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AT_2 receptor-mediated vasodilatation is unmasked by AT_1 receptor blockade in conscious SHR

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1 In the present study, we investigated the role of the angiotensin II type 2 receptor (AT_2) receptor in the regulation of regional haemodynamics in spontaneously hypertensive rats (SHR). We tested the hypothesis that AT_2 receptor activation directly causes vasodilatation.

2 Mean arterial pressure (MAP), renal, mesenteric and hindquarters flows and conductances were measured in various groups of conscious rats that received the following drug combinations on separate days: the AT_1 receptor antagonist, candesartan (5 or 10 μg kg^{-1} i.v.) alone, the AT_2 receptor agonist, CGP42112 (1 μg kg^{-1} min^{-1}) alone and candesartan plus CGP42112.

3 Low-dose candesartan (5 μg kg^{-1}) caused renal vasodilatation, while CGP 42112 alone caused minimal haemodynamic effects. In the presence of candesartan, CGP42112 caused a marked depressor effect together with generalised vasodilatation that was abolished by the coinfusion of the AT_2 receptor antagonist, PD123319 (50 μg kg^{-1} min^{-1}), with the candesartan and CGP42112 combination. PD123319, given alone, increased MAP and reduced renal and mesenteric conductances.

4 We also confirmed that the enhanced vasodilatation evoked by candesartan plus CGP42112 was not due to additional AT_1 receptor blockade, since angiotensin II-mediated vasoconstriction was inhibited by a similar magnitude in the combination treatment compared with candesartan alone. Analogous experiments in Wistar–Kyoto rats did not demonstrate significantly enhanced effects due to candesartan plus CGP42112.

5 Collectively, these data suggest that, in SHR, AT_2 receptors tonically modulate vascular tone and that direct AT_2 receptor-mediated vasodilatation was unmasked by AT_1 receptor blockade.
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Abbreviations: Ang II, angiotensin II; AT_2, angiotensin II type 2 receptor; AT_1, angiotensin II type 1 receptor; SHR, spontaneously hypertensive rats

Introduction

Angiotensin II (Ang II) acts at two main receptor subtypes: angiotensin II type 1 receptor (AT_1) or angiotensin II type 2 receptor (AT_2). AT_1 receptors are widely distributed throughout the body, including vascular smooth muscle, kidney, heart and the brain. AT_1 receptors are responsible for mediating most of the known actions of Ang II, including vasoconstriction, and AT_1-receptor antagonists are effective antihypertensive agents (de Gasparo *et al*., 2000). However, while the role of the AT_2 receptor is less well defined, it is now appreciated that this subtype is present in many tissues/organs in adulthood, albeit in less abundance (Matsubara, 1998). Moreover, a role for the AT_2 receptor in opposing the actions of AT_1 receptor stimulation has been implicated in growth and cardiovascular function (Matsubara, 1998; de Gasparo *et al*., 2000; Carey *et al*., 2001b; Widdop *et al*., 2003).

Studies in transgenic mice have suggested an inhibitory role of the AT_2 receptor in blood pressure control since basal blood pressure and/or pressor sensitivity evoked by Ang II was increased in mice when the AT_2 receptor gene had been disrupted (Hein *et al*., 1995; Ichiki *et al*., 1995) or decreased in mice with the overexpression of vascular AT_2 receptors (Tsutsumi *et al*., 1999).

In vivo, AT_1 receptor antagonists are associated with a rise in plasma Ang II concentration due to the inhibition of the AT_1 receptor-mediated negative feedback on renin release. Therefore, it has been suggested that, at therapeutic doses of AT_1 receptor antagonists, endogenous Ang II may stimulate unopposed AT_2 receptors, and so contribute to the decrease in blood pressure function (Matsubara, 1998; de Gasparo *et al*., 2000; Carey *et al*., 2001b; Widdop *et al*., 2003).

In a few instances, AT_2 receptor-mediated relaxation of isolated resistance arteries has been reported (Arima *et al*., 1997; Matrougui *et al*., 1999; Dimitropoulou *et al*., 2001; Widdop *et al*., 2002), although there is little direct evidence of AT_2 receptor-mediated vasodilatation *in vivo*. In this context, we have recently determined whether or not selective AT_2 receptor stimulation *in vivo* could alter blood pressure. Indeed, we found that selective AT_2 stimulation using the agonist CGP42112 facilitated the depressor response caused by the AT_1 receptor antagonist candesartan in conscious SHR (Barber *et al*., 1999).

Given that the measurement of blood pressure alone does not necessarily reflect direct vasodilatation, or where vasodilatation is actually occurring, we have extended our previous

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studies here by measuring regional haemodynamics in conscious rats. In doing so, we have tested the hypothesis that AT_2 receptor stimulation *in vivo* directly causes vasodilatation. This action has not previously been demonstrated in conscious rats and is a necessary first step in establishing whether or not AT_2 receptor stimulation (i) is likely to contribute to the antihypertensive effects of AT_1 receptor antagonists and (ii) represents a novel target site for the treatment of hypertension.

Therefore, we have determined the regional haemodynamic effect of the AT_1 receptor antagonist, candesartan (Li & Widdop, 1995; 1996), in the absence and presence of CGP42112, in spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats. A preliminary report of these results has previously been presented (Li & Widdop, 2001).

Methods

Adult male SHR, aged 19 weeks and weighing 330–350 g, were obtained from the Biological Research Laboratories at the Austin Hospital, Heidelberg, Melbourne, Australia, and maintained on a 12 h day/night cycle at 20–22°C with free access to food and water. All experiments were approved by the Monash University animal ethics committee and carried out in accordance with the guidelines of the National Health and Medical Research Council of Australia for the use of animals in research.

