ELSEVIER

European Journal of Pharmacology 378 (1999) 195–202

ejp

www.elsevier.nl/locate/ejphar

Interactions between AT_1 and AT_2 receptors in uterine arteries from pregnant ewes

Julie R. McMullen *, Karen J. Gibson, Eugenie R. Lumbers, Judith H. Burrell, June Wu

School of Physiology and Pharmacology, University of New South Wales, Sydney, NSW 2052, Australia

Received 9 February 1999; received in revised form 26 May 1999; accepted 18 June 1999

Abstract

This study was performed to investigate the roles of angiotensin receptors (AT_1 and AT_2) in the contractility of uterine arteries during normal pregnancy and after angiotensin II levels have been elevated. Pregnant ewes were given intravenous infusions of saline for 24 h (control) or angiotensin II (30 ng kg^{-1} min^{-1}) for 2 or 24 h. The contractile responses of uterine arterial rings to angiotensin II (4 μM) and antagonists were then examined in vitro. Most uterine arteries were relatively insensitive to the vasoconstrictor effects of angiotensin II. In rings from control ewes an angiotensin AT_2 antagonist enhanced ($P < 0.05$) the contractile responses to angiotensin II, suggesting that angiotensin AT_2 receptors inhibited the angiotensin AT_1 receptor mediated contractions. Uterine arterial rings from ewes given intravenous infusions of angiotensin II displayed greater ($P < 0.05$) contractile responses to angiotensin II in vitro compared to rings from control ewes. This was in part due to down regulation of angiotensin AT_2 receptors. Surprisingly, while performing these experiments a small number of ewes had uterine arteries which were ''hyperreactive'' to angiotensin II (contractile responses 6-fold greater). These ewes also had abnormal renin angiotensin systems and had some features which are characteristic of those seen in preeclampsia. The ''hyperreactivity'' of these arteries could only in part be explained by down regulation of angiotensin AT_2 receptors. It is concluded that in normal pregnancy angiotensin AT_2 receptors play a role in maintaining an adequate uterine blood flow for the fetus. When angiotensin II levels are elevated for a prolonged period this protective effect is lost partly because angiotensin AT_2 receptors are down regulated.

Keywords: Angiotensin AT_1 receptor; Angiotensin AT_2 receptor; Uterine artery; Pregnancy; (Ovine); Uteroplacental blood flow

1. Introduction

During pregnancy the activity of the renin angiotensin system is greatly elevated. Despite the rise in circulating angiotensin II, maternal blood pressure is normally not increased (Massani et al., 1967; Wilson et al., 1980). Pregnant women (Abdul-Karim and Assali, 1961) and late gestation sheep show a reduced vascular responsiveness to angiotensin II, and the ovine uteroplacental vascular bed is even more refractory to angiotensin II than the systemic vasculature (Rosenfeld and Naden, 1989). This refractoriness may reflect an important mechanism by which uteroplacental blood flow is normally maintained to supply the fetus with adequate nutrients and oxygen. When angiotensin II (< 60 ng kg^{-1} min^{-1}) was infused into pregnant sheep for 5–10 min uteroplacental blood flow increased or did not change (Naden and Rosenfeld, 1981; Rosenfeld and Gant, 1981; Rosenfeld and Naden, 1989; Clark et al., 1990; Magness et al., 1992). However, Stevens and Lumbers (1999) showed that although intravenous angiotensin II infused into pregnant ewes at 20–30 ng kg^{-1} min^{-1} initially (< 4 h) caused no change in uteroplacental blood flow, 16–24 h later uteroplacental blood flow had fallen and fetuses were hypoxaemic.

Angiotensin receptors are classified into two major subtypes (AT_1 and AT_2). Angiotensin AT_1 receptors mediate the vasoconstrictor actions of angiotensin II, whereas the role of angiotensin AT_2 receptors is unclear (Timmermans et al., 1993). Unlike most adult blood vessels, uterine arteries from pregnant sheep during late gestation contain a large proportion of angiotensin AT_2 receptors (70%; Burrell and Lumbers, 1997). It has been suggested that the high density of angiotensin AT_2 receptors relative to angiotensin AT_1 receptors at this stage of pregnancy might explain the refractoriness of uterine arteries to angiotensin II (Burrell and Lumbers, 1997). We postulated that the fall in uteroplacental blood flow observed by Stevens and

* Corresponding author. Tel.: +61-2-9385-3650; fax: +61-2-9385-1059

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00454-9

Lumbers (1999) after 16–24 h of angiotensin II infusions was in part due to down regulation of these angiotensin AT_2 receptors. This effect would not be evident during short term infusions (0–4 h) of angiotensin II. To test this hypothesis we studied the in vitro contractility of uterine arteries to angiotensin II and antagonists, obtained from pregnant ewes given intravenous infusions of saline (control) or angiotensin II for 2 h (short term infusion) or 24 h (long term infusion). We predicted that infusions of angiotensin II for 24 h would alter the contractility of uterine arteries to angiotensin II, resulting in arteries from these animals displaying greater contractile responses to angiotensin II in vitro than uterine arteries obtained from ewes treated with angiotensin II for 2 h or saline (control).

