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European Journal of Pharmacology 280 (1995) 195–203

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Renal pharmacology of GR138950, a novel non-peptide angiotensin AT_1 receptor antagonist

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Received 27 March 1995; accepted 31 March 1995

Abstract

This paper describes the renal pharmacology of the novel, specific, non-peptide angiotensin AT_1 receptor antagonist, GR138950 (1-[[3-bromo-2-[2-[[(trifluoromethyl) sulphonyl] amino] phenyl]-5-benzofuranyl] methyl]-4-cyclopropyl-2-ethyl-1*H*-imidazole-5-carboxamide). When administered to anaesthetised salt-replete dogs, GR138950 caused renal vasodilatation and significant increases in sodium and urine excretion. No change in glomerular filtration rate was observed indicating that the natriuresis was a consequence of inhibition of tubular sodium reabsorption. Qualitatively similar but less marked changes in renal function were observed in response to the angiotensin converting enzyme inhibitor, captopril, although in contrast to GR138950, captopril also caused a small but significant fall in mean blood pressure. Intra-renal artery infusion of exogenous angiotensin II resulted in dose-related renal vasoconstriction and decreases in urine excretion, sodium excretion, fractional excretion of sodium and glomerular filtration rate. These renal effects of angiotensin II were all markedly antagonised by GR138950. We conclude that GR138950 is an effective antagonist of the renal haemodynamic and excretory actions of endogenous and exogenous angiotensin II.

Keywords: Angiotensin AT_1 receptor antagonist; Captopril; Angiotensin converting enzyme inhibitor; Renal function; (Anesthetized dog)

1. Introduction

The renin-angiotensin system is physiologically important in the control of blood pressure and it is now recognised that overactivity of the system is implicated in the pathogenesis of essential hypertension. Moreover, angiotensin converting enzyme inhibitors, which prevent the formation of angiotensin II, are effective anti-hypertensive agents. An alternative and more specific approach to inhibit the activity of the renin-angiotensin system is to block angiotensin receptors, and this has led to the discovery of non-peptide angiotensin receptor antagonists. Two main subtypes of angiotensin receptors are presently recognised: angiotensin AT_1 and AT_2 receptors (Bumpus et al., 1991). No clear functional role for angiotensin AT_2 receptors has yet been identified. In contrast, angiotensin AT_1 receptors mediate the physiological effects of angiotensin II such as vasoconstriction and aldosterone release (Wong et al., 1990). GR138950 (1-[[3-bromo-2-[2-[[(trifluoromethyl) sulphonyl] amino] phenyl]-5-benzofuranyl] methyl]-4-cyclopropyl-2-ethyl-1*H*-imidazole-5-carboxamide; Fig. 1) is a recently discovered, potent and specific non-peptide angiotensin AT_1 receptor antagonist (Hilditch et al., 1993) which it is hoped will become useful in the treatment of essential hypertension. Given the profound effects of angiotensin II on renal function (Navar et al., 1991), and the importance of the kidneys in the long-term control of blood pressure (Guyton et al., 1980), the aim of this study was to carry out a detailed assessment of the effects of GR138950 on renal function, and determine whether GR138950 blocks the renal effects of angiotensin II in vivo. In addition, we have compared the renal effects of GR138950 with those of the angiotensin converting

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Elsevier Science B.V.
SSDI 0014-2999(95)00204-9

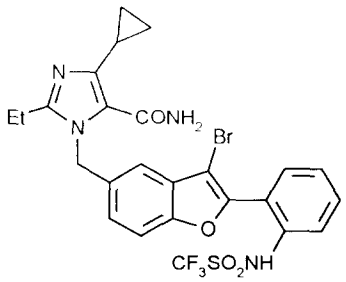

Fig. 1. Chemical structure of GR138950.

enzyme inhibitor, captopril, which is widely used clinically to reduce blood pressure.

