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Further investigations into the mechanism of the antihypertensive activity of the angiotensin AT_1 receptor antagonist, GR138950

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1 The angiotensin AT_1 receptor antagonist, GR138950, produces a long-lasting antihypertensive effect in conscious renal artery ligated hypertensive (RALH) rats but this effect does not correlate temporally with its antagonist profile against angiotensin II (AII). In the present experiments we have compared the inhibitory profiles of GR138950 and enalapril, against angiotensin I (AI), with their respective antihypertensive activities.

2 GR138950 (1 mg kg^{-1}, i.a.) and enalapril (3 mg kg^{-1}, i.a.) reduced blood pressure in RALH rats to a similar degree. Maximum reductions in blood pressure occurred approximately 5-24 h and 3-5 h after administration, respectively. The antihypertensive effect of GR138950 lasted for 24-48 h. However, the effect of enalapril lasted for only 5-24 h.

3 In conscious normotensive rats, inhibition of AI-induced pressor responses was maximal 1 h after systemic administration of GR138950 and enalapril. Dose-response curves to AI were displaced to the right, in a parallel manner, 1406 and 102 fold by GR138950 (1 mg kg^{-1}, i.a.) and enalapril (3 mg kg^{-1} i.a.), respectively. The inhibitory effect of enalapril lasted for <24 h whereas that of GR138950 lasted for up to 48 h.

4 Contractile responses to AI were extensively inhibited in aortae removed from either RALH rats or normotensive rats, 1 and 5 h after administration of GR138950 (1 mg kg^{-1}, i.a.). Responses were still significantly reduced 24 h after administration but had returned to control levels after 48 h. Enalapril pretreatment (3 mg kg^{-1}, i.a.) did not inhibit contractile responses to AI in aortae isolated from normotensive rats at any time point.

5 These experiments confirm that GR138950 is an effective and long-lasting antihypertensive agent. GR138950 was a more potent and longer lasting antagonist against AI than has previously been found against AII, and the duration of its antihypertensive activity coincides better with its blockade of responses to AI. Blockade of the effects of AII generated locally within the vascular wall might play an important role in the antihypertensive profile of GR138950.

Keywords: Conscious rats; blood pressure; hypertensive rats; angiotensin AT_1 receptors; angiotensin AT_1 receptor antagonist; angiotensin I; angiotensin II; rat isolated aorta; GR138950; enalapril

Introduction

GR138950 (Figure 1) is a potent, selective, and specific non-peptide antagonist at angiotensin AT_1 receptors in the rabbit isolated aorta, and is also a potent, long-lasting, orally active antihypertensive agent in rats in which blood pressure has been elevated as a result of activation of the renin-angiotensin system (Hilditch *et al.*, 1995). GR138950 and other similar compounds, such as losartan (Akers *et al.*, 1991) and GR117289 (Hilditch *et al.*, 1994), reduce blood pressure in renal artery-ligated hypertensive (RALH) rats but the time course of this effect does not coincide with that of their antagonist profile, against angiotensin II (AII)-induced pressor responses, in either normotensive rats or RALH rats. We have suggested that the antihypertensive activities of these compounds may reflect blockade of AT_1 receptors at sites additional to those in the vasculature (Hilditch *et al.*, 1994; 1995). These could include sites within the central nervous system. Alternatively, it might be that the time course of inhibition of pressor responses to exogenously administered AII does not fairly reflect blockade of vascular AT_1 receptors. It is possible that the transient vasoconstriction elicited by small bolus doses of systemically administered exogenous AII is mediated by AT_1 receptors located on the vascular smooth muscle cells closest to the vascular lumen, whereas the vasoconstriction mediated by sustained levels of endogenous AII, either circulating or generated locally, is mediated in a different compartment, perhaps deeper within the vessel wall.

Evidence has accumulated that major target organs for the physiological effects of AII have independent, local, endogenous renin-angiotensin systems. The essential components of the system, angiotensinogen, renin, angiotensin converting enzyme (ACE) and AII, have all been reported to exist in a variety of tissues including the brain, vascular smooth muscle and the heart. In particular, the kidney, adrenal gland, brain and heart, which make major contributions to cardiorenal homeostasis, all contain renin and angiotensinogen mRNAs (Dzau, 1987). The physiological implication of the local renin-angiotensin systems is that angiotensin-mediated functions of these target organs may be locally regulated, independent of circulating components. In addition, the vascular endothelium has been shown to be a major site for conversion of angiotensin I (AI) to AII by ACE located on its lumenal surface (Caldwell *et al.*, 1976). Furthermore, Kifor & Dzau (1987) have shown that bovine cultured aortic endothelial cells contain renin, angiotensinogen and angiotensins I, II and III, and angiotensins II and III are secreted. If this is a general feature of endothelial cells and occurs *in vivo* the vascular endothelium may be an important contributor to the activity of the local vascular renin-angiotensin system.

In this study, we have attempted to establish the extent to which blockade of responses to AII, generated locally in the

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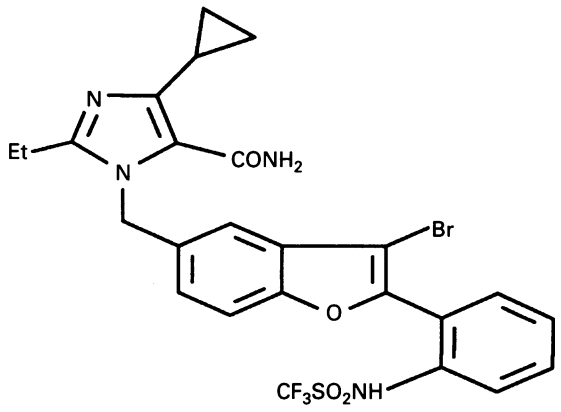

Figure 1 Chemical structure of GR138950.

vasculature, might contribute to the cardiovascular profile exhibited by GR138950. Preliminary findings of this study were reported to the British Pharmacological Society (Prior *et al.*, 1995).

