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The antihypertensive profile of the angiotensin AT_1 receptor antagonist, GR138950, and the influence of potential homeostatic compensatory mechanisms in renal hypertensive rats

[1,2,3]Ian K. Anderson & [1]Geoffrey M. Drew

[1]Disease Sciences, Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY

1 The cardiovascular profile of the angiotensin AT_1 receptor antagonist, GR138950, and the influence of potential compensatory homeostatic mechanisms on this profile, were investigated in renal artery ligated hypertensive (RALH) rats.

2 GR138950 caused a marked reduction in blood pressure associated with immediate tachycardia in conscious RALH rats. The antihypertensive action of GR138950 appeared biphasic; an immediate fall in blood pressure, which plateaued within 1 h, and which was followed by a further slow decline that reached maximum between 5–7 h after administration.

3 The tachycardia caused by GR138950 was attenuated by atenolol and was abolished by combined pretreatment with atenolol and atropine methyl nitrate. However, the antihypertensive profile of GR138950 was unchanged by these pretreatments.

4 The resting blood pressure and the antihypertensive effect of GR138950, in RALH rats, were unaffected by the vasopressin V_1 receptor antagonist, [β-mercapto-β,β-cyclopentamethylene propionyl[1]-O-Me-Tyr[2],Arg[8]]-vasopressin. Thus, vasopressinergic mechanisms are not involved in either maintaining blood pressure in RALH rats, or in compensating for the fall in blood pressure caused by GR138950.

5 In anaesthetized RALH rats, GR138950 caused a marked fall in blood pressure that was accompanied by an increase in heart rate along with sustained increases in renal and splanchnic sympathetic nerve activity.

6 In summary, the biphasic fall in blood pressure evoked by GR138950 in RALH rats can not be explained on the basis of changes in autonomic control of the heart, alteration of vasopressin-mediated vasoconstrictor mechanisms or overall suppression of central sympathetic outflow. Rather, increased vasoconstrictor tone might serve to oppose the initial fall in blood pressure.

Keywords: Renal hypertensive rats; blood pressure; heart rate; sympathetic nerve activity; angiotensin AT_1 receptors; GR138950; losartan; enalapril; vasopressin; angiotensin

Introduction

Angiotensin converting enzyme (ACE) inhibitors have become firmly established in the treatment of hypertension. It is generally assumed that they owe their clinical efficacy to preventing the conversion of angiotensin I to angiotensin II, although contributions from kinins and prostaglandins can not be discounted. As a natural progression, angiotensin receptor blocking agents have also been developed as antihypertensive agents. The antihypertensive action of such compounds has been attributed mainly to blocking the vasoconstrictor effects of endogenous angiotensin II at vascular AT_1 receptors. However, it has been noted that, amongst some agents of this class, including losartan and GR117289, there is a lack of a temporal relationship between their antihypertensive and their angiotensin AT_1 receptor blocking actions (Akers *et al*., 1991; Ohlstein *et al*., 1992; Drew, 1993; Hilditch *et al*., 1994). GR138950 is no exception; it is a potent and selective non-peptide antagonist at angiotensin AT_1 receptors *in vitro* and *in vivo* (Hilditch *et al*., 1995) which causes a marked antihypertensive effect in conscious rats in which blood pressure has been elevated following activation of the renin-angiotensin system (RAS) (Hilditch *et al*., 1995). However, the time-course of its antihypertensive effect in renal hypertensive rats (maximum fall in blood pressure occurs after 5–7 h) does not coincide with that of its antagonist profile against exogenous angiotensin I or angiotensin II-induced pressor responses in normotensive rats (maximal rightward shift of the dose-pressor response to angiotensin I or angiotensin II occurs at 1 h (Hilditch *et al*., 1995, 1996). Furthermore, the fall in blood pressure produced by GR138950 seems to occur in two phases; the first, rapidly developing phase occurs over the 30–60 min following systemic administration, and the second phase develops slowly and progressively reaching its nadir 7 h or more after administration (Anderson & Drew, 1997). A similar profile of action has been noted for some other AT_1 receptor antagonists, notably L-158,809 and EXP3174 which, like GR138950, do not depend upon metabolism for their activity (Hodges *et al*., 1992).

The explanation for the cardiovascular profile of GR138950 and other angiotensin AT_1 receptor antagonists is unknown but several mechanisms are possible (Hilditch *et al*., 1994, 1995). For example, in addition to blocking vascular angiotensin AT_1 receptors, it is possible that AT_1 receptor antagonists reduce blood pressure by blockade of AT_1 receptors other than those in the vasculature, or through mechanisms quite unrelated to AT_1 receptor blockade (Ohlstein *et al*., 1992). Alternatively, activation of compensatory cardiovascular mechanisms might oppose the fall in

[2]Current address: Cambridge Antibody Technology Ltd., The Science Park, Melbourn, Royston, Cambridgeshire, SG8 6JJ.
[3]Author for correspondence.

blood pressure elicited by such compounds, at least in the short term following their administration. In the present study, we have examined three possible mechanisms that might explain the cardiovascular profiles of AT_1 receptor antagonists, typified by GR138950 (and, in some experiments, losartan and the ACE inhibitor, enalapril, have been included for comparison).

The first of these mechanisms involves changes in autonomic control of the heart that might lead to a short term increases in cardiac output that would offset a decrease in vascular resistance caused by blockade of vascular AT_1 receptors. The subsequent recovery of cardiac output, to or below pre-drug levels as haemodynamic parameters slowly re-equilibrate, would then reveal the full extent of the vascular effects of AT_1 receptor blockade. In this context, it has been reported that, in conscious renal hypertensive rats, the AT_1 receptor antagonist, SK&F 108566, causes a fall in blood pressure which is mediated by a decrease in total peripheral resistance. This antihypertensive effect is offset by a reflex, heart rate driven increase in cardiac output (Brooks *et al*., 1992). Similar findings were reported for losartan in conscious rats with over-activation of the RAS (Batin *et al*., 1991). Therefore, blockade of these cardiac events might modify the antihypertensive profile of GR138950. This possibility was tested, in the present study, by examining the cardiovascular effect of GR138950 in RALH rats in which autonomic control of cardiac function was prevented by atenolol and atropine methyl nitrate.

The second mechanism that was investigated focused on the possible role of vasopressin in the regulation of blood pressure in RALH rats. The central administration of angiotensin II has been shown to release vasopressin, an effect mediated *via* AT_1 receptors (Hogarty *et al*., 1992). Furthermore, systemically administered angiotensin II has also been demonstrated to release vasopressin (Iovino & Steardo, 1984 Unger *et al*., 1988). The increased blood pressure in the RALH rat model seems to be largely dependent on increased circulating levels of angiotensin II (Wong *et al*., 1989a); thus, it is possible that angiotensin II-evoked vasopressin release contributes to the increased blood pressure in these animals. Therefore, it is possible that GR138950 may, in part, lower blood pressure in RALH rats by inhibiting the release of vasopressin following blockade of centrally located AT_1 receptors. The possible involvement of vasopressin in the hypertension caused by left renal artery ligation was investigated using the selective V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)AVP (Kruszynski *et al*., 1980; Manning & Sawyer, 1986).

The third possible mechanism that was investigated related to the effect of GR138950 on central sympathetic outflow. Angiotensin II has previously been shown to enhance sympathetic drive to the vasculature (Unger *et al*., 1985; Steckelings *et al*., 1992; Fink, 1997). Moreover, Fink *et al*. (1980) and Fink (1997) have suggested that there is a centrally mediated sympathetic vasoconstrictor component involved in the production of hypertension in high renin rat models. Interestingly, central application of angiotensin antagonists has been shown to reduce blood pressure (Yang *et al*., 1992) by inhibiting sympathoexcitatory vasomotor tone (Sakai & Dampney, 1990; Ito & Sved, 1996). Thus, the possibility exists that systemic administration of AT_1 receptor antagonists might act centrally to suppress sympathetic drive to the vasculature. This possibility was investigated, in the present study, by systemically administering GR138950 to anaesthetized RALH rats in which sympathetic tone was monitored by measuring changes in renal and splanchnic sympathetic nerve activity.

