^{-1} min^{-1}, i.r.a. At this higher dose, PD123177 caused a progressive antagonism of the renal vasoconstrictor effects of AII (Figure 6a and b), but still failed to inhibit the ability of AII to reduce urine output, sodium excretion (Figure 6c and d), fractional sodium excretion or GFR (data not shown).

Discussion

The present study investigated the role, and relative importance, of AT_1 versus AT_2 receptors in mediating the renal haemodynamic and excretory effects of AII *in vivo*. The drugs chosen to address this question were the AT_1 receptor antagonist, GR117289, and the AT_2 receptor antagonist, PD123177. Doses of these compounds were chosen which were predicted to be selective for AT_1 or AT_2 receptors, respectively (see Methods). Experiments were carried out in anaesthetized dogs, which provide stable conditions for leng-

Table 2 Basal values prior to infusion of angiotensin II (AII) alone, or AII in the presence of either GR117289 or PD123177

Parameter	*Experimental group*		
	AII alone ($n = 5$)	*AII in presence of GR117289* ($n = 5$)	*AII in presence of PD123177* ($n = 5$)
Mean blood pressure (mmHg)	116.6 ± 5.6	99.1 ± 8.9	110.8 ± 1.7
Renal vascular conductance (ml min^{-1} mmHg^{-1} × 10^{-3})	834 ± 152	954 ± 68	823 ± 96
Renal blood flow (ml min^{-1})	95.3 ± 14.2	93.9 ± 8.4	86.8 ± 7.7
Urine output (ml 10 min^{-1})	3.2 ± 0.5	6.3 ± 1.1	5.2 ± 1.0
Sodium excretion (μmol min^{-1})	89.6 ± 6.9	*135.2 ± 16.4	94.8 ± 8.1
Fractional sodium excretion (%)	2.6 ± 0.5	4.1 ± 0.7	2.4 ± 0.4
GFR (ml min^{-1})	24.3 ± 2.0	23.1 ± 2.6	22.4 ± 3.6

Values are arithmetic mean ± s.e.mean.
*$P < 0.05$, antagonist treated vs AII alone group. One way analysis of variance followed by Dunnett's multiple comparison test.

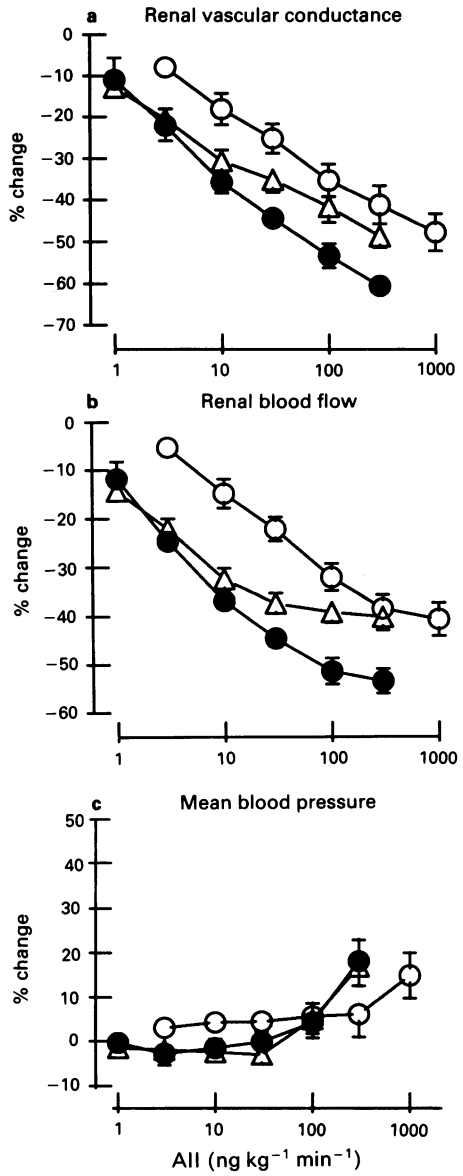

Figure 4 Effects of intra-renal artery infusion of angiotensin II (AII) on (a) renal vascular conductance, (b) renal blood flow, and (c) mean blood pressure in the absence (●, $n = 5$), and in separate groups of dogs, in the presence of the angiotensin AT_1 receptor antagonist, GR117289 (○, $n = 5$), or the AT_2 receptor antagonist, PD123177 (△, $n = 5$). See the text for antagonist doses. Values are mean % change ± s.e.mean from basal values (see Table 2).

thy renal function studies requiring the taking of multiple blood samples. In addition, preliminary radioligand binding data (unpublished observations) suggested that both AT_1 and AT_2 receptors are present in dog renal cortex.