Methods

Techniques used to measure blood pressure, to induce renal hypertension and to determine the antagonist effects of angiotensin receptor antagonists in conscious rats have been described in detail previously (Hilditch *et al.*, 1994), and will be given only briefly here.

Studies using conscious normotensive or hypertensive rats

Angiotensin-dependent hypertension was induced in male albino rats (Glaxo, AH/A, 260–370 g). Under isofluorane anaesthesia, the left renal artery was ligated close to its junction with the aorta and the rat left to recover for 5 days (Cangiano *et al.*, 1979). Blood pressure increased in 80–90% of rats over this period. Rats were considered to be hypertensive if their diastolic blood pressure (DBP) was greater than 135 mmHg. On the day before experimentation, a polythene catheter was inserted retrogradely into the left common carotid artery and was subsequently used for blood pressure recording and drug administration. The rats were allowed to recover from surgery for use on the following day. Arterial blood pressure was recorded from conscious, unrestrained normotensive or RALH rats. Control measurements of DBP were obtained 1 h before, and immediately prior to dosing with vehicle (0.1 ml 100 g^{-1}, distilled water), GR138950 (1 mg kg^{-1}) or enalapril (0.3–3 mg kg^{-1}), and 1, 3, 5, 24 and 48 h after dosing.

AI dose-ratio studies

Normotensive rat studies were carried out on male, albino rats (Glaxo, AH/A, 240–360 g). AI (0.1–30 ng kg^{-1}, i.a.) was administered to normotensive rats to produce dose-related increases in DBP. A period of approximately 3 min was left between successive doses to allow blood pressure to return to baseline. AI dose-response curves were constructed 1 h before, and immediately prior to administration of vehicle (distilled water, 0.1 ml 100 g^{-1}, i.a.), GR138950 (1 mg kg^{-1}, i.a.) or enalapril (3 mg kg^{-1}, i.a.). Further dose-response curves were subsequently constructed 1, 5, 24 and 48 h after vehicle or drug administration.

The inhibitory activity of GR138950 or enalapril, at each time point in each rat, was expressed in terms of the AI dose-ratio; that is, the ratio of equi-pressor doses of AI in the presence and absence of the antagonist/inhibitor calculated from linear portions of the dose-response curves at a response level between 20–35 mmHg. In all studies, geometric mean dose-ratios were calculated from the values obtained from individual rats.

Ex vivo *studies*

Initial studies were performed on aortic ring preparations prepared from cannulated RALH rats. Diastolic blood pressure was recorded from conscious rats prior to administration of vehicle (0.1 ml 100 g^{-1}, i.a.) or GR138950 (1 mg kg^{-1}, i.a.). One further recording was made 1, 5, 24 or 48 h after administration of vehicle or GR138950. Rats were then killed by concussion followed by cervical dislocation and their aortae removed and prepared as described below. Due to spontaneous activity encountered in these tissues, subsequent studies were performed on aortic ring preparations from normotensive rats in which vehicle or GR138950 was administered as described above.

Preparation of tissues

The thoracic aorta was removed, cleaned of adhering tissue and blood, and cut into 8 ring preparations, approximately 4–5 mm in width. The preparations were suspended in 10 ml organ baths containing Krebs-Henseleit solution (composition, mM: Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.5, HCO_3^- 25.0, $H_2PO_4^-$ 1.2, SO_4^{--} 0.6, D-glucose 11.1) maintained at 37°C and continuously bubbled with 95% O_2: 5% CO_2 to maintain a pH 7.4. Indomethacin (30 μM) was added to the Krebs-Henseleit solution to inhibit prostaglandin synthesis in the endothelium and vascular smooth muscle.

Preparations were mounted in the tissue baths by suspending them between two L-shaped stainless steel hooks. The lower hooks were attached to stationary supports at the bottom of the bath chambers. The upper hooks were attached to force-displacement transducers, connected to a chart recorder (Lectromed). Care was taken, during the preparation and mounting of the preparations, to preserve the integrity of the endothelium.

Experimental protocol

Preparations were washed and left to equilibrate for 60 min, under an initial resting tension of 1 g. A concentration-effect curve to AI was constructed. Responses to AI were obtained by adding a single concentration (0.1 nM–100 μM) to each bath to avoid the problems associated with tachyphylaxis (Eglème *et al.*, 1990). Each preparation from the same aorta was exposed to a different concentration of AI. Responses to AI were expressed as mg tension developed.

Subsequent to the addition of AI, the tissues were washed and left to re-equilibrate over the following 60 min, during which they were washed again at 10, 30 and 50 min. In one preparation from each aorta chosen at random, a contraction was obtained to the thromboxane A_2-mimetic, U-46619 (10 nM). The presence of a functional endothelium was tested for by the relaxation response to carbachol (1 μM; Furchgott & Zawadski, 1980). Relaxation of this one representative preparation was taken as an indication of an intact endothelium for the whole aorta.

Antagonist studies in vitro

These studies were performed on aortae from untreated normotensive rats. The preparations were set up as described previously. Seven preparations of the same aorta were incubated with vehicle or GR138950 (0.1–10 nM) for 2 h before obtaining an AI concentration-effect curve as described previously. A single concentration of AI was administered to each preparation; subsequently, the presence of an intact endothelium was investigated in 1 preparation as described above.

Statistical analysis

One way analysis of variance, incorporating Dunnett's test was used to determine the significance of difference between means. Results are expressed as arithmetic means ± s.e.mean, or geometric means and 95% confidence limits, for (n) experiments. A probability value (P) of ≤ 0.05 was considered to indicate a significant difference between groups.

Drugs used

The amorphous, water soluble potassium salt of GR138950 was synthesized in the Chemistry Division, Glaxo Research and Development Ltd. Indomethacin (Sigma Chemicals) was dissolved in 8% $NaHCO_3$ (1 ml) before addition to Krebs-Henseleit solution (5 l). U-46619 (Peninsula Chemicals), human AI (Sigma Chemicals or Peninsula Chemicals), human AII, carbachol and enalapril maleate (all Sigma Chemicals) were dissolved in distilled water. Doses of GR138950 and enalapril were corrected for the presence of the potassium salt or maleate salt, respectively.