Surgical procedures

In a two-stage operation, rats were implanted with pulsed Doppler flow probes (Crystal Biotec, Holliston, U.S.A.) and intravascular catheters, as described previously (Widdop *et al*., 1992; Li & Widdop, 1995; 1996). Briefly, rats were anaesthetised with methohexitone sodium (60 mg kg^{-1}, i.p., supplemented as required) and had flow probes placed around the left renal and superior mesenteric arteries and the distal abdominal aorta for the recording of renal, mesenteric and hindquarters Doppler shifts, respectively. After 1 week, rats were reanaesthetised (as above) and had two catheters inserted into the jugular vein and another was inserted into the carotid artery for the intravenous (i.v.) administration of drugs and the measurement of arterial blood pressure, respectively. The catheters were fed subcutaneously to exit at the same point as the probe wires. The latter were then soldered into a microconnector (Microtech Inc., Pennsylvania, U.S.A.) that was fitted to a harness that was worn by the rat. A flexible spring attached to the harness protected the catheters, all of which were connected to a counter-lever swivel system that allowed animals to move unrestrained around their individual home cages throughout the week-long experimental period, with free access to food and water. All variables were displayed on a MacLab-8 system (ADInstruments Pty Ltd, Sydney) interfaced with a Macintosh computer. Doppler shift is an index of blood flow, while regional vascular conductances were calculated by dividing the appropriate mean Doppler shift signal by mean arterial pressure (MAP). For clarity, only the regional conductance measurements are presented. Experiments were carried out 24–48 h after catheterisation.

Experimental protocols

The AT_1 receptor antagonist, candesartan, was given to separate groups of rats as an i.v. bolus at two different doses (see below); the AT_2 receptor agonist, CGP42112, was given as an infusion at 1 μg kg^{-1} min^{-1} for 4 h, which was previously shown to provide plasma concentrations that are highly selective for AT_2 receptors on the basis of binding data (Macari *et al*., 1993; 1994), while the AT_2 receptor antagonist, PD123319, was given at 50 μg kg^{-1} min^{-1} for 2 h, based on the previous studies (Siragy & Carey, 1997; Barber *et al*., 1999). In six separate groups of rats, basal haemodynamics were recorded over a 4-day protocol, in an analogous manner to our previous study (Barber *et al*., 1999), as outlined below. In groups 1 and 2, rats received a 4 h infusion ($\sim$1 ml kg^{-1} h^{-1}, i.v.) of saline (0.9% NaCl) on day 1, while on the subsequent 3 days, SHR were randomised to receive (i) candesartan at either 10 (group 1) or 5 (group 2) μg kg^{-1} as a bolus, plus a saline infusion for 4 h, (ii) candesartan plus an infusion of CGP42112 (1 μg kg^{-1} min^{-1}) for 4 h, and (iii) a 4 h CGP42112 infusion (1 μg kg^{-1} min^{-1}) alone. These studies indicated that a vasodilator effect of CGP42112 was unmasked using the lower dose of candesartan (see Results). Therefore, for group 3, additional SHR were randomised to receive (i) candesartan (5 μg kg^{-1} i.v. bolus) plus a saline infusion for 4 h, (ii) candesartan plus an infusion of CGP42112 (1 μg kg^{-1} min^{-1}) for 4 h, and (iii) candesartan plus an infusion of CGP42112 (1 μg kg^{-1} min^{-1} for 4 h) and an infusion of PD123319 (50 μg kg^{-1} min^{-1} for 2 h), and (iv) an infusion of PD123319 alone. We also confirmed the haemodynamic effect of PD123319 alone (see Results) in a separate group of SHR (group 4). Group 5 consisted of an identical protocol to group 3, but WKY rats were used. Finally, a separate group of SHR (group 6) were also subjected to similar protocols to groups 2 and 3, but in addition they were also given Ang II (15 ng kg^{-1} i.v.) at least 30 min before the commencement of a particular treatment and again after 30, 60 120, 240 and 300 min in order to study the time course of inhibition of Ang II-mediated vasoconstriction. In this group, the animals were randomised to receive each of the following treatments on a different day: CGP42112 or candesartan alone, candesartan plus CGP42112, or the combination of candesartan, CGP42112 and PD123319.

Materials

CGP42112 and candesartan were obtained from Norvartis (Switzerland) and AstraZeneca, (Sweden), respectively, while PD123319 was synthesised by CSIRO (Clayton, Australia). Ang II was purchased from Auspep (Melbourne, Australia).

Statistical analysis

For clarity, data on line graphs are presented as mean and vertical lines as group s.e.m., which was calculated from the equation EMS/n, where EMS is the error mean square from the analysis of variance (ANOVA) and n is the number of rats in each group (Li & Widdop, 1995; 1996). Changes in haemodynamic variables from pretreatment baseline on any given treatment day were analysed using repeated measures ANOVA. Differences between treatments and treatment/time interactions (using the Greenhouse–Geisser correction factor) were also analysed using repeated measures ANOVA

(Ludbrook, 1994). Statistical analysis was performed using Systat (version 9) and statistical significance was accepted as $P<0.05$.

Results

Baseline haemodynamic values were similar over the 4-day experimental period in each group of animals, indicating that individual daily treatments did not alter MAP and regional flows and conductances on subsequent days (Table 1).

Infusion of saline or CGP42112 alone had minimal effect on MAP, as noted previously (Barber *et al*., 1999), or on regional flows and conductances. At the higher dose, candesartan ($10\,\mu g\,kg^{-1}$) lowered MAP ($\sim$25 mmHg) and caused selective renal vasodilatation ($\sim$25 and 35% increases in renal flow and conductance, respectively, at 30–60 min), as we have described previously (Li & Widdop, 1996). However, this haemodynamic profile was not significantly different when CGP42112 was combined with candesartan (group 1 data not shown).

Using an identical 4-day protocol in SHR, the lower dose of candesartan ($5\,\mu g\,kg^{-1}$) still caused selective renal vasodilatation (increased conductance; $P<0.01$ for 60 min, ANOVA), although this effect was accompanied by only a slight fall in MAP. However, in the presence of candesartan, CGP42112 infusion caused a decrease in MAP as well as more prolonged renal vasodilatation ($P<0.01$ for 240 min, ANOVA) that was sustained for the duration of CGP42112 infusion (Figure 1). However, while the candesartan and CGP42112 combination increased renal and hindquarters conductances, only the antihypertensive effect ($P<0.01$, ANOVA) and hindquarters vasodilatation was significantly greater than AT_1 receptor blockade alone (Figure 1).