The AT_1 receptor antagonist, GR117289, reduced blood pressure, and caused renal vasodilatation, characterized by a sustained increase in renal blood flow. GR117289 did not cause any significant change in GFR, and since renal blood flow was increased, there was a tendency for filtration fraction to decline. Urine output, sodium excretion, and fractional excretion of sodium all showed a tendency to increase following GR117289, although the changes were not statistically significant. Taken together, these effects of GR117289 on basal renal function are consistent with blockade of the renal effects of AII, and other workers have obtained similar results in anaesthetized dogs when studying the effects of ACE inhibitors (Schmidt *et al.*, 1989), or the AT_1 receptor antagonist, losartan (Wong *et al.*, 1991a). Additionally, since GR117289 has been shown to be a very specific angiotensin receptor antagonist (Robertson *et al.*, 1992), its effects are most likely due to blockade of AT_1 receptors. In contrast to GR117289, the AT_2 receptor antagonist, PD123177, caused no significant change in renal haemodynamics, or sodium and urine excretion. It could be argued that the lack of effect of PD123177 may reflect the fact that there were low background levels of AII. However, this is highly unlikely since basal PRA values in dogs which received PD123177 were similar to those observed in GR117289-treated animals. Thus, these data suggest that the effects of endogenous AII on basal renal function in the dog are primarily mediated by AT_1 receptors.

Interestingly, although pre-drug PRA levels were not particularly raised, basal filtration fractions were high, suggesting that the kidneys of these animals may have been under a rather high degree of tonic vasoconstriction. This was possibly due to the fact that the experiments were carried out under anaesthesia. It would be interesting to determine if AT_1 and AT_2 receptor blockade has similar effects on renal function in conscious dogs.

In agreement with Gibson *et al.* (1991), the present data also suggest that AII feedback inhibition of renin release is mediated predominantly by AT_1 receptors, since a marked increase in PRA was observed following GR117289, while PD123177 had no significant effect. It is probable that the fall in blood pressure observed after GR117289 also contributed to an increase in renin release; however, previous reports (Schmid, 1972) show that falls in renal perfusion pressure within the limits of renal autoregulation do not markedly change PRA in anaesthetized dogs. Despite causing an increase in PRA, GR117289 reduced plasma aldosterone levels. This was probably a consequence of antagonism of the effects of endogenous AII in the adrenal cortex. Previous reports (Wong *et al.*, 1990) indicate that functionally important angiotensin receptors in the adrenal gland are of the AT_1 subtype.

It is now established that angiotensin AT_1 receptors occur in the proximal tubule, particularly in the S1 segment (Cogan, 1990; Xie *et al.*, 1990; Harris, 1992), where they play a physiological role in mediating the stimulatory effect of AII on sodium reabsorption. Thus, we were initially surprised that in the current study, a more marked diuretic/natriuretic response was not observed following the AT_1 receptor antagonist, GR117289. However, on closer consideration the results are probably not surprising for the following reasons. Firstly, GR117289 caused a marked and sustained fall in blood pressure. It is well established that falls in blood pressure *per se* result in reduced sodium and urine excretion (Guyton *et al.*, 1980). Secondly, the anaesthetized dogs used in this study received a saline infusion throughout the experiment to help achieve steady urine excretion and to provide a background on which to also study the renal effects of exogenous AII. Under these conditions it is unlikely that the renin-angiotensin system was exerting a great degree of tonic influence over sodium reabsorption. Indeed, previous studies in salt-replete dogs using either ACE inhibitors (Schmidt *et al.*, 1989) or saralasin (Lohmeier *et al.*, 1977), have also found that when blood pressure is reduced, these agents do not cause a significant diuresis/natriuresis. Moreover, in anaesthetized salt-replete guinea-pigs (Amrani *et al.*, 1991), and volume-expanded rats (Fenoy *et al.*, 1991), the AT_1 receptor antagonist, losartan, reduces blood pressure but does not cause a significant diuresis.

