British Journal of Pharmacology (1998) 125, 1429–1436
http://www.stockton-press.co.uk/bjp

Modulation of AT_1 receptor-mediated contraction of rat uterine artery by AT_2 receptors

[1]Adam S. Zwart, [1]Elizabeth A. Davis & [1,2]Robert E. Widdop

[1]Department of Pharmacology, Monash University, Clayton, Victoria 3168, Australia

1 The aim of this study was to characterize the angiotensin II receptors in isolated uterine arteries from non pregnant and pregnant rats, since it has been reported from binding studies that ovine uterine arteries contain AT_2 receptors.

2 Uterine arterial segments were obtained from virgin, non-pregnant and late pregnant (18–21 days) Sprague-Dawley rats and mounted in small vessel myographs. Concentration-response curves were constructed to angiotensin II (1 nM–10 μM) in the absence and presence of various angiotensin II receptor subtype selective compounds. These included losartan (AT_1 antagonist; 1, 10 and 100 nM), PD 123319 (AT_2 antagonist; 1 μM) and CGP 42112 (AT_2 agonist; 1 μM). Responses to angiotensin II were measured as increases in force (mN) and expressed as a per cent of the response to a K^+ depolarizing solution.

3 Losartan (1, 10 and 100 nM) caused significant concentration-dependent rightward shifts of the angiotensin II concentration-response curve in uterine arteries from non-pregnant and pregnant rats. The pA_2 values calculated from these data were 9.8 and 9.2, respectively, although the slope of the Schild plot in the non-pregnant group was less than unity.

4 PD 123319 (1 μM) caused significant 6- and 3 fold leftward shifts of the angiotensin II concentration-response curve in uterine arteries from non-pregnant and pregnant rats, respectively. In vessels from pregnant rats, PD 123319 also significantly increased the maximum response to angiotensin II.

5 CGP 42112 (1 μM) attenuated the response to angiotensin II of uterine arteries from non-pregnant rats. This was reflected by a 14 fold rightward shift of the angiotensin II concentration-response curve and a decrease in the maximum response. In uterine arteries from pregnant rats, CGP 42112 (1 μM) caused a 3 fold rightward shift of the angiotensin II concentration-response curve, but had no effect on the maximum response.

6 PD 123319 (1 μM) and CGP 42112 (1 μM) had no effect on the concentration-response curves to phenylephrine (PE) of uterine arteries from non-pregnant or pregnant rats. In addition, CGP 42112 (1 nM–1 mM) had no vasodilator effect on tissues precontracted with phenylephrine.

7 These results suggest that the contractile responses of the rat uterine artery are mediated by the AT_1 receptor. Furthermore, in this vascular preparation, the AT_2 receptor appears to inhibit the response mediated by the AT_1 receptor, although, this is not uniform between the non-pregnant and pregnant states.

Keywords: AT_2 receptor; AT_1 receptor; rat uterine artery; angiotensin II; pregnancy; PD123319; CGP 42112; losartan

Introduction

The renin-angiotensin system (RAS) is a complex system that plays an important role in blood pressure maintenance and volume homeostasis (Timmermans *et al*., 1992b). Currently, there are two main receptor subtypes, AT_1 and AT_2, which have been well described and to which angiotensin II displays similar affinity (de Gasparo *et al*., 1995).

AT_1 and AT_2 receptors exhibit a widespread central and peripheral distribution, although AT_1 receptors are responsible for most of the well known cardiovascular actions of angiotensin II (Guthrie, 1995). By contrast, the function of the AT_2 receptor is less well understood but it has been implicated in foetal growth (Cook *et al*., 1991) and in healing and repair (Timmermans *et al*., 1992a). However, there is increasing evidence that the AT_2 receptor may exert an inhibitory influence on AT_1 receptor-mediated stimulation (Widdop *et al*., 1994; Stoll *et al*., 1995a). For example, AT_2 receptor blockade disinhibited AT_1 receptor-mediated responses, including centrally-evoked drinking responses (Widdop *et al*., 1994) and endothelial cell proliferation (Stoll *et al*., 1995b), while an AT_2 receptor-mediated depressor response has also been implicated (Scheuer & Perrone, 1993; Munzenmaier & Greene, 1996).

In this context, a recent preliminary study suggested that the AT_2 receptor may exert a modulatory role on AT_1-receptor-mediated contraction of the ovine uterine artery (McMullen *et al*., 1996). These authors found that the contractile response to a single concentration of angiotensin II (1 μM) was enhanced in the presence of the AT_2 receptor antagonist, PD 123319 (1 μM). From these data, it was inferred that the AT_2 receptor may be inhibiting the contractile response mediated by the AT_1 receptor (McMullen *et al*., 1996). Interestingly, the uterine artery is one of the few vascular sites reported to contain AT_2 receptors (Cox *et al*., 1996a,b; Burrell & Lumbers, 1997); others include the ovine mammary artery (Cox *et al*., 1996a) and rat cerebral artery (Tsutumi & Saavedra, 1991) which contrasts with predominant AT_1 receptor subtype in most other systemic vasculature (Zhou *et al*., 1995; Cox *et al*., 1996a).

Therefore, the first aim of the present study was to examine the functional responses to angiotensin II in the rat uterine artery to confirm the preliminary results from studies using ovine uterine artery which demonstrated a functional interaction between AT_2 and AT_1 receptors, albeit using only one concentration of angiotensin II (McMullen *et al*., 1996).

[2]Author for correspondence.

During pregnancy, the uteroplacental circulation is even more refractory to the contractile responses to angiotensin II than the rest of the systemic circulation (Abdul-Karim & Assali, 1961; Naden & Rosenfeld, 1981; Rosenfeld-Naden, 1989). While the mechanisms for this effect is not fully understood, a recent study has shown that during pregnancy there was a marked increase in AT_2 receptor density in ovine uterine arteries (Burrell & Lumbers, 1997). By contrast, Cox *et al*. (1996a,b) reported no pregnancy-related changes in AT_2 binding in uterine arteries from women and sheep, although there was no assessment of angiotensin II reactivity in those tissues. Therefore, given the functional interaction between AT_2 and AT_1 receptors reported to occur in ovine uterine arteries (McMullen *et al*., 1996), the second aim of this study was to examine, at least in the rat uterine artery, if the attenuation of angiotensin II-mediated contraction during pregnancy was due, in part, to the increased inhibitory influence of the AT_2 receptor.