Given these synergistic effects, additional SHR (group 3) were given CGP42112 and candesartan, and a 2 h infusion of PD123319 ($50\,\mu g\,kg^{-1}\,min^{-1}$) was also included as part of the experimental protocol (Figure 2). As in the previous group, the CGP42112 and candesartan combination caused a greater antihypertensive effect compared with candesartan alone ($P<0.01$, ANOVA). Moreover, CGP42112 caused a sustained increase in renal conductance in the presence of candesartan ($P<0.05$, ANOVA) (Figure 2). Similarly, hindquarters conductance was again increased by the CGP42112 plus candesartan combination, as was mesenteric conductance (both $P<0.05$, ANOVA). In addition, the haemodynamic time course was followed after stopping the infusion of the AT_2 receptor agonist. Interestingly, after 1 h, baseline haemodynamics tended to normalize back towards the effect of AT_1 receptor blockade alone (Figure 2).

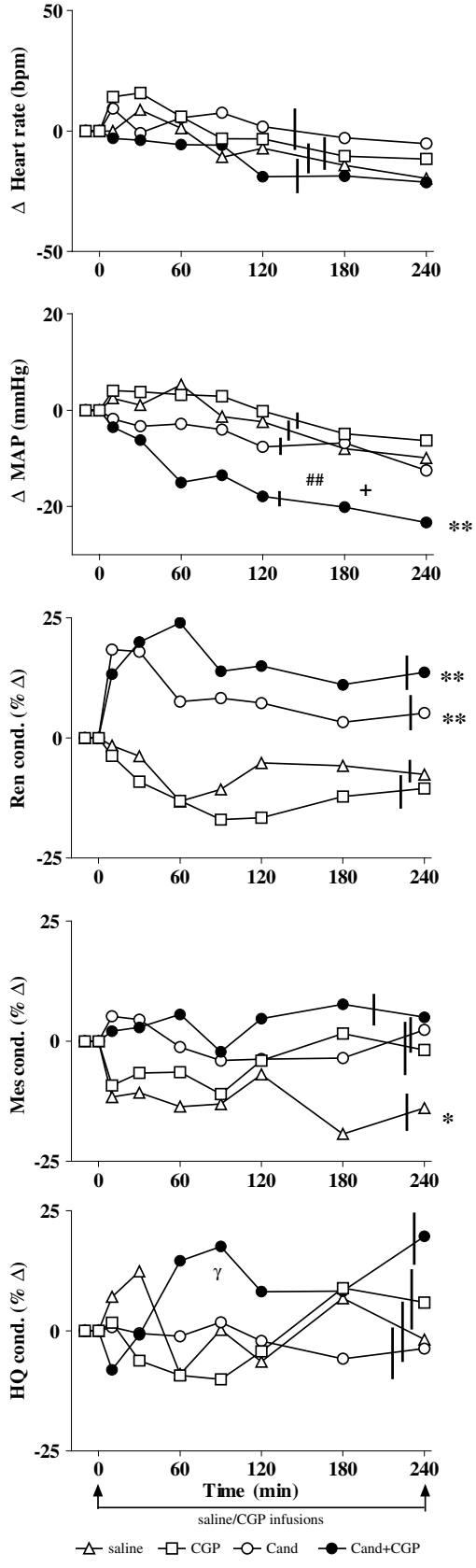
The AT_2 receptor antagonist PD123319 virtually abolished the haemodynamic profile evoked by CGP42112 in the presence of candesartan (Figure 2). PD123319 itself tended to cause a pressor response, although not significant, which

Table 1 Resting haemodynamic values recorded on different days prior to treatment indicated in separate groups of SHR and WKY rats

	MAP (mmHg)	*HR* (bpm)	*Ren flow* (kHz)	*Mes flow* (kHz)	*HQ flow* (kHz)	*Ren cond.*	*Mes cond.*	*HQ cond.*
Group 1 (SHR, n=7)								
Saline	173±8	359±5	2.9±0.6	8.5±1.0	2.0±0.4	17.0±3.4	49.1±4.7	11.4±2.2
CGP42112	163±6	352±11	3.8±1.1	8.7±1.2	2.3±0.4	24.5±7.2	55.0±8.6	14.6±2.8
Candesartan + saline ($10\,\mu g\,kg^{-1}$)	171±5	374±11	4.0±1.0	8.8±1.1	2.2±0.4	24.8±6.4	53.2±8.5	13.6±2.8
Candesartan + CGP	169±6	362±9	4.2±1.2	9.2±1.1	2.0±0.3	25.7±6.2	55.9±7.8	12.3±2.0
Group 2 (SHR, n=7)								
Saline	181±6	333±4	3.3±0.5	7.4±1.1	2.1±0.5	18.4±3.1	41.8±7.2	12±2.8
CGP42112	173±8	314±18	2.6±0.6	5.4±0.7	1.8±0.2	15.2±3.1	32.2±6.1	10.3±0.7
Candesartan + saline ($5\,\mu g\,kg^{-1}$)	170±4	327±15	3.5±0.5	7.1±1.0	2.5±0.5	21.0±3.1	42.6±6.8	14.9±3.2
Candesartan + CGP	174±3	333±14	3.8±0.5	6.6±0.8	1.7±0.5	21.5±2.8	38.1±4.7	9.4±2.6
Group 3 (SHR, n=10)								
Candesartan ($5\,\mu g\,kg^{-1}$)	174±7	342±16	3.3±0.5	6.2±0.8	1.6±0.2	19.9±3.5	33.9±4.5	8.9±1.1
Candesartan + CGP	180±6	336±11	3.2±0.7	6.0±0.7	1.6±0.3	18.7±4.6	33.3±3.9	8.8±1.5
Candesartan + CGP + PD	179±4	350±11	3.3±0.6	6.6±0.9	1.5±0.2	19.4±3.6	36.9±5.2	8.4±1.3
PD123319	165±5	328±10	3.0±0.8	7.7±1.1	1.7±0.2	19.0±5.8	46.5±6.7	10.3±1.4
Group 4 (SHR, n=10)								
Saline	171±4	339±9	4.4±0.7	5.7±0.7	2.0±.2	25.8±4.4	33.0±4.2	11.2±1.0
PD123319	163±4	340±9	5.0±1.1	5.7±0.8	2.1±0.3	30.8±6.2	34.8±4.4	12.9±2.0
Group 5 (WKY, n=8)								
Candesartan + saline ($50\,\mu g\,kg^{-1}$)	122±4	293±8	8.5±1.7	7.3±1.3	2.0±0.3	69.2±12.6	61.0±12.3	16.3±2.3
Candesartan + CGP	119±1	291±7	8.0±1.4	7.8±1.6	2.2±0.3	67.5±11.8	65.8±14.5	18.1±2.4
Candesartan + CGP + PD	113±3	287±8	7.1±1.3	8.0±1.3	1.9±0.3	63.3±12.0	73.0±13.1	17.3±2.8
PD123319	112±3	294±9	6.6±1.2	8.6±1.4	1.9±0.2	60.3±11.1	77.9±13.8	16.9±2.4
Group 6 (SHR, n=8)								
CGP42112	160±5	358±11	4.3±0.9	5.7±0.7	1.9±0.3	26.9±5.6	37.0±5.6	12.1±2.7
Candesartan + saline ($5\,\mu g\,kg^{-1}$)	165±4	358±7	4.7±0.7	5.9±0.6	2.2±0.3	28.4±3.9	35.5±3.8	13.8±1.8
Candesartan + CGP	172±3	348±10	4.7±0.9	5.8±0.6	2.0±0.3	27.3±5.1	33.6±3.3	11.8±1.7
Candesartan + CGP + PD	160±4	323±9	4.0±0.6	5.7±0.6	1.4±0.2	25.4±3.8	35.9±3.8	9.0±1.4

