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Comparison of angiotensin II type 1 receptor blockade and angiotensin-converting enzyme inhibition in pregnant sheep during late gestation

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1 The effects of antagonism of the maternal renin-angiotensin system (RAS) with either an angiotensin II type 1-(AT_1) specific receptor blocker (GR138950) or an angiotensin-converting enzyme (ACE) inhibitor (captopril) were compared in chronically-catheterised ewes and their foetuses during late gestation.

2 Daily from 127±1 days of gestation until parturition at 145±2 days, each ewe received i.v. either GR138950 (3 mg kg^{-1}; $n=10$), captopril (3 mg kg^{-1}; $n=6$) or an equivalent volume of vehicle solution (0.9% w/v saline; $n=10$).

3 Within 2 h of drug administration, GR138950 abolished the maternal, but not the foetal, pressor responses to angiotensin II (AII; 100–188 ng kg^{-1}, i.v.; $P<0.05$), whereas captopril abolished both the maternal and foetal pressor responses to angiotensin I (AI; 400–750 ng kg^{-1}, i.v.; $P<0.05$).

4 On the first day of treatment, maternal blood pressure decreased in all GR138950-treated (−21±4 mmHg; $P<0.05$) and captopril-treated (−13±5 mmHg; $P>0.05$) ewes at 2 h after drug administration. Captopril also significantly decreased foetal blood pressure by 5±1 mmHg ($P<0.05$). However, foetal blood pressure in the GR138950-treated animals remained unchanged. Maternal and foetal heart rates were unaffected by any treatment. Uterine blood flow was significantly reduced within 2 h of both GR138950 (−130±20 ml min^{-1}; $P<0.05$) and captopril (−72±16 ml min^{-1}; $P<0.05$) administration.

5 On the first day of treatment, maternal arterial haemoglobin (Hb) concentration and oxygen (O_2) content increased at 2 h in all GR138950-treated and captopril-treated ewes. Foetal arterial pH and oxygenation (O_2 content, O_2 saturation and PaO_2) were reduced by a similar extent in both groups of drug-treated ewes.

6 After one week of daily GR138950 administration, maternal blood pressure decreased from a pretreatment value of 96±5 mmHg on day 1 to 79±2 mmHg by day 7 ($P<0.05$). Captopril treatment had no long-term effect on maternal blood pressure. Although foetal blood pressure increased by 3±1 mmHg over a week of vehicle treatment ($P<0.05$), no significant differences were observed between the long-term changes in foetal blood pressure in all three groups of animals.

7 There were no long-term effects of drug administration on maternal Hb concentration or oxygenation, or on the foetal haematological parameters. However, changes in maternal $PaCO_2$ observed in the GR138950-treated (+1.4±0.5 mmHg; $P<0.05$) and captopril-treated (+3.3±1.1 mmHg; $P>0.05$) ewes were significantly different from those seen in the vehicle-treated animals ($P<0.05$).

8 There were no apparent adverse effects of maternal GR138950 or captopril treatment on foetal viability.

9 The present study demonstrated that administration of either GR138950 or captopril to pregnant ewes effectively blocked the maternal RAS, and caused hypotension and a decrease in uterine blood flow. However, only captopril appeared to cross the placenta to influence directly the RAS of the sheep foetus. This suggests that the fall in foetal oxygenation observed after AT_1-specific receptor blockade and ACE inhibition originates primarily from changes in the maternal and/or placental vasculature. Despite these changes, neither GR138950 nor captopril were detrimental to the outcome of pregnancy when foetal blood loss was kept to a minimum.

Keywords: Angiotensin II; AT_1 receptor antagonist; angiotensin-converting enzyme inhibitor; GR138950; captopril; blood pressure; foetus; pregnancy

Introduction

During pregnancy, the renin-angiotensin system (RAS) becomes more important in the homeostatic regulation of maternal cardiovascular and renal function, and increases in activity with gestational age in sheep (Fleischman *et al.*, 1975) and women (review: Skinner, 1993). Normally, adequate oxygen and nutrient delivery to the foetus is maintained in this situation by a decrease in the sensitivity of the systemic, including the uteroplacental, vasculature to the constrictive effects of angiotensin II (AII; sheep: Rosenfeld & Gant, 1981; Rosenfeld & Naden, 1989; women: Loquet *et al.*, 1990; Ito *et al.*, 1992). This refractoriness of the systemic vasculature to AII is not, however, as marked in women with pregnancy-

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induced hypertension (Gant *et al.*, 1973). Abnormalities within the RAS have, therefore, been implicated in the pathogenesis of pregnancy-induced hypertension.

Two types of anti-hypertensive drugs that act at different sites within the RAS have been developed. First, angiotensin-converting enzyme (ACE) inhibitors, such as captopril, prevent the conversion of the relatively inactive AI to vasoactive AII. However, a number of case studies have contraindicated the use of ACE inhibitors during human pregnancy because of adverse effects obtained in the foetus and newborn infant (review: Hanssens *et al.*, 1991). Maternal ACE inhibition has also been associated with foetotoxicity in a variety of experimental animals, including rabbits, guinea-pigs, baboons and sheep (Broughton Pipkin *et al.*, 1982; Thomas & Thompson, 1986; Harewood *et al.*, 1994). In sheep, the poor outcome of pregnancy may be due to the foetal hypotension, hypoxaemia and changes in renal function observed in pregnant ewes treated with captopril (Lumbers *et al.*, 1992; 1993). These changes in the sheep foetus may be due, at least in part, to the alterations in maternal cardiovascular function and placental perfusion observed after ACE inhibition (Lumbers *et al.*, 1992). However, since captopril readily crosses the ovine placenta, the responses seen in the foetus may also reflect direct inhibition of the foetal RAS, which is known to be active in cardiovascular homeostasis during late gestation. Captopril does not only act on the RAS. It inhibits bradykinin degradation, so allowing potential accumulation of a vasodilator, and inhibits uterine prostaglandin E synthesis (Ferris & Weir, 1983). Interpretation of the effects of captopril is, therefore, difficult. In addition, in a number of previous studies of maternal ACE inhibition, relatively large volumes of foetal blood were taken which would normally have activated the foetal RAS (Broughton Pipkin *et al.*, 1974) and, consequently, may have exacerbated the detrimental effects of captopril.