2. Materials and methods

2.1. Surgical preparation

Beagle dogs (10–12 kg) of either sex were anaesthetised with sodium pentobarbitone (30–40 mg/kg i.v.). Anaesthesia was maintained using a constant infusion of sodium pentobarbitone (5–10 mg/kg/h i.v., in a volume of 5–10 ml/h) administered into the right cephalic vein. Dogs were intubated and artificially respired. Body temperature was maintained at 37 ± 1° C using 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/min; in a volume of 0.03 ml/kg/min) via a cannula in the left femoral vein. Arterial blood pH, O_2 and CO_2 tensions were monitored using a Radiometer ABL30 acid-base analyser. Blood pressure was recorded using a Bell and Howell pressure transducer connected to a cannula inserted into the abdominal aorta via the right femoral artery. 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 = 0.86 mm), for the collection of urine. Urine output was measured gravimetrically. Left renal blood flow was measured using 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. This needle was kept patent with an infusion of saline (0.5 ml/min). During the surgical period, from the point at which the laparotomy was performed, dogs were saline-loaded (30 ml/kg at 0.25 ml/kg/min) via a cannula in the right femoral vein. The dogs were then maintained in a saline-loaded state by lowering the infusion rate to 0.07 ml/kg/min. This saline load was given to ensure a steady rate of urine production. Following the surgery, a bolus dose of creatinine (50 mg/kg i.v., volume = 5 ml) was administered followed by a continuous infusion (0.75 mg/kg/min i.v.) which was incorporated into the saline maintenance infusion described above.

2.2. Effects of saline alone

Following a post-surgical equilibration period of at least 60 min, a series of 10 min urine collection periods was started, while saline was infused (0.5 ml/min) into the left renal artery. At the end of each 10 min period, readings of cardiovascular parameters and urine output were made. Basal stability was considered to have been achieved when urine output and cardiovascular parameters showed less than 10% variation over three consecutive collection periods. The intra-renal saline infusion was then continued for ten consecutive 15 min urine collection periods.

At the midpoint of each urine 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 and Levy, 1973) and hence, creatinine clearance was used to estimate the glomerular filtration rate. Filtration fraction was obtained by dividing the glomerular filtration rate by the renal plasma flow (renal blood flow × 1 − haematocrit). For each collection period, sodium excretion and potassium excretion (μmol/min) were calculated by multiplying the urine output (ml/min) by the urine sodium and potassium concentration (μmol/ml), respectively. 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) and the glomerular filtration rate (ml/min). Alterations in fractional sodium excretion provide an index of changes in renal tubular sodium reabsorption. Changes in fractional sodium excretion are expressed in percentage terms.

At the end of each of the final three control periods, and the subsequent three 15 min collection periods a 1 ml arterial blood sample was obtained and placed on ice in a test tube containing 0.1 ml EDTA (100 mg/ml), to prevent clotting and further generation of angiotensin I. 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. Plasma renin activity was measured using commercially available radioimmunoassay kits from CIS (UK); SB-REN-1. Plasma renin activity values are expressed as ng angiotensin I formed per ml of plasma per hour at 37° C (ng angiotensin I/ml/h).

2.3. Effects of angiotensin II in the absence of antagonist

The same protocol was followed as in control experiments with the exception that after achieving baseline stability, the first dose of angiotensin II (1 ng/kg/min) was infused (0.5 ml/min) 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 3-fold whilst keeping the infusion rate constant. This procedure was subsequently repeated with several higher doses of angiotensin II (10–300 ng/kg/min). Angiotensin II mean dose-response curves were plotted.

2.4. Effects of captopril and GR138950 on basal renal function, and effects of GR138950 on the renal actions of angiotensin II

Choice of doses of GR138950 and captopril was based on previous experience. We have previously shown (Clark et al., 1993) that the non-peptide angiotensin AT_1 receptor blocker, GR117289, at a dose of 0.5 mg/kg i.v. + 1 μg/kg/min i.v., produced marked antagonism of the renal effects of angiotensin II in anaesthetised dogs. GR117289 has approximately 6-fold higher affinity for angiotensin AT_1 receptors than GR138950 (Robertson et al., 1992; Hilditch et al., 1993). Thus, to be confident of achieving a dose of GR138950 sufficient to block angiotensin AT_1 receptors, following the attainment of baseline stability, GR138950 was administered both as a bolus injection (1.0 mg/kg i.v.) and a constant infusion (10 μg/kg/min i.v.). Captopril was used at a dose (1 mg/kg i.v. + 20 μg/kg/min i.r.a.) which we have shown (Clark et al., 1991) to effectively block the renal vasoconstrictor response to angiotensin I in anaesthetised dogs. Infusions of captopril or GR138950 were maintained for the duration of the experiment.

