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Lack of a centrally-mediated antihypertensive effect following acute or chronic central treatment with AT_1-receptor antagonists in spontaneously hypertensive rats

M.W. Bunting & [1]R.E. Widdop

Department of Pharmacology, Monash University, Clayton, Victoria, 3168, Australia

1 The role of the central renin-angiotensin system in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR) was examined following acute and chronic intracerebroventricular (i.c.v.) infusions of angiotensin$_1$ (AT_1) receptor antagonists.

2 Groups of SHR were chronically instrumented for acute i.c.v. administration of the AT_1 receptor antagonists, losartan and CV-11974, on mean arterial blood pressure (MAP) and heart rate (HR). Other groups of SHR also had mini-osmotic pumps implanted for chronic i.c.v. infusion of CV-11974.

3 Initially both young (15-18 weeks, $n=8$) and old (25-29 weeks, $n=9$) SHR received acute i.c.v. injections of losartan (10 μg) while a third group of young SHR received CV-11974 (1 μg, $n=6$). In all three groups of SHR, MAP and HR did not change up to 24 h after antagonist injection. However, changes in MAP and HR in response to i.c.v. angiotensin II (AII, 100 ng) were abolished 15 min after administration of the AT_1 receptor antagonists. These responses had returned to control levels after 3 h in both groups given losartan but were still significantly depressed at 24 h in the CV-11974-treated group. By contrast, responses to i.v. AII (25 ng) before and 1 h after administration of AT_1 receptor antagonists were not significantly different.

4 For chronic studies, four groups of SHR received chronic i.c.v. infusion of either vehicle ($n=9$) or CV-11974 (1, 5 and 100 μg kg^{-1} day^{-1}) ($n=4$, 7 and 8 respectively) for 4 days. Baseline cardiovascular parameters were monitored daily together with changes in MAP and HR in response to both i.c.v. and i.v. AII (100 ng and 50 ng respectively) and i.v. phenylephrine (3 μg). Responses to i.c.v. carbachol (5 μg) were also recorded on day 4 while baroreflex function was assessed between days 1-3. In SHR treated chronically with i.c.v. vehicle or CV-11974, at 1 or 5 μg kg^{-1} day^{-1}, resting MAP and HR did not vary over the four day infusion period. However, SHR treated with 100 μg kg^{-1} day^{-1} CV-11974 had significantly lower MAP compared to vehicle-treated SHR. While there was some variation in resting HR, there were no differences between the drug-treated and vehicle-treated groups. Pressor responses following i.c.v. AII administration were slightly, but significantly, inhibited on days 3 and 4 in the low dose CV-11974-treated (1 μg kg^{-1} day^{-1}) SHR. However, these responses were abolished on all 4 days in the 5 and 100 μg kg^{-1} day^{-1} CV-11974-treated groups. By contrast, changes in MAP and HR following i.v. AII injection did not vary over the 4 day infusion between SHR treated with the 2 lowest doses of CV-11974 and the vehicle-treated group. However, in the high dose CV-11974-treated SHR (100 μg kg^{-1} day^{-1}), the cardiovascular effects of AII were abolished. In addition, phenylephrine (i.v.) and carbachol (i.c.v.) induced changes in MAP and HR were not significantly different in all four treatment groups. Similarly, baroreflex function was unaffected by i.c.v. infusion of 100 μg kg^{-1} day^{-1} CV-11974, except for a significant fall in BP_{50} which paralleled the fall in resting MAP.

5 Collectively, these results indicate that acute and chronic central AT_1 receptor antagonism does not lower MAP in conscious SHR in doses which only block central AII-induced pressor activity. Chronic central infusion of CV-11974 at sufficiently high doses will lower MAP, as has been reported by others, but not without the abolition of the peripheral effects of AII. Therefore it is most likely that peripheral AT_1 receptor blockade contributes to the hypotensive action of CV-11974 under these conditions.

Keywords: CV-11974; losartan; brain renin-angiotensin system; hypertension; spontaneously hypertensive rats

Introduction

It is now generally accepted that the brain has an intrinsic renin-angiotensin system (RAS) and that all the components necessary for its proper functioning, such as angiotensinogen, renin and angiotensin II (AII) receptors are present. Stimulation of AII receptors in the central nervous system results in a number of different physiological effects which can be cardiovascular, endocrine or behavioural in nature (Stecklings *et al.*, 1992). For example, administration of AII to the brain by intracerebroventricular (i.c.v.) injection results in pressor activity, stimulation of drinking and hypophyseal hormone release. In spontaneously hypertensive rats (SHR) an overactive brain RAS is thought to contribute to genetic hypertension. It has been demonstrated that the brains of SHR, compared to normotensive Wistar-Kyoto rats (WKY), have increased renin activity, increased rate of AII generation, increased angiotensin converting enzyme (ACE) activity, greater AII receptor density and binding, along with dysfunctional aminopeptidase, the enzyme responsible for the degradation of AII (Unger *et al.*, 1988). The SHR is generally considered a good experimental model of human essential hypertension (Phillips *et al.*, 1977; Trippodo & Frohlich, 1981).

This has lead to much speculation as to whether or not inhibition of brain AII can cause hypotension in SHR. Early work with central injections of peptide AII receptor antagonists yielded conflicting results with respect to their effect on resting blood pressure. Bolus i.c.v. injections of saralasin were

[1] Author for correspondence.

shown to lower baseline mean arterial blood pressure (MAP) in SHR (Sweet *et al.*, 1976; Phillips *et al.*, 1977; Mann *et al.*, 1978; Jensen *et al.*, 1988). Similarly, McDonald *et al.* (1980) showed chronic i.c.v. infusions of sarile also decreased MAP in SHR. However, others have demonstrated that both acute (Berecek *et al.*, 1991) and chronic (Bruner *et al.*, 1987) infusion of sarthran had no effect on MAP in genetically hypertensive rats. Angiotensin converting enzyme (ACE) inhibitors have also been shown to lower blood pressure when given centrally to SHR (Stamler *et al.*, 1980; Unger *et al.*, 1981; Okuno *et al.*, 1983; Phillips *et al.*, 1986).

With the development of nonpeptide AII receptor antagonists, the role of brain AII in tonic regulation of blood pressure can now be reassessed. It has been well established that most of the peripherally- and centrally-mediated effects of AII are inhibited by selective angiotensin$_1$ (AT_1) receptor antagonists such as losartan (Timmermans *et al.*, 1993). For example, AT_1 receptor antagonists administered i.c.v. will abolish the pressor response caused by AII given centrally (Kirby *et al.*, 1992; Beresford & Fitzsimons, 1992; Hogarty *et al.*, 1992; Toney & Porter, 1993a,b; Widdop *et al.*, 1993a), although, curiously, the AT_2 receptor antagonist PD 123319 has been shown to inhibit AII when given centrally but not peripherally (Widdop *et al.*, 1992; Widdop *et al.*, 1993a,b; Toney & Porter, 1993a,b). There is much debate as to whether or not AII receptor antagonists will decrease resting MAP when administered i.c.v to SHR.