A preliminary account of some of these observations has been presented to the British Pharmacological Society (Anderson & Hilditch, 1994).

Methods

Experiments were performed using male Allen & Hanbury/Albino (AH/A) rats (250–350 g). Rats were individually housed and maintained under a 12 : 12 h light-dark cycle (lights on at 06.00 h) with free access to food and water. Surgery was performed in two stages with 5 days between each stage and is described in detail elsewhere (Anderson & Drew, 1997). Briefly, renal hypertension was induced in all animals by ligation of the left renal artery. Five days after renal artery ligation (1 day before examination), rats were anaesthetized with sodium methohexitone (40 mg kg^{-1}, 1.3 ml kg^{-1} i.p.) before two intravenous catheters were implanted in the right jugular vein for drug administration and an intra-arterial catheter was placed in the distal abdominal aorta, *via* the caudal artery (for blood pressure and heart rate recordings). To maintain patency, when not in use, the arterial cannula was continuously infused (0.3 ml h^{-1}) with heparinized saline (15 u ml^{-1}) using an infusion pump (Razel). After each surgical stage wounds were closed and dusted with chlorotetracycline and the animals were also given an injection of procaine penicillin and dihydrostreptomycin (7 mg kg^{-1}, i.m.).

Experiments were performed with the animals in their home cages and they were given free access to food and water. Each animal wore a specially designed harness that was attached to a counterbalanced spring. The vascular catheters led out of the cage through the spring (for protection) and the arterial catheter connected *via* a watertight swivel (Instech, 375/22) to a pressure transducer (SensoNor 840). Arterial blood pressure was recorded and heart rate derived electronically from the blood pressure signal (Lectromed ECG amplifier). Drug or vehicle solutions were administered intravenously either by bolus injection (0.5 ml kg^{-1} over 1 min) or by intravenous infusion (0.3 ml h^{-1}). Injections of vehicle or drug were made when the animals were settled and when cardiovascular variables were stable.

Conscious RALH rats: Experimental protocols

Six days after ligation (1 day after cannulation), RALH rats were allowed to settle and baseline cardiovascular values were established. The patency of the intravenous catheters was confirmed following a bolus injection of saline (0.2 ml, i.v.). Experimental protocols began 10 min after this injection.

Group 1: Effect of GR138950, losartan or enalapril

A single dose of either GR138950 (1 mg kg^{-1}), losartan (3 mg kg^{-1}), enalapril (1 mg kg^{-1}) as well as vehicle for GR138950 (0.5 ml kg^{-1}, sodium bicarbonate and ethanol in distilled water) or vehicle for losartan and enalapril (0.5 ml kg^{-1}; distilled water) was administered i.v. and flushed in with a saline bolus (0.2 ml, i.v.). In each rat the cardiovascular response to a single dose of the test drug or vehicle was followed for up to 48 h. A dose of 1 mg kg^{-1} of GR138950 was selected for these experiments because experience showed that both the primary and secondary decreases in blood pressure were distinct and easily identified (see Figure 1). This choice of dose of GR138950 dictated those of losartan and enalapril; doses of

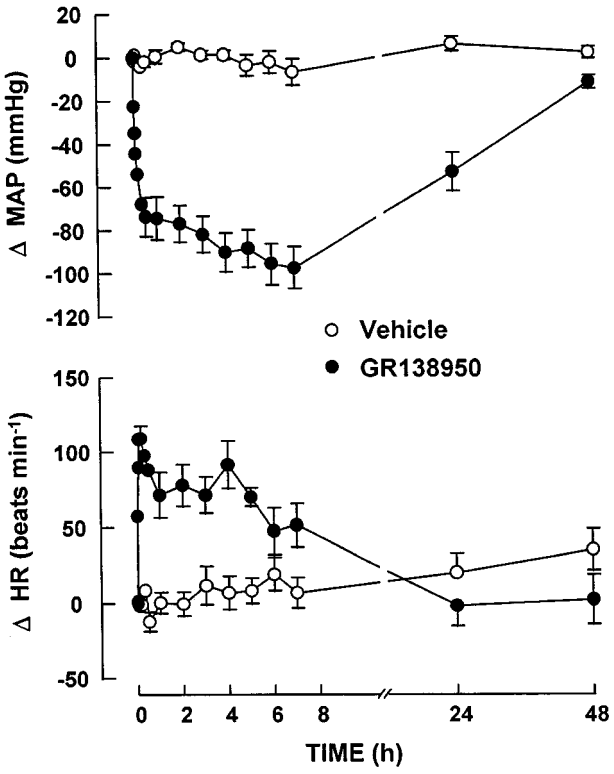

Figure 1 Conscious RALH rats. A comparison of the changes from baseline values over time (h) caused by vehicle (0.5 ml kg^{-1}, i.v., $n=6$), or GR138950 (1 mg kg^{-1}), i.v., $n=6$) in mean arterial pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical bars show s.e.mean. See Table 1 for statistical analysis.

these drugs were selected to produce comparable peak falls in blood pressure.

Group 2: Effects of β_1-adrenoceptor or combined β_1-adrenoceptor and muscarinic receptor blockade on the response to GR138950

An infusion of atenolol (0.5 mg kg^{-1} bolus, then 0.2 mg kg^{-1} h^{-1} infusion, i.v.) or a combination of atenolol (dose as above) and atropine methyl nitrate (atropine; 0.5 mg kg^{-1} bolus, then 0.1 mg kg^{-1} h^{-1} infusion, i.v.) was administered 30 min before the administration of GR138950 (1 mg kg^{-1}, i.v.) or vehicle (0.5 ml kg^{-1}, i.v.). Antagonist infusion was continued following administration of GR138950 or vehicle for the duration of the experiment. The vehicle treated animals provided time-matched control values for the cardiovascular changes caused by antagonist pretreatment. Blockade of cardiac β_1-adrenoceptors or muscarinic receptors was also evaluated in the vehicle control group. The blockade of β_1-adrenoceptors with atenolol was confirmed by examining the tachycardia caused by the administration of the β-adrenoceptor agonist, isoprenaline (10 ng kg^{-1}, i.v.) before, and 1 and 6.5 h after, administration of atenolol. The ability of atropine to block muscarinic receptors was tested by examining the vagally mediated bradycardia caused by activation of the cardiopulmonary afferents by the 5-HT_3 receptor agonist, phenylbiguanide (5 μg kg^{-1} i.v.; Bogle *et al.*, 1990) before, and 1 and 6.5 h after administration of the combination of atenolol and atropine. Atropine methyl nitrate was used because it does not penetrate the central nervous system (Brezenoff *et al.*, 1988).

Group 3: Effect of vasopressin V_1 receptor blockade on blood pressure and heart rate

An infusion of the V_1 receptor antagonist, $d(CH_2)_5$Tyr(-Me)AVP (20 mg kg^{-1} bolus, then 10 mg kg^{-1} h^{-1} infusion, i.v.) was administered for 6.5 h. After 30 min the vehicle for GR138950 was administered (0.5 ml kg^{-1}, i.v.). The blockade of V_1 receptors with $d(CH_2)_5$Tyr(Me)AVP was confirmed by examining the pressor response caused by vasopressin (10 ng kg^{-1}, i.v.) before, and 1 h and 6.5 h after administration of $d(CH_2)_5$Tyr(Me)AVP. In separate RALH rats $d(CH_2)_5$Tyr(Me)AVP (dose as above) was administered 30 min before the administration of GR138950 (1 mg kg^{-1}, i.v.). The infusion of $d(CH_2)_5$Tyr(Me)AVP was continued following administration of GR138950.