Thus, while the effects of GR117289 or PD123177 on basal function suggest that the renal effects of endogenous AII in the dog are mediated by AT_1 receptors, the scope of the results is slightly limited, as discussed above, by the experimental conditions (i.e. the data only relate to the role of AT_1 and AT_2 receptors under conditions of limited activation of the renin-angiotensin system). Thus, the second part of this study examined the effects of AT_1 or AT_2 receptor

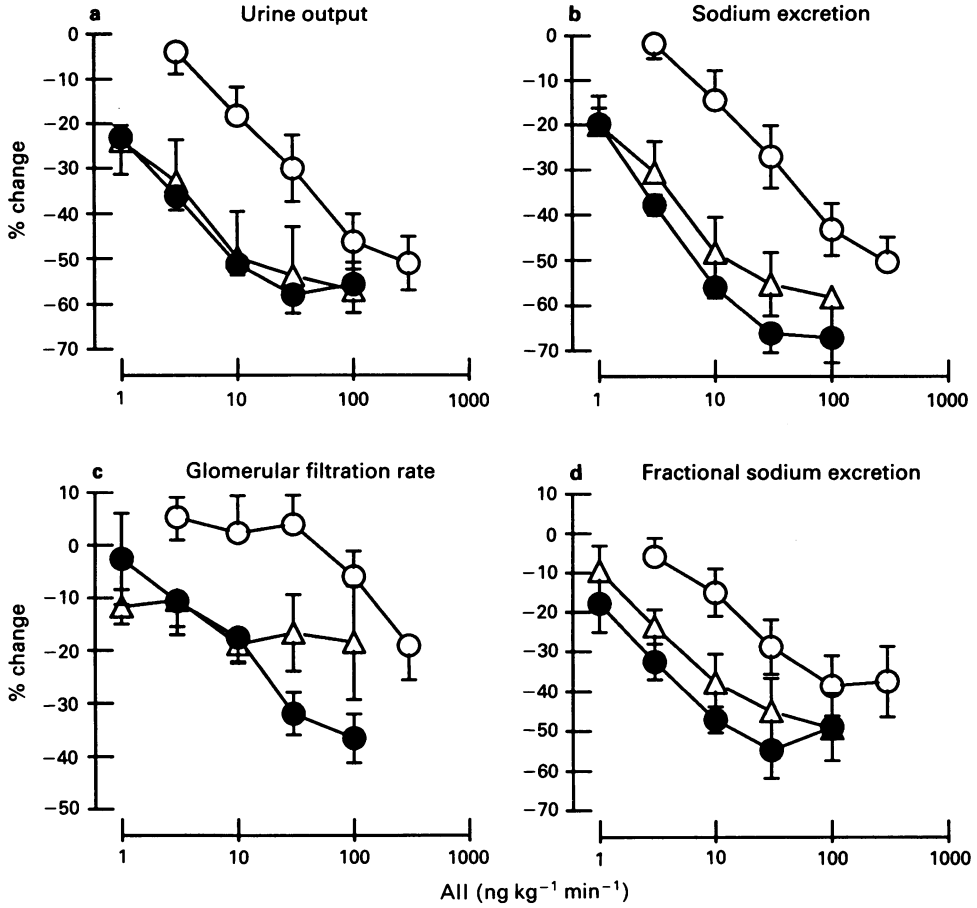

Figure 5 Effects of intra-renal artery infusion of angiotensin II (AII) on (a) urine output, (b) sodium excretion, (c) glomerular filtration rate, and (d) fractional sodium excretion, in the absence (●, $n = 5$), and in separate groups of dogs, in the presence of the angiotensin AT_1 receptor antagonist, GR117289 (○, $n = 5$), or the AT_2 receptor antagonist, PD123177 (△, $n = 5$). See the text for antagonist doses. Values are mean % change ± s.e.mean from basal values (see Table 2).

blockade on the renal haemodynamic and excretory effects of a wide dose-range of exogenous AII.

Local infusion of AII (1–300 ng kg^{-1} min^{-1}) into the renal artery resulted in dose-related renal vasoconstriction, characterized by decreased renal vascular conductance and blood flow. This represented a direct effect of the peptide on the renal vasculature since blood pressure was not increased, except at the highest dose where a small pressor response was observed. In addition to renal vasoconstriction, AII caused a dose-related reduction in urine output and sodium excretion. Since reductions in both GFR and fractional sodium excretion were observed, the AII-induced reduction in urine/sodium excretion was probably a consequence of both reduced filtered load and increased tubular sodium reabsorption. These well-established renal effects of AII are consistent with its role as a salt and water retaining hormone, and are almost certainly mediated by receptors at both vascular and proximal tubular sites (Navar *et al.*, 1991; Harris, 1992).