The second group of drugs which act on the RAS are antagonists that specifically block the AII type I (AT_1) receptor that mediates the vasoconstrictive action of AII (Bottari *et al.*, 1993). Since ACE inhibitors can also block bradykinin degradation and modulate prostaglandin synthesis, AT_1 receptor antagonists provide a more selective treatment for RAS-dependent hypertension (Timmermans *et al.*, 1993; MacFadyen & Reid, 1994), although they have not been used in pregnancy. Previous work has shown that administration of GR117289, an AT_1-specific receptor antagonist, to pregnant ewes causes transient maternal hypotension and reductions in placental perfusion and foetal oxygenation (Forhead *et al.*, 1995). However, the extent to which the foetal RAS is affected during long-term maternal AT_1 receptor blockade has not been examined. Furthermore, little is known about the comparative actions of the AT_1 receptor antagonists and ACE inhibitors during pregnancy. GR138950 is another potent AT_1-specific receptor antagonist which reduces the high blood pressure induced in rats by renal artery ligation, but which has negligible effects in normotensive animals (Hilditch *et al.*, 1995).

Hence, in the present study the effects of antagonising the maternal RAS with either GR138950 or captopril in chronically-catheterised, normotensive ewes in the short- and long-term during late gestation were compared. Haemodynamic and haematological responses to either AT_1-specific receptor blockade or ACE inhibition were determined in the pregnant ewe and foetus when relatively small blood samples were taken. The consequences of daily maternal administration of these RAS antagonists for foetal well-being and viability were also examined.

Methods

Animals

Twenty-six Welsh Mountain ewes (29–64 kg) of known gestational age were studied; all but two of the ewes (twins) carried single foetuses. The animals were housed within the laboratory animal house in individual pens, and were maintained on 200 g day^{-1} concentrates, with free access to hay, water and a salt-lick block. Food, but not water, was withheld for 18–24 h before surgery at 119 ± 1 days of gestation (term 145 ± 2 days).

Surgical procedures

Under halothane anaesthesia (1.5% in O_2/N_2O_2) with positive pressure ventilation, intravascular catheters were inserted into the maternal femoral artery and vein. Transonic flow probes (Transonic Systems Inc, New York, U.S.A.) were also implanted around the main branch of the uterine artery supplying the pregnant horn. In all of the foetuses, both femoral arteries and a branch of the femoral vein were catheterised as described perviously (Comline & Silver, 1972); an additional catheter was secured to the foetal hind-limb to monitor amniotic fluid pressure. All catheters and leads were exteriorised through the flank of the ewe and secured in a plastic bag sutured to the skin. The catheters were flushed daily with heparinised saline solution (100 iu ml^{-1} heparin in 0.9% w/v saline) from the day after surgery. Antibiotic (procaine penicillin: Depocillin, Mycofarm, Cambridge, U.K.) was administered i.m. to all ewes on the day of surgery and for three days thereafter. The foetuses were injected i.v. with 100 mg ampicillin (Penbritin, Beecham Animal Health, Brentford, U.K.) and 2 mg gentamycin (Frangen-100, Biovet Ltd, Mullingar, Ireland) at surgery and at the end of each session of blood pressure recording.

Experimental protocol

The animals were randomly assigned to vehicle ($n = 10$), GR138950 ($n = 10$) or captopril ($n = 6$) treatment groups. Daily from 127 ± 1 days of gestation until parturition, each ewe received i.v. either GR138950 (3 mg kg^{-1}), captopril (3 mg kg^{-1}) or an equivalent volume of vehicle solution (0.9% w/v saline). Haemodynamic and haematological parameters were monitored in all the ewes, and in the foetuses of 5 vehicle-treated, 6 GR138950-treated and 4 captopril-treated animals. The remaining foetuses were used as part of another study, and the data from these foetuses is not presented here.

Efficacy of blockade of the RAS Maternal and foetal arterial blood pressure responses to i.v. AII (vehicle and GR138950 groups) and AI (vehicle and captopril groups) were examined to determine the extent of blockade of the RAS. One day before treatment began, AI (400–750 ng kg^{-1}; 10 μg ml^{-1} in 0.9% w/v saline; Sigma Chemical Co, Poole, U.K.) and/or AII (100–188 ng kg^{-1}; 2.5 μg ml^{-1} in 0.9% w/v saline; Hypertensin, Ciba-Geigy Pharmaceuticals, Horsham, U.K.) were injected i.v. into both the ewe and the foetus; each challenge test was separated by 10 min. Foetal and maternal blood pressures were monitored throughout these response tests. This procedure was repeated at 0 h and 2 h after treatment on the second treatment day (ie, 24 h after the first treatment). Pressor response tests were examined in four vehicle-treated ewes and two of their foetuses, in four GR138950-treated ewes and two of their foetuses, and in four captopril-treated ewes and three of their foetuses.

Physiological responses to antagonism of the RAS In all animals, arterial blood samples were obtained daily from the ewe and foetus before treatment (1–2 ml at time 0 h). On the first day of the treatment, larger blood samples (5 ml from the foetus and 15 ml from the ewe) were taken immediately before (0 h) and 2 h after treatment. In all animals on day 1 of treatment, cardiovascular parameters, including uterine blood flow, were continuously monitored from 0.5 h before to 2.5 h after treatment. Measurements were confined to one foetus in the ewes carrying twin foetuses. Cardiovascular recordings were also made before the daily administration of either vehicle or drug on day 7 of treatment.

Physiological measurements

Maternal and foetal arterial blood pressures, amniotic fluid pressure and uterine blood flow were monitored by standard recording equipment (Lectromed UK Ltd, Letchworth, U.K.). Foetal blood pressure was expressed less the amniotic fluid pressure. Heart rates were monitored by a cardiotachometer triggered from the arterial pulse waves.