Following their administration, the effects of either GR138950 or captopril on renal haemodynamic and excretory function were monitored over three consecutive 15 min periods. In dogs treated with GR138950, angiotensin II (3–1000 ng/kg/min) was then administered as described above. Angiotensin II mean dose-response curves were plotted and compared with those obtained in antagonist-free dogs. Where appropriate, the degree of rightward displacement of agonist curves caused by the antagonist was estimated from linear parts of the mean dose-response curves.

2.5. Expression of results and statistical analysis

Drug-induced alterations in renal and cardiovascular variables have been expressed as mean percentage change ± S.E.M. along with basal values for reference. Data have been expressed in this way to facilitate the comparison of results (especially dose-response relationships) obtained from the different treatment groups. Changes in renal haemodynamics, excretory function or glomerular filtration rate refer to changes in the left kidney only.

2.6. Basal values

In experiments in which the effects of saline, angiotensin II alone, GR138950 or captopril were studied, the basal value was taken as the mean of the readings obtained at the end of each of the three 10 min collection periods, preceding infusion of drug or saline.

Where the effects of angiotensin II were studied in the presence of GR138950, the basal value was taken as that observed immediately prior to infusion of angiotensin II (i.e. 45 min post antagonist or saline).

2.7. Statistics

Changes in response to captopril or GR138950 were compared with those occurring over the corresponding time periods in the saline controls. Analysis of the data was carried out after its distribution had been assessed using the Wilk-Shapiro test of normality (Shapiro and Wilk, 1965). This was carried out using one-way analysis of variance followed by Dunnett's multiple comparison test. Dunnett's test is suitable for comparing changes at several time points in two treatment groups (GR138950 and captopril) with those observed in one control group.

The effects of saline, captropil or GR138950 on plasma renin activity 15, 30 and 45 min after administration were assessed by comparison with the mean value obtained from the preceding three 10 min collection periods in the corresponding groups (Student's *t*-test for paired data).

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

2.8. Drugs

Drugs used were angiotensin II (human sequence, Novabiochem); captopril (Sigma); creatinine hydro-

chloride (Sigma); GR138950 sodium salt (Glaxo Research and Development); pentobarbitone sodium (Rhône Poulenc). Drugs were dissolved and diluted in saline. Doses of drugs quoted in the text refer to the free compound or peptide.

3. Results

3.1. Effects of GR138950 and captopril on basal renal function

Basal values prior to administration of saline, GR138950 (1 mg/kg + 10 μg/kg/min i.v.) or captopril (1 mg/kg + 20 μg/kg/min i.r.a.) are shown in Table 1. No significant difference was observed between the two treatment groups for any parameter shown in Table 1 with the exception of plasma renin activity which was higher in the group of dogs which were destined to receive captopril.

Neither saline nor captropril significantly changed plasma renin activity 15, 30 or 45 min after administration. GR138950 caused a small but significant increase in plasma renin activity at 30, but not 15 or 45 min, after administration (from 1.9 ± 0.8 to 3.1 ± 0.8 ng angiotensin I/ml/h; data not shown).

Compared to the effects of saline, in the 45 min post-dose period, GR138950 caused a marked and sustained renal vasodilator response, characterised by increased renal vascular conductance and increased renal blood flow (Fig. 2), but did not reduce blood pressure. Captopril also caused renal vasodilatation over the 45 min post-dose period although renal blood flow was significantly increased 15 min post-dose only (Fig. 2). In contrast to GR138950, captopril caused a small but significant reduction in blood pressure.

In addition to renal vasodilatation, GR138950 also caused marked and sustained increases in urine output, sodium excretion, and fractional sodium excretion (Fig. 3). While captopril also tended to cause increases in these parameters (Fig. 3), the changes did not achieve statistical significance. Neither GR138950 nor captopril caused any significant change in potassium excretion (data not shown). The diuretic and natriuretic response to GR138950 occurred in the absence of any significant change in glomerular filtration rate (Fig. 4), although GR138950 did show a tendency to reduce filtration fraction (Fig. 4), and a statistically significant reduction was observed 45 min post-dose. Captopril had little effect on filtration fraction or glomerular filtration rate except immediately (15 min) after administration when a small but significant increase in glomerular filtration rate was observed (Fig. 4).