Results

Effects of GR138950 and enalapril in conscious RALH rats

Administration of vehicle (0.1 ml 100 g^{-1}, i.a.) had no significant effect ($P > 0.05$) on DBP in RALH rats over the 48 h study period whereas administration of GR138950 (1 mg kg^{-1}, i.a.) reduced DBP (Figure 2a). Diastolic blood pressure was significantly reduced ($P < 0.01$) 1, 3 and 5 h after dosing; the maximum reduction in DBP (62 ± 6 mmHg) occurred approximately 5 h after administration of GR138950. Twenty-four hours after administration of GR138950, DBP was still reduced ($P < 0.01$) but had returned to pre-dosing levels, 48 h after administration ($P > 0.05$).

Enalapril (0.3–3 mg kg^{-1}, i.a.) produced dose-related decreases in DBP (Figure 2b). The maximum reduction in blood pressure occurred between 3–5 h after dosing. The highest dose of enalapril (3 mg kg^{-1}) reduced DBP by 61 ± 10 mmHg, which was similar to the maximum effect achieved with GR138950 (1 mg kg^{-1}). Diastolic blood pressure was significantly reduced ($P < 0.01$) 1, 3 and 5 h after dosing. Twenty-four and 48 h after enalapril administration, DBP was not significantly different from control ($P > 0.05$). Enalapril (3 mg kg^{-1}) was chosen for subsequent comparison with GR138950 (1 mg kg^{-1}).

Effect of GR138950 and enalapril on AI pressor responses

AI (0.1–30 ng kg^{-1}, i.a.) produced dose-related increases in DBP (0–35 mmHg). Dose-response curves to AI were reproducible; dose-ratios varied by <2 fold at 1, 5, 24 or 48 h after vehicle administration (0.1 ml 100 g^{-1}, i.a.).

Administration of GR138950 (1 mg kg^{-1}, i.a.) inhibited the pressor responses to AI and produced rightward, parallel displacement of AI dose-response curves; although a true maximum could not, of necessity, be achieved in conscious rats, there was no evidence for suppression of the AI dose-response curve (Figure 3). The maximum inhibition occurred 1 h after administration. Twenty-four hours after the administration, the inhibitory effect of GR138950 was still evident but by 48 h after administration, pressor responses to AI had almost returned to pre-GR138950 levels. Enalapril (3 mg kg^{-1}, i.a.), like GR138950, also inhibited the pressor effect of AI. However, both the magnitude and the duration of the inhibitory effect were significantly less than those produced by GR138950. Results are shown in Table 1.

Inhibitory effect of GR138950 against AI in RALH isolated aorta

Administration of GR138950 (1 mg kg^{-1}, i.a.), but not vehicle (0.1 ml 100 g^{-1}, i.a.), to RALH rats reduced DBP measured 1, 5 and 24 h, but not 48 h after dosing (Table 2). The reductions in DBP at the individual time points were broadly similar to those observed, in preliminary experiments, when DBP was measured serially in each rat at intervals of 1, 3, 5, 24 and 48 h after vehicle or drug administration.

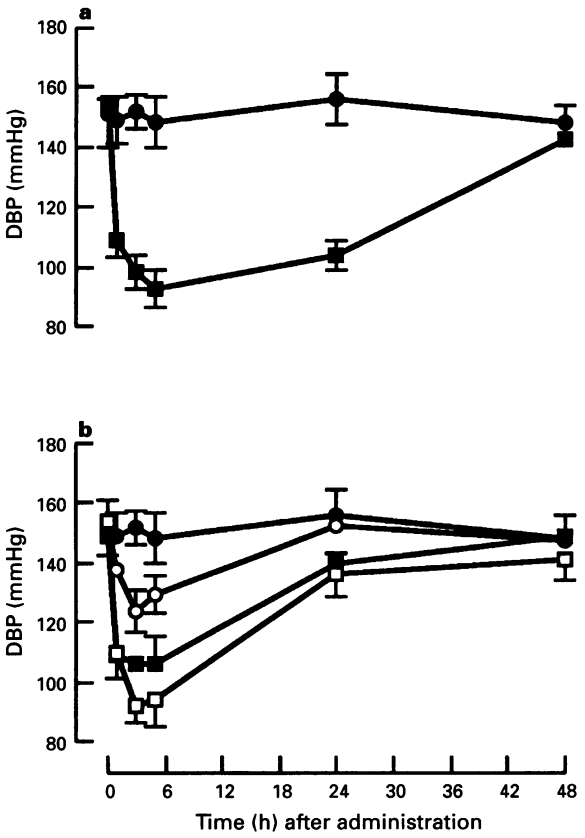

Figure 2 The effect of (a) vehicle (●, 0.1 ml 100 g^{-1}, i.a.) or GR138950 (■, 1 mg kg^{-1}, i.a.) or (b) vehicle (●, 0.1 ml 100 g^{-1}, i.a.) or enalapril (○, 0.3; ■, 1; □, 3 mg kg^{-1}, i.a.) on DBP in the conscious RALH rat. Results shown are group arithmetic means ± s.e.mean (n = 4–7). Some s.e.means omitted for clarity.

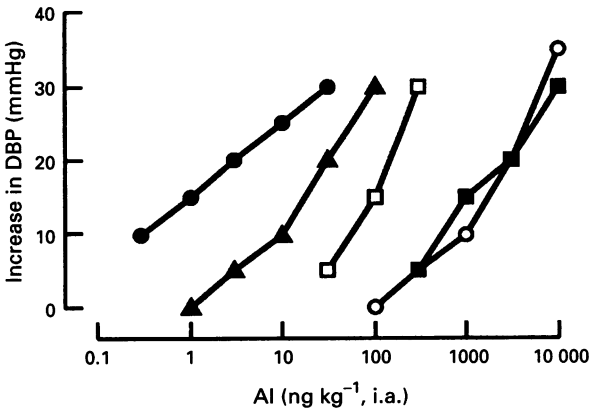

Figure 3 AI pressor responses before (●) and 1 (○), 5 (■), 24 (□) and 48 h (▲) after administration of GR138950 (1 mg kg^{-1}, i.a.) to the conscious normotensive rat. Results of a typical experiment are shown.