Anaesthetized RALH rats: Effect of GR138950 on sympathetic nerve activity

In RALH rats (6 days post-ligation) anaesthesia was induced with isoflurane (5% in oxygen, 0.4 l min^{-1} and nitrous oxide, 0.8 l min^{-1}) and maintained with α-chloralose (bolus 80 mg kg^{-1} then 10 mg kg^{-1} h^{-1} infusion, i.v.). α-Chloralose anaesthesia was used as it has been shown to preserve baroreceptor reflex mediated responses (Barringer & Buñag, 1990) and, in preliminary experiments in conscious RALH rats, hypertension was retained, albeit somewhat diminished, following administration of α-chloralose anaesthetic.

The left carotid artery was cannulated for the measurement of blood pressure. Blood pressure was measured using a pressure transducer (SensoNor 840) and heart rate was derived electronically from the blood pressure signal. The left jugular vein was cannulated for the administration of α-chloralose anaesthetic and drugs. A tracheal cannula was implanted. Body temperature was monitored by a rectal probe and maintained at 36–38°C with a homeothermic blanket system (Harvard). Rats were artificially ventilated (rate 70 min^{-1}, stroke volume 6 ml kg^{-1}) with oxygen-enriched room air by use of a positive pressure pump (Ugo Basile) and neuromuscular blockade was produced with decamethonium (3 mg kg^{-1} i.v.). Blood samples (100 μl; 2–4 per animal) were taken *via* a T-piece on the carotid arterial cannula and blood gases and pH were monitored with a pH/blood gas analyzer (ABL 30). Blood gases were maintained between 100–120 mmHg PO_2, 40–45 mmHg PCO_2 and pH 7.35–7.45. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml kg^{-1} h^{-1}) *via* the jugular vein with a solution consisting of 10 ml plasma substitute (Gelofusine), 10 ml distilled water, 0.04 g glucose, 0.168 g sodium bicarbonate and 30 mg decamethonium. This was to prevent the development of metabolic acidosis and to maintain blood volume and neuromuscular blockade.

Simultaneous whole nerve extracellular recordings were taken from the renal (post-ganglionic) and greater splanchnic (pre and postganglionic) sympathetic nerves. Renal nerve activity and splanchnic nerve activity were measured to allow evaluation of any differential changes in sympathetic outflow. The right kidney was exposed by a retroperitoneal approach and deflected laterally to reveal the right renal artery and renal nerve. The splanchnic nerve and coeliac ganglion were also exposed using this approach. Renal and splanchnic nerves were cleared of connective tissue and positioned on bipolar silver hook electrodes. Nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 100–700 Hz) and quantified by integrating the signal above

background noise over 5 s with a solid state integrator (Digitimer NL600). The validity of the integrator threshold setting was verified at the end of the experiment after the abolition of nerve activity following administration of pentobartibone sodium (20 mg per animal).

Sufficient depth of anaesthesia was confirmed by the stability of cardiovascular variables and the lack of corneal and toe pinch reflexes. After neuromuscular blockade, stability of sympathetic nerve activity, as well as heart rate and blood pressure, were used to indicate depth of anaesthesia.

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether heart rate, along with renal and splanchnic nerve activity, were increased by a reduction in blood pressure caused by sodium nitroprusside (2 μg kg^{-1}, 0.2 ml kg^{-1} i.v.). Only preparations with an intact baroreceptor reflex were subsequently used. Anaesthetized RALH rats with a basal mean arterial pressure less than 130 mmHg were excluded from the study. The renal nerve was considered to be postganglionic if the activity was abolished by hexamethonium (6 mg kg^{-1}, 0.6 ml kg^{-1}, i.v.) given at the end of each experiment. Arterial blood pressure, heart rate plus integrated renal and splanchnic nerve activity were displayed on a chart recorder (Gould 3800 series). Blood pressure and unfiltered nerve activity from renal and splanchnic nerves were recorded on electromagnetic tape.

After surgery, the preparation was allowed to stabilize for 30 min before the administration of saline (100 μl i.v.) to ensure the patency of the cannula. After a 10 min control period, a single dose of GR138950 (1 mg kg^{-1}, i.v.) or vehicle (0.5 ml kg^{-1}, i.v.) was given and the response was followed for 180 min. The loss of stability of nerve recording after 180 min in some preparations prevented recording of activity after this time.

Analysis of results

Baseline values were taken 1 min before the addition of drug or vehicle. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 1 min in arbitrary units and was expressed as the percentage change from baseline. The area over the curve (AOC) for mean arterial pressure (mmHG min) or area under the curve (AUC) for heart rate (beats), renal nerve activity (% min) and splanchnic nerve activity (% min) were determined. In conscious rats, reponses to drug or vehicle in the different treatment groups were compared using one way ANOVA and Duncan's multiple comparison test. In anaesthetized rats the response caused by GR138950 was compared to that for vehicle using Student's *t*-test for unpaired data. Changes in variables caused by pretreatments were compared to the pre-dose baseline using Student's *t*-test for paired data. All values are expressed as the mean $\pm$ s.e.mean; differences in the mean were taken as significant when $P<0.05$.

Drugs used

The drugs used were atenolol (Sigma, Poole, Dorset, U.K.); atropine methyl nitrate (Sigma, Poole, Dorset, U.K.); [β-mercapto-β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2, Arg^8]-vasopressin, (d(CH$_2$)$_5$Tyr(Me)AVP; Sigma, Poole, Dorset, U.K.); α-chloralose (Sigma, Poole, Dorset, U.K.); chlorotetracycline HCl (Aureomycin; Cyanamid, Wayne, New Jersey, U.S.A.); decamethonium bromide (Sigma, Poole, Dorset, U.K.); enalapril maleate (Sigma, Poole, Dorset, U.K.); Gelofusine (Consolidated Chem., Wrexham, Clwyd, U.K.); hexamethonium bromide (RBI, St. Albans, Hertfordshire, U.K.); isoflurane (Abbott Labs, Queensborough, Kent, U.K.); isoprenaline bitartrate (Sigma, Poole, Dorset, U.K.); losartan (DuP753; DuPont-Merck, Wilmington, DE, U.S.A.); methohexitone sodium (Eli Lilly, Basingstoke, U.K.); phenylbiguanide (ICN, Thame, Oxfordshire, U.K.); procaine penicillin/dihydrostreptomycin (Duphar + Strep; Solvay-Duphar, South Normington, Derbyshire, U.K.); sodium nitroprusside (Sigma, Poole, Dorset, U.K.).

GR138950, in the form of the amphoteric neutral compound, was prepared in the Chemistry Research Division, Glaxo Research & Development Ltd.

All drugs for intravenous administration were dissolved in 0.9% w/v saline with the following exceptions. GR138950 was dissolved in sodium bicarbonate (1 M; 5%; v/v) ethanol (absolute, 5%; v/v) in distilled water (90%; v/v). Losartan and enalapril were dissolved in distilled water. α-Chloralose was dissolved in borax (5% w/v di-sodium tetraborate in distilled water) to provide a concentrated stable solution (50 mg ml^{-1}) for infusion. All drug solutions were prepared on the day of the experiment and the doses given represent the amount of parent compound.

Results

Baseline values

Baseline values for mean arterial blood pressure and heart rate in conscious RALH rats for the various treatment groups are shown in Tables 1 and 2. α-Chloralose anaesthesia significantly reduced basal mean arterial pressure in RALH rats (conscious 172 ± 3 mmHg, $n=12$; anaesthetized 147 ± 6 mmHg, $n=10$; $P<0.01$, Student's *t*-test). However, anaesthetized RALH rats were still considered hypertensive since their blood pressure was significantly higher than that of α-chloralose anaesthetized normotensive animals (120 ± 3 mmHg, $n=7$; $P<0.01$, Student's *t*-test). Table 3 shows the baseline values for α-chloralose anaesthetized RALH rats.

Effect of drug vehicles in conscious RALH rats

In RALH rats, vehicle for GR138950 (sodium bicarbonate and ethanol in distilled water, 0.5 ml kg^{-1}, i.v., $n=6$; Figure 1) or vehicle for losartan and enalapril (distilled water; 0.5 ml kg^{-1}, i.v., $n=5$; Figure 2) caused little change in mean arterial blood pressure and heart rate and these parameters remained unchanged for the duration of the experiment (48 h).