The AT_1 receptor antagonist, GR117289, markedly antagonized the effects of exogenous AII over the entire dose-range tested, on renal haemodynamics, sodium and urine excretion, fractional sodium excretion, and GFR. In contrast to GR117289, the AT_2 receptor blocker, PD123177 (20 μg kg^{-1} min^{-1}), failed to antagonize the renal effects of AII over the dose-range 1–10 ng kg^{-1} min^{-1}. This suggests that over the latter dose-range the effects of AII on renal function are mediated exclusively by AT_1 receptors. Interestingly, however, while PD123177 still failed to inhibit the ability of higher doses of AII (30–300 ng kg^{-1} min^{-1}) to reduce urine output, sodium excretion and GFR, it did cause a small, but significant, inhibition of renal vasoconstrictor responses to AII. At a higher dose (50 μg kg^{-1} min^{-1}), PD123177 exerted further blockade of the renal vasoconstrictor effects of AII, but still caused no antagonism of the effects of the peptide on urine/sodium excretion or GFR. It is worth noting that these results relate only to the effects of exogenous infusions of AII, which may not completely mimic the effects of high levels of endogenous renal angiotensins. It would be interesting to compare these results with the effects of AT_1 and AT_2 receptor antagonists in this model under conditions of raised endogenous renal AII levels; for example, following salt depletion.

Taken together, the present results suggest that the effects of infusions of exogenous AII on renal haemodynamic and excretory function are predominantly mediated by AT_1 receptors. However, could the small inhibitory effect of PD123177 on AII-induced renal vasoconstriction indicate some functional significance for AT_2 receptors in the renal vasculature? Certainly, it is unlikely that the antagonism observed is due to PD123177 binding to AT_1 receptors, since (a) at the doses used it is improbable that PD123177 reaches high enough concentrations in the renal circulation to have affinity for AT_1 receptors, and (b) the compound caused no antagonism of the effects of AII on absolute/fractional sodium excretion or urine output; actions which are inhibited by AT_1 receptor antagonists such as losartan (Clark *et al.*, 1991) or GR117289. It is an intriguing possibility that functionally important AT_2 receptors may be present in the renal resistance vasculature. This possibility was previously raised by Widdop and co-workers (1992), who found that PD123177 caused inhibition of renal vasoconstrictor responses to AII in conscious rats, but only when given 24 h after the AT_1