Table 1 Angiotensin I dose-ratios after administration of vehicle, GR138950 or enalapril to conscious normotensive rats

	Hours after administration							
	1		*5*		*24*		*48*	
Vehicle	0.9	(0.6-1.3)	1.5	(0.9-2.6)	1.4	(0.3-7.5)	1.1	(0.3-4.4)
GR138950	1405.7	(457.2-4321.9)	1182.8	(403.4-3468.5)	98.5	(58.9-164.6)	3.9	(1.9-8.1)
Enalapril	102.0	(21.8-476.6)	15.8	(8.6-29.2)	0.8	(0.6-1.2)		

AI dose-ratios were obtained at various time intervals following intra-arterial administration of vehicle (0.1 ml 100 g^{-1}), GR138950 (1 mg kg^{-1}) or enalapril (3 mg kg^{-1}) to the conscious normotensive rat ($n=5-7$). Values shown are geometric means (95% confidence intervals).

Table 2 Effect of GR138950 on diastolic blood pressure (DBP) in conscious RALH rats

	Hours after administration							
	1		*5*		*24*		*48*	
	Basal	Δ	*Basal*	Δ	*Basal*	Δ	*Basal*	Δ
Vehicle	138±1	+1±6	144±5	+4±5	148±4	+8±6	160±3	−1±3
GR138950	149±4	−32±5	153±7	−43±7	154±2	−60±8	163±6	+1±7

Values shown are basal DBP (mmHg) and changes in this parameter at various time intervals following intra-arterial administration of vehicle (0.1 ml 100 g^{-1}) or GR138950 (1 mg kg^{-1}) to conscious RALH rats. After measurement of DBP, rats were killed and their thoracic aortae removed for *ex vivo* studies. Values shown are arithmetic means±s.e.mean ($n=4-6$).

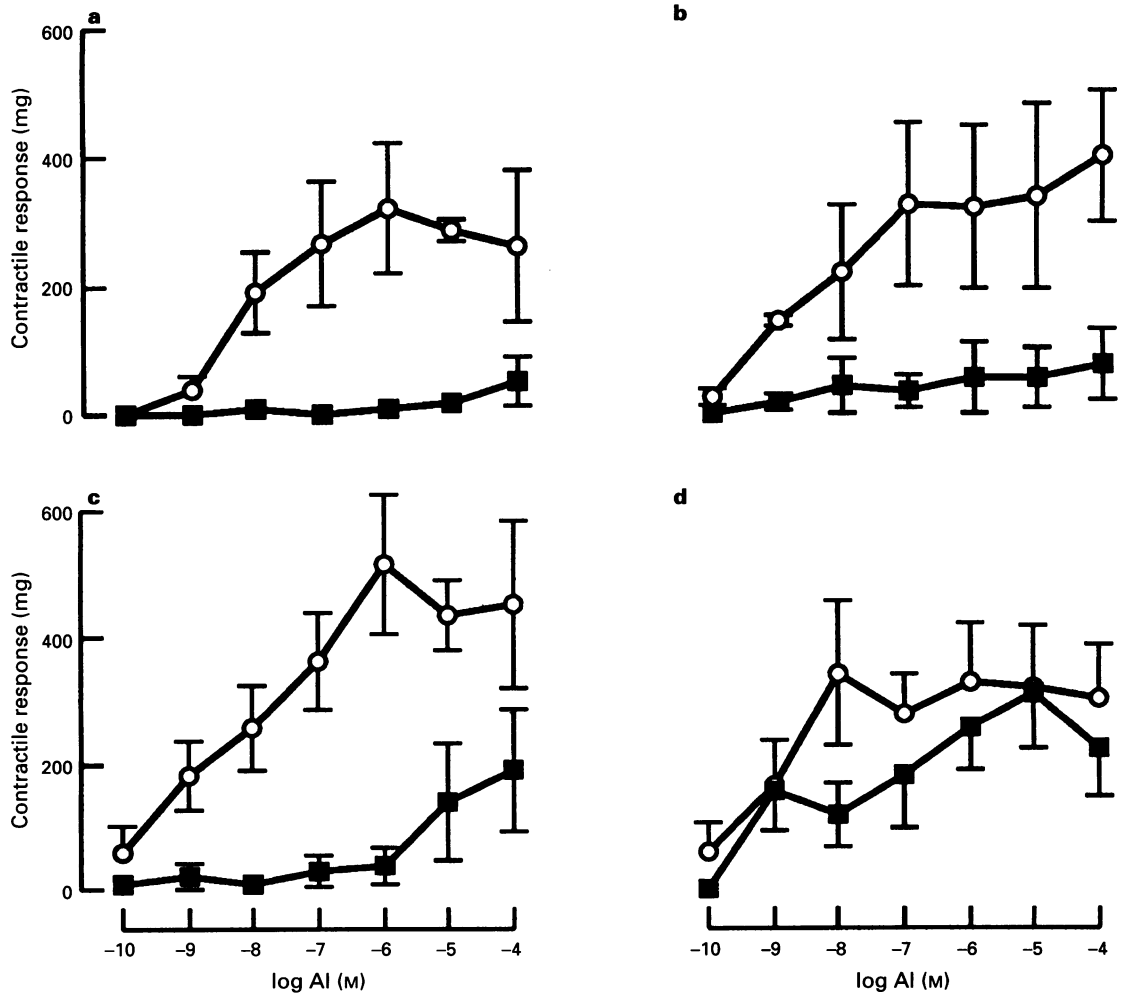

Figure 4 Contractile effects of AI in aortae isolated from RALH rats (a) 1 h, (b) 5 h, (c) 24 h and (d) 48 h after administration of vehicle (○; 0.1 ml 100 g^{-1}, i.a.) or GR138950 (■; 1 mg kg^{-1}, i.a.). Results shown for tension developed (mg) are group arithmetic means±s.e.mean ($n=4-6$). Some s.e.means are omitted for clarity.