2. Materials and methods

2.1. Materials

Stock solutions of 1 mM angiotensin II (Hypertensin, Ciba-Geigy, Basle, Switzerland), losartan (DuP 753, Du Pont Merck Pharmaceutical, Wilmington, DE, USA), PD 123319 ((*S*)1-[[4-(dimethylamino)-3-methylphenyl]-methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo-(4,5-*c*)pyridine-6-carboxylic acid, ditrifluoroacetate, monohydrate, Parke-Davis Pharmaceutical Research, Division of Warner Lambert, MI, USA), ATP (Sigma, St. Louis, MO, USA), and bradykinin (Sigma) were prepared in distilled water and kept at −20°C until use. All further dilutions of the above drugs and noradrenaline (Levophed, Sanofi Winthrop, Ermington, NSW, Australia) were made in distilled water just prior to use in in vitro experiments.

2.2. Animal care

Experiments were approved by the Animal Care and Ethics Committee, University of New South Wales. Pregnant merino ewes (50.0 ± 1.5 kg) were housed in metabolic pens in an air-conditioned room (18–23°C) with day/night cycles for 3–5 days. Each day they received water ad libitum, lucerne chaff (1200 g) and oats (100 g).

2.3. Intravenous infusions of saline or angiotensin II

The needle of an indwelling catheter (Cavafix, B. Braun Medical, Germany) was inserted rapidly through the skin of the ewe's neck into an external jugular vein. After securing the catheter to the ewe's neck it was connected to polyvinyl tubing (1.5 mm i.d., 2.7 mm o.d.). Ewes were randomly divided into three groups. Eleven ewes (120–130 days, full term 145–150 days) were infused with saline (0.15 M) for 24 h, seven ewes (118–127 days) were infused with angiotensin II (30 ng kg^{-1} min^{-1}, diluted with saline) for 2 h, and eight ewes (121–130 days) were infused with angiotensin II (30 ng kg^{-1} min^{-1}) for 24 h using a Braun infusion pump at 0.66 ml h^{-1} (Braun Perfusor, Melsungen, Germany). At the end of the infusion (0900–1100 h), a maternal blood sample was collected and the ewes were killed with an overdose of pentobarbitone sodium (approximately 3.5 g, Lethabarb Euthanasia Injection; Virbac (Australia), Peakhurst, NSW, Australia). The uterus was exposed via a flank incision. The main uterine arteries were carefully removed, taking care to avoid any stretch and placed into an ice cold Krebs solution of the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 5.0; aerated with 95% oxygen: 5% carbon dioxide (pH 7.4).

2.4. In vitro studies

The vessel was cleared of loose connective tissue and 12–16 ring segments (2–3 mm) were cut. Stainless steel hooks (0.45 mm o.d.) were inserted into the lumen of arterial rings. Gentle rubbing of the lumen with the hooks removed any functional endothelium. The rings were randomly suspended in 10 ml water jacketed organ baths filled with Krebs solution (pH 7.4), maintained at approximately 37°C. The preparations were connected to force transducers (Grass FT 0.03, Quincy, MA) and the isometric force was recorded using a MacLab/8-computer system (Analog Digital Instruments, Castle Hill, NSW, Australia).

2.5. Preliminary experiments

Uterine arterial rings developed tachyphylaxis to angiotensin II which lasted more than 2–3 h after the first dose of angiotensin II had been washed from the organ bath. Thus each ring could only be exposed to one concentration of angiotensin II. To determine the best concentration of angiotensin II, dose–response curves to angiotensin II were constructed using eight separate rings from a control (saline-infused) and eight rings from a 24 h angiotensin II-treated ewe. Since the response of rings to angiotensin II from control ewes was relatively small a near maximal concentration of angiotensin II was chosen (4 μM). The contractile responses of rings from the ewe infused with angiotensin II for 24 h were greater than the responses in rings from the saline treated ewe (Fig. 1a), but there were no differences in the responses to KCl (Fig. 1b).

Neither 10^{-6} nor 10^{-7} M amastatin or bestatin (aminopeptidase inhibitors) had any effect on angiotensin II contractile responses. Therefore, peptidase inhibitors were not used.

2.6. Experimental protocol

After an equilibration period (≥ 60 min), rings were stretched to an optimal resting tension of approximately

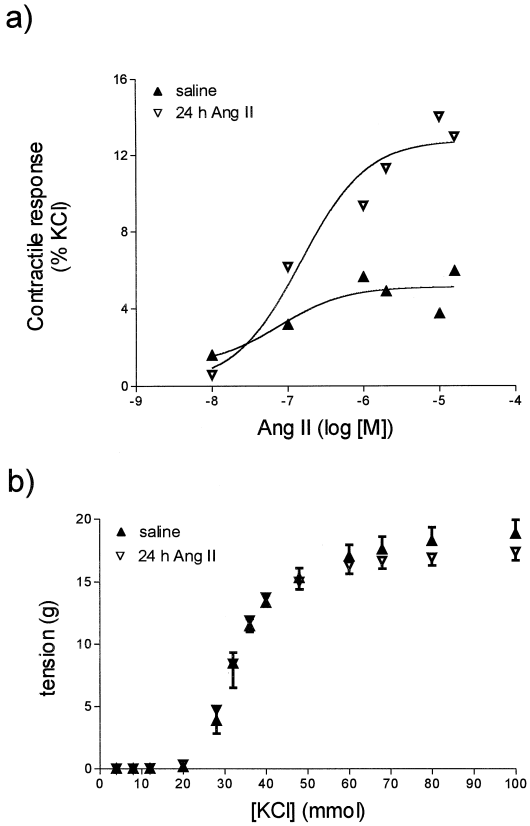

Fig. 1. (a) Angiotensin II dose–response curves constructed using uterine arterial rings from a saline and 24 h angiotensin II-treated ewe. Results are expressed as a percentage of the contraction obtained with 40 mM KCl. Each data point represents 1 or 2 ring preparations. (b) KCl cumulative dose–response curves constructed using rings from a saline ($n = 4$) and 24 h angiotensin II-treated (Ang II, $n = 5$) ewe. Values are means ± S.E.M., n refers to the number of ring preparations.