Methods

General procedures

Female Sprague-Dawley rats (weighing 200–250 g, virgin, non-pregnant and 350–400 g, late pregnant) were housed in standard rat cages at $21 \pm 3^{\circ}C$, with a 12 h light/dark cycle. Food and water were available *ad libitum*. The late pregnant rats were used on day 18–21 of the 22 day gestation period.

Animals were killed by stunning and exsanguination. In the case of the pregnant rats, the pups were quickly decapitated. The uterine horns, with their attached vasculature, were removed *en bloc* and placed immediately in ice-cold, oxygenated physiological salt solution (PSS; composition in mM: NaCl 119, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, ethylene-diaminetetracetic acid (EDTA) 0.027 and glucose 5.5).

Small sections of uterine artery containing endothelium were dissected free from surrounding connective tissue under a binocular dissection light microscope (Olympus SZ40) and set up in small vessel myographs as described previously (McPherson, 1992). Briefly, two 40 μm wires were threaded though the lumen of the vessel segment. One wire was attached to a stationary support driven by a micrometer, while the other was attached to an isometric force transducer. Data was recorded on a printer (Panasonic KX-P1180) and captured by the use of a data acquisition system (CVMS Version 2.0, World Precision Instruments, U.S.A.). The vessel segments were maintained in PSS at 37°C and aerated continuously with carbogen (95% O_2, 5% CO_2).

Following a 30 min equilibration period at 37°C and under zero force, the diameter of each vessel was normalized to an equivalent transmural pressure of 100 mmHg. Using the diameter of the vessel, calculated from the distance between the two mounting wires, a passive diameter-tension curve was constructed as described (Mulvany & Halpern, 1977; see McPherson, 1992 for details). From this curve the effective transmural pressure was calculated. The vessels were then set at tension equivalent to that generated at 0.7 times the diameter of the vessel at 100 mmHg (D100), which has been found previously to be optimal for active tension development in this vessel (Davis, 1994).

The tissues were equilibrated for 30 min after normalization before being contracted with a K^+ depolarizing solution (KPSS; composition in mM: KCl 123.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.027 and glucose 5.5). Once the response to KPSS had plateaued, the preparations were washed 3–4 times and rested for 20 min before an experimental protocol was commenced. In addition, a second KPSS response was performed at the completion of CR curves to check that antagonist treatment did not affect tissue reactivity in a nonspecific manner.

Effects of AT_1 and AT_2 compounds on contractile responses to angiotensin II

Cumulative (0.5 log unit) concentration-response (CR) curves were constructed to angiotensin II (0.1 nM–10 μM) in the absence of any ligand or after 30 min equilibration with either losartan (1, 10 and 100 nM), PD 123319 (1 μM) or CGP 42112 (1 μM). Only one CR curve was obtained per tissue, since preliminary studies had shown marked tachyphylaxis developed if a second CR curve to II was constructed (data not shown).

Effects of AT_2 compounds on contractile responses to phenylephrine

Cumulative (0.5 log unit) CR curves were constructed to phenylephrine (1 nM–0.1 mM) in the absence of any ligand or after 30 min equilibration with either PD 123319 (1 μM) or CGP 42112 (1 μM). Again, only one CR curve was obtained per tissue.

Effect of CGP 42112 on precontracted uterine arteries

In order to test for any direct vasodilator activity of CGP 42112, vessels were precontracted to 30–40% of their maximum contraction to KPSS with titrated concentrations of phenylephrine. When this response had plateaued, a cumulative (0.5 log unit) CR curve to CGP 42112 (1 nM–1 μM) was constructed. Spontaneous relaxation was measured in separate tissues which were similarly contracted with phenylephrine but not exposed to CGP 42112. At the completion of the CR curve (or at an equivalent time point), the maximum relaxation to acetylcholine (0.1 mM) was obtained.

Statistical analysis

Responses to contractile agents were expressed as a percentage of the KPSS-induced contraction. Log CR curves from individual tissues were grouped to generate mean log CR curves to angiotensin II. The effects of the various ligands on the responses to angiotensin II were analysed by comparing the CR curves to angiotensin II in the absence and presence of each ligand. CR curves were compared using linear regression analyses of the linear portion of the curves. Pairs of lines were tested for parallelism and coincidence, and the potency ratios with the 95% confidence limits (CL) was determined. These statistical procedures were carried out using a computer program based on procedures described in Documentia Geigy (Diem, 1970). From these data, pA_2 values for losartan were determined according to the method of Arunlakshana & Schild (1959). The effects of AT_2 compounds on the maximum response to angiotensin II were assessed using Student's unpaired *t*-test.

Relaxation responses to CGP 42112 were expressed as a percentage relaxation of the phenylephrine-induced precontraction (100%). The CR curves to CGP 42112 were then compared with the spontaneous relaxation of the phenylephrine-induced tone (over the same time period) using a two-

way analysis of variance (ANOVA) with repeated measures with a *post hoc* Newman-Keuls pairwise comparison test (Sigmastat, Jandel Scientific, U.S.A.). Data are presented as means ±s.e.mean. In all cases, statistical significance was accepted if $P<0.05$.

Drugs

Drugs and their sources were: acetylcholine HCl (Sigma), angiotensin II (American Peptide Co., U.S.A.), CGP 42112 (Novartis, Switzerland), losartan (DuPont Pharmaceuticals), PD 123319 (Parke Davis, U.S.A.) and phenylephrine HCl (Sigma). Stock solutions of all drugs were made up and diluted in distilled water.

Results

As expected, the diameter (D100) of the uterine arteries taken from the late pregnant rats (567±20 μm, $n=15$) was significantly greater than that of the vessels taken from non pregnant rats (391±7 μm, $n=12$; $P<0.05$, *t*-test). The KPSS-induced contraction of vessels from gravid animals (5.83±0.25 mN mm^{-1} wall length, $n=15$) was also greater than that of vessels from non gravid animals (4.75±0.40 mN mm^{-1}, $n=12$; $P<0.05$, *t*-test). When KPSS was repeated at completion of experiment, there was a small increase (~15%) in response compared with the first exposure, however, this effect was identical in tissues pretreated with either AT_1 or AT_2 compounds.