In aortae taken from rats 1, 5 24 or 48 h after administration of vehicle, AI (0.1 nM – 100 μM) produced concentration-related increases in resting tone. Maximal responses to AI were produced, usually, between 1 – 10 μM. Developed tension to AI (10 μM) in aortic rings prepared from rats receiving vehicle 1, 5, 24 and 48 h previously was 287 ± 17, 338 ± 145, 437 ± 53 and 323 ± 97 mg, respectively.

Contractile responses to AI (0.1 nM – 100 μM) were extensively inhibited in aortic rings prepared from rats treated with GR138950 (1 mg kg^{-1}) 1 or 5h previously. In preparations obtained from rats treated 24 h previously with GR138950, contractile responses to AI (0.1 nM – 1 μM) were still markedly inhibited but higher concentrations of AI (10 – 100 μM) produced small contractile responses. Contractile responses to AI were not inhibited in aortic rings prepared from rats treated 48 h previously with GR138950. Results are shown in Figure 4.

Inhibitory effects of GR138950 or enalapril against AI in normotensive rat isolated aorta

Administration of vehicle (0.1 ml 100 g^{-1}, i.a.), GR138950 (1 mg kg^{-1}, i.a.) or enalapril (3 mg kg^{-1}, i.a.) had little effect on DBP in normotensive rats (Table 3). In aorte taken from rats 1, 5, 24 or 48 h after administration of vehicle, AI (0.1 nM – 100 μM) produced concentration-related increases in resting tone. Maximal responses to AI occurred between 1 – 10 μM. Developed tension to AI (10 μM) in the aortae from

Table 3 Effect of GR138950 and enalapril on diastolic blood pressure (DBP) in conscious normotensive rats

	Hours after administration							
	1		*5*		*24*		*48*	
	Basal	Δ	*Basal*	Δ	*Basal*	Δ	*Basal*	Δ
Vehicle	118 ± 8	+0 ± 5	107 ± 6	+17 ± 2	124 ± 6	−18 ± 7	122 ± 3	−14 ± 5
GR138950	123 ± 4	−5 ± 6	121 ± 5	−9 ± 4	122 ± 6	−11 ± 5	123 ± 4	−18 ± 5
Enalapril	114 ± 5	−1 ± 2	123 ± 4	−2 ± 3	119 ± 5	+8 ± 1	125 ± 5	−21 ± 5

Values shown are basal DBP (mmHg) and changes in this parameter at various time intervals following intra-arterial administration of vehicle (0.1 ml 100 g^{-1}), GR138950 (1 mg kg^{-1}) or enalapril (3 mg kg^{-1}) to conscious normotensive rats. After measurement of DBP, rats were killed and their thoracic aortae removed for *ex vivo* studies. Values shown are arithmetic means ± s.e.mean (n = 4 – 8).

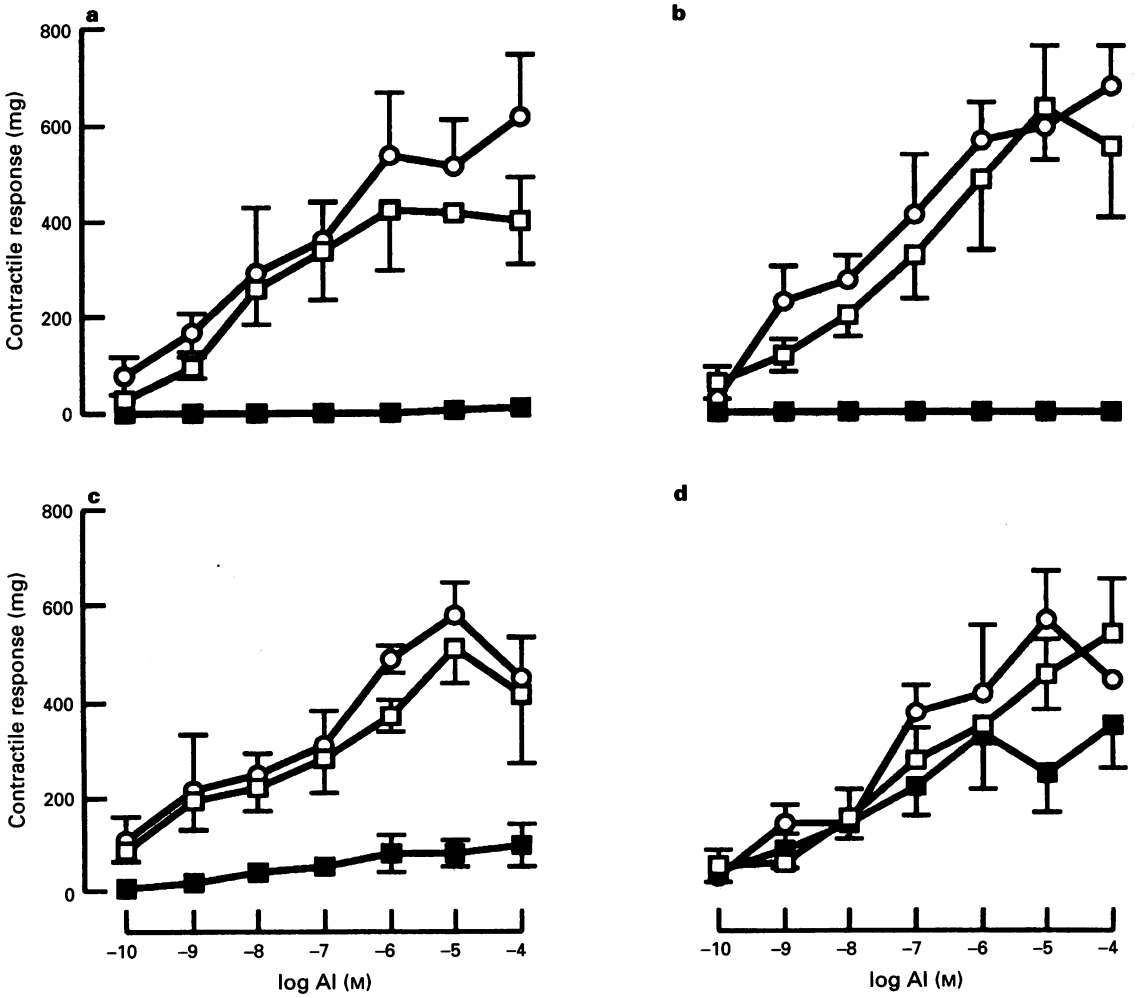

Figure 5 Contractile effects of AI in aortae isolated removed from normotensive rats (a) 1 h, (b) 5 h, (c) 24 h and (d) 48 h after administration of vehicle (○; 0.1 ml 100 g^{-1}, i.a.), GR138950 (■; 1 mg kg^{-1}, i.a.) or enalapril (□; 3 mg kg^{-1}, i.a.). Results shown for tension developed (mg) are group arithmetic means ± s.e.mean (n = 4 – 7). Some s.e.means are omitted for clarity.