Values are mean±s.e.m.; Ren Cond = renal conductance ($kHz\,mmHg^{-1}\times10^3$); Mes Cond = mesenteric conductance ($kHz\,mmHg^{-1}\times10^3$); HQ Cond = hindquarters conductance ($kHz\,mmHg^{-1}\times10^3$).

was accompanied by significant reductions in mesenteric flow and conductance (both $P<0.01$, ANOVA) and nonsignificant reductions in renal and hindquarters conductances (Figure 2).



PD123319 was also directly compared against a saline infusion arm in another group of SHR (group 4). In this group of SHR, PD123319 significantly increased MAP and reduced renal conductance relative to baseline (both $P<0.05$, ANOVA). Moreover, compared with the negligible effects of saline, the AT_2 receptor antagonist evoked a pressor response and renal as well as mesenteric vasoconstriction (reduced conductances) (all $P<0.05$, Figure 3).

The interaction between AT_2 receptor stimulation and AT_1 receptor blockade was also examined in WKY rats (group 5). In preliminary experiments, it was determined that a 10-fold greater dose of candesartan ($50\,\mu g\,kg^{-1}$) was required to evoke a small antihypertensive effect, which was used in an identical protocol to that used in SHR (group 3). Candesartan lowered MAP ($P<0.01$, ANOVA) and tended to increase renal conductance, while the candesartan plus CGP42112 combination reduced MAP and increased renal conductance (both $P<0.01$, ANOVA). However, the candesartan plus CGP42112 combination was not significantly different to candesartan alone with respect to changes in MAP, renal, mesenteric or hindquarters circulations (all $P>0.05$, RM ANOVA) (Figure 4).

In the last group of SHR (group 6), the haemodynamic effects of Ang II were examined in order to compare the inhibitory effect of each treatment on Ang II-mediated vasoconstriction. This peptide evoked a characteristic response consisting of a pressor effect together with renal and mesenteric vasoconstriction (decreased conductances) and hindquarters vasodilatation (increased conductance) (Figure 5), as described previously (Widdop *et al*., 1992; Li & Widdop, 1995; 1996). It is likely that the Ang II-mediated increase in hindquarters vasodilatation was due to reflex withdrawal of sympathetic tone in order to offset the pressor effect. In any case, CGP42112 did not alter Ang II-mediated vasoconstriction (results not shown), while candesartan generally inhibited responses over a 1–2 h period by ~30–40% (Figure 5). The candesartan plus CGP42112 combination caused a similar degree of inhibition of Ang II responses to that caused by AT_1 receptor blockade alone, over a similar time course (Figure 5).

Discussion

The main finding of the present study was that AT_2 receptor-mediated vasodilatation occurred in response to infusion with CGP42112, but only in the presence of AT_1 receptor blockade. To our knowledge, this study represents the first *in vivo* demonstration of direct AT_2 receptor-mediated vasodilatation in conscious rats and specifically in SHR. Moreover, tonic AT_2 receptor-mediated renal and mesenteric vasodilatation was apparent under control conditions since PD123319 evoked

Figure 1 Line graph showing changes ± group s.e.m. in MAP and renal, mesenteric and hindquarters conductances in response to 4-h infusions of saline, the AT_2 receptor agonist CGP42112 (CGP, $1\,\mu g\,kg^{-1}\,min^{-1}$), the AT_1 receptor antagonist, candesartan (Cand, $5\,\mu g\,kg^{-1}$, i.v.), or the Cand + CGP combination in SHR ($n=7$). $^*P<0.05$, $^{**}P<0.01$ for the overall effect of a particular treatment *versus* pretreatment baseline ; $^{\#}P<0.05$ for treatment effect, and $^{+}P<0.05$, $^{\gamma}P=0.07$ for treatment/time interaction, between Cand and Cand/CGP42112.

vasoconstriction in these regions, accompanied by a pressor effect, in response to this AT_2 receptor antagonist.

CGP42112 was used as the AT_2 receptor agonist because, when given alone, it is devoid of cardiovascular effects in anaesthetised rats at up to 100 times the dose used here (Macari *et al*., 1993; 1994), which we confirmed previously in SHR in which only blood pressure was measured (Barber *et al*., 1999). The dose of the AT_1 receptor antagonist, candesartan, was carefully titrated to produce a slow-onset depressor response, since we have shown that this protocol optimises the unmasking of AT_2 receptor-mediated depressor responses (Barber *et al*., 1999). In the present study, we used half the candesartan dose used previously in SHR in which only blood pressure was measured (Barber *et al*., 1999). We have extended our previous findings by demonstrating, for the first time, peripheral vasodilator effects of CGP42112 during AT_1 receptor blockade, as well as ruling out the possibility that it acted as an AT_1 receptor antagonist. That is, CGP42112 did neither lower MAP nor cause vasodilatation when given alone over the infusion period, nor did this compound inhibit the haemodynamic response to exogenous Ang II, nor increase the inhibitory effect of candesartan on Ang II-induced vasoconstriction when given in combination with the AT_1 receptor antagonist.