As with peptide AII receptor antagonists, initial central studies with nonpeptide AII receptor antagonists have also produced equivocal results. Depasquale *et al.* (1992) have demonstrated that losartan does not lower resting MAP in SHR over a 48 h period following i.c.v. administration. In contrast, Pare *et al.* (1993) recently demonstrated that losartan lowered resting MAP in SHR when administered i.c.v. but not until at least 18 h after administration. However, they claimed that the metabolite EXP 3174 did not affect resting MAP and that losartan may be converted to some other active metabolite. Interestingly, the central pressor effects of AII were purportedly not altered by losartan although no data were presented. Additionally, the delayed hypotensive effect of losartan was seen only in younger SHR (Pare *et al.*, 1993).

Therefore, the first aim of the present study was to investigate the acute effects of AT_1 receptor antagonists in conscious SHR. We chose losartan so as to compare with previous studies (Pare *et al.*, 1993) and another AT_1 receptor antagonist, CV-11974, which is at least 10 times more potent than losartan in peripheral studies (Shibouta *et al.*, 1993).

During the course of this study, it was shown that chronic (2 day) i.c.v. administration of CV-11974 (1 $\mu g\ kg^{-1}\ day^{-1}$) lowered MAP in SHR at a dose which had no effect following i.v. administration. Moreover, chronic (4 day) i.c.v. and i.v. infusion of CV-11974 at a higher dose (100 $\mu g\ kg^{-1}\ day^{-1}$) reduced MAP which was reversible following nephrectomy, but only in the i.v.-treated group of SHR. These data were suggestive of a centrally-mediated hypotensive effect of CV-11974, although the cardiovascular effects of AII were not shown (Kamitani *et al.*, 1994). Therefore, the second aim of this study was to document the chronic central effects of CV-11974 on basal MAP in conscious SHR and to determine the effectiveness of central, as well as peripheral, blockade of AII-mediated cardiovascular responses. We have previously shown that acute i.c.v. administration of an AT_1 receptor antagonist does not modify baroreflex function in conscious SHR (Bartholomeusz & Widdop, 1995). Therefore our third aim was to test baroreflex function in SHR chronically treated with i.c.v. CV-11974.

Methods

Surgical procedures

Male spontaneously hypertensive rats (SHR) were obtained from the Austin Hospital Biological Research Laboratories (Melbourne, Australia). Two groups of SHR, aged, 15–18 weeks (designated 'young') and 25–29 weeks (designated 'old'), weighing 250–350 g, underwent a two-stage operation, as described previously (Bartholomeusz & Widdop, 1995). Briefly, SHR were anaesthetized (sodium methohexitone 60 mg kg^{-1} i.p., supplemented as required) and a 23 gauge stainless steel guide cannula was implanted in the right lateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to the midline and 2.2 mm ventral to the skull surface) and fixed to the skull with screws and dental cement. A 31 gauge stainless steel stylet was inserted to extend 2 mm beyond the end of the guide cannula to ensure it remained patent. Following at least 5 days recovery, animals were briefly anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) and two catheters were implanted in the right jugular vein and a catheter was also inserted into the abdominal aorta via the caudal artery. After a further 24 h recovery, experiments were performed in conscious, unrestrained SHR, whereby continuous recordings were made of mean and phasic blood pressure and heart rate, and these were displayed on a polygraph (Grass Instruments Co., U.S.A.).

Intracerebroventricular injections were made through a 31-gauge stainless steel injector that extended 2 mm beyond the previously implanted guide cannula. The injector, attached via polyethylene tubing to a 5 μl microsyringe, was inserted into the guide cannula without handling the animals. Each injection of drug was given over a period of one minute in a 2 μl volume. MAP and HR were recorded for 30 min following AII injection during which time animals were denied access to water. When more than one injection of AII were made on a single day at least 2 h was left between successive injections. Correct i.c.v. cannula placement was confirmed at the end of each experiment by injecting 2 μl of 1% aqueous solution of Evans blue dye and examining the brain for staining of the ventricular spaces.

Protocol 1: effects of acute central administration of AT_1 receptor antagonists

Initially, the reproducibility of responses to i.c.v. AII (100 ng) was tested in 6 young SHR which were subsequently used in antagonist studies. To this end, 3 responses were obtained on the first experimental day at 2 h intervals, and a fourth response to i.c.v. AII was obtained 24 h later on day 2. For antagonist studies, MAP and HR were continuously recorded and the effect of i.c.v. AII was tested both before and at various times (15 min, 3 and 24 h) after a single i.c.v. dose of an AT_1 receptor antagonist. Losartan (10 μg i.c.v.) was given to both age groups of SHR while only young SHR were given CV-11974 (1 μg i.c.v.). In addition, responses to AII (25 ng i.v.) were also recorded before and 1 h after antagonist administration. This dose of losartan was chosen based on previous studies (Depasquale *et al.*, 1992; Toney & Porter 1993a,b), while CV-11974 was given at the lower dose as it is shown to be at least 10 times more potent than losartan (Shibouta *et al.*, 1993).

Protocol 2: chronic i.c.v. infusion of CV-11974 (1–100 $\mu g\ kg^{-1}\ day^{-1}$)

Surgical procedures for chronic infusions were similar to those already described except that a double i.c.v. cannula consisting of 2 lengths of 23 gauge stainless steel tubing (one bent at right angles which was fastened to a straight length with cyanoacrylic adhesive) was implanted. At the time of intravascular catheterisation (in these experiments the right carotid artery was cannulated), a mini-osmotic pump (Alzet model 2001, Alza Corp., U.S.A.) was implanted subcutaneously. The pump was connected via vinyl tubing to the curved guide cannula to allow the chronic i.c.v. infusion of drugs. This infusion system was buried under the skin, while acute i.c.v. injections could

still be made via the vertical, exposed cannula. SHR, 15–18 weeks of age, were given a constant i.c.v. infusion of either vehicle (5% 1 M Na_2CO_3/saline) or CV-11974 (1–100 $\mu g\ kg^{-1}\ day^{-1}$). MAP and HR were recorded 4–8 h after connection of the mini-osmotic pump ie. day 0, in an attempt to record these parameters before any significant drug effect. Thereafter, on days 1–4 after commencement of i.c.v. infusions, basal MAP and HR were recorded. In addition, the responses to central and peripheral injections of AII (100 ng and 50 ng respectively), as well as to phenylephrine (3 μg i.v.) were recorded. Carbachol (5 μg i.c.v.) was also given on day 4 to check for i.c.v. cannula placement and specificity of central responses.

In addition, baroreflex function was assessed once, usually between days 1 and 3, in SHR treated with either vehicle or 100 $\mu g\ kg^{-1}\ day^{-1}$ CV-11974 by constructing MAP-HR curves as described previously (Head & McCarty, 1987), by injecting phenylephrine (1–25 $\mu g\ kg^{-1}$ i.v.) and sodium nitroprusside (1–50 $\mu g\ kg^{-1}$ i.v.) alternately through separate venous catheters in order to raise or lower MAP by between 5 and 50 mmHg. A sigmoidal logistic equation was then fitted to the MAP and corresponding changes in HR:

$$\text{Heart rate} = P_1 + P_2/[1 + e^{P_3(MAP-P_4)}]$$

where P_1 is the lower HR plateau, P_2 is the HR range, P_3 is a curvature coefficient and P_4 is the BP_{50} value which is the MAP value at the midpoint of the HR range (Head & McCarty, 1987). The average gain (ie. slope) of the MAP-HR curve between the two inflection points is given by $-P_2 \times P_3/4.56$ and the upper plateau by $P_1 + HR$ range.