Effect of losartan on angiotensin II responses

Losartan (1, 10 and 100 nM), which did not affect resting baseline, caused parallel rightward shifts of the angiotensin II CR curves in uterine arteries from both non pregnant and pregnant rats, with no significant change in the maximum response achieved (Figure 1). From Schild plots of these data, the pooled pA_2 values for losartan were calculated to be 9.8 and 9.2 in uterine arteries from non-pregnant and pregnant rats, respectively. The slopes of the Schild plots obtained using data from the pregnant and non-pregnant rats were 1.01 and 0.78, respectively. While this is indicative of a conventional competitive antagonist in the former case, linear regression analysis revealed that the slope of the corresponding line from the non-pregnant group was significantly different ($P=0.019$), which may have led to an overestimation of the pA_2 value.

Effect of PD 123319 on angiotensin II and phenylephrine responses

In tissues from non-pregnant and pregnant rats, PD 123319 (1 μM) did not affect resting baseline but caused significant 6.1- (95% Confidence Limits: 12.2, 3.5) and 3.2- (95% Confidence Limits: 6.6, 1.7) fold parallel leftward shifts of the angiotensin II concentration-response curve, respectively (Figure 2). PD 123319 had no significant effect on the maximum response to angiotensin II of uterine arteries from non-pregnant rats (Figure 2). In contrast, in vessels from pregnant rats, the maximum response to angiotensin II increased significantly from 86.5% of the KPSS response in the absence of PD 123319 to 116.7±3.9% of the KPSS response in the presence of PD 123319 ($P<0.05$, *t*-test; Figure 2). Phenylephrine caused concentration-dependent contractile responses in uterine arteries from non-pregnant (Figure 3A) and pregnant (Figure 3B) rats. PD 123319 (1 μM) had no significant effect on the concentration response curves to phenylephrine obtained from either group of rats ($P>0.05$, ANOVA).

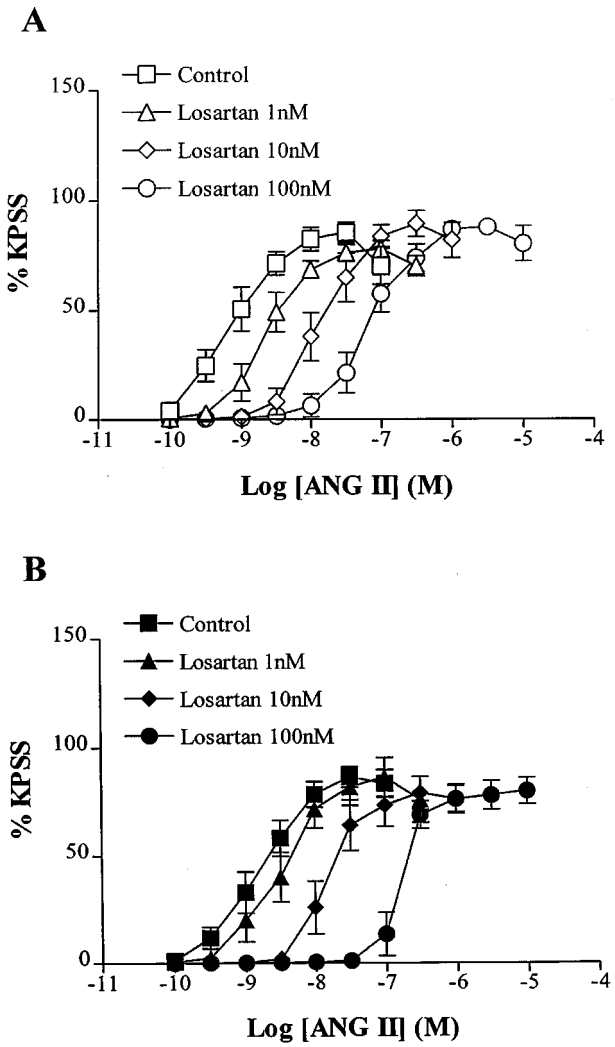

Figure 1 Effect of losartan (1, 10 and 100 nM) on the mean log concentration-response curves for angiotensin II in uterine arteries from (A) non-pregnant ($n=6-12$) and (B) late pregnant ($n=7-15$) rats. Responses are expressed as a per cent of the maximum response to KPSS; values are means and vertical lines show s.e.mean; n=number of vessels.

Effect of CGP 42112 on angiotensin II and phenylephrine responses

In uterine arteries from both non-pregnant and pregnant rats, CGP 42112 (1 μM) did not affect resting baseline but caused significant rightward shifts of the angiotensin II concentration-response curve (Figure 4). In tissues from non-pregnant rats CGP 42112 caused a 14.3- (95% Confidence Limits: 7.7, 25.0) fold rightward shift, while in preparations from pregnant rats, a 2.9- (95% Confidence Limits: 1.4, 5.9) fold parallel rightward shift was observed. In the tissues from non-pregnant rats, CGP 42112 significantly decreased the maximum response from 85.5±4.6% to 54.5±10.6%, of the KPSS response ($P<0.05$, *t*-test) (Figure 4A). In tissues from pregnant rats, however, CGP 42112 had no effect on the maximum response ($P>0.05$, *t*-test) (Figure 4B).

CGP 42112 (1 μM) had no significant effect on the phenylephrine concentration response curve obtained from uterine arteries from either group ($P>0.05$, ANOVA) (Figure 5).