1.5 g, and then exposed to a submaximal concentration (40 mM) of KCl. Once contractions had reached a plateau, the preparations were washed twice with Krebs solution and left for 30 min before another drug was given. Each ring was exposed to at least two more submaximal concentrations (40 mM) of KCl. The KCl response was used as a standard for comparison of all other contractile responses. Only rings in which the KCl responses were within 10% of each other were used (Svane et al., 1995).

After receiving at least three consecutive injections of KCl each ring received either angiotensin II (4 μM) alone, angiotensin II after prior incubation for 30 min with 1 μM losartan (angiotensin AT_1 receptor antagonist), or angiotensin II after prior incubation for 30 min with 1 μM PD 123319 (angiotensin AT_2 receptor antagonist). The concentrations of angiotensin II antagonists were based on preliminary experiments with losartan (10^{-8} to 10^{-5} M) and PD 123319 (10^{-8} to 10^{-5} M).

In some preparations cumulative dose–response curves to noradrenaline were constructed. These were obtained by the cumulative addition in 0.5 log increments of noradrenaline (range 10 nM–10 μM). Subsequent concentrations were added when the response produced by the previous concentration had plateaued.

The absence of endothelium was confirmed at the end of experiments by showing that 1 μM ATP (Zhang et al., 1995) or 1 μM bradykinin had no effect on rings preconstricted with noradrenaline. This was also confirmed histologically in some rings by staining sections with haematoxylin and eosin.

2.7. Plasma analyses

Maternal blood was spun for 10 min at $1000 \times g$. Plasma was removed and stored at −20°C. Plasma renin activity was measured as the rate of generation of angiotensin I by the action of endogenous renin on endogenous substrate at pH 7.4 and 37°C (Lumbers and Reid, 1977). Plasma sodium and potassium concentrations were measured using a Radiometer flame photometer (Model FLM3, Copenhagen, Denmark). Osmolality was measured by freezing point depression on a Fiske One-Ten Osmometer (Needham Height, MA, USA). Glucose and lactate measurements were made on a Glucose Lactate Analyzer YSI 2300 Stat (John Morris Scientific, Chatswood, NSW, Australia). Plasma noradrenaline and adrenaline levels were measured using high-performance liquid chromatography. Plasma cortisol levels were measured using a Cortisol [^{125}I]Radioimmunoassay kit (Orion Diagnostica, Espoo, Finland).

2.8. Statistical analysis

Responses to angiotensin II and antagonists, and noradrenaline were expressed as a percentage of the KCl response. All statistical analyses were performed using an IBM compatible personal computer and Statistical Package for the Social Sciences (SPSS/PC; SPSS, Chicago, IL, USA). Results are expressed as means ± standard error (S.E.M.). N refers to the number of animals and n refers to the number of uterine arterial ring preparations. When comparing groups statistical significance was determined using one-way analysis of variance (ANOVA). If the ANOVA showed significance ($P < 0.05$), it was followed by a Student–Newman–Keuls test. Significance level was $P < 0.05$.

Dose–response curves were analyzed by a curve fitting program, Prism (GraphPad Software, San Diego, CA, USA). Non-linear regression was performed using the sigmoidal concentration–response equation

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10^{\text{Log EC}_{50} - X}),$$

where Bottom is the Y value at the bottom plateau; Top is the Y value at the top plateau, and EC_{50} (concentration which produces 50% of the maximal effect) is the X value when the response is halfway between Bottom and Top.

3. Results

Rings from most uterine arteries (17 of 26 arteries) were relatively refractory to angiotensin II (4 μM). However, surprisingly there were a small number of arteries (9 of 26) which displayed ''hyperreactive'' responses to angiotensin II. The responses of rings to angiotensin II from individual arteries were averaged to determine whether there were two subpopulations of uterine arteries (independent of in vivo treatments). Seventeen uterine arteries had mean angiotensin II responses below 15% of KCl ($6.5 \pm 1.0\%$, range: 0.5–12.6%) whereas nine had mean angiotensin II responses above 25% ($40.2 \pm 5.0\%$, range: 27–75%). Since there was no overlap between the two groups it was clear that there were two subpopulations ($P < 0.001$, by non-paired Student's *t*-test) and these were called ''normal'' and ''hypercontracting''. The different contractilities of these arteries to angiotensin II in vitro were independent of the responses to KCl (''normal'': 11.3 ± 0.3 g, $n = 133$; ''hypercontracting'': 11.0 ± 0.4 g, $n = 63$). Angiotensin II dose–response curves from a control ewe with a ''normal'' contracting uterine artery and a control ewe with a ''hypercontracting'' uterine artery are shown in Fig. 2a. The hyperreactivity observed with angiotensin II was not observed with noradrenaline (Fig. 2b). Because of the large difference in angiotensin II induced contractilities of arteries from these two populations of sheep, separate analyses were used to examine the effects of the ewes' in vivo treatments (i.e., saline, 2 h angiotensin II or 24 h angiotensin II infusions) for each population.