Following completion of experiments on day 4 animals received a barbiturate overdose and the hearts were removed and left ventricular weight (mg kg^{-1} body weight) was recorded.

Statistical analysis

Changes in baseline MAP and HR values following administration of AT_1 receptor antagonists in both acute and chronic experiments, and responses to drug (eg. angiotensin and carbachol) over time, were analysed by one-way analysis of variance (ANOVA) with repeated measures. Comparison of baseline MAP and HR values, and of AII responses between various treatment groups in chronic experiments were analysed by a two-way ANOVA with repeated measures. ANOVA and post-hoc tests (Newman-Keuls) were done using a commercially available statistical package (CLR ANOVA) on an Apple Macintosh computer.

Values are given as means, and vertical error bar represents group standard errors, which were calculated using the equation $\sqrt{EMS/n}$, where EMS is the error mean square from the analysis of variance and n is the number of animals in the group.

Baroreflex parameters were analysed by non-paired Student's t test. Statistical significance was accepted at $P<0.05$.

Drugs

CV-11974 (2-ethoxy 1-[[2'(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl]-1H-benzimidazole-7-carboxylic acid) (Takeda Chemical Co., Japan) was dissolved in 5% 1 M Na_2CO_3/saline while losartan (DuPont, U.S.A.), angiotensin II (American Peptide Co., U.S.A.), phenylephrine (Koch-Light Laboratories, Colebrook, U.K.), sodium nitroprusside (Nipride, Roche, Sydney, Australia), carbachol (Sigma, St Louis, U.S.A.) and methohexitone sodium (Brietal Sodium, Eli Lilly, Sydney, Australia) were all dissolved in isotonic saline. Intracerebroventricular injections were made in a volume of 2 μl, while i.v. injections of AII were made in 50 μl and were flushed in with a further 0.1 ml saline.

Results

Protocol 1: Effects of acute central administration of AT_1 receptor antagonists

Resting values for MAP and HR were not significantly different between the 3 groups of SHR used in the acute studies (Table 1). Central administration of AII (100 ng i.c.v.) to conscious SHR resulted in highly reproducible pressor responses which lasted in excess of 30 min (Figure 1). For example, the fourth response to AII resulted in a significant increase in MAP (1–30 min, maximum = 43 ± 7 mmHg; $P<0.01$, ANOVA). There were no differences between the four responses to i.c.v. AII with respect to the pressor responses. However, following i.c.v. AII there were no significant changes in HR from resting values (−33 ± 25 beat min^{-1}) or between responses ($P>0.05$, ANOVA).

Acute i.c.v. administration of either losartan (10 μg) or CV-11974 (1 μg) in either age group had no effect on resting MAP or HR over a 24 h period following AT_1 antagonist administration ($P>0.05$, ANOVA) (Figure 2).

In both groups of SHR given losartan, the response to AII (100 ng i.c.v.) was abolished ($P<0.01$, ANOVA) when tested 15 min after antagonist administration (Figure 3), but had

Table 1 Resting values for mean arterial blood pressure (MAP) and heart rate (HR) in 3 separate groups of conscious SHR given i.c.v. AT_1 antagonists

	MAP (mmHg)	*HR* (beats min^{-1})
Losartan (10 μg)-Old ($n=8$)	175 ± 7	365 ± 14
Losartan (10 μg)-Young ($n=9$)	158 ± 4	351 ± 10
CV-11974 (1 μg)-Young ($n=6$)	158 ± 7	386 ± 6

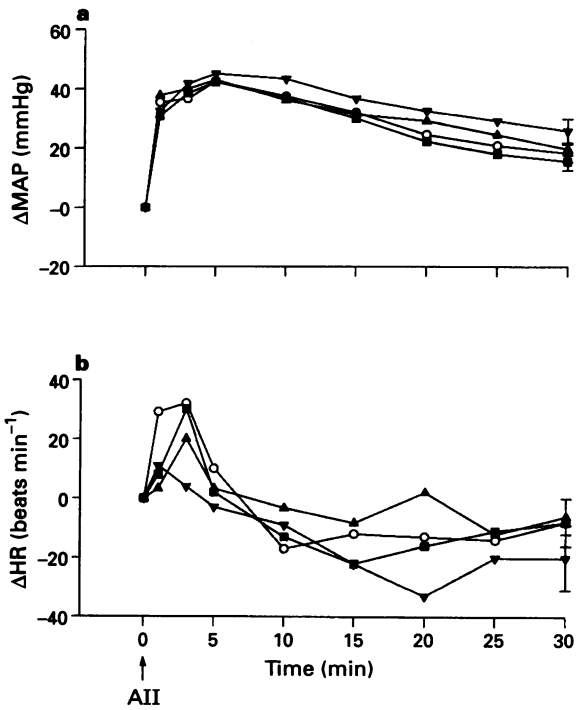

Figure 1 Time control data for changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) in conscious SHR ($n=6$) in response to angiotensin II (AII) (100 ng i.c.v. given at time 0 in figure), tested on 3 separate occasions at 2 h intervals (○, first; ■, second; ▲, third test) on the first experimental day and again (▼, fourth test) on the second experimental day. Values are mean with group s.e.mean shown by vertical lines.

returned to control levels at 3 h. In young SHR treated with CV-11974, the responses to i.c.v. AII (100 ng) were abolished at 15 min and 3 h ($P<0.01$) and still attenuated at 24 h ($P<0.05$, ANOVA) (Figure 3). There were no significant differences in HR responses either before or at the designated times after antagonist administration in all three groups of SHR (data not shown). In addition, there were no significant differences in the changes in MAP and HR in response to i.v. AII either before or 1 h after central AT_1 antagonist administration (Table 2).

Protocol 2: effects of chronic i.c.v. infusion of CV-11974 (1–100 μg kg^{-1} day^{-1})

Resting values for MAP and HR on day 0, ie. 4–8 h after connection of the mini-osmotic pump, were not different between those groups of SHR given chronic i.c.v. infusion of CV-11974 (1–100 μg kg^{-1} day^{-1}) and the SHR treated with vehicle ($P>0.05$, ANOVA) (Figure 4).

Effect on resting blood pressure and heart rate On day 1 (approximately 24 h after commencement of i.c.v. infusion) MAP had fallen slightly, but significantly, in the vehicle-treated SHR ($P<0.05$, ANOVA) which probably reflected full recovery from anaesthesia. Thereafter there were no changes in resting blood pressure in vehicle treated SHR on subsequent days (Figure 4). There were no changes in MAP over the 4 days in SHR treated with either 1 or 5 μg kg^{-1} day^{-1} CV-11974 and these values were not different to those in the vehicle treated group (Figure 4). However, resting MAP in the SHR treated with 100 μg kg^{-1} day^{-1} CV-11974 was significantly lower than the vehicle-treated group on days 1–4 ($P<0.01$, ANOVA) (Figure 4; Table 3). In contrast, there were no differences in resting HR values between the 3 SHR groups treated with CV-11974 (1–100 μg kg^{-1} day^{-1}) and the vehicle group over the four days. However, there were slight decreases on days 1–4 in the CV-11974 (100 μg kg^{-1} day^{-1}) and vehicle-treated SHR compared to their respective day 0 values ($P<0.05$; ANOVA) (Figure 4).