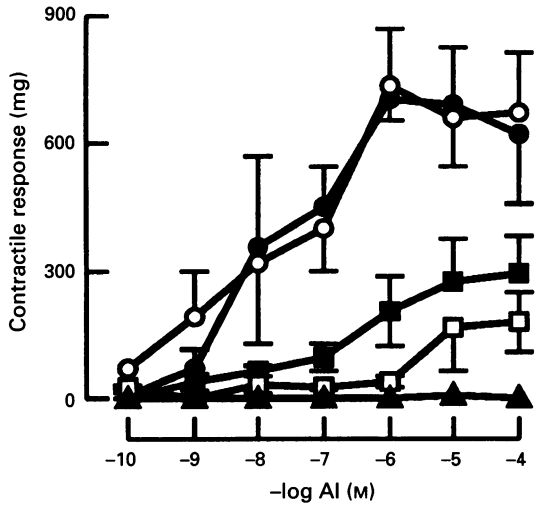

Figure 6 The effect of incubation (2 h) with vehicle (●) or GR138950 0.1 (○), 0.3 (■), 1 (□) and 10 (▲) nM on AI contractile responses in isolated aortae from untreated normotensive rats. Results shown for tension developed (mg) are group arithmetic means ± s.e.mean ($n=4$). Some s.e.means omitted for clarity.

rats treated with vehicle 1, 5, 24 and 48 h previously was 514±98, 520±61, 577±70 and 567±100 mg, respectively. Contractile responses to AI (0.1 nM–100 μM) were abolished or markedly inhibited in aortae from rats treated 1, 5 or 24 h previously with GR138950 (1 mg kg^{-1}). In aortae removed from rats treated 48 h previously with GR138950, contractile responses to AI were little reduced compared to their corresponding vehicle-treated controls. In aortae taken from enalapril-treated rats, contractile responses to AI (0.1 nM–100 μM) were generally unaffected at any time point. Results are shown in Figure 5.

Antagonist profile of GR138950 in normotensive rat aorta

AI (0.1 nM–1 μM) produced concentration-related increases in resting tone in aortae isolated from untreated normotensive rats; higher concentrations (10–100 μM) produced no greater effect. GR138950 (0.1 nM) had little effect on AI-evoked contractile responses. GR138950 (0.3 and 1 nM) caused a concentration-dependent inhibition of the contractile effect of AI and the maximum effect achieved with AI was progressively reduced. GR138950 (10 nM) abolished contractile responses to AI. Results are shown in Figure 6.

Discussion

GR117289 and GR138950 reduce blood pressure in rats in which it is elevated due to activation of the renin-angiotensin system (Hilditch *et al.*, 1993; 1994; 1995). However, their antagonist profiles against pressor responses produced by AII in conscious hypertensive and normotensive rats do not temporally correlate with their antihypertensive activities. Previously, in order to explain this disparity, we have suggested that compounds like GR117289 and GR138950 might interact with receptors within the central nervous system, or deep within the vascular wall where they might inhibit the contribution made by the local renin-angiotensin system to the regulation of vascular tone (Hilditch *et al.*, 1994; 1995). In the present experiments, we have attempted to establish the extent to which blockade of AII, generated locally in the vasculature, plays a role in this disparity. To this end, we have investigated the time course over which GR138950 reduces pressor responses produced by systemically administered AI in conscious normotensive rats; we have assumed that this reflects antagonism largely, if not exclusively, of AII generated by ACE and/or other enzymes mainly in the vasculature. Previous studies (Campbell *et al.*, 1990; Campbell, 1993) have shown that AII is the major metabolite of AI and that shorter fragments (e.g. the heptapeptide, angiotensin III) are produced only in small quantities. This, and the fact that the shorter fragments are weak vasoconstrictor agents, makes it unlikely that they contribute much to the pressor response evoked following administration of AI. We have compared the time course over which GR138950 reduced pressor responses evoked by administration of AI with that seen when AII was used (Hilditch *et al.*, 1995). In addition, we have evaluated the ability of GR138950 to inhibit vasoconstrictor responses to AI, *ex vivo*, in aortae removed from hypertensive and normotensive rats at various intervals after dosing with the antagonist. Parallel experiments were performed with the ACE inhibitor, enalapril, to compare the antihypertensive and AI inhibitory profiles of the two compounds.