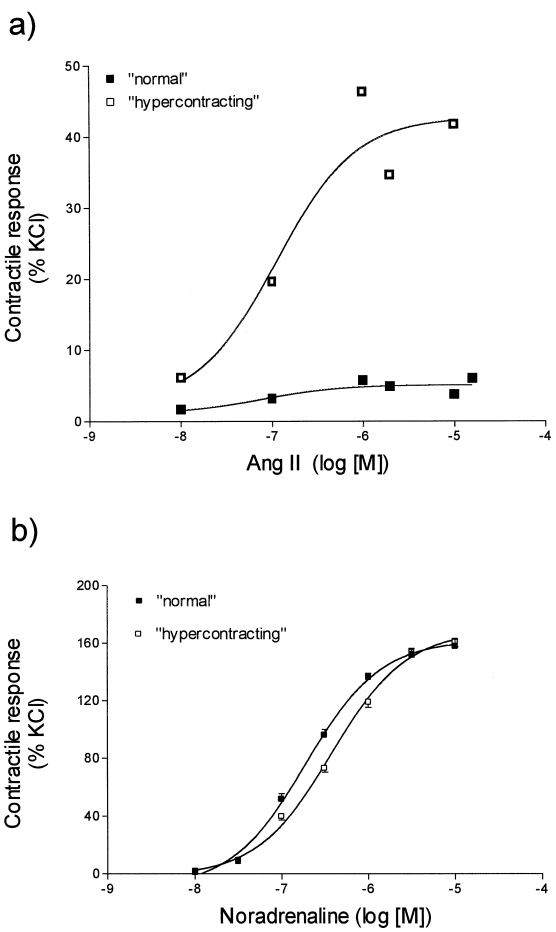

Fig. 2. (a) Angiotensin II (Ang II) dose–response curves in rings from a 24 h saline-infused ewe with a ''normal'' contracting uterine artery and a 24 h saline-infused ewe with a ''hypercontracting'' artery. Each data point represents 1 or 2 ring preparations. (b) Cumulative dose–response curves to noradrenaline (10 nM–10 μM) of rings from ''normal'' contracting uterine arteries ($n = 33$ at each concentration of noradrenaline, $N = 5$) and ''hypercontracting'' uterine arteries ($n = 66$ at each concentration, $N = 7$). Values are mean ± S.E.M., *N* refers to the number of animals and *n* refers to the number of ring preparations.

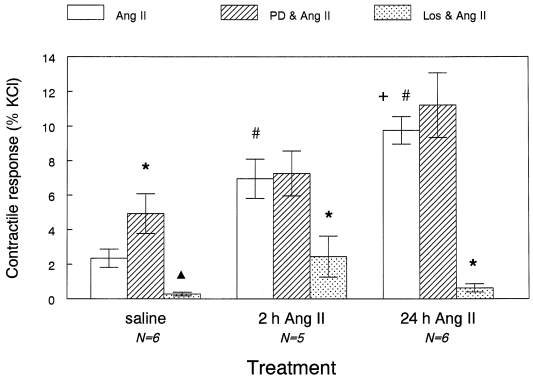

Fig. 3. Contractile responses of ''normal'' uterine arteries to angiotensin II (Ang II, 4 μM) alone (open rectangle; ewes infused with saline, $n = 24$; angiotensin II for 2 h, $n = 24$; angiotensin II for 24 h, $n = 19$) and in the presence of 1 μM PD 123319 (rectangle with diagonal lines; saline, $n = 18$; 2 h angiotensin II, $n = 13$; 24 h angiotensin II, $n = 9$) or 1 μM losartan (rectangle filled; saline, $n = 10$; 2 h angiotensin II, $n = 8$; 24 h angiotensin II, $n = 8$). Results are expressed as mean ± S.E.M. *N* refers to the number of animals and *n* refers to the number of ring preparations. *: $P < 0.05$ compared to angiotensin II alone, ▲: $P < 0.05$ compared to angiotensin II alone (significant by non-paired Student's *t*-test only), #: $P < 0.05$ compared to angiotensin II response in saline treated ewes, +: $P < 0.05$ compared to angiotensin II response in 2 h angiotensin II-treated ewes.

3.1. ''Normal'' contracting uterine arteries

The contractile responses of rings from 2 and 24 h angiotensin II-treated ewes to angiotensin II (4 μM) were greater than that of rings from saline-infused ewes ($P < 0.05$, Fig. 3). Also, the contractile response of rings from 24 h angiotensin II-treated ewes was greater than that of rings from 2 h angiotensin II-treated ewes ($P < 0.05$). There were no differences in the responses of rings to KCl (saline: 11.3 ± 0.5 g, $n = 52$; angiotensin II treatment for 2 h: 12.0 ± 0.4 g, $n = 45$; and angiotensin II treatment for 24 h: 10.6 ± 0.4 g, $n = 36$).