Arterial blood samples were analysed for pH and gas tensions (PaO_2 and $PaCO_2$) by an ABL330 Radiometer analyser, and for O_2 saturation and haemoglobin (Hb) concentration by an OSM2 Hemoximeter (Radiometer, Copenhagen, Denmark). The remainder of the blood was placed in EDTA-containing tubes and centrifuged at 1000 *g* and 4°C for 10 min; the plasma aliquots were stored at −20°C to await analysis.

At delivery, lamb weight, sex and viability were noted. Crown-rump length (CRL), and kidney and adrenal weights were measured within 24 h of parturition when the lamb was killed by a lethal dose of barbiturate (sodium pentobarbitone: Pentoject, Animalcare Ltd, York, U.K.).

Drugs

GR138950 (1-[[3-bromo-2-[2-[[(triflurormethyl) sulphonyl] amino] phenyl]-5-benzofuranyl]methyl]-4-cyclopropyl-2-ethyl-1*H*-imidazole-5-carboxamide; Hilditch *et al.*, 1995) was supplied by Glaxo Wellcome Research and Development, and had an assigned purity of 90.5% w/w. Both GR138950 and captopril ([2S]-1-[3-mercapto-2-methylpropionyl]-L-proline; Sigma Chemical Co, Poole, U.K.) were freshly prepared every 3–4 days and stored at 4°C.

Statistical analysis

All data are presented as mean values ± s.e.mean. Pressor responses to exogenous AI or AII were expressed as a percentage change from the basal blood pressure; differences within and between the treatment groups were assessed by paired and unpaired *t* tests, respectively. Significant changes in the physiological parameters measured at 0 h and 2 h after vehicle or drug administration on the first day of treatment were determined by paired *t* tests. Within each treatment group, the long-term changes in the basal values between day 1 and day 7 were assessed by paired *t* tests. On day 7 of treatment, data were only available from 9 vehicle-treated, 8 GR138950-treated and 4 captopril-treated ewes, and were statistically compared to the data obtained from the same animals on day 1. Likewise, on day 7 of maternal vehicle treatment, data were only available from 4 of the 5 foetuses studied on day 1. One-way ANOVA and Fisher's test were used to detect any differences in the basal values, and in the short-term and long-term changes, between the three treatment groups. Differences of $P<0.05$ were regarded as significant.

Results

Efficacy of blockade of the RAS

The maternal pressor responses to AII and AI were abolished at 2 h after administration of GR138950 and captopril, respectively, on the second day of drug treatment (Figure 1a and b). In the GR138950-treated ewes, the maternal pressor responses to AII were similar before and 24 h after administration of the first dose of GR138950 (Figure 1a). By contrast, the maternal pressor response to AI observed at 24 h after the first administration of captopril was significantly smaller than that observed before treatment began ($P<0.05$; Figure 1b). Vehicle treatment had no effect on the maternal pressor responses to AII and AI (Figure 1).

In the foetuses studied, maternal GR138950 and vehicle treatment had no effect on the foetal pressor response to AII. However, the foetal pressor response to AI was abolished within 2 h of maternal administration of captopril, but not vehicle. Individual foetal blood pressure responses to AII and AI in vehicle-treated and drug-treated animals are shown in Figure 2.

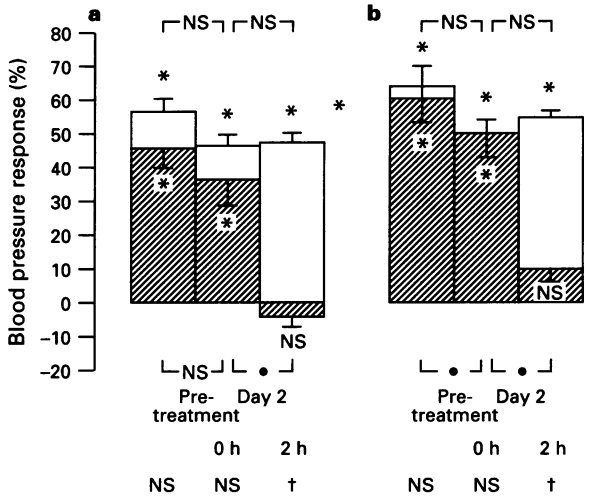

Figure 1 Mean (±s.e.mean) maternal blood pressure responses to (a) i.v. angiotensin II (AII) in GR138950-treated ($n=4$) and vehicle-treated ($n=4$) ewes, and to (b) i.v. AI in captopril-treated ($n=4$) and vehicle-treated ($n=4$) ewes. Responses observed in vehicle-treated and drug-treated animals are denoted as open and hatched columns, respectively. Significant pressor responses are indicated above or in each column (*$P<0.05$; NS, not significant). For each challenge, significant differences between the pressor responses observed in the vehicle-treated and drug-treated groups are shown below the labels (†$P<0.05$). Significant within-group differences between the pretreatment and the day 2 pressor responses at 0 h, and between the 0 h and 2 h pressor responses on day 2, are shown above the columns for the vehicle-treated ewes and below for the drug-treated ewes (•$P<0.05$).

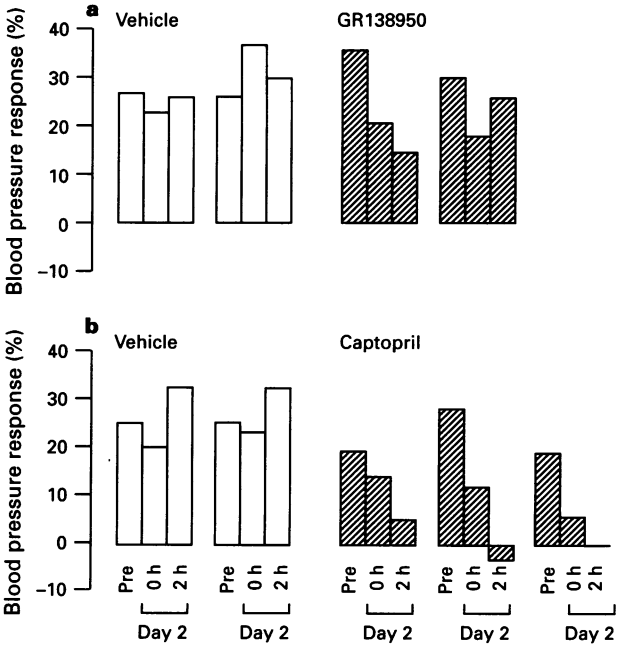

Figure 2 Individual foetal blood pressure responses to (a) i.v. angiotensin (AII) in GR138950-treated ($n=2$) and vehicle-treated ($n=2$) animals, and to (b) i.v. AI in captopril-treated ($n=3$) and vehicle-treated ($n=2$) animals. Responses observed in vehicle-treated and drug-treated animals are denoted as open and hatched columns, respectively.