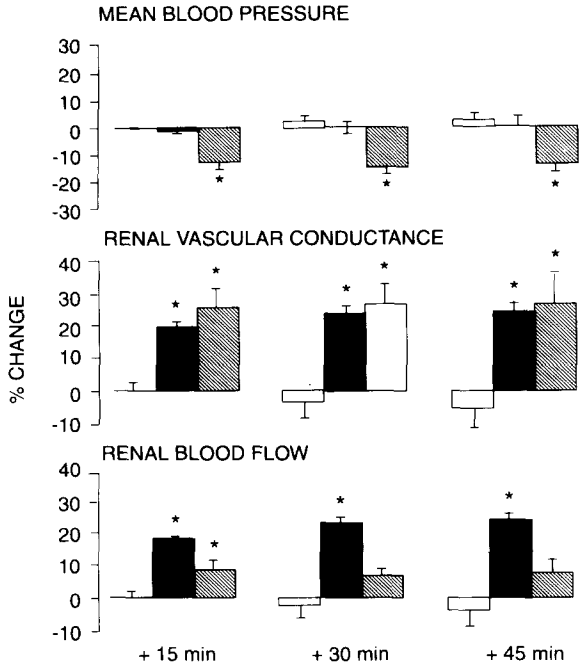

Fig. 2. Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR138950, 1 mg/kg + 10 μg/kg/min i.v. (solid columns, $n = 4$), or the angiotensin converting enzyme inhibitor, captopril, 1 mg/kg + 20 μg/kg/min i.r.a. (shaded columns, $n = 5$) on mean blood pressure, renal vascular conductance, and renal blood flow. Columns represent mean percent change ± S.E.M. from basal values (see Table 1). * $P < 0.05$, Dunnett's test, drug treated groups vs. saline controls.

Table 1
Basal values *prior* to infusion of saline, GR138950 or captopril

Parameter	Experimental group		
	Saline alone ($n = 4$)	GR138950 ($n = 4$)	Captopril ($n = 5$)
Mean blood pressure (mm Hg)	113.1 ± 7.4	121.8 ± 5.9	111.4 ± 3.1
Renal vascular conductance (ml/min/mm Hg × 10^{-3})	791.0 ± 31.0	778.0 ± 67.0	889.0 ± 23.0
Renal blood flow (ml/min)	88.8 ± 3.3	94.3 ± 8.2	99.8 ± 5.7
Urine output (ml/10 min)	5.1 ± 0.5	3.1 ± 0.8	3.3 ± 0.4
Sodium excretion (μmol/min)	118.4 ± 9.6	81.8 ± 18.1	82.9 ± 10.2
Fractional sodium excretion (%)	3.0 ± 0.2	2.2 ± 0.4	2.2 ± 0.3
Glomerular filtration rate (ml/min)	27.2 ± 1.0	23.8 ± 1.4	24.8 ± 0.6
Filtration fraction (%)	46.7 ± 1.6	41.4 ± 2.3	41.2 ± 2.4
Plasma renin activity (ng angiotensin I/ml/h)	1.5 ± 0.3	1.9 ± 0.8	* 4.0 ± 0.7 [a]

All values are arithmetic mean ± S.E.M. [a] $P < 0.05$; ANOVA/Dunnett's multiple comparison test.

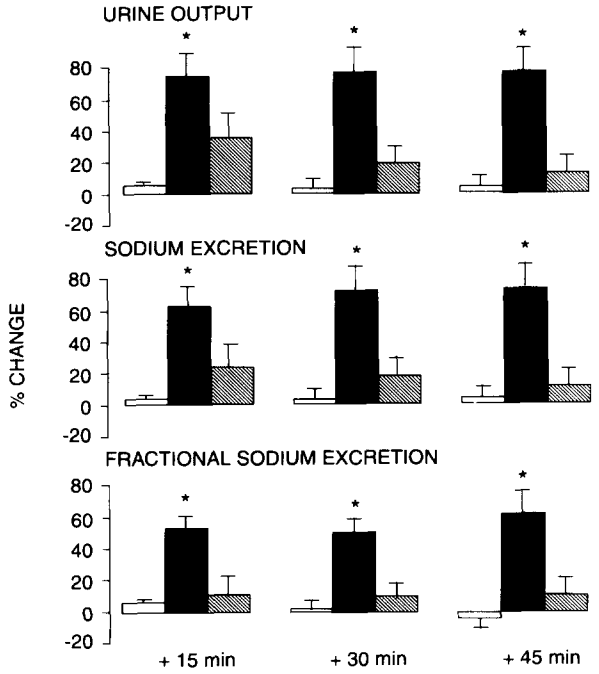

Fig. 3. Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR138950, 1 mg/kg + 10 μg/kg/min i.v. (solid columns, $n = 4$), or the angiotensin converting enzyme inhibitor, captopril, 1 mg/kg + 20 μg/kg/min i.r.a. (shaded columns, $n = 5$) on urine output, sodium excretion, and fractional excretion of sodium. Columns represent mean percent change ± S.E.M. from basal values (see Table 1). * $P < 0.05$, Dunnett's test, drug treated groups vs. saline controls.