In the present study, the AT_2 receptor antagonist PD123319 reversed the accelerated depressor effect and vasodilatation observed with the candesartan plus CGP42112 combination, thus confirming the involvement of AT_2 receptor-mediated vasodilatation, as we have reported previously when measuring blood pressure alone (Barber *et al*., 1999). Similarly, Carey *et al*. (2001a) recently reported that CGP42112 reduced blood pressure in conscious rats and that this effect was blocked by PD123319. However, no haemodynamics were reported by these authors.

As we reported previously (Barber *et al*., 1999), when candesartan is given at a dose that markedly reduces blood pressure, the addition of CGP42112 was unable to further lower blood pressure or haemodynamics in the present study. This finding suggests that the depressor effect is already maximal in SHR (Barber *et al*., 1999). Alternatively, there may be a greater increase in plasma levels of Ang II with the higher candesartan dose (due to either blockade of the negative feedback on renin release or because of a larger depressor effect) that results in increased competition between endogenous Ang II and CGP42112 at the AT_2 receptor.

An alternative approach to stimulating AT_2 receptors has been to infuse Ang II in the presence of AT_1 receptor blockade (Munzenmaier & Greene, 1996; Gohlke *et al*., 1998). Gohlke *et al*. (1998) found that AT_2 receptor stimulation increased aortic cyclic GMP content; however, any potential blood pressure reductions caused by this protocol were presumably

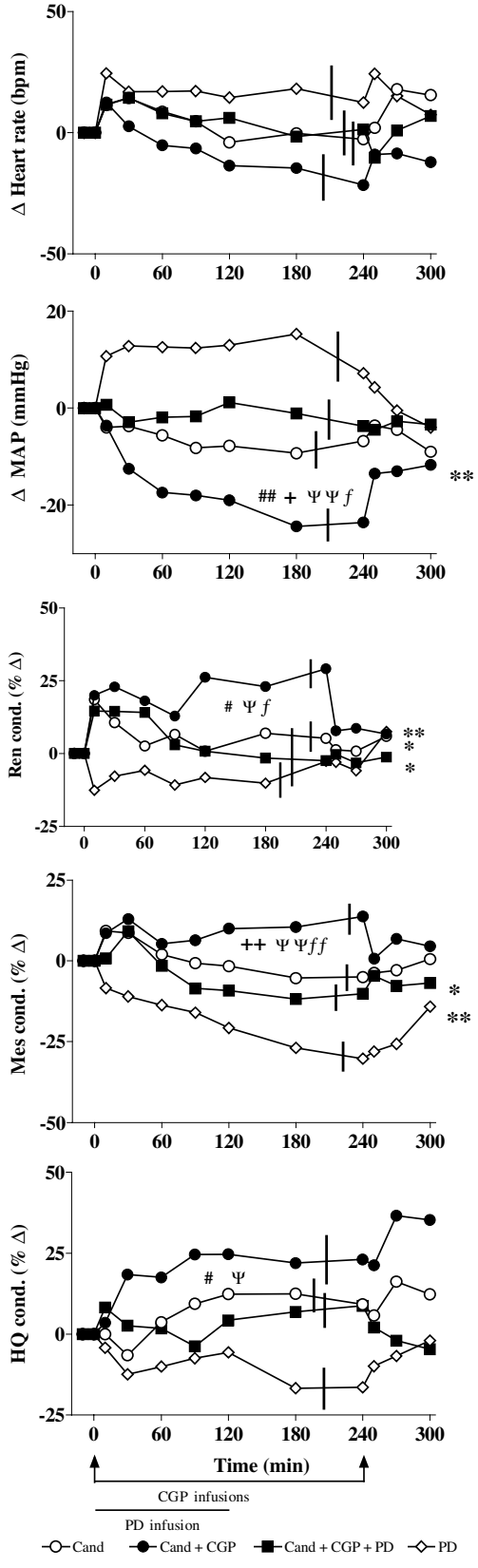


Figure 2 Line graph showing changes ± group s.e.m. in MAP, renal, mesenteric and hindquarters conductances in response to the AT_1 receptor antagonist, candesartan (Cand, 5 $\mu g\,kg^{-1}$, i.v.), the combination of candesartan and the AT_2 receptor agonist CGP42112 (1 $\mu g\,kg^{-1}\,min^{-1}$ for 4 h) (Cand + CGP), the combination of candesartan, CGP42112 and the AT_2 receptor antagonist PD123319 (PD, 50 $\mu g\,kg^{-1}\,min^{-1}$ for 2 h) (Cand + CGP + PD) or PD123319 alone in SHR ($n = 10$). $^{*}P<0.05$, $^{**}P<0.01$ for the overall effect of a particular treatment *versus* pretreatment baseline; $^{\#}P<0.05$, $^{\#\#}P<0.01$ for treatment effect, and $^{+}P<0.05$, $^{++}P<0.01$ for treatment/time interaction, between Cand and Cand and CGP42112; $^{\psi}P<0.05$, $^{\psi\psi}P<0.01$ for treatment effect, and $^{f}P<0.05$, $^{ff}P<0.01$ for treatment/time interaction, between Cand plus CGP42112 and Cand plus CGP plus PD.