Short-term responses to antagonism of the RAS

Cardiovascular responses No significant differences in pre-treatment maternal blood pressure were observed between the three groups of ewes (Figure 3a). On the first day of treatment, a decrease in maternal blood pressure was observed in all GR138950-treated and captopril-treated ewes at 2 h after drug administration (Figure 3a). However, because of the inter-animal variation, the mean decrement was only significant in the GR138950-treated group (-21 ± 4 mmHg; $P<0.05$; Figure 3a). The mean decreases in maternal blood pressure seen at 2 h were similar in the two groups of drug treated ewes, and were significantly different from the negligible change observed after vehicle administration ($P<0.05$; Figure 3a). Basal blood pressure in the foetuses was similar in all animals, and was unaffected by maternal GR138950 or vehicle administration (Figure 3b). In the captopril-treated ewes, foetal blood pressure significantly decreased by 5 ± 1 mmHg at 2 h after maternal drug administration ($P<0.05$; Figure 3b). The mean decrement was significantly different from the changes seen at 2 h in the vehicle-treated and GR138950-treated animals ($P<0.05$; Figure 3b),

Basal heart rates in both the ewes and the foetuses were similar in the three groups (Table 1). In addition, there were no significant changes in maternal or foetal heart rate at 2 h after treatment in any of the groups of ewes (Table 1).

There were no significant differences in the basal values of uterine blood flow between the three treatment groups (Figure 3c). On the first day of treatment, uterine blood flow significantly decreased at 2 h after treatment in both groups of drug-treated ewes ($P<0.05$; Figure 3c). No significant change in uterine blood flow was observed in the vehicle-treated animals (Figure 3c). GR138950 induced a greater reduction in uterine blood flow than captopril (-130 ± 20 ml min^{-1} *versus* -72 ± 16 ml min^{-1}, respectively; $P<0.05$; Figure 3c), and these mean changes were both significantly different from the change observed in the vehicle-treated ewes ($P<0.05$; Figure 3c).

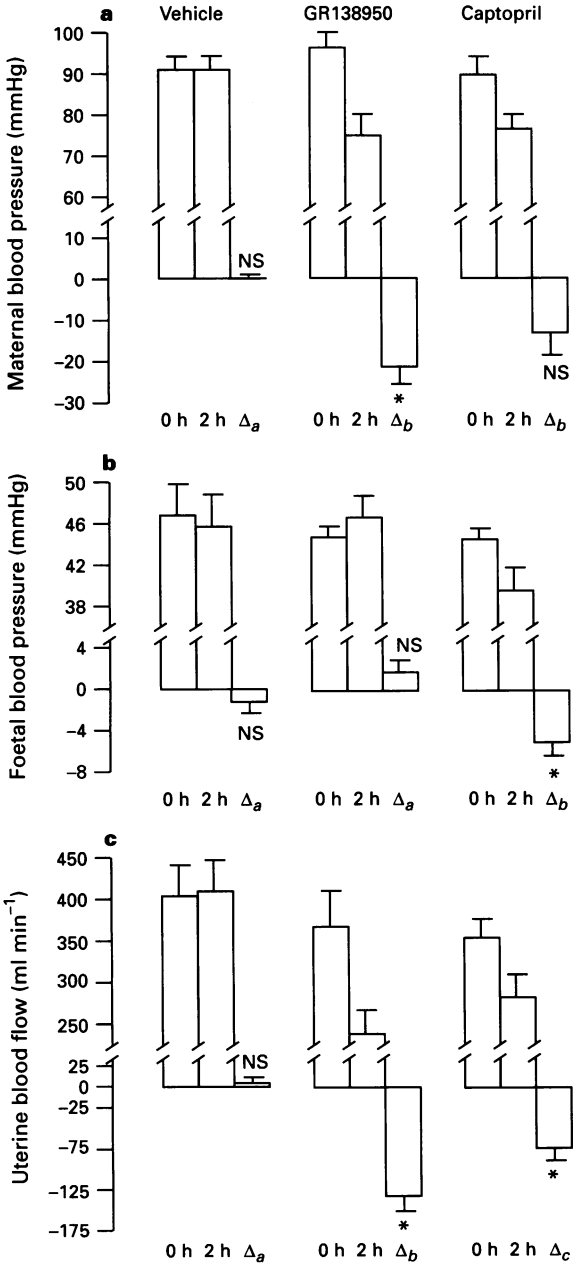

Figure 3 Mean (±s.e.mean) (a) maternal and (b) foetal blood pressure, and (c) uterine blood flow, at 0 h and 2 h after maternal administration of vehicle ($n=10$ ewes; $n=5$ foetuses), GR138950 ($n=10$ ewes; $n=6$ foetuses) and captopril ($n=6$ ewes; $n=4$ foetuses) on the first day of treatment. Uterine blood flow was measured in nine vehicle-treated, nine GR138950-treated and six captopril-treated ewes. Significant changes are indicated at each column (*$P<0.05$; NS, not significant). Statistical comparisons between the mean changes at 2 h post-treatment are shown below the columns; values with differing subscripts are significantly different ($P<0.05$).

Haematological responses On the first day of treatment, maternal Hb concentration increased in all GR138950-treated ($+0.7 \pm 0.1$ mmol l^{-1}; $P<0.05$) and captopril-treated ($+0.5 \pm 0.2$ mmol l^{-1}; $P>0.05$) ewes at 2 h after drug administration (Table 1). The mean increments were similar in the two drug-treated groups, and were significantly different from the mean change observed in the vehicle-treated ewes ($P<0.05$; Table 1). Foetal Hb concentration was unaffected by treatment in any of the groups of animals (Table 1).