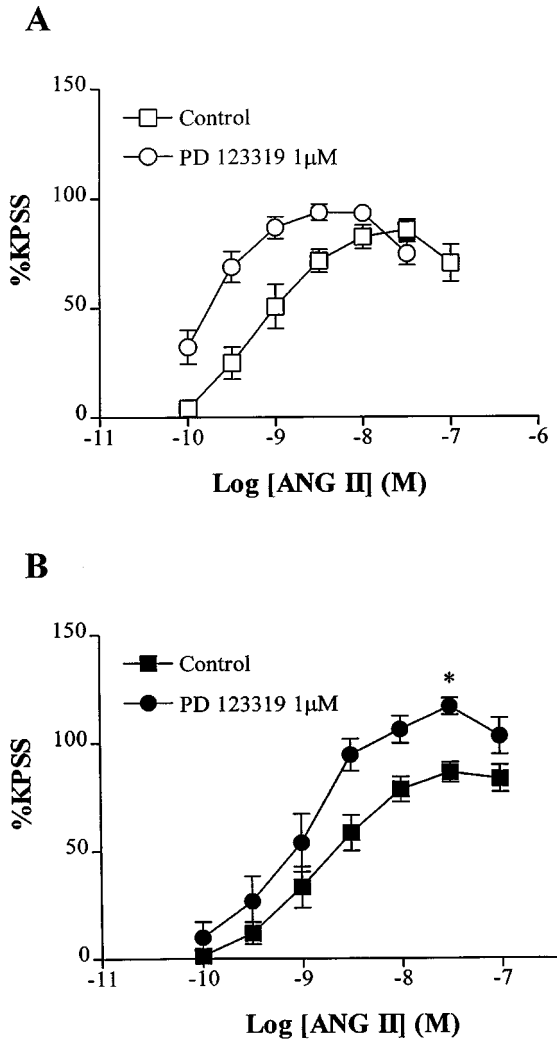

Figure 2 Effect of PD 123319 (1 μM) on the mean log concentration-response curves to angiotensin II in uterine arteries from (A) non-pregnant ($n=7-12$) and (B) late pregnant ($n=10-15$) rats. Responses are expressed as a per cent of the maximum response to KPSS; values are means and vertical lines show s.e.mean; $n=$number of vessels. $^*P<0.05$ for maximum response versus control.

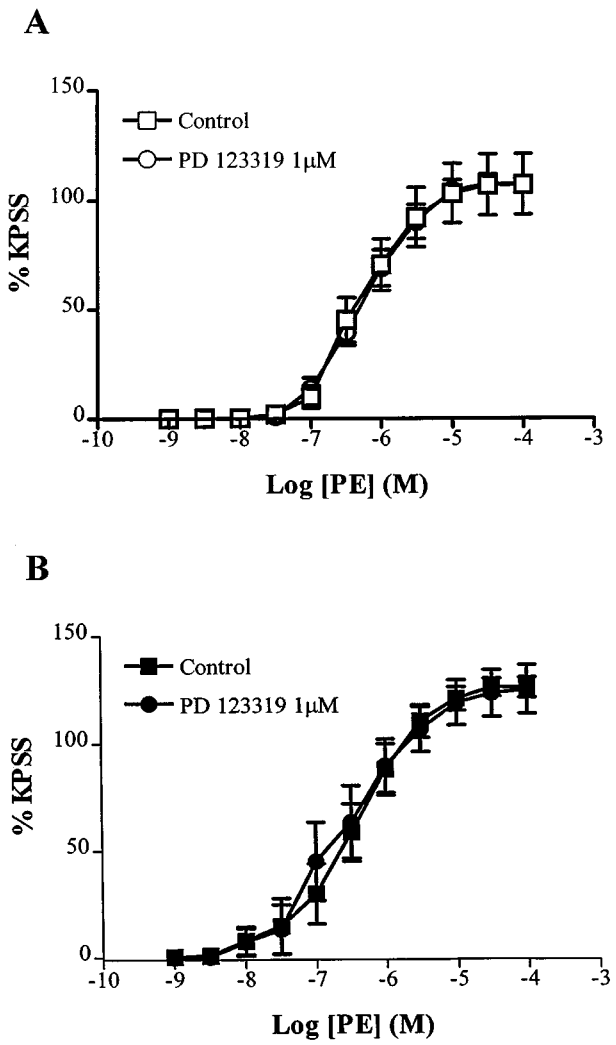

Figure 3 Effect of PD 123319 (1 μM) on the mean log concentration-response curves to phenylephrine (PE) in uterine arteries from (A) non-pregnant ($n=6-12$) and (B) late pregnant ($n=6$) rats. Responses are expressed as a per cent of the maximum response to KPSS; values are means and vertical lines show s.e.mean; $n=$number of vessels.

Effects of CGP 42112 on preparations precontracted with phenylephrine

The effect of CGP 42112 (1 nM – 1 μM) added to precontracted uterine arteries from non-pregnant and late pregnant rats was not significantly different from the spontaneous relaxation which occurred in the corresponding time control vessels ($P>0.05$, ANOVA; Figure 6). The presence of a functional endothelium, however, was indicated by the marked relaxant response to ACh (0.1 mM) in the uterine arteries used as time controls (relaxation of $91.6\pm5.8\%$ ($n=9$) in non-pregnant and $92.8\pm6.3\%$ ($n=7$) in pregnant rats) or those exposed to CGP 42112 (relaxation of $92.3\pm4.9\%$ ($n=10$) in non-pregnant and $93.2\pm7.0\%$ ($n=7$) in pregnant rats).

Discussion

The novel findings of the present study were that the AT_2 receptor modulates the AT_1-mediated contractile effects of angiotensin II in the rat isolated uterine artery, however this modulation is not uniform between the non-pregnant and pregnant states.

Uterine arteries from non-pregnant rats

Losartan caused a concentration-dependent rightward shift of the CR curves to angiotensin II in uterine arteries from non-pregnant rats. The pA_2 value estimated for losartan from this data was 9.8, which is within the range of values reported for the AT_1 receptor antagonist in other vascular preparations and therefore suggests a similar AT_1 receptor affinity at this vascular site (Boulanger *et al.*, 1995; Chiu *et al.*, 1990). However, it must be kept in mind that, in the present study, the slope of the Schild plot for losartan obtained from the uterine arteries of non-pregnant rats was less than unity and therefore the calculated pA_2 must be accepted with some caution. By contrast, the AT_2 receptor antagonist, PD 123319, caused an increase in sensitivity to angiotensin II, reflected in the 6 fold leftward shift of the angiotensin II CR curve. These findings concur with the preliminary study by McMullen *et al.* (1996), who found that PD 123319 increased