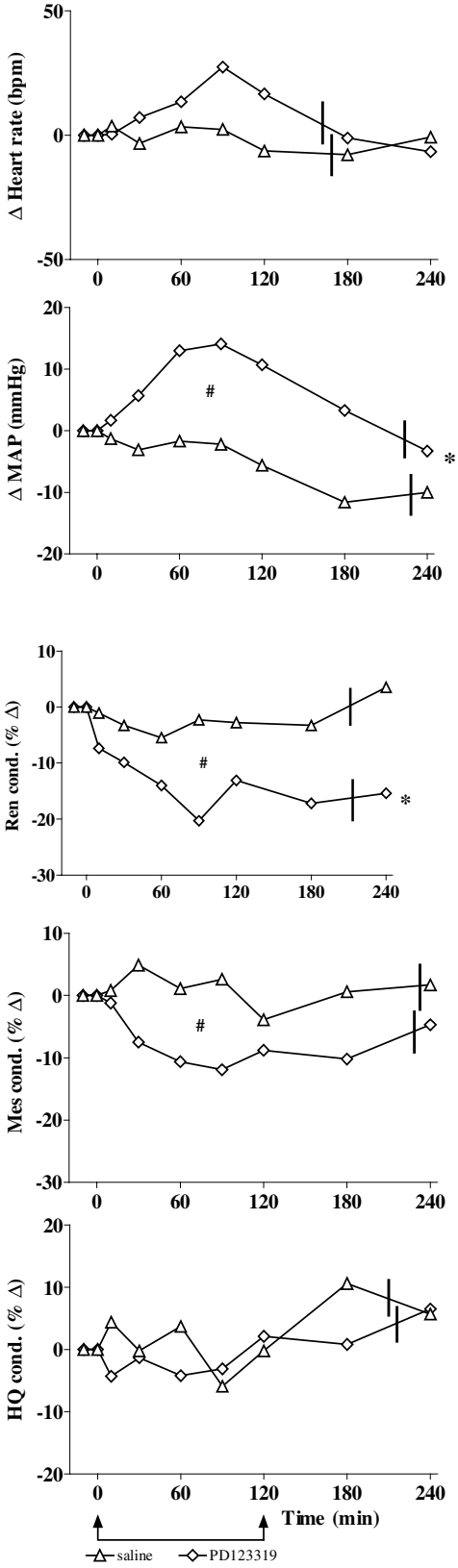


Figure 3 Line graph showing changes ± group s.e.m. in MAP and renal, mesenteric and hindquarters conductances in response to infusions of saline and the AT_2 receptor antagonist PD123319 (PD, 50 $\mu g\,kg^{-1}\,min^{-1}$) in SHR ($n=10$). $^*P<0.05$, for the overall effect of a particular treatment *versus* pretreatment baseline; $^{\#}P<0.05$, for treatment effect between saline and PD123319.

offset by direct vasoconstriction caused by infusion of a large dose of Ang II, since the depressor effect of losartan combined with AngII was less than with losartan alone in stroke-prone SHR. However, Schuijt *et al*. (1999) recently reported that AT_2 receptor stimulation (Ang II infused in the presence of AT_1 receptor blockade) did not evoke vasodilatation measured at limited time intervals using radioactive microspheres in anaesthetised rats. Interestingly, this same group has now reported that AT_2 receptor-mediated coronary vasodilatation, but not renal vasodilatation, occurred in rats, when tested 3–4 weeks after myocardial infarction (Schuijt *et al*., 2001). Again, these studies were limited to a single time point, at the end of a 10 min Ang II infusion, in anaesthetized rats and do not provide continuous haemodynamic measurements as in the current study.

Consistent with a vasodilator role for the AT_2 receptor, we also established for the first time that the AT_2 receptor antagonist PD123319 *per se* exerted haemodynamic effects consisting of modest pressor activity and renal and mesenteric vasoconstriction. Owing to limited drug supplies, few studies have examined the effect of PD123319 alone. Earlier studies (Macari *et al*., 1993; 1994) indicated that, at the present dose, PD123319 exerts no cardiovascular effects *per se*, although these studies were performed in anaesthetized rats. In contrast, our data obtained in conscious SHR suggest that the AT_2 receptor tonically modulated vascular tone in the renal and mesenteric circulations, which we confirmed in two separate groups of animals in which PD123319 was infused. The importance of renal AT_2 receptors for renal vasodilatation was also recently inferred in a study in which antisense oligodeoxynucleotide directed against the AT_2 receptor mRNA was infused into the renal interstitium in rats, since this perturbation caused an increase in blood pressure (Moore *et al*., 2001). Our data would support an AT_2 receptor vasodilator role in both renal and mesenteric vasculatures, which were measured simultaneously.

Thus, our results are consistent with *in vitro* studies identifying a vasorelaxant role for AT_2 receptors (Arima *et al*., 1997; Matrougui *et al*., 1999; Dimitropoulou *et al*., 2001; Widdop *et al*., 2002). There is also an emerging concept that an additional depressor effect due to AT_2 receptor stimulation during AT_1 receptor blockade may play a role in the beneficial effects of AT_1 receptor antagonists (Matsubara, 1998; de Gasparo *et al*., 2000; Carey *et al*., 2001b; Widdop *et al*., 2003). In this context, it has recently been reported that the cardiovascular effects of the AT_1 receptor antagonist losartan were blocked by the coadministration of the AT_2 receptor antagonist PD123319 in rats with heart failure (Liu *et al*., 1997). More recently, it has been reported that acute administration of PD123319 reversed both the acute antihypertensive effect (using tail cuff measurements) and elevated levels of cGMP, NO and bradykinin in renal interstitial fluid caused by AT_1 receptor blockade in renal wrap and salt-restricted rats (Siragy & Carey, 1999; Siragy *et al*., 2000). While this limited number of studies are persuasive (although not usually tested in a chronic setting, see Widdop *et al*., 2003), these previous studies have not directly assessed the extent of AT_2 receptor-mediated vasodilatation *per se*, as we have carried out in the current study. Indeed, the current study has unequivocally demonstrated an AT_2 receptor-mediated vasodilatation in conscious SHR, which confirms previous studies in which a vasodilator action was assumed from blood

pressure measurements (Barber *et al*., 1999; Siragy & Carey, 1999; Tsutsumi *et al*., 1999; Siragy *et al*., 2000; Moore *et al*., 2001; Carey *et al*., 2001a). Further studies are required to assess directly the potential involvement of AT_2 receptors in the haemodynamic effects of AT_1 receptor antagonists.