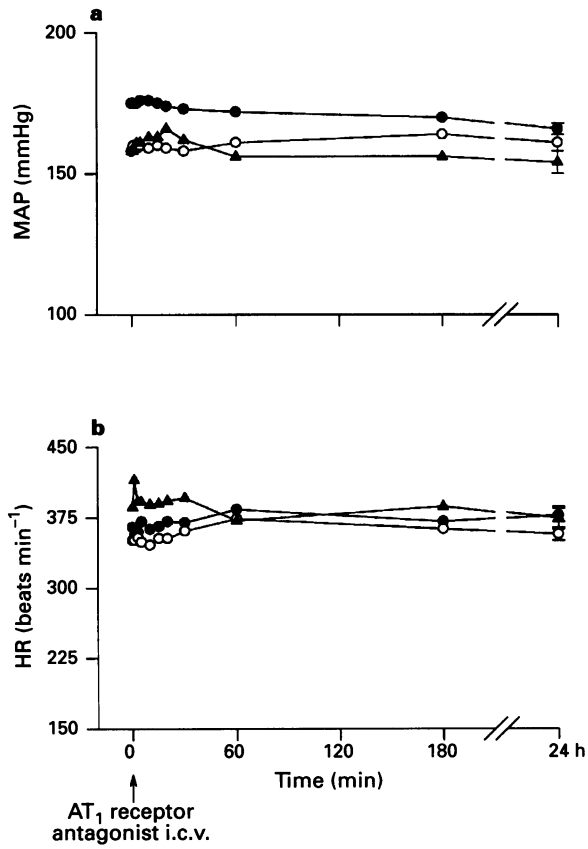

Figure 2 Effect of losartan (10 μg i.c.v.) in young adult SHR (○, 15–18 weeks old, $n=9$) and older SHR (●, 25–29 weeks old, $n=8$) and of CV-11974 (1 μg i.c.v., $n=6$) in (▲) young adult SHR on (a) resting mean arterial pressure (MAP) and (b) heart rate (HR). Values are mean with group s.e.mean shown by vertical lines.

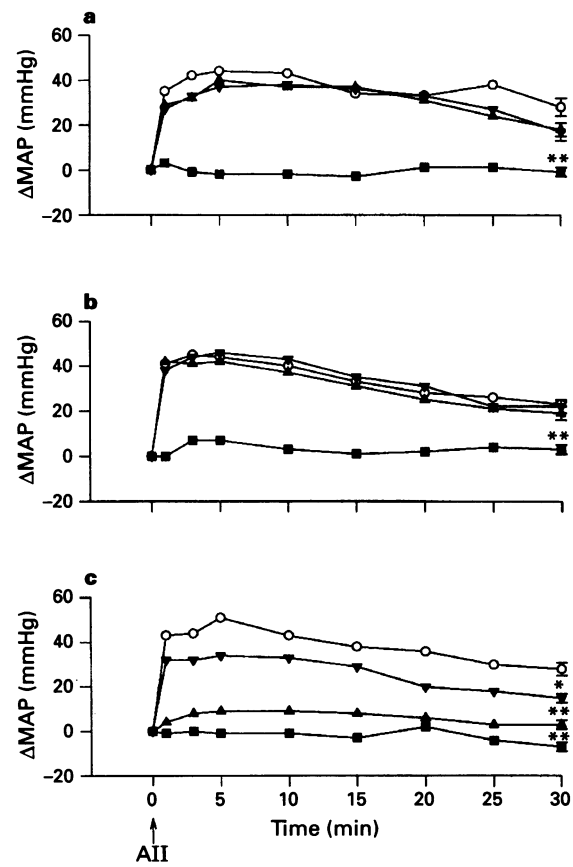

Figure 3 Group data for changes in mean arterial pressure (MAP) in response to angiotensin II (AII) (100 ng i.c.v. given at time 0 in figure) before (○) and 15 min (■), and 3 h (▲) and 24 h (▼) after either losartan (10 μg i.c.v.) in (a) older ($n=8$) and (b) younger ($n=9$) SHR or (c) CV-11974 (1 μg i.c.v.) in younger SHR ($n=6$). Values are mean with group s.e.mean shown by vertical lines. *$P<0.05$; **$P<0.01$ for entire response versus control response (i.e. pre-antagonist) (ANOVA).

Table 2 Changes in mean arterial blood pressure (MAP) and heart rate (HR) in response to i.v. AII (25 ng) before, and 1 h after acute administration of i.c.v. AT_1 antagonists

	ΔMAP (mmHg)		*ΔHR* (beats min^{-1})	
	Pre-antagonist	1 h	Pre-antagonist	1 h
Losartan (10 μg)-Old ($n=6-8$)	44±5	47±5	−39±9	−43±7
Losartan (10 μg)-Young ($n=6-9$)	58±10	43±2	−34±10	−43±14
CV-11974 (1 μg)-Young ($n=3-6$)	32±2	28±4	−17±3	−32±6

Effect on i.c.v. AII-induced pressor responses As expected, reproducible pressor responses to AII (100 ng i.c.v.) were obtained on each of the four days in the vehicle-treated SHR (Figure 5). In SHR treated with the lowest dose of CV-11974 (1 μg kg^{-1} day^{-1}), similar pressor responses to i.c.v. AII were obtained compared to the vehicle-treated SHR on days 1 and 2 however, these were attenuated subsequently on days 3 and 4 compared to the vehicle-treated SHR ($P<0.01$, ANOVA) (Figure 5). In contrast, MAP responses to i.c.v. AII were abolished with respect to vehicle on days 1–4 in SHR treated with either 5 or 100 μg kg^{-1} day^{-1} CV-11974 ($P<0.01$, ANOVA) (Figure 5). Changes in HR evoked by i.c.v. AII were small and variable in the vehicle-treated SHR and were also negligible in each group of SHR treated with CV-11974 (data not shown).

Table 3 Resting values† for mean arterial blood pressure (MAP) and heart rate (HR) in four separate groups of conscious SHR which were treated chronically with either i.c.v. vehicle or CV-11974

	MAP (mmHg)	*HR* (beats min^{-1})
Vehicle ($n=9$)	174±7	351±7
CV-11974 (1 μg kg^{-1} day^{-1}) ($n=4$)	169±14	380±14
CV-11974 (5 μg kg^{-1} day^{-1}) ($n=7$)	170±6	374±15
CV-11974 (100 μg^{-1} kg^{-1} day^{-1}) ($n=8$)	133±6**	348±15

**$P<0.01$ versus vehicle (ANOVA).
†Values are those obtained on day 1, i.e. 24h after commencement of i.c.v. infusions.

Effect of i.v. AII and phenylephrine administration on MAP and HR AII (50 ng i.v.) caused an increase in MAP and corresponding decrease in HR of approximately 50 mmHg and 50 beats min^{-1} respectively on all four days of experimentation (Figure 6). These responses did not vary between SHR chronically infused with i.c.v. vehicle, 1 or 5 μg kg^{-1} day^{-1} CV-11974. However, in the SHR treated with the highest dose of CV-11974 (100 μg kg^{-1} day^{-1}), MAP and HR changes in response to i.v. AII were markedly attenuated on each of the four days compared to the vehicle group ($P<0.01$, ANOVA) (Figure 6).