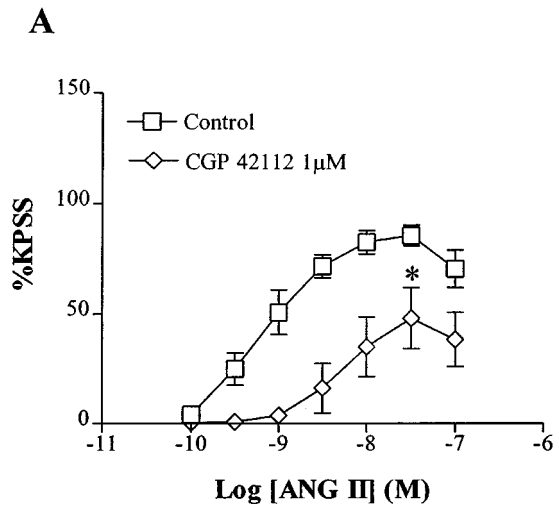

Figure 4 Effect of CGP 42112 (1 μM) on the mean log concentration-response curves to angiotensin II in uterine arteries from (A) non-pregnant ($n=7-12$) and (B) late pregnant ($n=5-15$) rats. Responses are expressed as a per cent of the maximum response to KPSS; values are means and vertical lines show s.e.mean; $n=$number of vessels. $^*P<0.05$ for maximum response versus control.

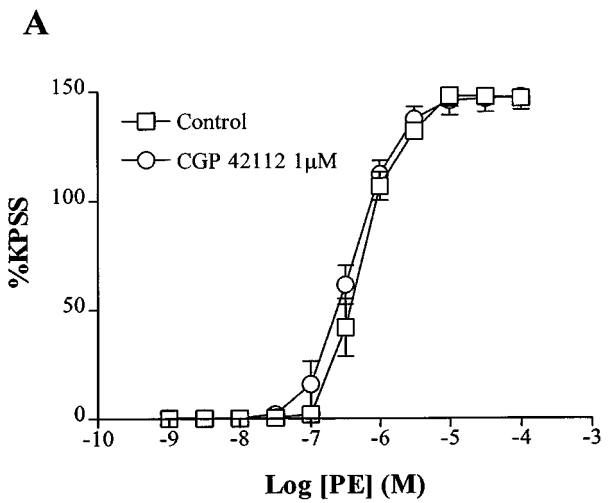

Figure 5 Effect of CGP 42112 (1 μM) on the mean log concentration-response curves to phenylephrine (PE) in uterine arteries from (A) non-pregnant ($n=5-6$) and (B) late pregnant ($n=6$) rats. Responses are expressed as a per cent of the maximum response to KPSS; values are means and vertical lines show s.e.mean; $n=$number of vessels.

the contractile response to a single concentration of angiotensin II (1 μM) in uterine arteries from pregnant sheep, although they did not report data from non-pregnant sheep.

These results implicate an inhibitory role of the AT_2 receptor which may counteract the excitatory effect of AT_1 receptor mediated stimulation (McMullen *et al.*, 1996) and are consistent with increasing evidence of opposing actions of AT_1 and AT_2 receptors in the context of proliferative (Stoll *et al.*, 1995b; Munzenmaier & Greene, 1996) and vasoactive (Scheuer & Perone, 1993; Munzenmaier & Greene, 1996) effects.

CGP 42112, which is considered to be an AT_2 receptor agonist (Brechler *et al*, 1993; Tsuzuki *et al.*, 1996) caused a decreased sensitivity to angiotensin II, as seen by the 14 fold rightward shift of the angiotensin II CR curve with a decreased maximum response. This effect was, in fact, the opposite to that observed with PD 123319 and suggests that CGP 42112 was acting as an AT_2 receptor agonist to inhibit AT_1-mediated contraction of uterine arteries. Importantly, we found that neither PD 123319 nor CGP 42112 affected the CR curve to phenylephrine or potassium, indicating that these compounds did not alter the smooth muscle contractility non-specifically, and thus appear to act exclusively on AT_2 receptors. To our knowledge, there are no previous reports describing an inhibitory effect of CGP 42112 on angiotensin II-mediated contraction. Moreover, this is the first demonstration of opposing effects of PD 123319 and CGP 42112 in vasculature *in vitro*.

Uterine arteries from pregnant rats

Pregnancy is associated with substantial remodelling of the uteroplacental vasculature (Rosenfeld *et al.*, 1974; Rosenfeld, 1977; Griendling *et al.*, 1985), and this was evident in the increase in the internal diameter (D100) of the uterine arteries taken from the pregnant compared to the non-pregnant rats. Moreover, these vessels also showed an increased response to the K^+ depolarizing solution, which is in agreement with studies using ovine uterine arteries (Annibale *et al.*, 1989). Therefore, agonist-induced responses were expressed as a per cent of the KPSS response.

Similar directional changes were observed using the angiotensin II ligands in uterine arteries from pregnant rats