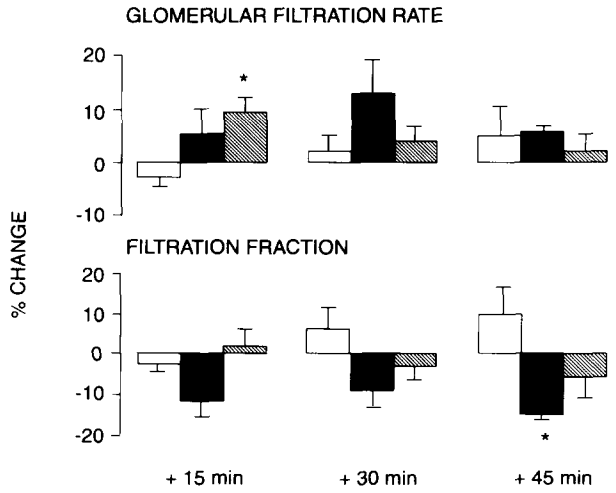

Fig. 4. Effects of saline (open columns, $n = 4$), the angiotensin AT_1 receptor antagonist, GR138950, 1 mg/kg + 10 μg/kg/min i.v. (solid columns, $n = 4$), or the angiotensin converting enzyme inhibitor, captopril, 1 mg/kg + 20 μg/kg/min i.r.a. (shaded columns, $n = 5$) on glomerular filtration rate, and filtration fraction. Columns represent mean percent change ± S.E.M. from basal values (Table 1). * $P < 0.05$, Dunnett's test, drug treated groups vs. saline controls.

3.2. *Effects of GR138950 on the renal actions of angiotensin II*

The effects of intra-renal artery infusion of angiotensin II were determined in the absence of antagonist and, in a separate group of dogs, in the presence of GR138950 (1 mg/kg + 10 μg/kg/min i.v.). To facilitate the comparison of dose-response curves in the absence and presence of antagonist, the data are expressed as percent change from basal values. Table 2 shows the basal values obtained prior to angiotensin II infusion in the two groups of dogs. There was no significant difference between corresponding basal values in the two groups of animals. It should be noted that the effects of angiotensin II in this model have previously been reported (Clark et al., 1993).

Renal haemodynamics

In antagonist-free animals, intra-renal artery infusion of angiotensin II (1–300 ng/kg/min) resulted in dose-related renal vasoconstriction, as indicated by decreases in renal vascular conductance and renal blood flow (Fig. 5). These effects of angiotensin II occurred in the absence of any change of blood pressure except at the highest dose (300 ng/kg/min), where a pressor response was observed (Fig. 5). GR138950 antagonised the renal vasoconstrictor effects of angiotensin II, causing an approximate 22- and 31-fold rightward displacement of the angiotensin II mean dose-response curves for renal vascular conductance and renal blood flow,

Table 2
Basal values *prior* to infusion of angiotensin II alone, or angiotensin II in the presence of GR138950

Parameter	Experimental group	
	Angiotensin II alone ($n = 5$)	Angiotensin II in presence of GR138950 ($n = 4$)
Mean blood pressure (mm Hg)	116.6 ± 5.6	122.1 ± 4.2
Renal vascular conductance (mm Hg/ml/min × 10^{-3})	834.0 ± 152.0	963.0 ± 88.0
Renal blood flow (ml/min)	95.3 ± 14.2	117.0 ± 8.7
Urine output (ml/10 min)	3.2 ± 0.5	5.5 ± 1.6
Sodium excretion (μmol/min)	89.6 ± 6.9	137.3 ± 28.2
Fractional sodium excretion (%)	2.6 ± 0.5	3.6 ± 0.6
Glomerular filtration rate (ml/min)	24.3 ± 2.0	25.1 ± 1.3
Plasma renin activity (ng angiotensin I/ml/h)	1.7 ± 0.4	2.7 ± 0.3

All values are arithmetic mean ± S.E.M.