Interestingly, there was some variation in the vasodilator effect of CGP42112 between different groups of SHR, although the depressor response was similar between groups. Likewise, there were similar vasoconstrictor effects of PD123319 between different groups of SHR, although there were slight variations in the statistical significance achieved. These finding are likely to reflect the lower expression level of AT_2 receptors than AT_1 receptors in the vasculature (Viswanathan *et al*., 1991; Munzenmaier & Greene, 1996; Matsubara, 1998; Nora *et al*., 1998; Touyz *et al*., 1999; Bonnet *et al*., 2001; Dimitropoulou *et al*., 2001), which can make it difficult to detect AT_2 receptor-mediated vasodilatation. For example, AT_2 receptor-mediated relaxation is readily detected using a pressure myograph (Matrougui *et al*., 1999; Dimitropoulou *et al*., 2001; Widdop *et al*., 2002), but this is not the case using a conventional myograph (Zwart *et al*., 1998). In the present study, the vasodilator effects of CGP42112 were maintained during the infusion but tended to wane immediately after the CGP42112 infusion was stopped. We have not attempted to perform AT_2 receptor-mediated dose–response relationships *in vivo*, since we were mindful of avoiding potential AT_1 receptor effects of CGP42112 (Macari *et al*., 1993; 1994). However, we have previously examined the concentration-dependent relaxation effects of CGP42112 (and Ang II) in more rigorously controlled *in vitro* experiments (Widdop *et al*., 2002). These data indicate that, unlike AT_1 receptor-mediated contraction, AT_2 receptor-mediated relaxation does not exhibit desensitisation (Widdop *et al*., 2002), which agrees with the current study in that vasodilatation did not wane during CGP42112 infusion (although it was relatively short lived after infusion was stopped).

As already mentioned, another factor that may influence the magnitude of AT_2 receptor responses *in vivo* is the reflex increase in Ang II levels caused by AT_1 receptor blockade. Bonnet *et al*. (2001) recently reported that Ang II infusions increased AT_2 receptor expression in vasculature. While we did not measure plasma levels of Ang II, it is possible that variable increases in flow and conductance evoked by CGP42112 may reflect the influence of circulating levels of Ang II on the less abundant AT_2 receptor. In any case, incremental increases in vascular conductances evoked by CGP42112, across a wider range of vascular beds than were measured in the present study, are likely to contribute to the blood pressure-lowering effect of CGP42112.

Another unique finding of the present study was that the time course of inhibition of Ang II-mediated vasoconstrictor responses was not temporally related to baseline haemodynamic changes evoked by the drug combinations. The low dose of candesartan caused ~30–40% block of Ang II-mediated vasoconstrictor responses that was maximal at 1–2 h, and this

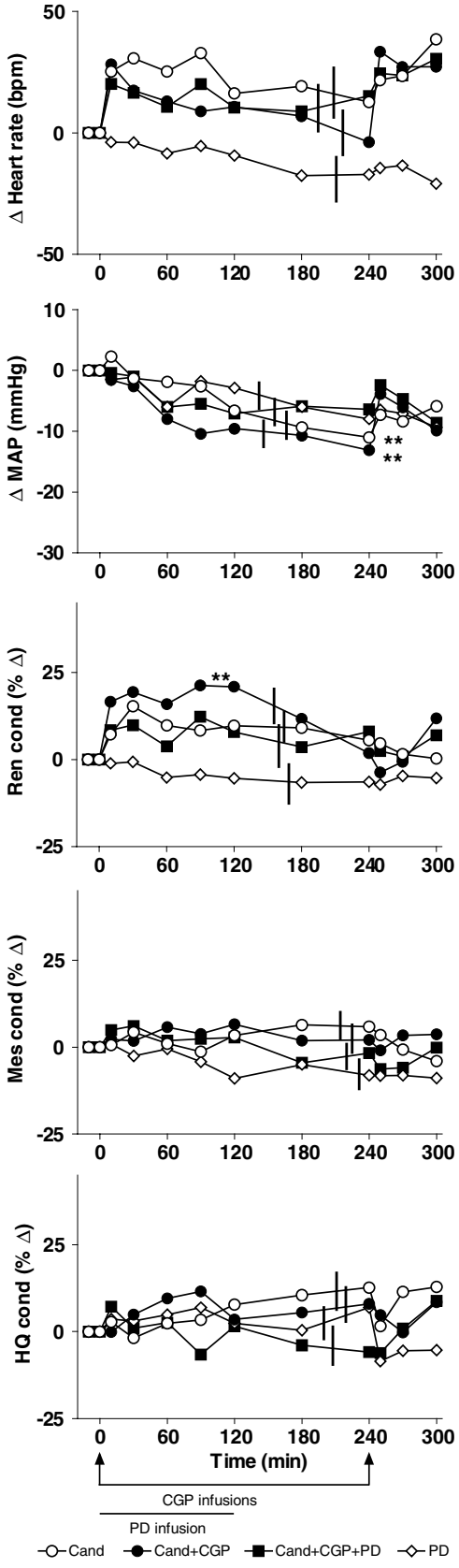


Figure 4 Line graph showing changes ± group s.e.m. in MAP and renal, mesenteric and hindquarters conductances in response to the AT_1 receptor antagonist, candesartan (Cand, 50 $\mu g\,kg^{-1}$, i.v.), the combination of candesartan and the AT_2 receptor agonist CGP42112 (1 $\mu g\,kg^{-1}\,min^{-1}$ for 4 h) (Cand + CGP), the combination of candesartan, CGP42112 and the AT_2 receptor antagonist PD123319 (PD, 50 $\mu g\,kg^{-1}\,min^{-1}$ for 2 h) (Cand + CGP + PD) or PD123319 alone in WKY rats ($n = 8$). $^{**}P < 0.01$ for the overall effect of a particular treatment *versus* pretreatment baseline.