Effect of GR138950 in RALH rats

GR138950 (1 mg kg^{-1}, i.v.; $n=6$) caused a rapidly developing decrease in mean blood pressure that amounted to -74 ± 9 mmHg over the first 30 min following administration; thereafter, a further slow decline was observed which reached maximum of -97 ± 9 mmHg 6–7 h after administration (Table 1). Twenty four hours after administration of GR138950, mean arterial blood pressure was still reduced compared to that in vehicle treated rats but had returned close to baseline levels 48 h after administration (Figure 1). The fall in blood pressure caused by GR138950 was accompanied with significant (see Table 1) tachycardia. Maximum tachycardia (109 ± 8 beats min^{-1}) was observed 5–10 min following administration. The tachycardia was maintained for at least 7 h but had returned to baseline levels after 24 h (Figure 1).

Effect of losartan in RALH rats

Losartan (3 mg kg^{-1} i.v., $n=5$) caused an immediate fall in mean arterial blood pressure which was associated with a marked increase in heart rate. These responses were significantly different from vehicle treated animals but not significantly different from those observed for GR138950 (Table 1). The fall in mean arterial blood pressure occurred in two phases; an initial rapid fall was observed within 30 min of administration (-52 ± 7 mmHg at 10 min) and this was followed by a slowly developing reduction which attained nadir (-94 ± 7 mmHg) after 6 h. Mean arterial blood pressure was reduced 24 h after administration but had returned to baseline levels 48 h after administration (Figure 2). The tachycardia caused by losartan was maximal 3 min after administration (183 ± 27 beats min^{-1}) and was maintained for at least 7 h. Heart rate had returned to baseline levels by 24 h (Figure 2).

Effect of enalapril in RALH rats

Administration of enalapril (1 mg kg^{-1} i.v., $n=6$) caused a significant fall in blood pressure (compared to vehicle, Table 1) which was immediate in onset; blood pressure reached its nadir (-85 ± 7 mmHg) after 2 h, after which it recovered progressively and had returned to pretreatment levels within 24 h (Figure 2, Table 2). The fall in blood pressure caused by enalapril was associated with a significant increase in heart rate (Table 1). Tachycardia was maximal after 10 min (159 ± 28 beats min^{-1}) and was maintained above baseline levels for at least 7 h. Mean arterial blood pressure and heart rate had returned to baseline levels 24 h after treatment with enalapril (Figure 2).

Therefore, in this model of renal hypertension, drugs that inhibit the RAS reduce blood pressure, and this effect is accompanied by tachycardia, but their profiles differ somewhat, in that a second phase seems to contribute to the antihypertensive effect of the AT_1 receptor antagonists. This phenomenon was investigated in more detail using GR138950.

Effect of pretreatment with atenolol on the response to GR138950 in RALH rats Atenolol (0.5 mg kg^{-1} bolus, then 0.2 mg kg^{-1} h^{-1} infusion, i.v.) had no effect on resting mean arterial blood pressure but caused a significant fall in heart rate in RALH rats (Table 2). After this initial change, mean arterial blood pressure and heart rate remained stable

Table 1 Baseline values and changes in mean arterial blood pressure (MAP) and heart rate (HR) in conscious RALH rats following drug or vehicle treatment

Treatment group	n	*MAP* (mmHg)	*HR* (beats min^{-1})	*MAP AOC$_{0-48\,h}$* (mmHg min $\times 10^3$)	*HR AUC$_{0-6\,h}$* (beats $\times 10^3$)
Vehicle 0.5 ml kg^{-1}	6	171 ± 4	382 ± 22	5.6 ± 4.2	4.1 ± 2.2
GR138950 1 mg kg^{-1}	6	174 ± 5	378 ± 16	163.4 ± 17.1**	46.8 ± 17.4*
dH_2O 0.5 ml kg^{-1}	5	169 ± 7	380 ± 20	13.1 ± 4.4	3.0 ± 1.2
Losartan 3 mg kg^{-1}	5	170 ± 5	347 ± 23	$156.7\pm22.6^{\tau}$	$49.6\pm8.3^{\tau}$
Enalapril 1 mg kg^{-1}	6	175 ± 4	356 ± 18	$77.5\pm10.4^{\tau}$	$36.5\pm9.5^{\tau}$

Changes in MAP and HR as shown as AOC and AUC, respectively. The effects of GR138950, losartan or enalapril were compared to those of their respective vehicle control using one way ANOVA and Duncan's test. *$P<0.05$; **$P<0.01$ compared to vehicle (for GR138950); $^{\tau}P<0.01$ compared to distilled water (dH_2O). There were no significant changes in the HR response to any antihypertensive agent over 48 h, the HR AUC values shown are up to 6 h after administration.

Table 2 Baseline values and changes in mean arterial pressure (MAP) and heart rate (HR) in conscious RALH rats caused by GR138950 or vehicle in the various pretreatment groups

		MAP			*HR*		
Treatment	n	*Basal* (mmHg)	*30 min* (mmHg)	*AOC$_{0-6\,h}$* (mmHg min $\times 10^3$)	*Basal* (beats min^{-1})	*30 min* (beats min^{-1})	*AUC$_{0-6\,h}$* (beats $\times 10^3$)
Without pretreatment							
Vehicle	6	171 ± 4	–	0.8 ± 0.5	382 ± 22	–	4.1 ± 2.2
GR138950	6	174 ± 5	–	29.2 ± 3.1	378 ± 16	–	27.1 ± 3.6
Atenolol pretreatment							
Vehicle	5	164 ± 4	154 ± 5	1.7 ± 0.6	379 ± 20	338 ± 6*	2.2 ± 1.5
GR138950	7	172 ± 6	165 ± 6	24.8 ± 1.0 n.s.	362 ± 12	330 ± 5*	$8.5\pm2.3^{\tau}$
Atenolol and atropine methyl nitrate pretreatment							
Vehicle	5	168 ± 4	159 ± 5*	1.2 ± 0.3	376 ± 14	397 ± 12	0.3 ± 0.2
GR138950	5	174 ± 3	168 ± 2	28.0 ± 1.9 n.s.	428 ± 19	402 ± 16	$0.9\pm0.8^{\tau}$
d(CH$_2$)$_5$Tyr(Me)AVP pretreatment							
Vehicle	6	166 ± 8	172 ± 6	1.6 ± 0.6	415 ± 27	392 ± 26	2.7 ± 1.5
GR138950	4	168 ± 9	166 ± 7	23.9 ± 3.5 n.s.	394 ± 42	384 ± 46	25.5 ± 12.3 n.s.

Baseline values and changes in MAP and HR 30 min after the administration of atenolol (0.5 mg kg^{-1}, then 0.2 mg kg^{-1} h^{-1}, i.v.) atenolol plus atropine (0.5 mg kg^{-1}, then 0.1 mg kg^{-1} h^{-1}, i.v.) or d(CH$_2$)$_5$Tyr(Me)AVP (20 μg kg^{-1}, then 10 μg kg^{-1} h^{-1}, i.v.) in conscious RALH rats which subsequently received GR138950 (1 mg kg^{-1}, i.v.) or vehicle (0.5 ml kg^{-1}, i.v.). Changes caused by the pretreatments were compared to baseline values using Student's *t*-test for paired data; *$P<0.05$. The affect of the pretreatments on the response to GR138950 (without pretreatment) was evaluated by comparing the AOC or AUC values (for MAP or HR, respectively) using one way ANOVA and Duncan's test; $^{\tau}P<0.01$; n.s., not significant. Animals treated with vehicle provided time-matched control data for the pretreatments and these pretreatments were found not to significantly affect MAP or HR when compared to vehicle alone.

for up to a further 6 h after administration of vehicle for GR138950 (Figure 3). In these vehicle control experiments, isoprenaline (10 ng kg^{-1} i.v., $n=5$) caused a small decrease in blood pressure and a tachycardia (maximum change 82 ± 7 beats min^{-1}) which was almost abolished 1 h (11 ± 6 beats min^{-1}) and 6.5 h (2 ± 2 beats min^{-1}) after atenolol administration.