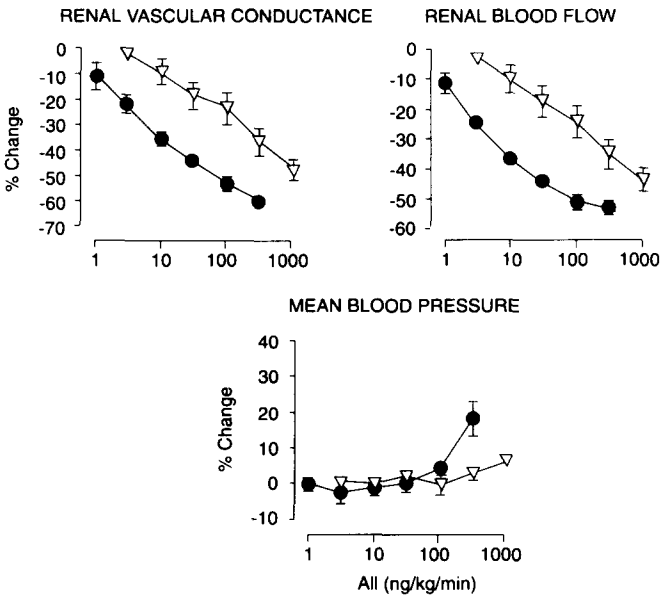

Fig. 5. Effects of intra-renal artery infusion of angiotensin II on renal vascular conductance, renal blood flow, and mean blood pressure in the absence (●, $n = 5$), and in a separate group of dogs, in the presence of GR138950 (1 mg/kg + 10 μg/kg/min i.v.; ▽, $n = 4$). Values are mean percent change ± S.E.M. from basal values (Table 2).

respectively (Fig. 5). No significant pressor response was observed to high doses of angiotensin II (300 and 1000 ng/kg/min) in the presence of GR138950 (Fig. 5).

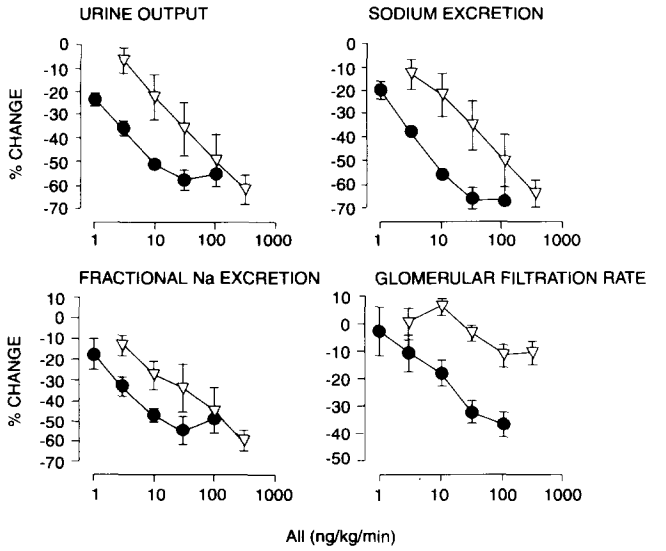

Fig. 6. Effects of intra-renal artery infusion of angiotensin II on urine output, sodium excretion, fractional sodium excretion and glomerular filtration rate, in the absence (●, $n = 5$), and in a separate group of dogs, in the presence of GR138950 (1 mg/kg + 10 μg/kg/min i.v.; ▽, $n = 4$). Values are mean percent change ± S.E.M. from basal values (Table 2).

Renal excretory function

Changes in renal perfusion pressure can result in alterations in renal tubular function. To avoid this complicating factor, the following results describe the effects of non-pressor doses of angiotensin II on renal function, in the absence and presence of GR138950.

In the absence of antagonist, angiotensin II (1–100 ng/kg/min) caused marked dose-related reductions in urine output and sodium excretion which occurred concomitantly with falls in fractional sodium excretion and glomerular filtration rate (Fig. 6). GR138950 antagonised the effects of angiotensin II on these parameters (Fig. 6). Angiotensin II mean dose-response curves for urine output, sodium excretion, and fractional sodium excretion were displaced approximately 10-, 12-, and 8-fold, respectively, to the right of those observed in the absence of antagonist (Fig. 6). GR138950 also markedly inhibited the ability of angiotensin II to reduce glomerular filtration rate, causing an approximate 29-fold rightward displacement of the angiotensin II mean dose-response curve (Fig. 6).