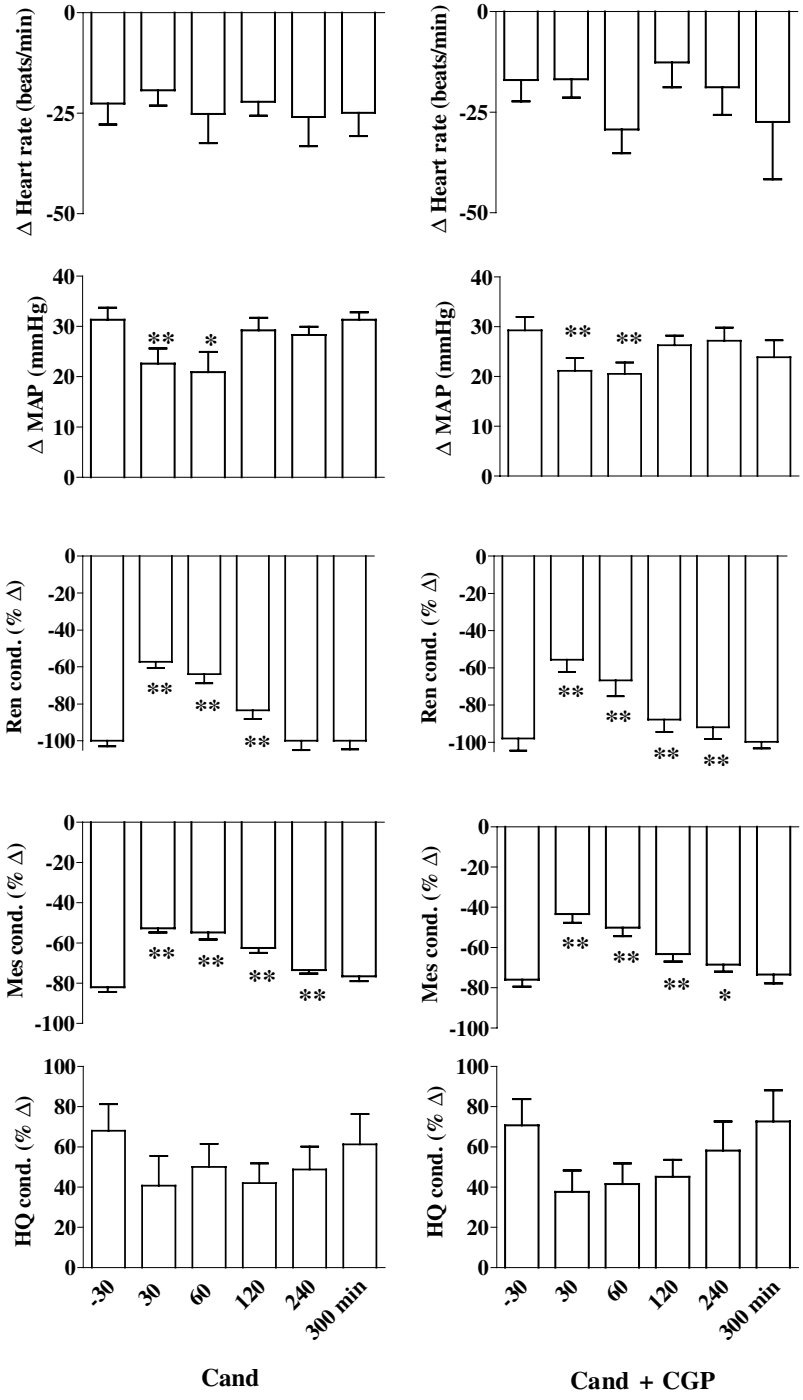


Figure 5 Bar graph showing changes ± s.e.m. in MAP and renal, mesenteric and hindquarters conductances in response to Ang II (15 ng kg^{-1} i.v.), given before (−30 min) and up to 300 min after treatment with either candesartan (Cand, 5 μg kg^{-1}, i.v., left panels) or the candesartan and CGP42112 combination (1 μg kg^{-1} min^{-1}, right panels) in SHR ($n=8$). *$P<0.05$, **$P<0.01$ *versus* respective control (−30 min). Note that CGP42112 alone did not inhibit Ang II responses over the same time period (data not shown).

AT_1 receptor blocking effect was not substantially altered by the combinations of either candesartan and CGP42112 or candesartan plus CGP42112 plus PD123319. In contrast, CGP42112 alone did not inhibit Ang II responses, whereas the AT_2 receptor-mediated vasodilator effect during AT_1 receptor blockade was maximal at 2–4 h, which is consistent with an additional (AT_2 receptor) vasodilator component, which contributed to the overall haemodynamic profile. In the present study, we deliberately used animals with intact reflexes to allow comparisons in a physiologically relevant state. While the hindquarters effects of Ang II may involve a reflex component, further experiments in autonomically blocked animals would be required to determine the effects of Ang II without the influence of cardiovascular reflexes. However, it is unlikely that a major reflex component has interfered with the time course study reported here, since the largest basal

haemodynamic changes to CGP42112 occurred at a time (2–4 h) when Ang II-mediated pressor and vasoconstrictor responses had virtually normalised, strikingly demonstrating a dissociation between AT_1 receptor blockade and basal haemodynamics.

Finally, we noted previously that the effect of AT_2 receptor stimulation on basal blood pressure was observed in SHR but not WKY, which may suggest that covert AT_2 receptor-mediated vasodilatation occurs as a consequence of hypertension (Barber *et al*., 1999) and is consistent with enhanced expression of this subtype in SHR (Otsuka *et al*., 1998). Alternatively, it may be more difficult to detect small AT_2 receptor-mediated vasodilator effects in normotensive rats with a lower basal blood pressure. Indeed, at 10 times the dose used in SHR, candesartan caused only a small reduction in blood pressure in WKY rats, together with a trend to cause renal vasodilatation. While these effects were slightly greater in the presence of CGP42112, there were no significant differences between the treatments, indicating a lack of a clear AT_2 receptor effect in WKY rats. Moreover, PD123319 itself caused peripheral vasoconstriction in SHR but not WKY rats, which would further support a predominant vasodilator role of AT_2 receptors in the hypertensive strain.

In conclusion, the unmasking of the antihypertensive and vasodilator effect of CGP42112 by candesartan in conscious SHR suggests that AT_2 receptors do play a modulatory role in blood pressure regulation. While these data suggest that an AT_2 receptor component should be considered as a potential complementary effect that may contribute to the therapeutic action of AT_1 receptor antagonists, additional chronic experiments are required to confirm this hypothesis. In any case, the present study would suggest the AT_2 receptor should be considered as a novel target for the treatment of cardiovascular disease.

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