Antihypertensive and AI inhibitory effects of GR138950

GR138950 (1mg kg^{-1}, i.a.) reduced DBP in RALH rats. The maximum effect occurred approximately 5 h after administration. Diastolic blood pressure was still significantly reduced 24 h after administration but had recovered to pretreatment levels by 48 h after administration. Intra-arterial administration of GR138950 (1 mg kg^{-1}) to conscious, normotensive rats inhibited pressor responses to AI. GR138950 produced rightward, parallel displacements of AI dose-response curves; they were displaced 1406, 1183, 99 and 4 fold at 1, 5, 24 and 48 h after administration, respectively. Consequently, the AI inhibitory effect of GR138950 was maximal 1 h after administration and of long duration, lasting between 24–48 h. In previous studies, administration of the same dose of GR138950 (1 mg kg^{-1}, i.a.) inhibited pressor responses to AII in conscious normotensive rats (Hilditch *et al.*, 1993; 1995). Responses were, again, maximally displaced 1 h after administration, but only by 76 fold. In addition, the antagonist effect of GR138950 against AII was of shorter duration (7–24 h). GR138950, therefore, appears to be a more effective and longer lasting antagonist against AI- than AII-evoked pressor responses in normotensive rats. One possible explanation for this finding is that, as well as blocking AT_1 receptors, GR138950 also inhibits the conversion of AI to AII via ACE. This is most unlikely, however, because GR138950, in concentrations up to 100 nM (~100 times higher than its K_B for AT_1 receptors; Hilditch *et al.*, 1995), has little or no inhibitory activity against human ACE *in vitro* (unpublished observation). In keeping with our results, Ohlstein *et al.* (1992) reported that, 24 h after administration of losartan to renin-dependent hypertensive rats, the pressor response to a single dose of AI was still substantially inhibited, although the response to AII had returned to pre-losartan levels. Like GR138950, losartan does not inhibit ACE (Chiu *et al.*, 1990). Thus, the ability to inhibit differentially responses to AI and AII does not appear to be restricted to GR138950.

The inhibitory effect of GR138950 against AI was also investigated in aortae removed from rats pretreated with GR138950 (1 mg kg^{-1}, i.a.). These studies were performed on several serial ring preparations, each receiving a single concentration of AI in order to avoid tachyphylaxis. This technique has been used successfully by other groups (e.g. Gruetter *et al.*, 1988) and Eglème *et al.* (1990) have reported that responses to AI are dependent upon its conversion to AII via ACE. In our first series of experiments, aortae were removed from vehicle or GR138950-treated RALH rats. However, several of these preparations exhibited spontaneous activity and had to be discarded; those that were retained showed considerable variability in their responsiveness to AI. Consequently, further experiments were performed using aortae from normotensive rats. These preparations displayed little or no spontaneous activity. In aortae removed from vehicle-

treated RALH or normotensive rats, AI produced concentration-related increases in resting tone. Tension was increased to a greater degree by AI (10 μM) in aortae from normotensive rats (514–577 mg) than from RALH rats (287–437 mg); the maximum response was produced, usually, by 1–10 μM AI in aortae from hypertensive and normotensive rats. It is possible that the smaller responses to AI in aortae from RALH rats is a consequence of the elevated levels of endogenous AII to which these animals had been exposed. Whether or not this is the explanation, the overall profile of GR138950 was similar in aortae obtained from normotensive or RALH rats; that is, GR138950 abolished responses to AI 1 and 5 h after administration of the antagonist. Slight recovery, only, was seen at 24 h but responses had almost fully recovered by 48 h. This time course broadly paralleled that of the antihypertensive activity of GR138950.

The AI inhibitory profile of GR138950, administered directly to the Krebs-Henseleit solution bathing rings of aortae obtained from untreated normotensive rats, was almost identical, qualitatively, to that observed in preparations obtained from rats pretreated with GR138950. That is, GR138950 (0.1–10 nM) caused a concentration-dependent suppression, rather than rightward displacement, of the AI concentration-response curve. This is similar to its profile against AII in rabbit isolated aorta (Hilditch *et al.*, 1995), although the extent of suppression of the maximum response to AI was more marked in the rat; the reason for this is not known.

Antihypertensive and AI inhibitory effects of enalapril

Enalapril (0.3–3 mg kg^{-1}, i.a.) produced dose-related decreases in DBP in RALH rats. The maximal reduction in DBP produced by enalapril (3 mg kg^{-1}, i.a.) occurred 3–5 h after administration. However, DBP was little reduced 24 h after administration. Previous studies in our laboratories using the same dose of enalapril, resulted in a greater peak fall in DBP in RALH rats, with maximum effects occurring 5–7 h after administration (Travers & Hilditch, 1992). However, the duration of the antihypertensive effect of enalapril, even in those studies was less than 24 h. Enalapril, therefore, appears to be a less potent and shorter lasting antihypertensive agent than GR138950.

Administration of enalapril (3 mg kg^{-1}, i.a.) to conscious, normotensive rats markedly inhibited pressor responses to AI, thus confirming that responses to AI are dependent upon conversion to AII, via ACE. Enalapril displaced AI dose-response curves by 102 and 16 fold to the right in a parallel manner, 1 and 5 h after administration, respectively. No inhibition of AI responses was evident 24 h after administration. In this respect, as well as in its antihypertensive activity, enalapril was less potent and shorter lasting than GR138950.

In contrast to GR138950, enalapril pretreatment (3 mg kg^{-1}, i.a.) did not inhibit contractile responses to AI in aortae isolated from inhibitor pretreated normotensive rats. Enalapril is a potent ACE inhibitor and might have been expected to prevent conversion of AI to AII, *ex vivo*, particularly at times when its antihypertensive effect was most marked. There are several possible explanations for the fact that it did not: (i) Enalapril does not inhibit ACE in vascular smooth muscle or endothelial cells, but does so in blood; this would account for its effects *in vivo*. (ii) AI can be converted to AII in isolated blood vessels via alternative pathways that do not involve ACE and are not, therefore, susceptible to inhibition by enalapril. Several serine proteases, including tonin, cathepsin G and chymotrypsin can convert AI to AII (Campbell, 1993). However, neither alternative seems likely in view of the fact that Eglème *et al.* (1990) have shown that addition of enalaprilat (the di-acid active metabolite of enalapril) to the bathing medium completely suppressed the responses to AI in rat isolated aortic rings, and that the ACE responsible is located in the vessel wall, rather than in the endothelial cells. (iii) Enalaprilat, formed *in vivo* following administration of enalapril, may not penetrate deep into the vascular smooth muscle, so that the subsequent conversion of AI to AII in blood vessels removed from enalapril-pretreated rats, proceeds unimpaired. (iv) Enalaprilat may penetrate the vascular wall and inhibit ACE *in vivo*, but after removal of the aorta and its subsequent immersion in a large volume of Krebs-Henseleit solution followed by washing, enalaprilat may dissociate from the enzyme and establish a new equilibrium. The residual ACE-inhibitory activity in the vascular smooth muscle might not then be sufficient to prevent local conversion of AI to AII. Whatever the explanation for the failure of enalapril pretreatment of rats to prevent the vasoconstrictor response produced by the administration of AI to subsequently isolated aortic rings, the results obtained with enalapril contrast sharply with those obtained with GR138950. The long-lasting effects of GR138950 in isolated aortic rings probably reflects its high affinity for the vascular AT_1 receptor and/or its lipophilic nature, which might result in its retention within the membrane lipid. A similar phenomenon has been proposed for other AT_1 receptor antagonists (Panek *et al.*, 1995). In any event, it is clear that GR138950 exerts long lasting blockade of AII generated locally, within the vascular wall, from exogenously administered AI.