In the presence of atenolol, GR138950 (1 mg kg^{-1}, i.v.; $n=7$) caused a fall in mean arterial blood pressure which was not significantly different from that observed in non-pretreated animals (Figure 3, Table 2). However, the tachycardia caused by GR138950 was significantly attenuated in the presence of atenolol (Figure 3, Table 2).

Effect of combined pretreatment with atenolol and atropine on the response to GR138950 in RALH rats

Combined intravenous pretreatment with atenolol (0.5 mg kg^{-1} bolus, then 0.2 mg kg^{-1} h^{-1} infusion) and atropine (0.5 mg kg^{-1} bolus, then 0.1 mg kg^{-1} h^{-1} infusion) had no significant effect on resting heart rate or mean arterial pressure (Table 2). Furthermore, mean arterial blood pressure and heart rate remained stable for up to 6 h after administration of vehicle for GR138950 (Figure 4). In control experiments, the vagally mediated bradycardia, caused by stimulation of the cardiopulmonary afferents by phenylbiguanide (5 μg kg^{-1}, i.v., $n=3$) was blocked in the presence of the

Table 3 Baseline values and changes in MAP, HR, and renal and splanchnic nerve activity caused by GR138950 or vehicle in anaesthetized RALH rats

Anaesthetized RALH rats	*Vehicle* 0.5 ml kg^{-1} $n=5$	*GR138950* 1 mg kg^{-1} $n=5$
Basal MAP (mmHg)	146 ± 9	148 ± 10 n.s.
Basal HR (beats min^{-1})	420 ± 11	417 ± 9 n.s.
MAP $AOC_{0-180\ min}$ (mmHg min $\times 10^3$)	1.5 ± 0.6	9.6 ± 2.1*
HR $AUC_{0-30\ min}$ (beats $\times 10^3$)	0.3 ± 0.1	1.4 ± 0.4*
RNA $AUC_{0-180\ min}$ (% min $\times 10^3$)	2.5 ± 1.1	25.1 ± 7.4*
SNA $AUC_{0-180\ min}$ (% min $\times 10^3$)	0.8 ± 0.6	52.0 ± 21.1*

Baseline values and changes (as described by AOC or AUC values) in mean arterial pressure (MAP), heart rate (HR), Renal nerve activity (RNA) and splanchnic nerve activity (SNA) in vehicle and GT138950 groups were compared using Student's *t*-test for unpaired data. *$P<0.05$; n.s., not significant.

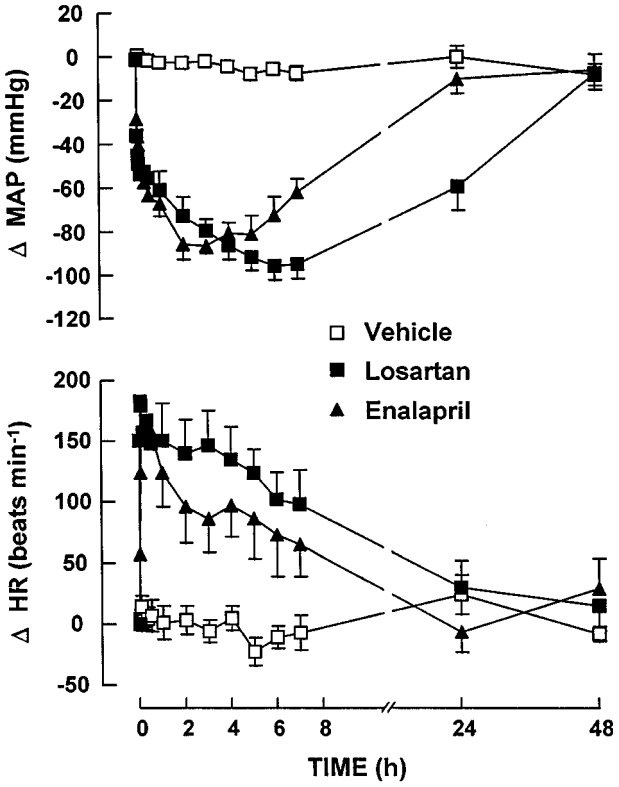

Figure 2 Conscious RALH rats. A comparison of the changes from baseline values over time (h) caused by vehicle (0.5 ml kg^{-1} i.v., $n=5$), losartan (3 mg kg^{-1} i.v., $n=5$), or enalapril (1 mg kg^{-1} i.v., $n=6$) in mean arterial pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical bars show a s.e.mean. See Table 1 for statistical analysis.

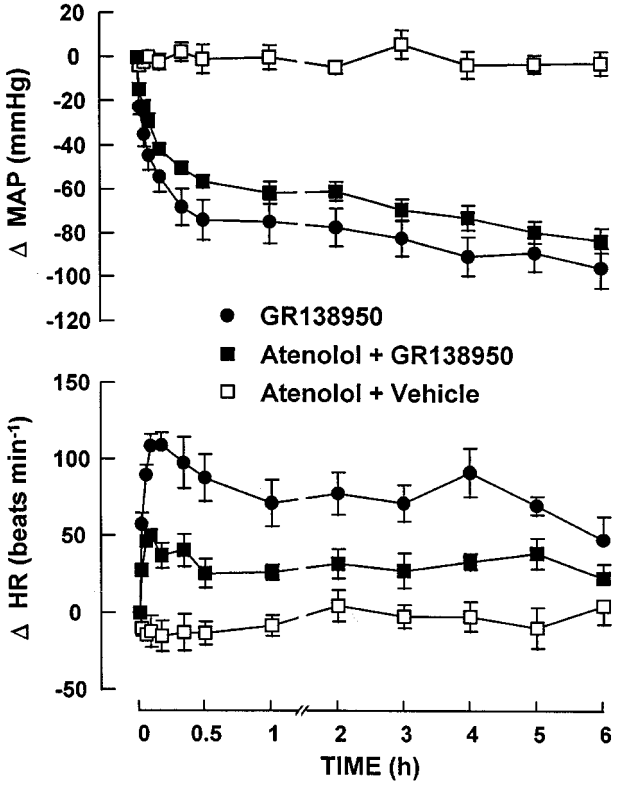

Figure 3 Conscious RALH rats. A comparison of the changes from baseline or post-pretreatment values over time (h) caused by GR138950 1 mg kg^{-1} i.v. in the absence ($n=6$) and presence of atenolol (0.5 mg kg^{-1} bolus, then 0.2 mg kg^{-1} h^{-1} infusion, i.v.; $n=7$) in mean arterial pressure (MAP) and heart rate (HR). The effects of vehicle in the presence of atenolol over the same time period are shown ($n=5$). Each point represents the mean value and the vertical bars show s.e.mean. See Table 2 for statistical analysis.