Injection of phenylephrine (PE, 3 μg i.v.) caused similar MAP increases as AII (50 ng i.v.), however, the corresponding bradycardia was much greater than that evoked by AII. In contrast to the AII data, changes in MAP and HR in response to PE were not different between all four treatment groups on days 1–4, apart from a slight augmentation of the pressor response on day 4 in the 100 μg kg^{-1} day^{-1} CV-11974-treated SHR ($P<0.01$, ANOVA) (Figure 7).

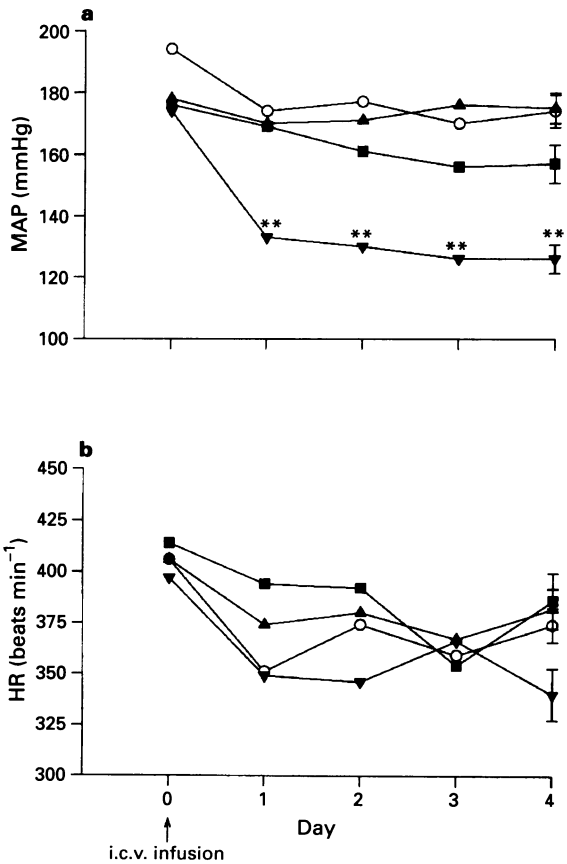

Figure 4 Effect of chronic i.c.v. infusion of vehicle (1 μl h^{-1}, ○, $n=9$) and CV-11974 at 1 (■, $n=4$), 5 (▲, $n=8$) and 100 (▼, $n=8$) μg kg^{-1} day^{-1} in conscious SHR on resting (**a**) mean arterial pressure (MAP) and (**b**) heart rate. Day 0 represents baseline MAP and HR values ~4–8h after connection of osmotic minipump. Values are mean with group s.e.mean shown by vertical lines. **$P<0.01$ versus vehicle (ANOVA).

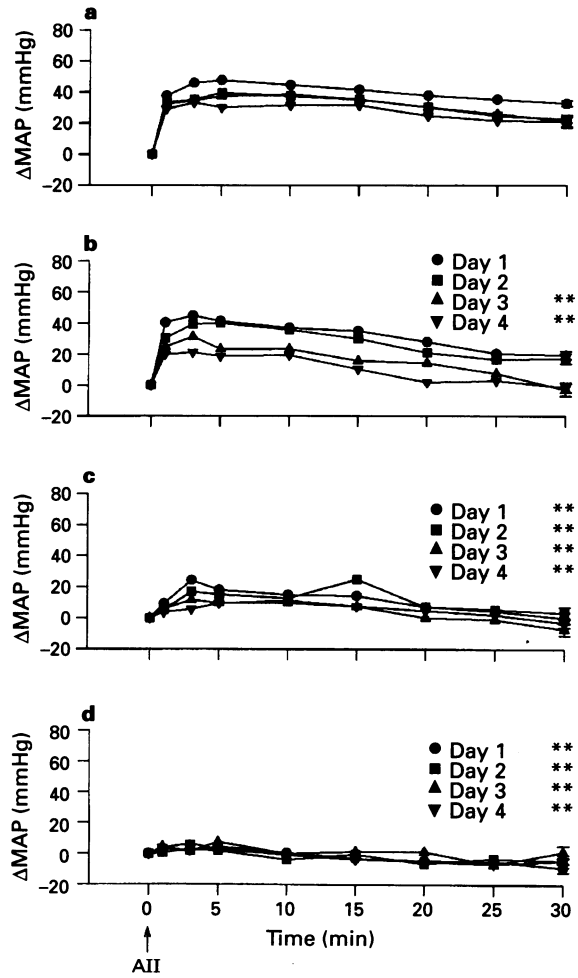

Figure 5 Group data for changes in mean arterial pressure (MAP) in response to angiotensin II (AII) (100 ng i.c.v. given at time 0 in figure), tested 1 (●), 2 (■), 3 (▲) and 4 (▼) days after commencement of i.c.v. infusion of (a) vehicle ($n=9$) and (b) 1 ($n=4$), (c) 5 ($n=8$) and (d) 100 ($n=8$) μg kg^{-1} day^{-1} CV-11974 in conscious SHR. Values are mean with group s.e.mean shown by vertical lines. **$P<0.01$ versus AII response on respective days in vehicle-treated SHR (ANOVA).

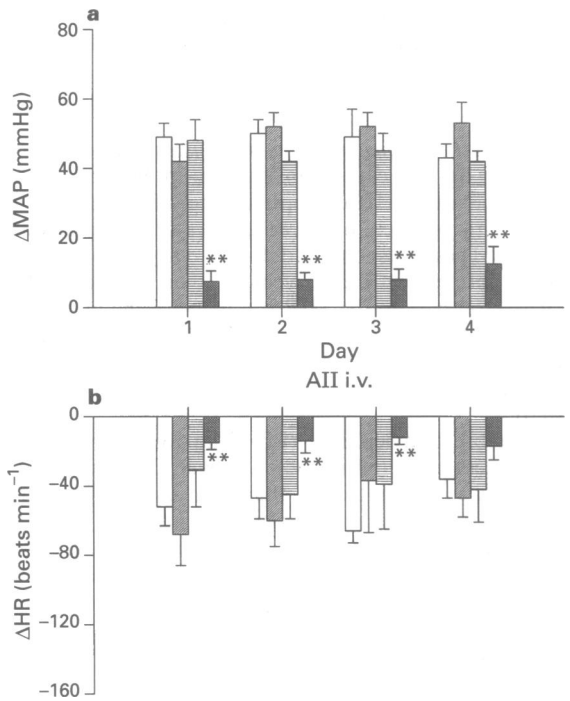

Figure 6 Group data for maximum changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) in response to angiotensin II (AII) (50 ng i.v.) tested on days 1–4 after commencement of i.c.v. infusion of vehicle (open columns, $n=9$) and 1 (diagonally-hatched columns, $n=4$), 5 (horizontal hatched columns, $n=7$) and 100 (solid-hatched columns, $n=8$) $\mu g\,kg^{-1}\,day^{-1}$ CV-11974 in conscious SHR. Values are mean with s.e.mean shown by vertical lines. $^{**}P<0.01$ versus AII on respective days in vehicle-treated SHR (ANOVA).