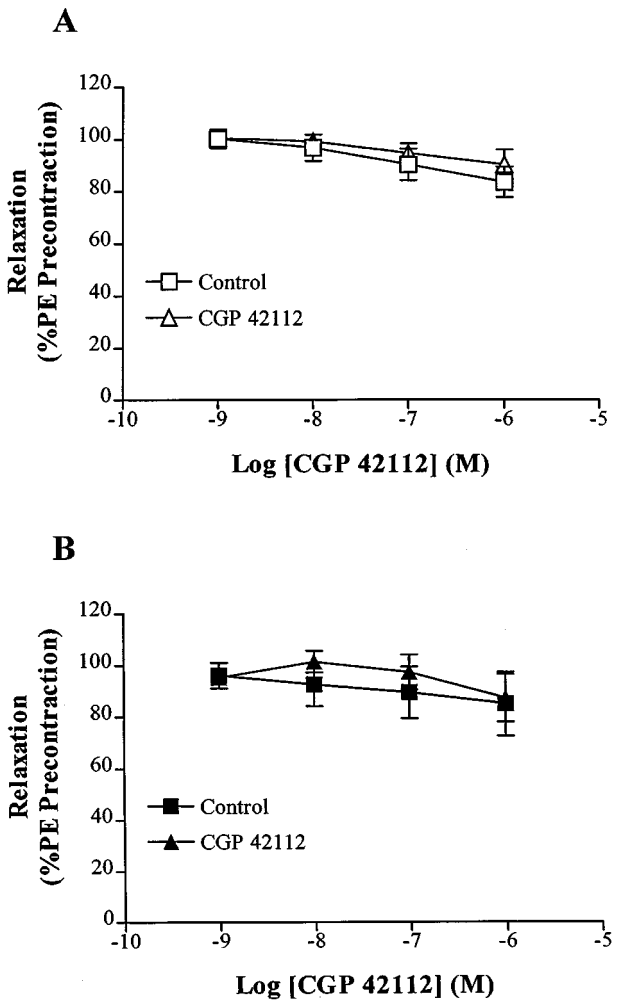

Figure 6 Mean log concentration response curves to CGP 42112 (1 nM – 1 μM) on precontracted uterine arteries from (A) non-pregnant ($n=9-10$) and (B) late pregnant ($n=7$) rats. Also shown is the spontaneous relaxation of time control tissues over the same period but in the absence of CGP 42112. Responses are expressed as a percentage relaxation of the phenylephrine-induced contraction; values are means and vertical lines show s.e.mean; $n=$number of vessels.

compared with non-pregnant rats, although there were subtle differences between the two states. For examples, the pA_2 for losartan obtained using uterine arteries from pregnant rats (9.2) was similar to that from the vessels from non-pregnant rats and the slope of unity for the Schild plot was indicative of a true competitive AT_1 receptor antagonist. The small differences in the pA_2 values for losartan between the groups may reflect an overestimation in the non-pregnant group because the slope of the Schild plot for losartan in this group was less than unity, for reasons which are not immediately obvious. In addition, Cox *et al.* (1996a,b) found no differences in AT_1 binding characteristics in uterine arteries from non-pregnant and pregnant sheep and women. Saturation binding using uterine arteries from non-pregnant and pregnant ewes revealed similar K_D values at the AT_1 receptor (Burrell & Lumbers, 1997), although competition binding in the same study revealed an increase in the IC_{50} value of losartan associated with pregnancy in ovine uterine arteries, which suggests a decrease in AT_1 receptor affinity (Burrell & Lumbers, 1997). In the present study, PD 123319 and CGP 42112 caused leftward and rightward shifts, respectively, of the CR curve to angiotensin II, similarly to in the non-pregnant state. However, in the case of PD 123319, there was a smaller (3 fold) leftward shift but an increased maximum response to angiotensin II, while, for CGP 42112, there was only a small (3 fold) rightward shift in the CR curve to angiotensin II with no depression of maximum response in uterine arteries from pregnant rats.

The reason for the difference in AT_2 receptor modulation between non-pregnant and pregnant rats is not immediately obvious, but may involve altered AT_2 receptor number and/or affinity. In this context, Burrell & Lumbers (1997) found an increase in AT_2 receptor density and affinity in uterine arteries from pregnant, compared with non-pregnant, sheep. By contrast, Cox *et al.* (1996a,b) found that the ratio of AT_2/AT_1 receptors ($\sim$85%/15%) in uterine arteries of sheep and women did not change during pregnancy. The reason for the discrepancy between these two laboratories is not clear, but may relate to differences in the ligand used and methodological procedures employed (Burrell & Lumbers, 1997).

Interestingly, there is a downregulation of myometrial AT_2 receptors during pregnancy (Cox *et al.*, 1993; de Gasparo *et al.*, 1994). Furthermore, angiotensin II-induced myometrial contraction could only be demonstrated in uterine smooth muscle obtained in the pregnant state, suggesting that there was an association between increased AT_1 receptor-mediated contraction and downregulation of myometrial AT_2 receptors (Cox *et al.*, 1993, 1996b). In this context, Burrell & Lumbers (1997) speculated that an up-regulation of AT_2 receptors may contribute to the reduced sensitivity of uterine vasculature to angiotensin II during pregnancy. While our data showing PD 123319 potentiated the maximum response to angiotensin II in uterine arteries from pregnant rats fits with this interpretation, the smaller degree of the leftward shift of the angiotensin II CR curve caused by PD 123319 does not. Furthermore, a greater inhibitory action of AT_2 receptors on AT_1 receptors during pregnancy is difficult to reconcile with the proposed AT_2 receptor agonist action of CGP 42112, particularly since it failed to modify the maximum response to angiotensin II in uterine arteries from pregnant rats despite having a marked inhibitory effect in the non-pregnant state. Interestingly, a high affinity AT_2 binding site was observed in uterine arteries from pregnant, but not non-pregnant ewes (Burrell & Lumbers, 1997). Therefore, unless the pharmacodynamics of CGP 42112 changed during pregnancy, it would appear that the AT_2 receptor is not directly responsible for the attenuation of angiotensin II-mediated uterine vasoconstrictor responses during pregnancy, at least in the rat. In any case, the present study has clearly shown a modulatory action of AT_2 ligands on an AT_1 receptor-mediated contractile response. However, the situation is quite different in myometrial smooth muscle which also contains predominantly AT_2 receptors (Cox *et al.*, 1993, 1996a,b; de Gasparo *et al.*, 1994; Burrell & Lumbers, 1997), since there was no AT_2/AT_1 receptor interaction observed in ovine or human myometrial strips (Cox *et al.*, 1993, 1996b). This conclusion was based on the fact that PD 123319 failed to modify a near-maximal angiotensin II-evoked contraction of myometrial strips, although it should be noted that the AT_2 receptor antagonist was only incubated for 4 min prior to angiotensin II administration.

Given that CGP 42112 reduced the contractile response to angiotensin II, we also examined whether or not a direct relaxant effect of CGP 42112 could be demonstrated in this preparation. However, in uterine arteries from non-pregnant and pregnant rats, we could not demonstrate a direct relaxant effect of CGP 42112. This was despite an almost complete relaxation of these vessels by acetylcholine indicating the

presence of a functional endothelium. Nevertheless, it is possible that the endothelium may be involved in the AT_2 receptor modulation that we have observed since, in addition to AT_1 receptors, AT_2 receptors are also found on rat endothelial cells (Stoll *et al*., 1995a,b) which may be linked to vasodilator function (Wiemer *et al*., 1993). Clearly, future experiments will need to address the exact localization of AT_1 and AT_2 receptors in rat uterine arteries, as well as the role of endothelium in the functional setting. Interestingly, previous autoradiographic studies have identified AT_2 receptors in medial smooth muscle of endothelium-denuded ovine uterine arteries (Cox *et al*., 1996a), while pregnancy increased the expression of AT_1 receptors in the endothelium of the same blood vessel (Bird *et al*., 1997).