Losartan (1 μM) inhibited angiotensin II induced contractions in all groups ($P < 0.05$, Fig. 3). By contrast PD 123319 (1 μM) enhanced the response to angiotensin II of rings from control ewes from $2.3 \pm 0.5\%$ to $4.9 \pm 1.2\%$

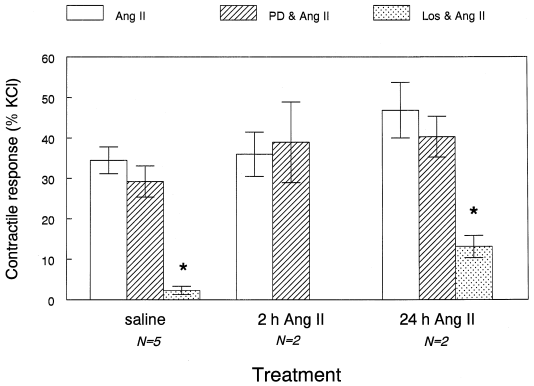

Fig. 4. Contractile responses of ''hypercontracting'' uterine arteries to angiotensin II (Ang II, 4 μM) alone (open rectangle; ewes infused with saline, $n = 21$; angiotensin II for 2 h, $n = 4$; angiotensin II for 24 h, $n = 8$) and in the presence of 1 μM PD 123319 (rectangle with diagonal lines; saline, $n = 12$; 2 h angiotensin II, $n = 3$; 24 h angiotensin II, $n = 3$) or 1 μM losartan (rectangle filled; saline, $n = 9$; 24 h angiotensin II, $n = 3$). Results are mean ± S.E.M. N refers to the number of animals and n refers to the number of ring preparations. *: $P < 0.05$ compared to angiotensin II alone.

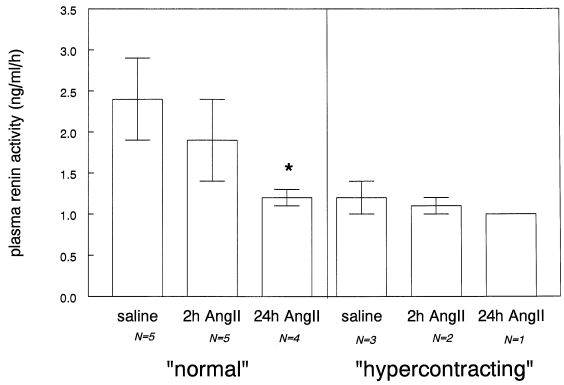

Fig. 5. Plasma renin activity in ewes with ''normal'' and ''hypercontracting'' uterine arteries during infusions of saline, angiotensin II (Ang II) for 2 h and angiotensin II for 24 h. Values are mean ± S.E.M. N refers to the number of animals. *: $P < 0.05$ compared to saline-infused ewes with ''normal'' contracting uterine arteries.

($P < 0.05$, Fig. 3). PD 123319 did not alter the contractile response to angiotensin II of rings from ewes infused with angiotensin II for 2 or 24 h (Fig. 3).

3.2. ''Hypercontracting'' uterine arteries

In contrast to ''normal'' contracting uterine arteries, there were no differences between the contractile responses of ''hypercontracting'' rings to angiotensin II (4 μM) from any of the three treatments (Fig. 4). Losartan (1 μM) inhibited these angiotensin II induced contractions ($P < 0.05$, Fig. 4), but PD 123319 (1 μM) had no effect in rings from any of the three treatments.

3.3. Plasma analyses

Maternal plasma noradrenaline, adrenaline, cortisol, lactate, glucose, osmolality, sodium and potassium concentrations, and sodium potassium ratio were similar in the two populations and were not affected by the infusions of angiotensin II (Table 1). Plasma renin activity was the only parameter which showed any significant differences. Plasma renin activity was greatest in saline treated ewes with ''normal'' contracting arteries (Table 1, Fig. 5). In the ''normal'' population, plasma renin activity tended to be lower in 2 h angiotensin II-treated ewes and was significantly lower in 24 h angiotensin II-treated ewes ($P < 0.05$). Ewes with ''hypercontracting'' arteries had very similar plasma renin activities to those found in 24 h

Table 1
Plasma analyses. Plasma renin activity (PRA), noradrenaline (NA), adrenaline (ADR), cortisol (CORT), lactate, glucose, osmolality (osM), sodium (Na) and potassium (K) concentrations, and sodium potassium ratio (Na/K) in saline, 2 h angiotensin II-treated and 24 h angiotensin II-treated ewes with ''normal'' and ''hypercontracting'' uterine arteries

	Normal			Hypercontracting		
	Saline ($N = 5$)	2 h Ang II ($N = 5$)	24 h Ang II ($N = 4$)	Saline ($N = 3$)	2 h Ang II ($N = 2$)	24 h Ang II ($N = 1$)
PRA (ng ml^{-1} h^{-1})	2.4 ± 0.5	1.9 ± 0.5	1.2 ± 0.1[a]	1.2 ± 0.2	1.1 ± 0.1	1.0
NA (nmol/l)	2.7 ± 0.7	2.4 ± 1.1	2.2 ± 1.0	2.6 ± 0.8	2.1 ± 0.5	2.6
ADR (nmol/l)	0.9 ± 0.3	1.6 ± 0.6	1.5 ± 1.0	1.3 ± 0.4	2.3 ± 2.2	1.1
CORT (nmol/l)	75 ± 13	88 ± 11	91 ± 18	51 ± 9	85 ± 41	38
lactate (mmol/l)	5.8 ± 0.4	7.0 ± 1.2	7.5 ± 0.7	7.6 ± 0.7[b]	5.0 ± 1.5	6.5
glucose (mmol/l)	3.4 ± 0.3	3.9 ± 0.1	4.2 ± 0.3	4.7 ± 0.8[b]	4.4 ± 1.4	3.5
Osm (mosM/kg)	295 ± 4	303 ± 2	304 ± 9	297 ± 6.0[b]	297 ± 4	295
Na (mmol/l)	154 ± 5	159 ± 4	142 ± 7	143 ± 8[b]	152 ± 11	147
K (mmol/l)	4.1 ± 0.1	4.5 ± 0.3	4.0 ± 0.4	4.1 ± 0.2[b]	4.2 ± 0.0	4.3
Na/K	37 ± 1	36 ± 4	37 ± 5	35 ± 0[b]	37 ± 5	35

[a] $P < 0.05$ compared to saline-treated ewes with ''normal'' contracting uterine arteries. Values are means ± S.E.M. Number of animals, N, as shown at the top of each column unless otherwise indicated.
[b] $N = 2$.

angiotensin II-treated ewes with ''normal'' contracting uterine arteries (Table 1, Fig. 5).