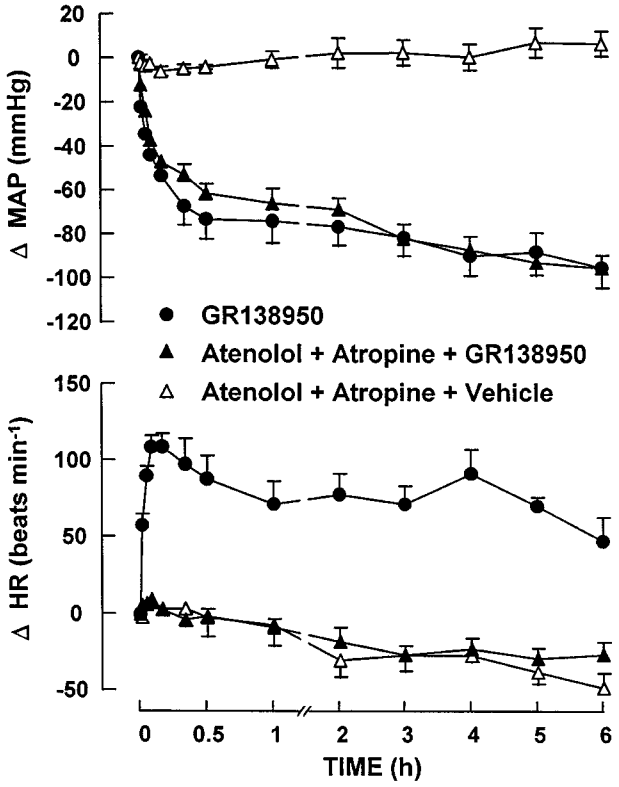

Figure 4 Conscious RALH rats. A comparison of the changes from baseline or post-pretreatment values over time (h) caused by GR138950 1 mg kg^{-1} i.v. in the absence ($n=6$) and presence of a combination of atropine methyl nitrate and atenolol (0.5 mg kg^{-1} bolus, then 0.1 mg kg^{-1} h^{-1} infusion and 0.5 mg kg^{-1} bolus, then 0.2 mg kg^{-1} h^{-1} infusion, respectively, i.v.; $n=5$) in mean arterial pressure (MAP) and heart rate (HR). The effects of the combination of atropine methyl nitrate and atenolol alone over the same time period are shown ($n=5$). Each point represents the mean value and the vertical bars show s.e.mean. See Table 2 for statistical analysis.

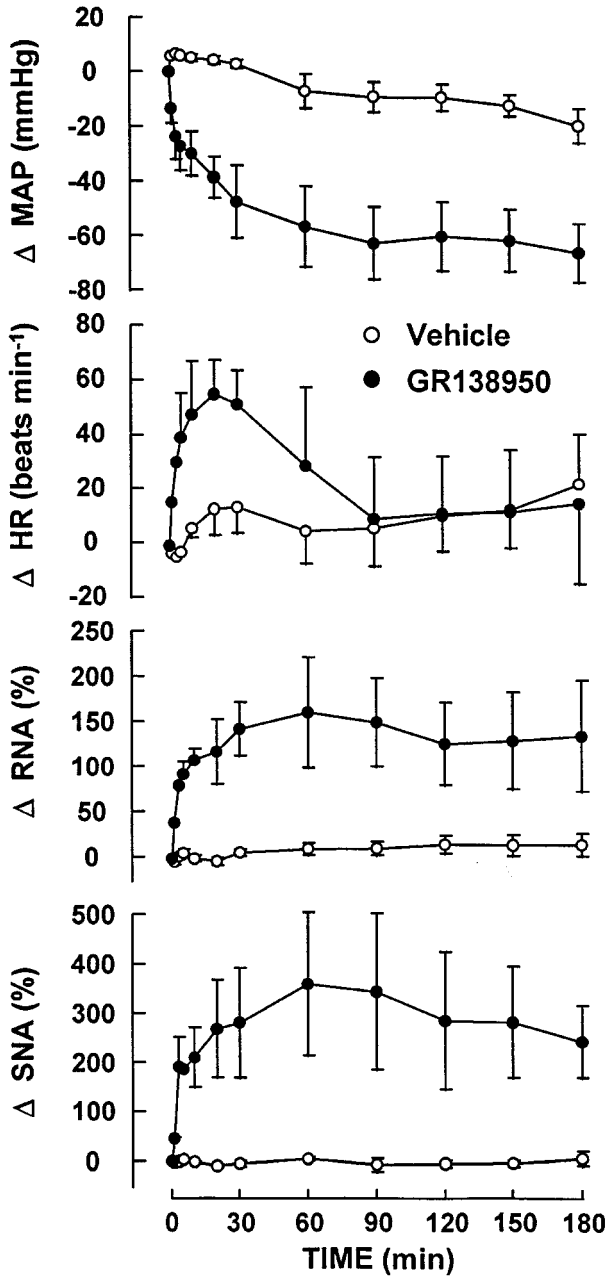

Figure 5 α-Chloralose anaesthetized RALH rats. A comparison of the changes from baseline values over time (min) caused by vehicle (0.5 ml kg^{-1}, i.v.; $n=5$) or GR138950 (1 mg kg^{-1}, i.v.; $n=5$) in mean arterial pressure (MAP), heart rate (HR), renal nerve activity (RNA) and splanchnic nerve activity (SNA). Each point represents the mean value and the vertical bars show s.e.mean. See Table 3 for statistical analysis.

combination of atenolol and atropine (before -117 ± 33, and 1 h -3 ± 3 and 6.5 h -2 ± 2 beats min^{-1} after treatment).

In the presence of atenolol and atropine, GR138950 (1 mg kg^{-1}, i.v., $n=6$) caused a fall in mean arterial blood pressure which was not significantly different from the antihypertensive response to GR138950 in non-pretreated animals (Figure 4, Table 2). However, the tachycardia caused by GR138950 was abolished by combined administration of atenolol and atropine (Figure 4, Table 2).

Cardiovascular effects of the vasopressin V_1 receptor antagonist, d(CH$_2$)$_5$Tyr(Me)AVP in RALH rats and its effect on the response to GR138950

In RALH rats, d(CH$_2$)$_5$Tyr(Me)AVP (20 μg kg^{-1} bolus, then 10 μg kg^{-1} h^{-1} infusion, i.v.; $n=6$) did not affect resting mean arterial pressure or heart rate for up to 6.5 h after administration (Table 2). Vehicle for GR138950 administered 30 min after d(CH$_2$)$_5$Tyr(Me)AVP had little effect. In these animals, the pressor response (maximum change 17 ± 4 mmHg) caused by administration of vasopressin (10 ng kg^{-1} i.v., $n=5$) was blocked 1 h (-2 ± 2 mmHg) and 6.5 h (2 ± 2 mmHg) after treatment with d(CH$_2$)$_5$Tyr(Me)AVP.

In a further four animals, pretreated with d(CH$_2$)$_5$Tyr(Me)AVP (20 μg kg^{-1} bolus, then 10 μg kg^{-1} infusion, i.v.; see Table 2 for baseline values and changes caused by the pretreatment), GR138950 (1 mg kg^{-1}, i.v.) caused an immediate fall in mean arterial blood pressure associated with tachycardia not significantly different to that observed in non-pretreated animals (Table 2).

Effect of GR138950 on sympathetic nerve activity in anaesthetized RALH rats

Vehicle for GR138950 (0.5 ml kg^{-1} i.v.; $n=5$) had little effect on mean arterial blood pressure, heart rate and renal or splanchnic nerve activity and these variables remained largely stable for the duration of the experiment (180 min; Figure 5).

GR138950 (1 mg kg^{-1} i.v.; $n=5$) caused a significant fall in mean arterial pressure (compared to vehicle, see Table 3) which was immediate in onset, maximal after 90 min (maximum fall -63 ± 13 mmHg) and was maintained for the duration of the experiment (180 min). In addition, GR138950 caused a significant increase in heart rate (compared to vehicle, see Table 3) which was maximal (56 ± 12 beats min^{-1}) within 20 min of administration. The tachycardia was not maintained and returned to baseline values 90 min after GR138950 administration. GR138950 caused significant increases in both renal and splanchnic nerve activity which were maximal after 60 min ($162\pm61\%$ and $360\pm145\%$, respectively, Table 3). In contrast to the tachycardia, the increase in sympathetic nerve activity was sustained for the duration of the experiment (Figure 5).