Relationship between the antihypertensive activity and AI inhibitory effects of GR138950

Figure 7 shows the relationship between the antihypertensive activity of GR138950 and its inhibitory effect against AI-evoked pressor responses. The same relationship between antihypertensive activity and blockade of AII pressor activity, derived from previously published data (Hilditch *et al.*, 1995), is also shown. Clearly, there was a poor correlation between the antihypertensive and AII antagonist activities of GR138950. In particular, blood pressure was still substantially reduced 24 h after administration of GR138950, despite the fact that there was no significant blockade of pressor responses to AII. However, when similar experiments were performed with AI in place of AII, a better relationship was revealed. Despite this, it is noticeable that the fall in blood pressure observed 1 h after administration of GR138950 was less than might have been expected, given the extent of the inhibition of the pressor responses produced following administration of AI

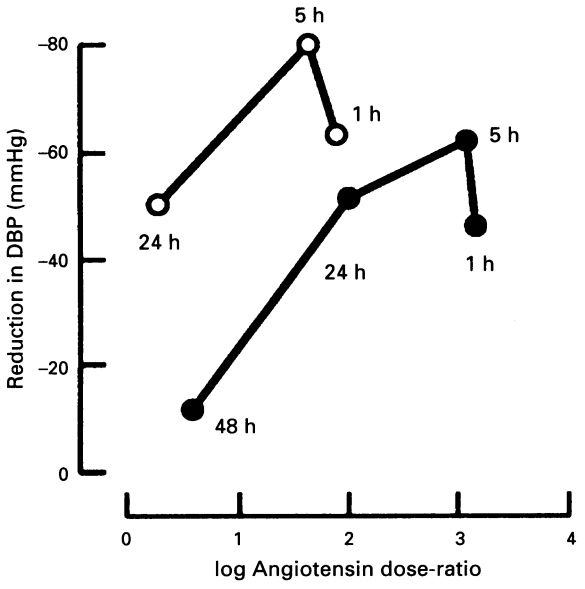

Figure 7 Relationship between the antihypertensive activity of GR138950 and its inhibitory effect against AI-evoked pressor responses (this study; ●) and AII-evoked pressor responses (Hilditch *et al.*, 1995; ○).

at this time. The same was true in experiments in which enalapril was used in place of GR138950; it was also the case when GR138950 (Hilditch *et al.*, 1995), GR117289 or losartan (Drew, 1993) were used to antagonize AII. We have no simple explanation for these findings but we may speculate that, soon after administration of the antagonists to RALH rats, reflex mechanisms are activated that attempt to oppose the reduction in blood pressure. The effectiveness of these mechanisms may decline over the next few hours, allowing the progressive expression of the antihypertensive activity of the antagonists.

Why is GR138950 more effective and longer lasting against pressor responses produced following administration of AI than AII? One hypothesis that may explain these findings is that the pressor responses produced by bolus doses of exogenous AII (previously reported by Hilditch *et al.*, 1995) might be mediated via a different population of vascular AT_1 receptors from those that mediate responses produced by AII generated *in vivo* following conversion, via ACE, from AI. For example, small doses of AII, given by intra-arterial bolus, may act at AT_1 receptors on smooth muscle cells that lie, predominantly, nearest to the vascular lumen. In contrast, as shown by the results obtained with enalapril, AI given by the same route is first metabolised, presumably to AII (and possibly other fragments) before it acts. Although some AI will be converted to AII by ACE in plasma and on endothelial cells, some may penetrate deeper into the vascular wall where it will be converted to AII, which then acts locally. Following systemic administration, plasma levels of GR138950 are high initially but fall rapidly; the plasma half-life in rats is about 3 h (Judd *et al.*, 1994), and after 24 h the level is too low to exert any appreciable blockade of responses to bolus doses of AII. However, the evidence from experiments performed in isolated aortic rings show that the amount of GR138950 retained within the vascular smooth muscle 24 h after dosing is sufficient to antagonize the AII generated locally from exogenous AI. Furthermore, the data presented in Figure 6 suggest that the local concentration of GR138950 within the vascular smooth muscle, 24 h after systemic administration, is similar to that attained after 2 h incubation with 1–3 nM GR138950 added to the solution bathing aortic rings obtained from untreated rats.

If the data obtained from the experiments using aortic rings can be extrapolated to the blood vessels responsible for regulating blood pressure, the residual activity of GR138950 in vascular smooth muscle 24 h after administration could account for its long-lasting antihypertensive activity. It is generally accepted that a local renin-angiotensin system is present in the vascular wall, and that it probably contributes to the maintenance of blood pressure. Consequently, the decrease in blood pressure produced by GR138950 (and similar compounds) in RALH rats may reflect blockade of AII generated from AI locally, in the vascular wall, as well as of that circulating in blood, albeit perhaps with different time courses.

In conclusion, GR138950 is a long-lasting antihypertensive agent in rats in which blood pressure is elevated by activation of the renin-angiotensin system. For the most part, the duration of its antihypertensive activity correlates better with its ability to inhibit pressor responses evoked by administration of AI, rather than AII, in conscious rats, and with its ability to inhibit vasoconstrictor responses to AI in vascular smooth muscle isolated from GR138950 pretreated rats. Thus, interaction with locally generated, as well as plasma-borne, AII probably contributes to the antihypertensive activity of GR138950.

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