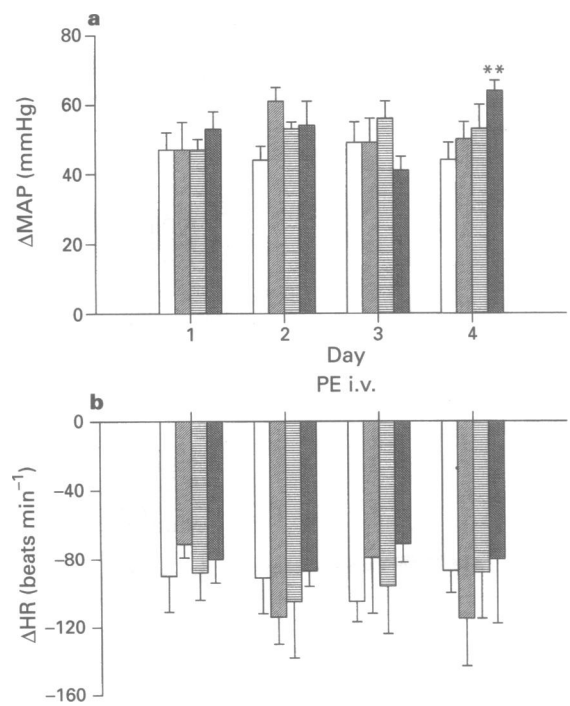

Figure 7 Group data for changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) in response to phenylephrine (PE) (3 μg i.v.) tested on days 1–4 after commencement of i.c.v. infusion of vehicle (open columns, $n=9$) and 1 (diagonally-hatched columns, $n=4$), 5 (horizontal hatched columns, $n=7$) and 100 (solid-hatched columns, $n=8$) $\mu g\,kg^{-1}\,day^{-1}$ CV-11974 in conscious SHR. Values are mean with s.e.mean shown by vertical lines. $^{**}P<0.01$ versus PE on day 4 in vehicle-treated SHR (ANOVA).

Effect of i.c.v. carbachol on MAP and HR Carbachol (5 μg i.c.v.) caused marked pressor and tachycardic responses when tested on day 4 in vehicle-treated SHR. However, it was found that the effect of i.c.v. carbachol in all three groups of SHR treated with CV-11974 (1–100 $\mu g\,kg^{-1}\,day^{-1}$) was similar to the vehicle-treated group (Figure 8).

Effect on baroreflex function Baroreflex function was tested once between days 1 and 3 of protocol in SHR treated with either vehicle or 100 $\mu g\,kg^{-1}\,day^{-1}$ CV-11974. Pressor and depressor responses to phenylephrine and sodium nitroprusside respectively, were obtained, and corresponding HR values were used to construct MAP-HR curves. Chronic i.c.v. infusion of the AT_1 receptor antagonist, CV-11974, caused a leftward shift in the baroreflex curve compared with the vehicle-treated SHR (Figure 9). This resulted in a significant decrease ($P<0.01$, non-paired t test) in the BP_{50} of the 100 $\mu g\,kg^{-1}\,day^{-1}$ CV-11974-treated group which paralleled the fall in resting MAP (Table 4 and Figure 9). Other baroreflex parameters (eg. upper and lower HR plateaus, HR range and average gain) were not significantly altered (Table 4).

Left ventricular weight Left ventricular weights in the vehicle and CV-11974 (100 $\mu g\,kg^{-1}\,day^{-1}$) treated group were 879 ± 39 and 872 ± 29 mg respectively, or when expressed per body weight, 2.89 ± 0.07 and 2.93 ± 0.12 mg g^{-1} respectively, which were not significantly different ($P>0.05$).

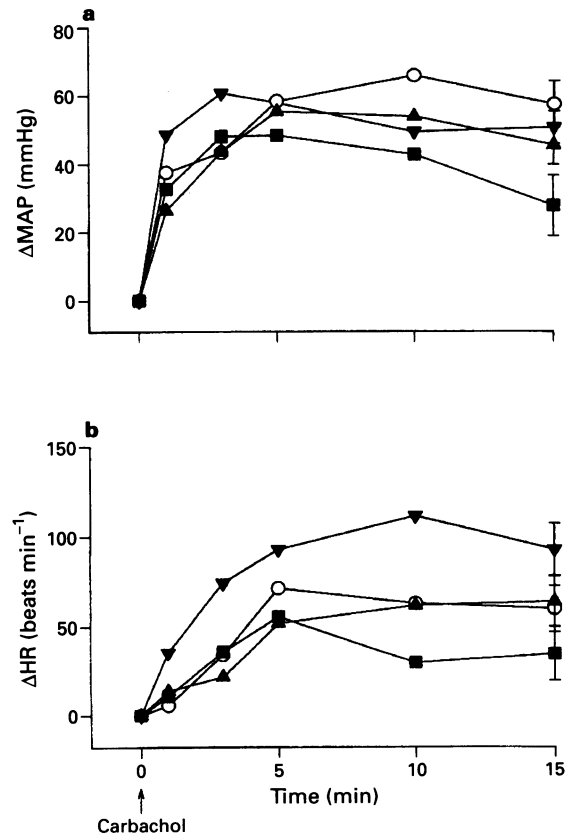

Figure 8 Group data for changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) in response to carbachol (5 μg i.c.v.) tested on day 4 after commencement of i.c.v. infusion of vehicle (○, $n=7$) and 1 (■, $n=3$), 5 (▲, $n=6$) and 100 (▼, $n=7$) $\mu g\,kg^{-1}\,day^{-1}$ CV-11974 in conscious SHR. Values are mean with group s.e.mean shown by vertical lines.

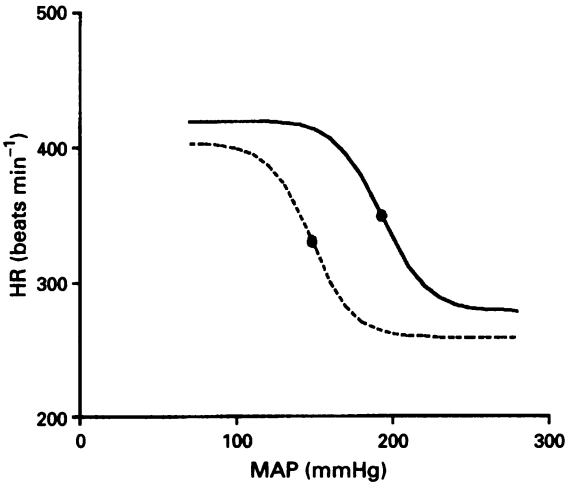

Figure 9 Mean baroreflex mean arterial pressure-heart rate (MAP-HR) curves tested between days 1–3 after commencement of i.c.v. infusion of vehicle (solid line, $n=9$) and 100 $\mu g\,kg^{-1}\,day^{-1}$ CV-11974 (dashed line, $n=8$) in conscious SHR. (●) Resting values.