Discussion

The cardiovascular profiles of GR138950, losartan and enalapril were compared in RALH rats, in which the hypertension is largely attributable to elevated plasma angiotensin II levels (Wong *et al*., 1989a). GR138950, losartan and enalapril caused marked reductions in blood pressure associated with immediate tachycardia in RALH rats. The antihypertensive action of GR138950 and losartan appeared biphasic; an immediate fall in blood pressure which plateaued within 1 h and was followed by a further slow decline which reached maximum between 5–7 h after administration of GR138950, and lasting >24 h. This general profile of action has been noted for several other angiotensin AT_1 receptor antagonists (Hodges *et al*., 1992). In contrast, enalapril, though reducing blood pressure to a similar extent to GR138950 and losartan, exhibited a monophasic profile, blood pressure failing to its nadir around 2 h after administration, followed by a steady, progressive recovery. These observations confirm earlier studies with GR138950 (Hilditch *et al*., 1995; 1996) and losartan (Akers *et al*., 1991; Ohlstein *et al*., 1992) in which the maximum antihypertensive effect occurs between 5–7 h. Moreover, the antihypertensive effect of GR138950 in unrestrained, conscious RALH rats (described in the present study) does not coincide, temporally, with the maximum block of vascular AT_1 receptors (described previously by Hilditch *et al*., 1995; 1996; see Introduction).

Interestingly, GR138950, losartan or enalapril caused marked increases in heart rate that attained maximum soon after administration (5–10 min) with a duration of at least 7 h. In previous studies in RALH rats, AT_1 receptor antagonists, such as GR138950 (Hilditch *et al*., 1995), GR117289 (Hilditch *et al*., 1994), losartan (Wong *et al*., 1990), L-158,809 (Siegl *et al*., 1992) or ABBOTT-81282 (Lee *et al*., 1993), failed to cause increases in heart rate. However, in keeping with the present work, other studies have reported tachycardia accompanying the antihypertensive response to several AT_1 receptor antagonists (including losartan, Wong *et al*., 1990; Batin *et al*., 1991; Niederberger *et al*., 1995: SK&F108566, Brooks *et al*., 1992: valsartan, Criscione *et al*., 1993). These contrasting findings are best explained by the different handling and blood pressure recording techniques employed in these studies; tachycardia was observed in those studies employing recording techniques without the need to handle the animals. It should be noted that, although tachycardia accompanies the blood pressure fall caused by AT_1 receptor antagonists in hypertensive rat models, there were no changes in heart rate in humans in which blood pressure was reduced by AT_1 receptor antagonists (e.g. Tsunoda *et al*., 1993; Bindschedler *et al*., 1997).

Twenty-four hours after administration of GR138950 or losartan, heart rate had returned to baseline levels despite a significant residual antihypertensive response at this time. Similar observations have been made by Niederberger *et al*. (1995) for the cardiovascular response to losartan in two-kidney, one-clip (2K1C) hypertensive rats. Thus, it appears that the cardiac baroreceptor reflex may have been reset in animals treated with GR138950 or losartan. It is not known whether blockade of AT_1 receptors, *per se*, mediated the resetting of the reflex, or whether it was a progressive change induced by lowering the blood pressure (Edmunds *et al*., 1990).

Acute reflex increases in heart rate leading to secondary increases in cardiac output have been observed in renal hypertensive rats treated with SK&F 108566 (Brooks *et al*., 1992) or in Brattleboro rats (water-deprived to activate the RAS) treated with losartan (Batin *et al*., 1991). It is possible that the immediate fall in blood pressure caused by AT_1 receptor blockade in hypertensive rats is offset by these reflex changes in cardiac function and, consequently, the magnitude of the initial blood pressure fall might be smaller than would otherwise be seen in the absence of cardiac reflex changes. If this is so, it might help explain why blood pressure nadir does not coincide, temporally, with maximum AT_1 receptor blockade by GR138950 and similar compounds. Thus, prevention of any such compensatory mechanisms might reveal the full extent of the blood pressure lowering effects of acute blockade of vascular AT_1 receptors and align it more closely with inhibition of pressor responses to exogenous angiotension II. To examine this possibility, cardiac β_1-adrenoceptors and muscarinic receptors were blocked prior to treatment with GR138950. Atenolol, alone, reduced resting heart rate and markedly reduced the tachycardia produced by intravenous injection of isoprenaline. It was thus considered to have extensively blocked β_1-adrenoceptors, however, it only attenuated the tachycardia caused by GR138950. In contrast, the tachycardia caused by GR138950 was abolished by the combination of atenolol and atropine methyl nitrate. In preliminary experiments, the dose of atropine methyl nitrate used was shown to all but abolish the vagally mediated bradycardia, caused by phenylbiguanide-induced stimulation of cardiopulmomary afferents, for the duration of the experiments. Interestingly, the combination of atenolol and atropine methyl nitrate had no significant effect on resting heart rate, probably because the effects of the individual agents just offset each other. Taken together, the results suggest that the tachycardia caused by GR138950 was mediated indirectly, through the stimulation of sympathetic drive and withdrawal of vagal drive to the heart. It is likely that this tachycardia was elicited following activation of the baroreceptor reflex arc, as a consequence of the initial rapid fall in blood pressure caused by GR138950. A similar mechanism is likely to explain the tachycardia induced by losartan and enalapril in this model. In contrast, the blood pressure fall caused by GR138950 was unaffected by atenolol or antenolol combined with atropine. Therefore, we are able to conclude that a reflex increase in heart rate and (potentially) cardiac output, elicited by GR138950, does not seem to oppose the acute antihypertensive effect of this agent. However, one shortcoming of these experiments is that we did not measure cardiac output *per se*. Therefore, we can not exclude the possibility that changes in cardiac output mediated through other mechanisms, such as changes in venous return or cardiac contractility, could have influenced the overall effect of GR138950 on blood pressure. If this is true, however, it must be independent of autonomic

control of cardiac function. It should also be noted that these studies do not preclude a reflex increase in sympathetic vasoconstrictor tone to oppose the initial blood pressure fall caused by GR138950. This reflex vasoconstrictor action could account for the cardiovascular profile of GR138950 if, after initial activation, it declined progressively over the next several hours. Finally, it might seem surprising that atenolol, itself, did not reduce blood pressure in RALH rats, given its widespread use as an antihypertensive agent. However, it is well established that β-adrenoceptor blocking agents do not lower blood pressure in many animal models of hypertension, including renal hypertensive rats, particularly after single dose administration (Buckingham & Hamilton, 1979).

The existence of a central RAS has been established and angiotensin-containing pathways associated with brain regions involved in cardiovascular control have been described (Unger *et al*., 1988). Stimulation of brain angiotensin receptors causes, amongst others, cardiovascular effects. For example, administration of angiotensin II into the lateral cerebral ventricle has been shown to cause an increase in blood pressure, and vasopressin release has been shown to contribute to this pressor response (Steckelings *et al*., 1992). Furthermore, systemically administered angiotensin II has been demonstrated to release vasopressin *via* an action at the subfornical organ (a circumventricular organ bordering the third ventricle) which projects to the vasopressin producing neurones of the supraoptic nucleus and paraventricular nucleus of the anterior hypothalamus (Iovino & Steardo, 1984; Unger *et al*., 1988). It is possible that the elevated circulating levels of angiotensin II, present in RALH rats, stimulated the release of vasopressin which contributed to the hypertension in these animals. Angiotensin AT_1 receptor antagonists have been shown to inhibit release of vasopressin elicited by both central injection of angiotensin II (Hogarty *et al*., 1992). Therefore it is possible that GR138950 might, in part, lower blood pressure in RALH rats by inhibiting the release of vasopressin as a result of blockade of centrally located AT_1 receptors. The possibility was investigated using the selective V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)AVP (Kruszynski *et al*., 1980; Manning & Sawyer, 1986). The idea behind these experiments was that prior removal of any contribution of angiotensin II-driven vasopressin release to the hypertension in these rats would not only affect resting blood pressure, but also modify the profile (both shape and magnitude) of the fall in pressure caused by subsequent administration of GR138950. $d(CH_2)_5$Tyr(Me)AVP, at a dose which abolished the pressor response to injected vasopressin (present study; Buñag & Miyajima, 1984), did not affect resting blood pressure or the cardiovascular response to GR138950 in conscious RALH rats. Therefore, vasopressin release does not seem to contribute to the hypertension seen in this model and, hence, there is no evidence to suggest that GR138950 inhibits angiotensin II-stimulated vasopressin release in RALH rats.