AT_1/AT_2 receptor interactions

The implication from the present study of an interaction between AT_1 and AT_2 receptors is a relatively new concept that has developed from a number of *in vivo* studies. Widdop *et al*. (1994) reported that PD 123177, an AT_2 antagonist, potentiated the AT_1 receptor mediated drinking caused by an intracerebroventricular injection of angiotensin II in rats. These data were confirmed by Höhle *et al*. (1995), who also showed that PD 123177 potentiated AT_1-mediated vasopressin release. Furthermore, in a study of pressure-natriuresis in rats, PD 123319 increased diuresis and natriuresis by acting as an AT_2 receptor antagonist, while CGP 42112 acted as an AT_2 receptor agonist to decrease urine flow and natriuresis (Lo *et al*., 1995). In a recent study, Munzenmaier & Greene (1996) reported that rats co-infused with angiotensin II and PD 123319 had a greater rise in blood pressure than those infused with angiotensin II alone, suggesting AT_2 receptor activation caused inhibition of the AT_1-mediated pressor response. Moreover, it was reported that co-infusion of losartan and angiotensin II resulted in a greater depressor response than losartan alone, implicating an AT_2-vasodilator effect (Munzenmaier & Greene, 1996). However, it must be noted that this previous study reported a control dose of losartan which was 10 fold lower than the combination, which makes any interpretation of direct AT_2 receptor-mediated vasodilatation difficult. Nevertheless, these previous data are consistent with our *in vitro* data since PD 123319 potentiated angiotensin II-mediated contractile responses while AT_2 receptor-mediated relaxation was not evident. The fact that there was modulation of AT_1 receptor-mediated contraction by AT_2 receptors in uterine arteries (current study), but not uterine smooth muscle (Cox *et al*., 1996a,b), may suggest differences between types of smooth muscle or reflect the use of AT_2 receptor antagonist under non-equilibrium conditions in the latter case. To our knowledge, apart from the preliminary data obtained from pregnant ewes (McMullen *et al*., 1996), there are no other similar functional studies using isolated vasculature for comparison.

In conclusion, the present study has found that both AT_1 and AT_2 receptors play important functional roles in the rat uterine artery. The AT_1 receptor is responsible for the contractile response evoked by angiotensin II, while the AT_2 receptor appears to inhibit the response mediated by the AT_1 receptor. However, the AT_2 receptor does not uniformly regulate these responses between non-pregnant and pregnant rats and is not likely to mediate the insensitivity to angiotensin II in uterine vasculature during pregnancy. Further studies are required to elucidate the mechanism of this receptor interaction and its role in pregnancy.

We would like to thank Dr M. de Gasparo (Novartis, Switzerland), Dr J. Keiser (Parke Davis, U.S.A.) and Dr R. Smith (Du Pont, U.S.A.) for their gifts of CGP 42112, PD123319 and losartan, respectively. This work was supported by the grants from the National Health and Medical Research Council of Australia, of which R.E.W. is a Research Fellow.

References

ABDUL-KARIM, R. & ASSALI, N.S. (1961). Pressor response to angiotensin in pregnant and non-pregnant women. *Am. J. Obstet. Gynecol.,* **82,** 246–251.

ANNIBALE, D.J., ROSENFELD, C.R. & KAMM, K.E. (1989). Alterations in vascular smooth muscle contractility during ovine pregnancy. *Am. J. Physiol.,* **256,** H1282–H1288.

BIRD, I.M., ZHENG, J., CALE, J.M. & MAGNESS, R.R. (1997). Pregnancy induces an increase in angiotensin II type-1 receptor expression in uterine but not systemic artery endothelium. *Endocrinology,* **138,** 490–498.

BOULANGER, C.M., CAPUTO, L. & LÉVY, B.I. (1995). Endothelial AT_1-mediated release of nitric oxide decreases angiotensin II contractions in rat carotid artery. *Hypertension,* **26,** 752–757.

BRECHLER, V., JONES, P.W., LEVENS, N.R., DE GASPARO, M. & BOTTARI, S. (1993). Agonistic and antagonistic properties of angiotensin analogs at the AT_2 receptor in PC12W cells. *Regul. Pept.,* **44,** 207–213.

BURRELL, J.H. & LUMBERS, E.R. (1997). Angiotensin receptor subtypes in the uterine artery during ovine pregnancy. *Eur. J. Pharmacol.,* **330,** 257–267.

CHIU, A.A., MCCALL, D.E., PRICE, W.A., WONG, P.C., CARINA, D.J., DUNCIA, J.V., WEXLER, R.R., YOO, S.E., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990). Nonpeptide angiotensin II receptor antagonists VII. Cellular and biochemical pharmacology of DuP 753, an orally active anti-hypertensive agent. *J. Pharmacol. Exp. Ther.,* **252,** 711–718.

COOK, V.I., GROVE, K.L., MCMENAMIN, K.M., CARTER, M.R., HARDING, J.W. & SPETH, R.C. (1991). The AT_2 angiotensin receptor subtype predominates in the 18 day gestational foetal rat brain. *Brain Res.,* **560,** 334–336.

COX, B.E., IPSON, M.A., SHAUL, P.W., KAMM, K.E., & ROSENFELD, C.R. (1993). Myometrial angiotensin II receptor subtypes change during ovine pregnancy. *J. Clin. Invest.,* **92,** 2240–2248.

COX, B.E., ROSENFELD, C.R., KALINYAK, J.E., MAGNESS, R.R. & SHAUL, P.W. (1996a). Tissue specific expression of vascular smooth muscle angiotensin II receptor subtypes during ovine pregnancy. *Am. J. Physiol.,* **271,** H212–H221.

COX, B.E., WORK, R.A. & ROSENFELD, C.R. (1996b). Angiotensin II receptor characteristics and subtype expression in uterine arteries and myometrium during pregnancy. *J. Clin. Endocrin. Metab.,* **81,** 49–58.