The mean increments in maternal arterial O_2 content in the GR138950-treated and captopril-treated ewes were similar, and were significantly greater than the mean change seen in the vehicle-treated ewes ($P<0.05$; Table 1). Foetal arterial O_2 content decreased in all but one of the drug-treated animals yet, because of wide individual variation, the mean decrements in foetal O_2 content were not significant in either group (Table 1). However, the mean reductions in foetal O_2 content observed in the drug-treated animals were significantly different from that seen after vehicle administration ($P<0.05$; Table 1). At 2 h after drug administration, foetal O_2 saturation was decreased in the GR138950-treated ($-10.6 \pm 3.1\%$; $P<0.05$) and captopril-treated ($-10.1 \pm 4.0\%$; $P>0.05$) animals (Table 1). In addition, decreases in foetal PaO_2 were observed within 2 h of maternal administration of captopril (-2.9 ± 0.6 mmHg; $P<0.05$) and GR138950 (-2.4 ± 1.1 mmHg; $P>0.05$; Table 1). No significant changes were observed in maternal O_2 saturation or PaO_2 at 2 h after treatment in any of the groups of ewes (Table 1).

Foetal $PaCO_2$ was significantly increased at 2 h after maternal administration of captopril, but not GR138950 or vehicle ($P<0.05$; Table 1). Foetal pH significantly decreased at 2 h in both drug-treated groups ($P<0.05$; Table 1), but remained unchanged in the vehicle-treated animals (Table 1). However, no significant differences were observed in the foetal $PaCO_2$ or pH responses between the three groups of animals (Table 1). A small, but significant, decrease in maternal $PaCO_2$ was observed at 2 h after administration of GR138950, but not vehicle or captopril ($P<0.05$; Table 1). Maternal pH was unaffected by vehicle or drug treatment (Table 1).

Long-term responses to antagonism of the RAS

Cardiovascular responses Daily GR138950 administration for 7 days reduced maternal blood pressure from a pre-treatment value of 96 ± 5 mmHg to 79 ± 2 mmHg ($P<0.05$; Table 2).

Table 1 Maternal and foetal heart rates, and haematological parameters at 0 h and 2 h after maternal administration of vehicle, GR138950 and captopril on the first day of treatment

		Maternal			*Foetal*		
		Vehicle	*GR138950*	*Captopril*	*Vehicle*	*GR138950*	*Captopril*
		($n=10$)	($n=10$)	($n=6$)	($n=5$)	($n=6$)	($n=4$)
Heart rate	0 h	99±7	101±6	96±4	162±7	160±5	162±5
(beats min^{-1})	2 h	94±5	105±8	101±4	152±15	185±14	165±11
	Change	−5±2	+4±4	+5±3	−10±9	+25±13	+3±7
Hb conc (mmol l^{-1})	0 h	4.8±0.2	4.4±0.3	4.7±0.2	6.5±0.4	5.8±0.3	5.7±0.5
	2 h	4.7±0.2	5.1±0.4	5.2±0.2	6.9±0.4	6.0±0.3	5.9±0.6
	Change	$-0.1\pm0.1_a$	$+0.7\pm0.1^*_b$	$+0.5\pm0.2_b$	+0.4±0.2	+0.2±0.1	+0.2±0.1
O_2 content	0 h	4.81±0.23	4.59±0.30	4.71±0.22	3.69±0.32	3.63±0.28	3.43±0.42
(mmol l^{-1})	2 h	4.67±0.22	5.21±0.39	5.26±0.28	3.94±0.28	3.12±0.19	2.88±0.39
	Change	$-0.14\pm0.07_a$	$+0.62\pm0.13^*_b$	$+0.55\pm0.20_b$	$+0.25\pm0.19_a$	$-0.51\pm0.23_b$	$-0.55\pm0.23_b$
O_2 saturation (%)	0 h	$99.7\pm0.9_a$	$104.2\pm1.4_b$	$101.8\pm1.1_{ab}$	57.1±3.1	63.5±4.2	60.1±3.8
	2 h	99.2±1.1	103.1±1.3	101.8±1.3	56.7±2.3	52.9±4.4	50.0±6.6
	Change	−0.5±0.3	−1.1±0.7	0±0.3	−0.4±1.7	−10.6±3.1*	−10.1±4.0
Pa O_2 (mmHg)	0 h	99.1±1.6	102.3±2.2	99.0±1.2	$20.7\pm2.3_a$	$28.0\pm0.9_b$	$27.2\pm0.4_b$
	2 h	97.4±1.8	101.7±3.4	100.8±2.0	20.6±2.4	25.6±1.6	24.3±0.8
	Change	−1.7±1.7	−0.6±2.0	+1.8±2.7	−0.1±0.3	−2.4±1.1	−2.9±0.6*
Pa CO_2 (mmHg)	0 h	37.1±1.0	36.3±0.9	37.3±0.9	52.6±1.0	52.8±2.5	52.9±1.0
	2 h	36.7±0.8	35.1±0.9	36.9±1.1	52.5±0.7	53.6±1.8	55.6±1.3
	Change	−0.4±0.5	−1.2±0.5*	−0.4±0.9	−0.1±1.1	+0.8±0.9	+2.7±0.4*
pH	0 h	7.487±0.009	7.494±0.007	7.496±0.016	7.366±0.009	7.364±0.011	7.370±0.006
	2 h	7.470±0.005	7.480±0.011	7.465±0.015	7.367±0.005	7.349±0.010	7.358±0.008
	Change	−0.017±0.009	−0.014±0.011	−0.031±0.030	+0.001±0.012	−0.015±0.004*	−0.012±0.003*

Data shown are means±s.e.mean. Significant changes are indicated as superscripts (*$P<0.05$). Statistical comparisons between the basal values, and between the mean changes at 2 h post-treatment, are shown as subscripts: values with differing subscripts are