Table 4 Baroreflex curve parameters for conscious SHR chronically treated with i.c.v. vehicle or CV-11974 (100 $\mu g\ kg^{-1}\ day^{-1}$)

Parameter	*Vehicle* ($n=9$)	*CV-11974* (100 $\mu g\ kg^{-1}\ day^{-1}$) ($n=8$)
Gain (beats min^{-1} $mmHg^{-1}$)	−2.12 ± 0.28	−2.20 ± 0.30
Upper plateau (beats min^{-1})	414 ± 12	406 ± 11
Lower platea (beats min^{-1})	278 ± 12	258 ± 14
BP_{50} (mmHg)	193 ± 6	147 ± 8**
HR range (beats min^{-1})	142 ± 7	148 ± 14

**$P<0.01$ versus vehicle, Gain is the average slope of the MAP-HR curve between the two inflection points, BP_{50} is the MAP at the midpoint of the HR range

Discussion

This study examined the likelihood of a central component of the acute and chronic hypotensive effect of nonpeptide AT_1 AII receptor antagonists. The main findings were that chronic, but not acute, AT_1 receptor antagonists lowered MAP in SHR, although the resultant hypotension following chronic administration may not necessarily involve a central action.

In this study, acute i.c.v. injection of either losartan or CV-11974 in either young or old SHR (15–18 and 25–29 weeks old, respectively, as defined by Pare *et al.*, 1993) failed to lower blood pressure over the following 24 h. In both groups of SHR given losartan, the MAP and HR responses to i.c.v. AII given fifteen minutes after antagonist injection were abolished; however, AII-evoked pressor responses had returned to control values three hours later. The time course of central AT_1 receptor blockade was very similar to our previous observations in normotensive rats (Widdop *et al.*, 1993a,b). In the SHR given i.c.v. CV-11974, the MAP and HR responses to i.c.v. AII were not only abolished at fifteen minutes and 3 h after antagonist injection, but were also slightly, but significantly, reduced 24 h later. These data are consistent with the greater potency of CV-11974 over losartan (Shibouta *et al.*, 1993). However, the responses to AII given i.v. both before and 1 h after either antagonist were not different. Therefore despite a functional blockade of central AT_1 receptors by the AT_1 receptor antagonists used in all three groups of SHR, there was no change in resting MAP or HR.

These data correlate with those of DePasquale *et al.* (1992), who also demonstrated that, immediately following i.c.v. losartan injections, responses to i.c.v. AII were abolished but the responses to i.v. AII were unaffected in SHR. No changes in MAP or HR were observed up to 48 h after giving losartan. However, they did report a transient increase in MAP immediately following losartan administration which was not observed in the present study. In contrast, Pare *et al.* (1993) have shown that following acute i.c.v. administration of the AT_1 receptor antagonists, losartan and L 158809, MAP fell significantly 18 h after antagonist administration in young but not old SHR, and that this hypotensive effect persisted for several days. Moreover, they found that EXP 3174, the active metabolite of losartan, and the AT_2 receptor antagonist, PD 123319, had no hypotensive properties when given centrally, and concluded that there may in fact be a third AII receptor subtype in the brain which is insensitive to both AT_1 and AT_2 receptor antagonists but is recognised by losartan. These authors also hypothesized that, due to a lack of effect of EXP 3174, losartan may be metabolized to some other active metabolite in the brain. Unfortunately, considering losartan decreased MAP, Pare *et al.* (1993) did not administer AII i.v. to test for a possible peripheral AT_1 receptor blockade. Moreover, it was stated that the central response to AII was not antagonized by losartan. This is not surprising since the dose of AII used (20 μg) was approximately 200–500 times that used in the present and previous studies (Kirby *et al.*, 1992; Depasquale *et al.*, 1992; Widdop *et al.*, 1993a,b) and would most likely displace losartan from AT_1 receptor sites. In addition, the pressor response evoked by i.c.v. AII was much larger in WKY compared with SHR (Pare *et al.*, 1993), which contrasts with most other studies which documented a heightened centrally-mediated pressor effect of AII in SHR (see Unger *et al* 1988). This may point to a difference in the strain of SHR used by Pare *et al.* (1993). Alternatively, losartan may have reached AII selective sites which were not accessed in other studies. In this context, it has been shown that microinjections of losartan into the anterior, but not posterior, hypothalamus decreased MAP over a 1 h period immediately following administration in salt-sensitive SHR (Yang *et al.*, 1992). However, these results are yet to be confirmed. More recently, Gyurko *et al.* (1993) have observed an antihypertensive effect in SHR following central administration of antisense oligodeoxynucleotides to the AT_1 receptor, although no data were presented on the time course of onset or duration of this hypotension.

Thus, the available evidence is inconclusive as to whether or not AT_1 receptor antagonists unequivocally lower MAP following acute central administration. In the present study, in doses which were sufficient to abolish the centrally-mediated pressor effects of AII, the acute i.c.v. injections of losartan and CV-11974 did not alter resting MAP or HR.

Recently, Kamitani *et al.* (1994) demonstrated that CV-11974 (1–100 $\mu g\ kg^{-1}\ day^{-1}$) infused centrally decreased MAP in SHR. These authors hypothesized that the hypotension seen following chronic i.c.v. CV-11974 administration is a centrally-mediated effect, on the basis of two observations. Firstly, they saw significant falls in MAP following 2-day i.c.v. infusions at a dose (1 $\mu g\ kg^{-1}\ day^{-1}$) which had no effect on MAP when given i.v. for the same period. Secondly, both 4-day i.v. and i.c.v. infusion of CV-11974 (100 $\mu g\ kg^{-1}\ day^{-1}$) significantly lowered MAP in both groups of SHR. However, following bilateral nephrectomy, MAP returned to normal in the i.v. but not centrally, treated group.

In contrast, our study showed different results with respect to both of these points. We found that the lowest dose of CV-11974 (1 $\mu g\ kg^{-1}\ day^{-1}$), which was infused i.c.v. for 4 days failed to lower MAP over this time. Indeed, after the first 2 days of i.c.v. infusion, there was no evidence of central AT_1 receptor blockade on the basis of i.c.v. AII-induced pressor responses. By days 3 and 4, there was only a modest attenuation of i.c.v. AII responses but with no concomitant hypotension. In those SHR which received the highest dose of

CV-11974 (100 $\mu g\ kg^{-1}\ day^{-1}$), there was in fact a reduction in resting MAP. However, in addition to achieving a continuous abolition of i.c.v. AII-induced MAP and HR responses, we also observed chronic blockade of the effects of peripherally administered (i.v.) AII. This observation suggests that, at the dose used, there was peripheral leakage of CV-11974 during the central infusion. Thus, the present results clearly indicate that there was a substantial peripheral blockade of vascular AT_1 receptors following central infusion of CV-11974. As expected, the pressor response evoked by i.v. phenylephrine was unaffected in the same SHR. Kamatani *et al.* (1994) did not check for central or peripheral AT_1 receptor blockade. Instead, their evidence was based on the fact that the same i.c.v. dose of CV-11974 as used here caused hypotension which was not reversed following nephrectomy, performed on day 2 of the chronic CV-11974 infusion. On the other hand, the CV-11974-induced hypotension following i.v. infusion was in fact reversed following nephrectomy.