4. Discussion

GR138950 is a novel, specific, non-peptide angiotensin AT_1 receptor antagonist, which produces a long-lasting reduction in blood pressure in animal models of hypertension (Hilditch et al., 1993), an effect which is almost certainly due to the ability of the drug to block the activity of the renin-angiotensin system. Given the important role of the kidneys in the long-term control of blood pressure (Guyton et al., 1980), it is important to assess the effects of any potential anti-hypertensive drug on renal function. This is especially true for drugs like GR138950 which act by blocking the activity of the renin-angiotensin system, since angiotensin II causes renal vasoconstriction and stimulates tubular sodium reabsorption (Navar et al., 1991), and failure to block these effects would be predicted to attenuate the anti-hypertensive efficacy of any such agent. Consequently, this study aimed to examine the renal pharmacology of GR138950 in anaesthetised dogs. The renin-angiotensin system is not markedly activated in the majority of essential hypertensives; therefore, to parallel this situation, the experiments were carried out in salt-replete animals. In the initial part of the study, we examined the effects of GR138950 on basal renal function in comparison with the angiotensin converting enzyme inhibitor, captopril, which is in common clinical use as an anti-hypertensive.

Administration of GR138950 resulted in renal vasodilatation which was accompanied by a marked increase in sodium and urine excretion. Glomerular filtration rate was not significantly changed by GR138950 and since fractional excretion of sodium was markedly

increased, the diuresis/natriuresis almost certainly resulted from inhibition of tubular sodium reabsorption. Given the specificity of action of GR138950 (Hilditch et al., 1993), these effects of GR138950 most likely reflect antagonism of the renal vasoconstrictor and direct renal tubular effects of endogenous angiotensin II. Moreover, these renal effects of GR138950 are similar to those reported from other studies in salt-replete anaesthetised dogs using structurally diverse angiotensin AT_1 receptor antagonists such as losartan (Wong et al., 1991; Chan et al., 1992) and GR117289 (Clark et al., 1993).

Since captopril also blocks the activity of the renin-angiotensin system it would be predicted to produce similar renal effects to angiotensin AT_1 receptor antagonism. In a previous study using anaesthetised dogs, Wong et al. (1991) reported this to be the case for losartan and captopril. In the present study, the renal effects of captopril were broadly similar to, although not as marked as, those of GR138950. Thus, captopril caused renal vasodilatation and showed a tendency to increase sodium and urine excretion although the changes did not achieve statistical significance. The fact that, unlike GR138950, captopril did not evoke a significant diuretic and natriuretic response is perhaps not surprising, since captopril also caused a significant reduction in blood pressure which per se would be expected to decrease sodium and urine excretion (Guyton et al., 1980). Indeed, previous studies with angiotensin converting enzyme inhibitors in salt-replete dogs (Schmidt et al., 1989) or the angiotensin AT_1 antagonist, losartan, in volume expanded rats (Fenoy et al., 1991) have found that these compounds do not produce an acute diuretic response when blood pressure is also reduced.

The fact that captopril, but not GR138950, evoked an acute vasodepressor response in the current study may reflect the fact that, by chance, basal activation of the systemic renin-angiotensin system was somewhat higher in the dogs which received captopril (4.0 ± 0.7 ng angiotensin I/ml/h) in comparison with those which received GR138950 (1.9 ± 0.8 ng angiotensin I/ml/h). Thus, the lack of a vasodepressor response to GR138950 was most likely due to a lack of endogenous angiotensin II-induced arteriolar tone in the systemic vasculature. Consistent with this view was the observation in the second part of this study that, in the same group of dogs in which GR138950 failed to reduce blood pressure, it nevertheless antagonised pressor responses to exogenous angiotensin II, suggesting that the compound was blocking vascular AT_1 receptors outside the kidney. An alternative explanation for the blood pressure lowering effect of captopril is that it has cardiovascular effects independent of angiotensin converting enzyme inhibition. Vollmer et al. (1978) reported that captopril (SQ14,225) reduced blood pressure in pentobarbital-anaesthetised dogs, even after nephrectomy or blockade of angiotensin receptors with Sar^1Ala^8-angiotensin II. They proposed that extra-renal kallikrein may form physiologically important amounts of kinins and that they exerted vasodilator activity when their breakdown was prevented by captopril. Miura et al. (1982) came to similar conclusions. Such an effect would be consistent with observation, in the present experiments, that captropril reduced blood pressure but did not affect renin activity presumably because the volume loading had largely suppressed renin release and its feedback control. Mimram et al. (1980) suggested that the endogenous kallikrein-kinin system played a part in the antihypertensive effect of captropil in patients with renin-dependent hypertensin. However, the extent to which kinins contributed to the vasodepressor response to captopril, or indeed its effects on basal renal function, in the present experiments, remains unknown.