4. Discussion

Three important and novel findings emerged from this study. First, blocking angiotensin AT_2 receptors in the vascular smooth muscle of uterine arteries from control animals enhanced in vitro angiotensin contractile responses. This suggests angiotensin AT_2 receptors inhibited the vasoconstrictor effects of angiotensin II mediated via angiotensin AT_1 receptors. Second, prolonged intravenous infusions of angiotensin II in pregnant ewes enhanced the contractility of their uterine arteries to angiotensin II in vitro. Further, angiotensin AT_2 blockade in vitro did not enhance the contractile responses of these uterine arterial rings to angiotensin II, suggesting that the increased reactivity of these vessels was due in part to down regulation of angiotensin AT_2 receptors. Finally, we found a subpopulation of pregnant ewes with abnormal renin angiotensin systems and uterine arteries which showed a hyperreactivity to angiotensin II.

Due to angiotenisn II induced tachyphylaxis and the refractory nature of the uteroplacental vasculature to angiotensin II, some technical constraints occurred with regard to the in vitro studies. Angiotensin II induced tachyphylaxis (acute and rapid desensitisation) is a recognised in vitro phenomenon (Sim and Kuttan, 1992). Because uterine arterial rings developed tachyphylaxis to angiotensin II we were limited to a single concentration of angiotensin II on each ring. This combined with animal limitations prevented the construction of dose–response curves routinely. Also, because the rings did not maintain a sustained contraction to angiotensin II cumulative dose–response curves were not possible. The refractory nature of the uteroplacental vasculature meant that the angiotensin II responses of rings from ''normal'' arteries was small, even to near maximal concentrations. Therefore it was not feasible to study rings with intact endothelium. Cox et al. (1996a) showed that angiotensin II caused increases in ovine uterine artery prostacyclin (potent vasodilator) synthesis which was mediated by endothelial AT_1 receptors. Thus in this study we have only examined the effects of drugs on the vascular smooth muscle of uterine arteries.

4.1. Ewes with ''normal'' contracting uterine arteries

The uterine arteries eliciting contractions to angiotensin II less than 15% (of KCl) were classified as ''normal'' because most arteries fell into this category, and based on in vivo studies we expected uterine arteries to be relatively refractory to angiotensin II. Maternal plasma renin activity was used as an index of the activity of the maternal endogenous renin angiotensin system. As predicted, ewes given an infusion of angiotensin II for 24 h had lower plasma renin activities compared to control (saline treated) ewes (Katz and Malvin, 1993).

The mean angiotensin II induced contraction in rings from ewes infused with angiotensin II for 24 h was approximately 4 times greater than the response observed in rings from saline-infused animals (Fig. 3). The contractile response of rings from 2 h angiotensin II-treated ewes was also greater than rings from control arteries ($P < 0.05$), however there was greater variation between ewes in this group. Some arteries responded to angiotensin II in a manner similar to those of control animals while others responded like arteries from 24 h angiotensin II-treated animals. The increase in the in vitro reactivity of uterine arteries to angiotensin II from 24 h angiotensin II-treated ewes would explain, at least in part the fall in uterine blood flow observed by Stevens and Lumbers (1999) after 16 h of an angiotensin II infusion.

The mechanisms via which intravenous infusions of angiotensin II caused an increased reactivity of uterine arteries to angiotensin II in vitro were studied using angiotensin AT_1 and AT_2 antagonists. Most actions of angiotensin II are mediated by the angiotensin AT_1 receptor subtype (Timmermans et al., 1993). Since angiotensin II contractile responses were inhibited ($P < 0.05$) by losartan in the present study, contraction of uterine arterial vascular smooth muscle was mediated by angiotensin AT_1 receptors.

In contrast to most other adult blood vessels, the angiotensin AT_2 receptor subtype is the dominant angiotensin II receptor expressed in ovine uterine arteries during late gestation (Cox et al., 1996a,b; Burrell and Lumbers, 1997). The physiological role of the angiotensin AT_2 receptor is not well defined, though an inhibitory or vasodilator role for angiotensin II via the angiotensin AT_2 receptor has been suggested (Hein et al., 1995; Höhle et al., 1995; Ichiki et al., 1995; Nossaman et al., 1995; Munzenmaier and Greene, 1996). In the present study the angiotensin AT_2 antagonist, PD 123319, enhanced ($P < 0.05$) the angiotensin II induced response of arterial rings from saline-infused ewes by more than 100% (Fig. 3). This suggests that the angiotensin AT_2 receptor inhibited the contractile response mediated by the angiotensin AT_1 receptor. Since we first reported this data (McMullen et al., 1996), Zwart et al. (1998) have repeated our experiments on rat uterine arteries using the same concentration of PD 123319 (1 μM) and their results support our original findings. They found a 3-fold leftward shift of the angiotensin II curve and an increase in the maximum response by about 30% in the presence of the angiotensin AT_2 antagonist (Zwart et al., 1998). Although studies in rat aortic rings have failed to show any interaction between angiotensin AT_2 and AT_1 receptors in arterial vascular smooth muscle preparations (Li et al., 1995), this may be due to a lower density of angiotensin AT_2 receptors in this tissue (Viswanathan et al., 1991).