Nephrectomy is well known to abolish the antihypertensive effect of AT_1 receptor antagonists administered i.v., thus indicating that kidney-derived renin is important for their action (Wong *et al.*, 1990). It is important to realise that MAP is well maintained following nephrectomy because other vasoconstrictor mechanisms compensate for the loss of circulating renin. The central effect of AII is mediated via an increase in sympathetic nerve activity and vasopressin release (Reid, 1984; Unger *et al.*, 1988). Therefore, in the study by Kamitani *et al.* (1994), it is possible that any compensatory increase in sympathetic drive following kidney removal was inhibited during the chronic i.c.v. infusion of CV-11974 so that blood pressure remained depressed following nephrectomy. In contrast, peripheral AT_1 receptor blockade would be less likely to interfere with centrally mediated compensatory mechanisms, resulting in restoration of blood pressure after nephrectomy. Unfortunately, Kamitani *et al.* (1994) did not provide any data concerning responses to both i.c.v. and i.v. AII prior to bilateral nephrectomy, at a time when both i.c.v. and i.v. infusions of CV-11974 lowered MAP by approximately the same amount (ie. 40 mmHg). Considering our data demonstrated inhibition of peripheral AII responses during chronic central infusion of CV-11974 (at the same dose used by Kamitani *et al.* in the nephrectomy experiments), it is difficult to attribute the hypotensive effect of CV-11974 to a central effect.

Further evidence to support our claim can be seen in experiments where we felt it was necessary to choose a dose of the AT_1 receptor antagonist which would abolish the central, but not the peripheral, actions of AII. This was achieved by using 5 $\mu g\ kg^{-1}\ day^{-1}$ CV-11974; however, this also failed to lower MAP. Thus, our data strongly indicate that central AT_1 receptors are not involved in tonic regulation of MAP in conscious SHR, at least when CV-11974 was chronically infused. It is difficult to reconcile these results with those of Kamitani *et al.* (1994). However, as previously discussed, it is conceivable that in their study, removal of both kidneys caused a compensatory increase in brain RAS activity in SHR which in turn unmasked a central inhibitory effect of CV-11974, albeit under somewhat nonphysiological conditions.

Previous studies in which the effects of chronic central infusions of peptide AII receptor antagonists were examined have also yielded conflicting results. McDonald *et al.* (1980) claimed that chronic i.c.v. infusion of the peptide AII receptor antagonist sarile lowered MAP in SHR. However, others have shown that chronic central administration of the peptide AII antagonist sarthran had no antihypertensive effect even after five days treatment in SHR (Bruner *et al.*, 1987).

In the present study, another central pressor agent, carbachol, was used to examine the specificity of CV-11974. Unlike the complete abolition of the response to i.c.v AII, chronic central infusion of CV-11974 at the highest dose of 100 $\mu g\ kg^{-1}\ day^{-1}$ had no effect on the pressor or tachycardic response to i.c.v. carbachol. These data contrast with those of Gruber *et al.* (1992) who found that peptide AII receptor antagonists significantly inhibited (by approximately 70%) the pressor response to i.c.v. carbachol. From their observations they speculated that there is a central angiotensinergic pathway regulating sympathetic outflow and hence any responses to drugs with central pressor activity would be blocked by an AII receptor antagonist. While the same dose of carbachol (5 μg) was used in both studies, Gruber *et al.* (1992) achieved maximum pressor responses (approximately 30 mmHg) in normotensive rats which were about half that seen from our work with SHR. Therefore, it is possible a strain difference could account for this discrepancy. In any case, in the present study, CV-11974 caused a specific blockade of AII responses only.

It has been shown that central administration of both ACE inhibitors and peptide AII receptor antagonists enhance baroreflex function (Buñag *et al.*, 1990; Berecek *et al.*, 1991); however, the effects of AT_1 receptor antagonists on baroreflex have been less studied. Bartholomeusz and Widdop (1995) showed that acute central injections of the AT_1 receptor antagonists, EXP 3174, had no effect on resting MAP or baroreflex function. In contrast, subcutaneous treatment with the same drug for fifteen days lowered MAP and normalised baroreflex function (i.e. enhanced the vagal component of heart rate range) in conscious SHR to levels observed in normotensive WKY rats. Considering the inhibitory nature of AII on baroreflex, and the fact that in SHR this reflex is impaired, the effect of chronic central AT_1 receptor blockade by CV-11974 on baroreflex function was also examined.

Baroreflex testing was performed only in the SHR chronically treated with the highest dose of CV-11974 (100 $\mu g\ kg^{-1}\ day^{-1}$) since it became clear that there were no changes in resting MAP in the other treatment groups. There was a significant decrease in BP_{50} in the CV-11974 versus the vehicle-treated group, which paralleled the fall in the resting MAP. However, upper and lower HR plateaus, range and gain of the baroreflex curve were all unaffected in the CV-11974-treated group. This indicates that reflex changes in HR due to MAP variations were not different between the two groups but, rather, baroreflex activity had been reset to a lower pressure threshold. We did not test baroreflex function in SHR given the dose of CV-11974 (5 $\mu g\ kg^{-1}\ day^{-1}$) which only blocked central AII responses. It is conceivable that this dose may have affected baroreflex curve parameters, although this would seem unlikely for several reasons. Firstly, the high dose of CV-11974 shifted the baroreflex curve in parallel with the reduction in MAP but did not alter baroreflex curve parameters. Secondly, it has previously been demonstrated that acute blockade of central AT_1 receptors in SHR also failed to modify baroreflex function (Bartholomeusz & Widdop, 1995).

Interestingly, chronic, systemic treatment with the AT_1 receptor antagonist, EXP 3174, normalised baroreflex function in SHR which usually exhibit a deficit in the bradycardic arm of the reflex (Bartholomeusz & Widdop, 1995). These data are consistent with the study by Minami & Head (1993) who showed similar results when using chronic, oral treatment with an ACE inhibitor. However, in the present study, we found no evidence of vagal enhancement of baroreflex function, although the baroreflex curve was reset (i.e. left-shifted) by a similar magnitude as that observed with subcutaneous EXP 3174 treatment (Bartholomeusz & Widdop, 1995). One possible reason for this discrepancy could be the role of cardiac hypertrophy in baroreflex function, since restoration of baroreflex function in SHR following ACE inhibition was correlated with the regression of cardiac hypertrophy (Minami & Head, 1993). It has also been suggested that in hypertensive models with reversed hypertension, the recovery of baroreflex is slower than the fall in MAP, and instead parallels a decrease in cardiac hypertrophy (Edmunds *et al.*, 1990). In the present study, there was no difference in left ventricular weight between the CV-11974- and vehicle-treated SHR, which could, in part, explain the similar baroreflex parameters in the 2 groups of SHR. Given that we have observed peripheral leakage of CV-11974 during central infusion, it is likely that an extended period of time without AII would be necessary before any

changes in baroreflex parameters (e.g. increased heart rate range) would be observed. However, this awaits further investigation.

In conclusion, we have shown that acute or chronic AT_1 receptor antagonism, in doses which only blocked the central pressor actions of AII, did not reduce resting MAP in conscious SHR. Larger doses of CV-11974 chronically infused i.c.v. did in fact lower MAP. However, this was accompanied by marked inhibition of both the central and peripheral effects of AII, the latter of which is likely to have contributed to the hypotensive action of CV-11974. Additionally, at this time, baroreflex activity was reset in parallel with the fall in MAP. Collectively, these results do not provide any evidence for a modulatory influence of brain RAS in tonic cardiovascular control in SHR.

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