DAVIS, E.A. (1994). Effects of passive resting tension on reactivity of isolated uterine arteries from pregnant and non-pregnant rats. *Can. J. Physiol. Pharmacol.,* **72** (Suppl 4): P2.1.5.

DE GASPARO, M., HUSAIN, A., ALEXANDER, W., CATT, K.J., CHIU, A.T., DREW, M., GOODFRIEND, T.L., HARDING, J.W., INAGAMI, T. & TIMMERMANS, P.B.M.W.M. (1995). Proposed update of angiotensin receptor nomenclature. *Hypertension,* **25,** 924–927.

DE GASPARO, M., WHITEBREAD, S., KALENGA, M.K., DE HERTOGH, R., CREVOISIER, P. & THOMAS, K. (1994). Down regulation of the angiotensin II receptor subtype AT_2 in human myometrium during pregnancy. *Regul. Pept.,* **53,** 39–45.

DIEM, K. (1970)(ed). Scientific Tables. In: *Documenta Geigy* 6th, ed, Geigy Pharmaceuticals, Australasia.

GRIENDLING, K.K., FULLER, E.O. & COX, R.H. (1985). Pregnancy-induced changes in sheep uterine and carotid arteries. *Am. J. Physiol.,* **248,** H658–H665.

GUTHRIE, G.P. (1995). Angiotensin receptors: Physiology and pharmacology. *Clin. Cardiol.,* **18** (Suppl III): III29–III34.

HÖHLE, S., SPITZNAGEL, H., RASCHER, W., CULMAN, J. & UNGER, T. (1995). Angiotensin AT_1 receptor-mediated vasopressin release and drinking are potentiated by an AT_2 receptor antagonist. *Eur. J. Pharmacol.,* **275,** 277–282.

LO, M., LIU, K., LANTELME, P. & SASSARD, J. (1995). Subtype 2 of angiotensin II receptors controls pressure-natriuresis in rats. *J. Clin. Invest.,* **95,** 1394–1397.

MCMULLEN, J.R., GIBSON, K.J. & LUMBERS, E.R. (1996). Angiotensin II and uteroplacental blood flow. *Proc. Aust. Soc. Clin. Exp. Pharmacol. Toxicol.,* **3,** 54.

MCPHERSON, G.A. (1992). Optimal conditions for assessing vascular reactivity in small resistance arteries in the small vessel myograph. *Clin. Exp. Pharmacol. Physiol.,* **19,** 815–825.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.,* **41,** 19–26.

MUNZENMAIER, D.H. & GREENE, A.S. (1996). Opposing actions of angiotensin II on microvascular growth and arterial blood pressure. *Hypertension,* **27 (2),** 760–765.

NADEN, R.P. & ROSENFELD, C.R. (1981). Effect of angiotensin II on uterine and systemic vasculature in pregnant sheep. *J. Clin. Invest.,* **68,** 469–474.

ROSENFELD, C.R. (1977). Distribution of cardiac output in ovine pregnancy. *Am. J. Physiol.,* **232,** H231–H235.

ROSENFELD, C.R. & NADEN, R.P. (1989). Uterine and nonuterine vascular responses to angiotensin II in ovine pregnancy. *Am. J. Physiol.,* **257,** H17–H24.

ROSENFELD, C.R. & MORRISS, F.H., MAKOWSKI, E.L., MESCHIA, G. & BATTAGLIA, F.C. (1974). Circulatory changes in the reproductive tissues of ewes during pregnancy. *Gynecol. Invest.,* **5,** 252–268.

SCHEUER, D.A. & PERONE, M.H. (1993). Angiotensin type 2 receptors mediate depressor phase of biphasic pressure response to angiotensin. *Am. J. Physiol.,* **264,** R917–R923.

STOLL, M., MEFFERT, S., STROTH, U. & UNGER, T. (1995b). Growth or antigrowth: angiotensin and the endothelium. *J. Hypertens,* **13,** 1529–1534.

STOLL, M., STECKELINGS, U.M., PAUL, M., BOTTARI, S.P., METZGER, R. & UNGER, T. (1995a). The angiotensin AT2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J. Clin. Invest.,* **95,** 651–657.

TIMMERMANS, P.B.M.W.M., BENFIELD, P., CHIU, A.T., HERBLIN, W.F. WONG, P.C. & SMITH, R.D. (1992a). Angiotensin II receptors and functional correlates. *Am. J. Hypertens.,* **5,** 221S–235S.

TIMMERMANS, P.B.M.W.M., WONG, P.C., CHIU, A.T. & HERBLIN, W.F. (1992b). Nonpeptide angiotensin II receptor antagonists. *Trends Pharmacol. Sci.,* **12,** 55–62.

TSUTUMI, K. & SAAVEDRA, J.M. (1991). Characterization of AT_2 angiotensin II receptors in rat anterior cerebral arteries. *Am. J. Physiol.,* **261,** H667–H670.

TSUZUKI, S., EGUCHI, S. & INAGAMI, T. (1996). Inhibition of cell proliferation and activation of protein tyrosine phosphatase mediated by angiotensin II type 2 (AT_2) receptor in R3T3 cells. *Biochem. Biophys. Res. Commun.,* **228,** 825–830.

WIDDOP, R.E., GARDINER, S.M. & BENNETT, T. (1994). Effects of angiotensin II AT_1- or AT_2-receptor antagonists on drinking evoked by angiotensin II or water deprivation in rats. *Brain Res.,* **648,** 46–52.

WIEMER, G., SCHÖLKENS, B.A., BUSSE, R., WAGNER, A., HEITSCH, H. & LINZ, W. (1993). The functional role of angiotensin II-subtype AT2-receptors in endothelial cells and isolated ischemic rat hearts. *Pharm. Pharmacol. Lett.,* **3,** 24–27.

ZHOU, J., ALLEN, A.M., ALCORN, D., ALDRED, G.P., MACGREGOR, D.P. & MENDELSOHN, F.A.O. (1995). Distribution of angiotensin II receptors. In *Hypertension: Pathophysiology, diagnosis and management*. ed. Laragh, J.H. & Brenner, B.M. 1739–1762. New York: Raven Press Ltd.

(Received May 5, 1998
Revised July 2, 1998
Accepted July 21, 1998)