Table 2 Maternal and foetal heart rates, and haematological parameters on days 1 and 7 before maternal administration of vehicle, GR138950 and captopril

			Maternal			*Foetal*	
		Vehicle	*GR138950*	*Captopril*	*Vehicle*	*GR138950*	*Captopril*
		($n=9$)	($n=8$)	($n=4$)	($n=4$)	($n=6$)	($n=4$)
Blood pressure (mmHg)	Day 1	90±4	96±5	93±6	47±4	45±1	45±1
	Day 7	87±4	79±2	92±3	50±4	43±3	41±5
	Change	$-3\pm2_a$	$-17\pm4^*_b$	$-1\pm5_a$	+3±1*	−2±3	−4±5
Heart rate (beats min^{-1})	Day 1	101±7	105±5	94±6	160±9	160±5	162±5
	Day 7	101±6	111±5	102±4	149±4	144±4	150±5
	Change	0±7	+6±6	+8±9	−11±6	−16±6*	−12±10
Hb conc (mmol l^{-1})	Day 1	4.8±0.2	4.4±0.4	4.8±0.1	6.5±0.5	5.8±0.3	5.7±0.5
	Day 7	4.5±0.2	4.2±0.3	4.7±0.2	6.0±0.7	5.8±0.3	6.0±0.7
	Change	−0.3±0.2	−0.2±0.2	−0.1±0.1	−0.5±0.2	0±0.2	+0.3±0.3
O_2 content (mmol l^{-1})	Day 1	4.79±0.26	4.59±0.38	4.88±0.20	3.84±0.37	3.63±0.28	3.43±0.42
	Day 7	4.57±0.24	4.31±0.32	4.74±0.19	3.49±0.50	3.59±0.22	2.91±0.40
	Change	−0.22±0.18	−0.28±0.15	−0.14±0.02*	−0.35±0.31	−0.04±0.30	−0.52±0.36
O_2 saturation (%)	Day 1	$99.7\pm1.0_a$	$105.2\pm1.6_b$	$101.7\pm1.4_{ab}$	59.2±2.9	63.5±4.2	60.1±3.8
	Day 7	101.3±0.8	103.1±1.1	101.9±1.1	58.1±3.4	62.3±2.7	49.5±6.7
	Change	+1.6±0.8	−2.1±1.1	+0.2±1.7	−1.1±4.5	−1.2±4.9	−10.6±7.0
PaO_2 (mmHg)	Day 1	99.3±1.7	102.0±2.7	100.1±0.5	21.2±2.8	27.5±1.4	27.2±0.4
	Day 7	101.6±2.2	101.7±2.8	14.5±4.4	21.2±2.1	27.0±0.8	26.8±2.1
	Change	+2.3±2.4	−0.3±2.5	+4.4±4.7	0±2.9	−0.5±0.9	−0.4±2.0
PaCO_2 (mmHg)	Day 1	37.5±0.6	36.3±1.1	37.3±1.2	52.7±1.3	52.8±2.5	52.9±1.0
	Day 7	$36.0\pm0.8_a$	$37.7\pm1.2_{ab}$	$40.6\pm0.4_b$	51.8±0.9	52.4±2.1	57.7±2.2
	Change	$-1.5\pm0.3^*_a$	$+1.4\pm0.5^*_b$	$+3.3\pm1.1_b$	−0.9±2.1	−0.4±0.7	+4.8±3.1
pH	Day 1	7.487±0.013	7.496±0.007	7.492±0.021	7.367±0.013	7.364±0.011	7.370±0.006
	Day 7	7.495±0.009	7.480±0.012	7.449±0.014	7.338±0.002	7.356±0.008	7.343±0.015
	Change	+0.008±0.012	−0.016±0.014	−0.043±0.017	−0.028±0.011	−0.008±0.014	−0.027±0.020

Data shown are means±s.e.mean. Significant changes are indicated as superscripts (*$P<0.05$). Statistical comparisons between the basal values, and between the mean changes on day 7, are shown as subscripts: values with differing subscripts are significantly different ($P<0.05$).

The mean long-term decrement in maternal blood pressure seen in the GR138950-treated ewes was significantly different from the negligible changes observed in the vehicle-treated and captopril-treated groups ($P<0.05$; Table 2). Mean foetal blood pressure was similar on days 1 and 7 of GR138950 and captopril treatment (Table 2), but in the vehicle-treated animals, basal foetal blood pressure was greater on day 7 than on day 1 ($P<0.05$; Table 2).

On day 7 of vehicle and drug treatment, no significant changes in basal maternal heart rate were observed from day 1 (Table 2). After a week of maternal GR138950 administration, basal foetal heart rate was significantly lower on day 7 than on day 1 ($P<0.05$; Table 2), but did not differ from the unchanged basal values observed in the other treatment groups on day 7.

Between treatment days 1 and 7, there were no significant differences in the mean basal uterine blood flow in ewes administered either vehicle (419 ± 41 ml min^{-1} on day 1 *versus* 401 ± 30 ml min^{-1} on day 7; $n=8$), GR138950 (336 ± 14 ml min^{-1} *versus* 356 ± 45 ml min^{-1}l $n=7$) or captopril (341 ± 32 min min^{-1} *versus* 389 ± 28 ml min^{-1}; $n=4$).

Haematological responses No significant changes in the basal values of maternal Hb concentration, O_2 saturation, PaO_2 and pH were observed between days 1 and 7 in any of the three treatment groups (Table 2). In the captopril-treated ewes, basal maternal O_2 content significantly decreased on day 7 ($P<0.05$; Table 2), but the mean long-term decrement did not differ from the negligible changes observed after GR138950 and vehicle treatment (Table 2). Basal maternal $PaCO_2$ differed between days 1 and 7 in both the vehicle and GR138950, but not the captopril, groups ($P<0.05$; Table 2). The long-term changes in maternal $PaCO_2$ were significantly different between the vehicle-treated and drug-treated ewes ($P<0.05$; Table 2). Basal maternal $PaCO_2$ on day 7 was also significantly different between the vehicle-treated and captopril-treated ewes ($P<0.05$; Table 2). For all three treatment groups, the basal foetal values of Hb concentration, O_2 content, O_2 saturation, PaO_2, $PaCO_2$ and pH observed on day 7 were not significantly different from those seen on day 1 (Table 2).