In uterine arterial rings from ewes infused with angiotensin II for 2 or 24 h, PD 123319 had no effect on the angiotensin II induced response. This suggests that there may have been a smaller percentage of AT_2 receptors in arteries from angiotensin II-treated ewes (i.e., angiotensin II caused down regulation of AT_2 receptors). Relatively fewer angiotensin AT_2 receptors to suppress the angiotensin AT_1 receptor mediated contraction could explain the increased reactivity to angiotensin II observed in arterial rings from angiotensin II-treated ewes. Down regulation of AT_2 receptors is partly substantiated by preliminary data showing that uterine arteries from ewes treated with angiotensin II for 24 h had lower AT_2 mRNA levels compared to arteries from control animals. There were no differences in the expression of AT_1 mRNA (McMullen et al., 1999a). Ouali et al. (1997) showed that angiotensin AT_2 receptors and mRNA decrease in bovine adrenal cells after exposure to angiotensin II for 6 h.

4.2. Ewes with ''hypercontracting'' uterine arteries

The mean angiotensin II induced contractility of ''hypercontracting'' uterine arteries was 6-fold greater than that elicited in ''normal'' contracting uterine arteries. This effect was specific to angiotensin II in that the responses to KCl and noradrenaline were not affected (Fig. 2). Blockade of angiotensin AT_2 receptors had no effect on angiotensin II induced contractile responses of ''hypercontracting'' arteries. The large contractility of these arteries, combined with their inability to respond to PD 123319 may in part be explained by down regulation of angiotensin AT_2 receptors. This is supported by recent results, showing that ''hypercontracting'' uterine arteries from control animals tended to have lower angiotensin AT_2 mRNA levels compared to ''normal'' uterine arteries from control animals, while there was no difference in angiotensin AT_1 mRNA levels (McMullen et al., 1999b). However, down regulation of AT_2 receptors is unlikely to account entirely for the hyperreactivity observed in these arteries since they were very much more reactive to angiotensin II than any vessels from ewes with ''normal'' uterine arteries.

The different reactivity of uterine arteries to angiotensin II in vitro was not the only distinguishing feature observed in the 2 populations of sheep. Ewes with ''hypercontracting'' arteries also tended to have lower plasma renin activities, higher adrenal AT_1 mRNA, and lower adrenal and left ventricle AT_2 mRNA levels than saline-infused ewes with ''normal'' contracting uterine arteries (McMullen et al., 1999b). Together, this suggests that these animals had very different profiles in terms of their renin angiotensin systems.

The reason why this subpopulation of ewes existed is unknown. However, the nine ewes with ''hypercontracting'' uterine arteries were experimented on at the end of the breeding season. A possible explanation, may be that these ewes experienced an environmental insult that permanently altered the balance of their endocrine systems, resulting in abnormal activity of the renin angiotensin system. It is interesting to note that two distinct features of ewes with ''hypercontracting'' arteries, i.e., low plasma renin activities and an increased sensitivity to angiotensin II, are also features of preeclampsia (August, 1993). The ''hyperreactivity'' of these uterine arteries to angiotensin II may also suggest that these ewes had low uterine blood flows as occurs with preeclampsia. Blood pressure and uterine blood flow were not measured in our study because general anaesthesia or major abdominal surgery can result in down regulation of angiotensin AT_2 receptors, as a consequence of raised levels of ACTH and glucocorticoids (Burrell and Lumbers, 1997). Therefore, we can only speculate that ewes with ''hypercontracting'' uterine arteries may have had a preeclampsia-like disorder.

In conclusion, during normal pregnancy the angiotensin AT_2 receptor may help suppress angiotensin AT_1 receptor mediated vasoconstriction of the uterine artery and play a role in the regulation of uterine blood flow. However, long term (24 h) elevation of angiotensin II above levels that normally occur during pregnancy causes increased reactivity of uterine arteries to angiotensin II and loss of this protective effect. This is in part due to down regulation of angiotensin AT_2 receptors.

Acknowledgements

This study was supported by the National Health and Medical Research Council of Australia. J.R. McMullen was supported by an Australian Postgraduate Award. We would like to thank Ciba-Geigy Australia for their gift of Hypertensin, DuPont Merck Pharmaceutical for their gift of losartan, Ms. J. Flux (Department of anatomy, University of NSW) for histological detection of endothelium, Ms. V. Lazzaro (Renal Diagnostic Laboratory, Royal Prince Alfred Hospital, Camperdown) for measuring plasma catecholamines, and H. Theile (Endocrinology, Prince of Wales Hospital, NSW, Australia) for measuring plasma cortisol levels.

References

Abdul-Karim, R., Assali, N.S., 1961. Pressor response to angiotonin in pregnant and nonpregnant women. Am. J. Obstet. Gynecol. 82, 246–251.

August, P., 1993. The renin–angiotensin–aldosterone system in hypertension in human pregnancy. In: Robertson, J.I., Nicholls, M.G. (Eds.), The Renin–Angiotensin System, Chap. 52. Gower Medical Publishing, London.

Burrell, J.H., Lumbers, E.R., 1997. Angiotensin receptor subtypes in the uterine artery during ovine pregnancy. Eur. J. Pharmacol. 330, 257–267.

Clark, K.E., Irion, G.L., Mack, C.E., 1990. Differential responses of uterine and umbilical vasculatures to angiotensin II and norepinephrine. Am. J. Physiol. 259, H197–H203.