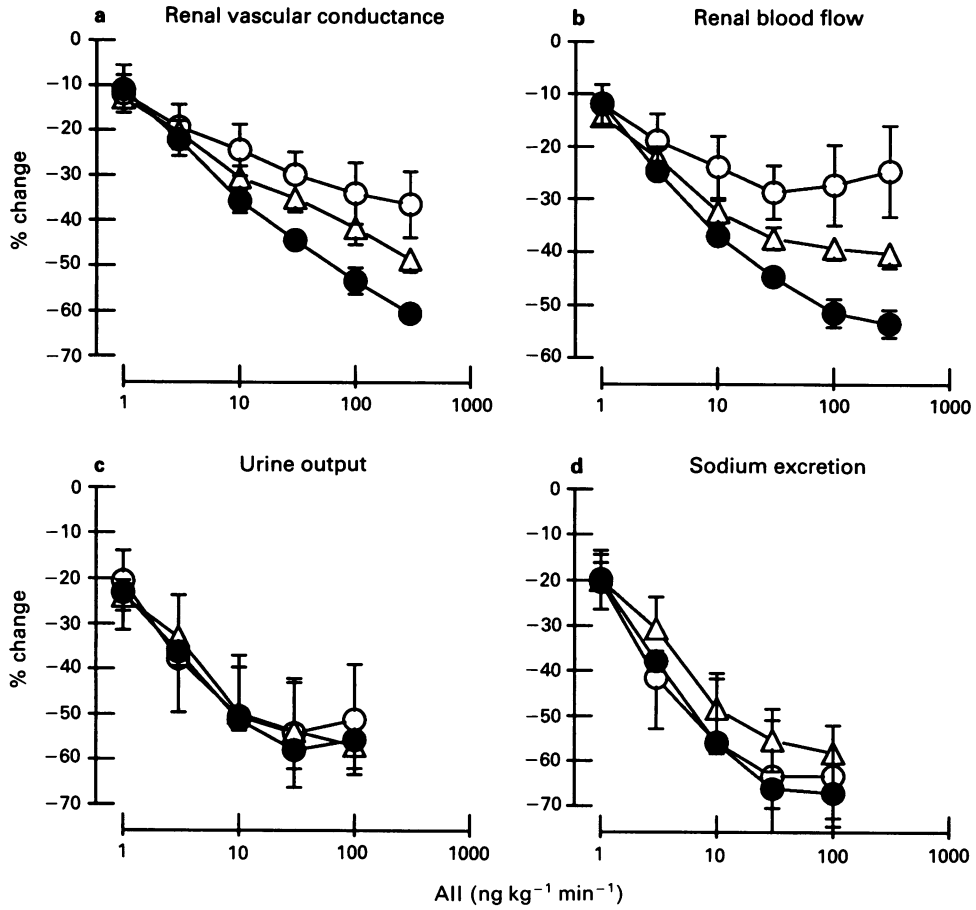

Figure 6 Effects of intra-renal artery infusion of angiotensin II (AII) on (a) renal vascular conductance, (b) renal blood flow, (c) urine output, and (d) sodium excretion, in the absence (●, $n = 5$), and in separate groups of dogs, in the presence of the angiotensin AT_2 receptor antagonist, PD123177 (Δ, 20 μg kg^{-1} min^{-1}, n = 5; ○, 50 μg kg^{-1} min^{-1}, $n = 4$). Values are mean % change ± s.e.mean from basal values.

receptor antagonist, EXP3174. Thus, these workers suggested that their results may have been due to displacement of EXP3174 by PD123177 from plasma protein binding sites, which does seem likely since binding studies (Edwards *et al.*, 1992; Sechi *et al.*, 1991) have failed to find a significant population of AT_2 receptors in rat kidney. In contrast, it is interesting to note that using autoradiography, Grone *et al.* (1992) have shown that AT_2 binding sites are present in the human pre-glomerular vasculature. As yet, there have been no reports on the distribution of AT_1 and AT_2 binding sites in the dog kidney, although preliminary observations (unpublished) within our own laboratory do suggest that AT_2 binding sites are present in dog renal cortex.

However, while it is possible that functionally important AT_2 receptors are present in the canine kidney, other explanations of the data deserve comment. Firstly, since few groups have been able to identify any antagonism by PD123177 of functional responses to AII, there has been little need to investigate the specificity of action of the drug. Thus, PD123177 could have had a non-specific spasmolytic action in the present study. This seems unlikely, however, since PD123177, at both doses tested, had no effect on basal blood pressure or renal vascular conductance. In addition, Wong and co-workers (1990), have shown that pressor responses to AII in pithed rats are unaffected by PD123177 at a dose of 100 mg kg^{-1}. Moreover, it has also been shown in anaesthetized dogs (Wong *et al.*, 1991b), that renal vasoconstrictor responses to noradrenaline were unaffected by PD123177 at doses up to 100 μg kg^{-1} min^{-1}; five times the dose used in the present study. Another explanation for the data is that more than two subtypes of angiotensin receptors are present in the kidney. In 1987, Douglas proposed two subtypes of renal angiotensin receptors; one subtype which primarily used adenylate cyclase as a transducer, while the other subtype is mainly coupled to phospholipase C. Since there is no evidence to suggest that AT_2 receptors are coupled via either of these transducers, it is possible that in addition to AT_2 receptors, there are AT_1 receptor subtypes, or atypical angiotensin receptors (neither AT_1 nor AT_2), present in the renal vasculature.

In conclusion, this study has shown that the renal effects of both endogenous and low doses of exogenous AII are mediated by angiotensin AT_1 receptors, since they are sensitive to blockade by the AT_1 antagonist, GR117289, but not the AT_2 receptor antagonist, PD123177. However, PD123177 did inhibit renal vasoconstrictor responses to high doses of AII. This observation could indicate heterogeneity of functionally important renal vascular angiotensin receptors and warrants further investigation.

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