Perinatal viability There were no apparent adverse effects of long-term maternal GR138950 or captopril administration on gestational length or foetal survival in the foetuses presented in this study. One of the vehicle-treated ewes carrying twin foetuses had difficulties at delivery and both foetuses died as a consequence. Irrespective of maternal treatment, all the live lambs were viable and suckled normally. The outcome of pregnancy and the morphometric measurements obtained from the lambs delivered to vehicle-treated, GR138950-treated and captopril-treated ewes are shown in Table 3. No significant differences in lamb bodyweight, CRL or organ weights were observed between drug-treated and vehicle-treated animals.

Table 3 Neonatal outcome in vehicle-treated, GR138950-treated and captopril-treated animals

	Vehicle	*GR138950*	*Captopril*
Number of lambs	5	6	4
Delivery (days)	145 ± 2	143 ± 2	137 ± 2
Survival at delivery (%)	80	100	100
Lamb body weight (kg)	2.93 ± 0.19	2.89 ± 0.29	2.71 ± 0.19
Sex	2F/3M	3F/3M	3F/1M
CRL (cm)	46 ± 2	47 ± 2	44 ± 1
Kidney weight (g)	10.0 ± 0.8	12.8 ± 2.2	11.2 ± 3.4
Adrenal weight (mg)	373 ± 4	404 ± 61	439 ± 117

Discussion

The results of the present study show that, in the pregnant ewe, antagonism of the maternal RAS with either an AT_1-specific receptor blocker or an ACE inhibitor causes maternal hypotension, a fall in uterine blood flow and increases in maternal Hb and O_2 content within 2 h of drug administration. These short-term changes were accompanied by a reduction in foetal pH and oxygenation. In the long-term, treatment with either GR138950 or captopril for 7 days had no effect on uterine blood flow, or maternal and foetal haematological parameters, although AT_1-specific receptor antagonism did produce a sustained reduction in maternal blood pressure. However, none of the drug-induced changes appeared to have a detrimental effect on foetal viability or on the outcome of pregnancy in the present study.

The short-term reductions in maternal blood pressure observed after antagonism of the maternal RAS in the present study are consistent with previous findings that administration of ACE inhibitors during pregnancy lowers blood pressure in a variety of species (Olsson *et al.*, 1984; Harewood *et al.*, 1994; August *et al.*, 1995). In these earlier studies, ACE inhibition had a greater depressor effect in pregnant, than non-pregnant, animals which led to the suggestion that the RAS may become more prominant in cardiovascular homeostasis during pregnancy (Broughton Pipkin & O'Brien, 1978). The current observation that the fall in maternal blood pressure was similar in ewes treated with either GR138950 or captopril indicates that the AT_1 receptor, in peripheral and central locations, probably mediates the increased activity of the RAS during pregnancy. After a week of drug treatment, antagonism of the AT_1 receptor by GR138950 was more effective than ACE inhibition in the long-term reduction of maternal blood pressure. In part, this may reflect differences in drug clearance as preliminary measurements suggest that GR138950 accumulates in the maternal circulation over the 7 days of treatment (G Evans, unpublished observations). Alternatively, the non-specific effects of ACE inhibition may counteract the anti-hypertensive actions in the long-term

Clear differences were evident in the actions of maternal GR138950 and captopril treatment on the foetal cardiovascular system. While captopril appeared to cross the ovine placenta rapidly to block the foetal pressor response to AI, the foetal pressor response to AII remained intact in the GR138950-treated animals. Also, within 2 h of maternal drug administration, a hypotensive response was observed in the foetuses of the captopril-treated, but not GR138950-treated, animals. These findings suggest, in common with previous observations (Lumbers *et al.*, 1992; Stevenson *et al.*, 1995), that captopril administration to the pregnant ewe directly antagonizes the foetal RAS. In contrast, GR138950 behaved in a similar manner to losartan, another AT_1-specific receptor antagonist, in that both drugs effectively blocked the maternal, but not the foetal, pressor response to AII in pregnant sheep (Stevenson *et al.*, 1995).

There are a number of possible explanations for the negligible effect of maternal GR138950 treatment on the cardiovascular system of the sheep foetus. First, GR138950 may not be rapidly transferred across the placenta to the sheep foetus. Although GR138950 can be detected in the foetal circulation within 2 h of drug administration to the pregnant ewe, the concentrations in the foetus were 70–725 times lower than those measured in the ewe at the time that the maternal pressor response was blocked (G Evans, unpublished observations). Concentrations of GR138950 may, therefore, have been insufficient to saturate the binding sites responsible for the maintenance of basal blood pressure and the pressor response to AII in the sheep foetus. There is a evidence, however, that GR138950 accumulates in the foetal circulation over the 7 days of maternal treatment (G Evans, unpublished observations). Indeed, there were indications in the present study that the normal progressive increase in

foetal blood pressure that occurs with gestational age (Boddy *et al.*, 1974), and that was demonstrated in the vehicle-treated animals, was abolished in both groups of drug-treated animals.

Second, AII receptors in the sheep foetus may differ from those in the adult and, therefore, may bind GR138950 less effectively. In the rat foetus, the predominant AII receptor subtype in a variety of tissues is the AT_2 receptor although AT_1 mRNA is exclusively expressed in the major foetal blood vessels (Tsutsumi *et al.*, 1991; Shanmugam *et al.*, 1994). AT_1 receptors have also been located in foetal blood vessels in the human placenta (Kalenga *et al.*, 1991), but little is known about the AII receptors present in the vasculature of the sheep foetus. In contrast to ACE inhibition, blockade of the AT_1 receptor would have little, if any, effect on the foetal cardiovascular system if AII acts via the AT_2 receptors *in utero*. However, direct administration of losartan to the sheep foetus reduces foetal blood pressure (Stevenson & Lumbers, 1994) and suppresses the foetal pressor response to exogenous AII (Stevenson *et al.*, 1995) which suggests that the vasoconstrictive action of AII is mediated via the AT_1, or an AT_1-like, receptor subtype.