If a lack of background activation of the renin-angiotensin system was responsible for the lack of a depressor response to GR138950, why then was the compound able to cause a marked renal vasodilatation and diuresis? An answer to this paradox probably lies in the emerging evidence that the level of activation of the systemic renin-angiotensin system may not accurately reflect angiotensin II levels in the kidney, where the peptide plays an important role in the local control of renal function. Indeed, Seikaly et al. (1990) have demonstrated renal levels of angiotensin II far in excess of those found in plasma. The present data are in agreement with the studies of Chan et al. (1992) and Bovee et al. (1991) who reported that the angiotensin AT_1 receptor antagonist, losartan, preferentially enhanced renal haemodynamic and excretory function in anaesthetised and conscious dogs. It is interesting that renal angiotensin II appears to play an important sodium retaining role in the dog even under salt-replete conditions.

Having found evidence to suggest that GR138950 blocks the renal effects of endogenous angiotensin II, we went on to substantiate that GR138950 was effectively blocking renal angiotensin receptors. Thus, we examined how renal responses to infusions of exogenous angiotensin II were affected by the antagonist. This second phase of the study also provided us with information on how a wide range of renal concentrations of angiotensin II were affected by GR138950. Infusions of angiotensin II were given directly into the renal artery so that the direct effects of the peptide on renal function could be observed without any complicating changes in renal perfusion pressure.

As we have previously reported (Clark et al., 1993), local infusion of angiotensin II (1–100 ng/kg/min) into the renal artery resulted in marked dose-related renal vasoconstriction, characterised by decreased re-

nal vascular conductance and blood flow. This reflected a direct effect of the peptide on the renal vasculature, since blood pressure was not increased. GR138950 markedly and competitively antagonised the renal vasoconstrictor effects of angiotensin II, confirming its ability to block renal vascular angiotensin AT_1 receptors. Moreover, while in the absence of GR138950, the highest dose of angiotensin II (300 ng/kg/min) caused a pressor response (~ 10–15 mm Hg), neither this dose nor the higher dose of 1000 ng/kg/min evoked a pressor response following GR138950. The pressor response to high doses of angiotensin II almost certainly reflects vasoconstriction not only in the kidney, but also in other vascular beds following escape of the peptide to the systemic circulation. Thus, the ability of GR138950 to inhibit these pressor responses suggests that the lack of a depressor response to GR138950 per se (discussed above) does not reflect a reduced affinity of the compound for angiotensin AT_1 receptors in extra-renal vascular beds.

In addition to renal vasoconstriction, angiotensin II (1–100 ng/kg/min i.r.a.) caused a dose-related decrease in urine output and sodium excretion. Over the same dose range, angiotensin II also reduced glomerular filtration rate. Thus, decreased filtered load may partly account for the reduced urine/sodium excretion. However, this decrease in urine and sodium excretion was also partly due to increased tubular reabsorption, since marked decreases in fractional sodium excretion were observed. The mechanism of angiotensin II-induced stimulation of tubular sodium reabsorption most likely reflects both a direct effect on angiotensin receptors in the proximal tubule (Harris and Young, 1977; Navar et al., 1991; Romero et al., 1991), and an indirect effect due to the ability of the peptide to decrease renal blood flow (see review by Hall and Guyton, 1990). As well as inhibiting the haemodynamic effects of angiotensin II, GR138950 also markedly and competitively antagonised the ability of angiotensin II to decrease urine output, sodium excretion, fractional sodium excretion and glomerular filtration rate. Taken together, these data strongly suggest that GR138950 blocks renal angiotensin AT_1 receptors at both vascular and tubular sites.

In conclusion, this study has demonstrated that the specific angiotensin AT_1 receptor blocker, GR138950, is an effective antagonist of the renal haemodynamic and excretory actions of endogenous and exogenous angiotensin II. Thus, the renal pharmacology of GR138950 is consistent with its potential use as an anti-hypertensive agent.

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