In a different context, it is possible that the acute fall in blood pressure, caused by GR138950 could, itself, be the stimulus for the release of vasopressin (Bennett & Gardiner, 1985) in an attempt to maintain homeostasis. However, the observation that the antihypertensive profile of GR138950 in the presence of $d(CH_2)_5$Tyr(Me)AVP was almost superimposible on that in its absence (data not shown) suggests that this is unlikely. Thus, not only does vasopressin not seem to contribute to maintenance of hypertension, or to the antihypertensive effect of GR138950, in RALH rats, but it does not seem to exert any compensatory influence following a marked decrease in arterial pressure resulting from AT_1 receptor blockade either.

As well as stimulating vasopressin release, central administration of angiotensin II increases central sympathetic flow. Furthermore, Fink *et al*. (1980) and Fink (1997) have suggested that there is a central component to the production of hypertension in high renin rat models and have demonstrated the involvement of the area postrema in hypertension induced following chronic intravenous administration of a subpressor dose of angiotensin II (Fink *et al*., 1987) or unilateral renal artery constriction (2K1C rats; Fink *et al*., 1986). Furthermore, tonic stimulation of central AT_1 receptors involved in cardiovascular regulation has been demonstrated. Yang *et al*. (1992) have shown that microinjection of losartan into the anterior hypothalamus causes a reduction in blood pressure as well as inhibiting the pressor response produced by microinjection of angiotensin II to this site. Moreover, there is evidence that angiotensin II tonically excites rostral ventrolateral medulla (RVLM) neurones, and AT_1 receptor antagonists injected into the RVLM cause significant falls in blood pressure and sympathetic nerve activity (Sakai & Dampney, 1990; Averill *et al*., 1994). Thus, it is possible that angiotensin AT_1 receptor antagonists lower blood pressure in RALH rats, at least in part, by preventing the neurogenic vasoconstriction mediated through the action of angiotensin II at centrally located AT_1 receptors. In this respect, EXP3174 administered intravenously blocked the pressor response mediated by microinjection of angiotensin II into the area postrema (Gorbea-Oppliger & Fink, 1995). However, in the present study, systemic administration of GR138950 caused sustained increases in renal and splanchnic sympathetic nerve activity as well as tachycardia, in anaesthetized RALH rats. As baroreceptor function was maintained in these anaesthetized RALH rats, it is likely that the generalized sympathoexcitation and tachycardia resulted from activation of the baroreceptor reflex arc in response to the antihypertensive effect of GR138950. Thus, systemic administration of GR138950 does not appear to cause a centrally mediated suppression of sympathetic vasomotor tone. In fact, an increase in sympathetic nerve-mediated vasoconstriction in renal and mesenteric vascular beds is likely and it is possible that this action may oppose the initial blood pressure fall produced by GR138950. The AT_1 receptor antagonists, losartan and CV-11974 have recently been reported to cause reflex increases in sympathetic nerve activity in conscious renal hypertensive rats (2K1C; Niederberger *et al*., 1995) or spontaneously hypertensive rats (Takishita *et al*., 1994), respectively. These studies support our findings with GR138950.

Despite the evidence presented above, our studies in anaesthetized rats, and the deductions drawn from them, are open to criticism in at least two aspects; the rats were anaesthetized and the time course of the experiments was shorter than those in conscious RALH rats. It is clear that anaesthesia, alone, reduced blood pressure in RALH rats. This could simply reflect a generalized suppression of central sympathetic outflow, but it might specifically reflect inhibition of that component of sympathetic drive that is believed to be driven by elevated circulating levels of angiotensin II. Chloralose was chosen as the anaesthetic for these experiments because it is generally believed to have much less effect than many other anaesthetic agents on the integrity of baro-receptor mechanisms. Although our data do not allow us to establish whether chloralose impaired any central component of the hypertension mediated by elevated circulating levels of angiotensin II in RALH rats, it might be pertinent that Ito & Sved (1996) reported that bilateral injection of (non-peptide) angiotensin receptor antagonists into the rostral ventrolateral medulla, a major cardiovascular control region, markedly

reduced resting blood pressure in chloralose anaesthetized rats. Co-administration of angiotensin II with one of these antagonists (Sar^1Thr^8 angiotensin II) nullified the hypotensive effect of the antagonist but, in separate experiments, had no effect on the hypotensive effect of muscimol injected into the same region of the medulla. The authors concluded that tonic stimulation of angiotensin receptors in the rostral ventrolateral medulla was largely responsible for vasomotor drive emanating from this region. Although it might not be appropriate to compare, directly, these findings with our own, they nevertheless illustrate preservation of at least some angiotensin-driven central sympathetic outflow in rats under chloralose anaesthesia.

The time course over which recording of sympathetic nerve activity in anaesthetized rats was shorter than that over which the full magnitude of the antihypertensive response to GR138950 developed. Nevertheless it encompassed the time ($\sim$60 min after administration) at which the initial, rapidly developing fall in blood pressure gave way to the onset of the secondary, progressive and sustained fall in presssure. Over the next 2 h, during which the secondary component of the antihypertensive effect was developing in conscious RALH rats, there was no indication of a significant reduction in the already elevated level of efferent sympathetic nerve activity; even 3 h after administration of GR138950, renal and splanchnic nerve activity were 100 and 200% higher than prior to drug administration. Xu & Brooks (1996) reported that losartan reduced blood pressure and increased central sympathetic outflow in conscious rats fed on a low salt diet (to activate their RAS); restoration of their blood pressure, to pre-losartan levels, by infusion of a vasopressor agent was followed by a reduction in efferent sympathetic nerve activity to below pre-losartan levels. These findings led the authors to suggest that, although overall efferent sympathetic nerve activity was increased during the losartan-induced hypotension, there was an underlying component, driven by angiotensin II that was blocked by losartan. This component could be revealed only when the baroreceptor mediated global increase in activity was eliminated. It is of interest to note that the sympathoexcitation caused by the AT_1 receptor antagonists was less than that generated by a comparable fall in blood pressure elicited by the calcium channel blocker, nicardipine (Takashita *et al*., 1994) or the nitric oxide donor, sodium nitroprusside (Niederberger *et al*., 1995). Taking these data collectively, we can not exclude the possibilities that we failed to identify an angiotensin II-driven increase in central sympathetic outflow, either because of the use of anaesthesia in these experiments or, alternatively, because this component was present, but masked by a much greater baroreceptor-mediated increase in sympathetic activity. What we can say, however, is that the progressive fall in blood pressure that starts approximately 1 h after administration of GR138950 does not seem to be attributable to a widespread suppression of sympathetic nerve activity, at least over the next 2 h.

In conclusion, GR138950 caused a long-lasting, biphasic reduction in blood pressure which was associated with a tachycardia. The tachycardia resulted from an increase cardiac sympathetic drive and withdrawal of cardiac vagal drive. Blockade of these cardiac changes did not influence the antihypertensive profile of GR138950. Furthermore, the activity of GR138950 can not be ascribed to blockade of centrally mediated, angiotensin II-dependent increases in vasopressin release or in efferent sympathetic nerve activity. On the contrary, GR138950 increased overall central sympathetic outflow. This might have been expected to compensate for the antihypertensive effect of AT_1 receptor blockade and oppose the fall in blood pressure seen in the first hour or so after administration of GR138950. Accordingly, it might seem surprising that blood pressure fell so precipitously during this time. It must be remembered, however, that interference with the function of the RAS by ACE inhibition (Hatton & Clough, 1982), antibodies to angiotensin II (Wong *et al*., 1989b) or AT_1 receptor blockade (Moreau *et al*., 1993) markedly impairs the vasoconstrictor response to stimulation of the peripheral sympathetic nervous system. Thus, the RAS seems to play a permissive role in the vasoconstrictor function of the sympathetic nervous system, and withdrawal of this support might render this compensatory mechanism much less effective than it would otherwise be.

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