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., Word, R.A., Rosenfeld, C.R., 1996b. Angiotensin II receptor characteristics and subtype expression in uterine arteries and myometrium during pregnancy. J. Clin. Endocrinol. Metab. 81, 49–58.

Hein, L., Barsh, G.S., Pratt, R.E., Dzau, V.J., Koblika, B.K., 1995. Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice. Nature 377, 744–747.

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.

Ichiki, T., Labosky, P.A., Shiota, C., Okuyama, S., Imagawa, Y., Fogo, A., Niimura, F., Ichikawa, I., Hogan, B.L.M., Inagami, T., 1995. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. Nature 377, 748–750.

Katz, S.A., Malvin, R.L., 1993. Renin secretion: control, pathways, and glycolysation. In: Robertson, J.I., Nicholls, M.G. (Eds.), The Renin–Angiotensin System, Chap. 24. Gower Medical Publishing, London.

Li, Q., Zhang, J., Pfaffendorf, M., Van Zwieten, P.A., 1995. Comparative effects of angiotensin II and its degradation products angiotensin III and angiotensin IV in rat aorta. Br. J. Pharmacol. 116, 2963–2970.

Lumbers, E.R., Reid, G.C., 1977. Effects of vaginal delivery and caesarian section on plasma renin activity and angiotensin II levels in human umbilical cord blood. Biol. Neonat. 31, 127–134.

Magness, R.R., Rosenfeld, C.R., Faucher, D.J., Mitchell, M.D., 1992. Uterine prostaglandin production in ovine pregnancy: effects of angiotensin II and indomethacin. Am. J. Physiol. 263, H188–H197.

Massani, Z.M., Sanguinetti, R., Gallegos, R., Raimondi, D., 1967. Angiotensin blood levels in normal and toxemic pregnancies. Am. J. Obstet. Gynecol. 99, 313–317.

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, (Abst).

McMullen, J.R., Gibson, K.J., Lumbers, E.R., 1999a. AT_2 receptors may play a role in maintaining uterine blood flow. FASEB J. 13 (4), A431, (Abst) Part I (366.5).

McMullen, J.R., Gibson, K.J., Lumbers, E.R., 1999b. A preeclampsia-like disorder in ewes?. FASEB J. 13 (4), A431, (Abst) Part I (366.6).

Munzenmaier, D.H., Greene, A.S., 1996. Opposing actions of angiotensin II on microvascular growth and arterial blood pressure. Hypertension 27, 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, 468–474.

Nossaman, B.D., Feng, C.J., Kaye, A.D., Kadowitz, P.J., 1995. Analysis of responses to Ang IV: effects of PD-123319 and DuP-753 in the pulmonary circulation of the rat. Am. J. Physiol. 268, L302–L308.

Ouali, R., Berthelon, M.C., Bégeot, M., Saez, J.M., 1997. Angiotensin II receptor subtypes AT1 and AT2 are down-regulated by angiotensin II through AT1 receptor by different mechanisms. Endocrinology 138, 725–733.

Rosenfeld, C.R., Gant, N.F., 1981. The chronically instrumented ewe: a model for studying vascular reactivity to angiotensin II in pregnancy. J. Clin. Invest. 67, 486–492.

Rosenfeld, C.R., Naden, R.P., 1989. Uterine and nonuterine vascular responses to angiotensin II in ovine pregnancy. Am. J. Physiol. 257, H17–H24.

Sim, M.K., Kuttan, S.C., 1992. Effects of noradrenaline and prostaglandin F2α on angiotensin-induced contraction and tachyphylaxis in rat aortic rings. Pharmacol. Toxicol. 70, 60–64.

Stevens, A.D., Lumbers, E.R., 1999. The effects of long term infusions of angiotensin II into the pregnant ewe on uterine blood flow and on the fetus. J. Cardiovasc. Pharmacol., in press.

Svane, D., Kahr, O., Hansen, V.B., Holm-Nielsen, P., Forman, A., 1995. Angiotensin-converting enzyme activity and contractile effects of angiotensin I and II in human uteroplacental arteries. Am. J. Obstet. Gynecol. 172, 991–997.

Timmermans, P.B.M.W.M., Wong, P.C., Chiu, A.T., Herblin, W.F., Benfield, P., Carini, D.J., Lee, R.J., Wexler, R.R., Saye, J.A.M., Smith, R.D., 1993. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol. Rev. 45, 205–251.

Viswanathan, M., Tsutsumi, K., Correa, F.M.A., Saavedra, J.M., 1991. Changes in expression of angiotensin receptor subtypes in the rat aorta during development. Biochem. Biophys. Res. Commun. 179, 1361–1367.

Wilson, M., Morganti, A.A., Zervoudakis, I., Letcher, R.L., Romney, B.M., Von Oeyon, P., Papera, S., Sealey, J.E., Laragh, J.H., 1980. Blood pressure, the renin–aldosterone system and sex steroids throughout normal pregnancy. Am. J. Med. 68, 97–104.

Zhang, L., Pearce, W.J., Longo, L.D., 1995. Noradrenaline-mediated contractions of ovine uterine artery: role of inositol 1,4,5-trisphosphate. Eur. J. Pharmacol. 289, 375–382.

Zwart, A.S., Davis, E.A., Widdop, R.E., 1998. Modulation of AT_1 receptor-mediated contraction of rat uterine artery by AT_2 receptors. Br. J. Pharmacol. 125, 1429–1436.