The third possible explanation for the negligible effect of GR138950 on basal blood pressure *in utero* is that the foetal RAS may not have been active in cardiovascular control at the time of maternal drug adminstration. This seems unlikely, considering the fall in foetal blood pressure observed in the captopril-treated animals. However, other investigators have shown both hypotensive (Broughton Pipkin & O'Brien, 1978; Iwamato & Rudolph, 1979) and negligible (Rankin & Phernetton, 1978) blood pressure responses to direct antagonism of the foetal RAS using saralasin, a non-specific AII receptor antagonist, which suggests that the level of activity of the foetal RAS before treatment may be critical in determining the observed response.

In the present study, uterine blood flow was shown to decrease within 2 h of either GR138950 or captopril administration to the pregnant ewes. These observations are in agreement with previous studies with AT_1 receptor blockers (Forhead *et al.*, 1995) and ACE inhibitors (Lumbers *et al.*, 1992). AII receptors have been localised in the ovine uteroplacental circulation (Mackanjee *et al.*, 1991), and AII infusions have dose-dependent effects on blood flow and vascular resistance in the pregnant uterus (Bruce *et al.*, 1981; Clarke *et al.*, 1990). During AII infusion in pregnant dogs, an increase in uterine vascular resistance and a decrease in uterine blood flow has been demonstrated in animals in which the uterine perfusion pressure was experimentally maintained at pretreatment values and was prevented from rising in response to the AII-induced increase in systemic blood pressure (Woods, 1993). The fall in uterine blood flow during antagonism of the maternal RAS may, therefore, reflect both the decrease in systemic pressure and a change in the relative resistances of the uteroplacental and systemic circulations. In the present study, the drug-induced reduction in uterine blood flow was less in the captopril-treated ewes than in the GR138950-treated animals. During AT_1-specific receptor blockade, there is an increase in plasma AII concentrations (Broughton Pipkin *et al.*, 1994) which may, in turn, stimulate unblocked AT_2 receptors. Although the function of these receptors in the adult animal remains unclear, AT_2 receptors have been found in the smooth muscle of uterine, but not systemic, arteries in pregnant ewes (Cox *et al.*, 1994). The differences in response of uterine blood flow to GR138950 and captopril treatment may, therefore, result from the contribution made by the AT_2 receptors to uterine vascular resistance, as AT_1-specific receptor blockade might enhance AT_2 receptor activity while ACE inhibition would reduce activation of both AT_1 and AT_2 receptors. Alternatively, these differences may be due to the non-specific effects of ACE inhibition, such as a local increase in concentrations of vasodilator substances such as bradykinin. Indeed, the decrease in uterine blood flow produced in pregnant rabbits by AII receptor blockade is reduced when the AII receptor antagonist is given in combination with an ACE inhibitor (Albertini *et al.*, 1980).

In addition to effects on the cardiovascular system, the RAS has an important role in the expansion of maternal blood volume that occurs during pregnancy. Increased production of AII during pregnancy stimulates secretion of aldosterone which, in turn, causes sodium and water reabsorption. In the pregnant ewe, antagonism of the RAS by either AII receptor blockade (Broughton Pipkin *et al.*, 1994) or ACE inhibition (Broughton Pipkin *et al.*, 1982; Broughton Pipkin & Wallace, 1986) has been shown to lead to a decrease in plasma aldosterone concentration in the maternal circulation. Consequently, the short-term increases in arterial Hb concentration and, thereby, O_2 content observed in both GR138950-treated and captopril-treated ewes probably reflect a decrease in plasma volume produced by the drug-induced decrease in plasma aldosterone concentration.

Although different cardiovascular responses to maternal GR138950 and captopril treatment were evident in the sheep foetus, both drugs produced remarkably similar changes in the foetal haematological parameters. If there is indeed inadequate placental transfer of GR138950 to antagonize the foetal RAS, the reductions in foetal pH and oxygenation seen with 2 h of maternal drug administration probably occurred secondarily to the drug-induced decrease in uterine blood flow and the consequential changes in umbilical O_2 delivery. Indeed, this would be consistent with previous studies in which direct antagonism of the foetal RAS with saralasin (Iwamoto & Rudolph, 1979) or captopril (Robillard *et al.*, 1983) produced hypotension without influencing foetal pH or blood gas status. However, a decrease in foetal pH and Pa_{O_2} has been observed after administration of losartan to the sheep foetus (Stevenson & Lumbers, 1994), which suggests that the changes in pH and Pa_{O_2} observed in the present study may not originate entirely from alterations in maternal placental perfusion.

Changes in placental perfusion have been proposed as one cause of the perinatal mortality associated with ACE inhibition. However, although marked short-term changes in uterine blood flow and foetal oxygenation were induced by GR138950 and captopril in the present study, there was no evidence to suggest any detrimental consequences for foetal viability. The drug-treated ewes studied here carried single foetuses which were normal and healthy. Poor foetal outcome after maternal ACE inhibition does seem more likely in animals with multiple offspring (Broughton Pipkin *et al.*, 1982; Thomas & Thompson, 1986; Harewood *et al.*, 1994). The experimental protocol used in the present study also sought to minimize blood loss and possible activation of the foetal RAS. Stressful situations *in utero*, such as haemorrhage and, indeed, maternal hypertension, are known to activate the foetal RAS (Iwamoto & Rudolph, 1981; Brar *et al.*, 1987), and antagonism of the RAS in these situations may be more deleterious to foetal well-being. Further investigations are required to determine whether the poor perinatal outcome associated with the use of ACE inhibitors occurs only in stressed foetuses that are relying on a functional RAS for survival. In addition, a number of other factors may be responsible for the difference in foetal viability observed between previous work and the present study. Influences such as drug dosage, length of treatment, foetal well-being and gestational age at drug exposure need to be examined in greater detail before the risks associated with RAS antagonism during pregnancy can be clearly defined.

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