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The effect of enalapril (MK421), an angiotensin converting enzyme inhibitor, on the conscious pregnant ewe and her foetus

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1 The effects of enalapril, an angiotensin converting enzyme (ACE) inhibitor, on maternal and foetal blood pressure, heart rate and components of the renin-angiotensin-aldosterone system were studied in 9 chronically-cannulated pregnant ewes and their foetuses.

2 Six ewes received 1 mg kg^{-1} enalapril i.v. while 3 were given 2 mg kg^{-1}. Although the initial fall in blood pressure was slightly greater in the higher dose group, there was substantial overlap of data. The pressor response to angiotensin I, assessing ACE activity, was abolished within 10 min of administration, and did not recover during 3 h of observation. Maternal systolic and diastolic pressures reached a nadir 90 min after administration ($P < 0.001$, $P < 0.002$ respectively). The maximum tachycardia was seen at 60 min ($P < 0.05$).

3 The foetuses of the ewes given 1 mg kg^{-1} enalapril showed no change in systolic or diastolic blood pressure or heart rate. Those of the ewes given the higher dose showed late-onset hypotension, coincident with the lowest maternal blood pressures.

4 Maternal plasma renin concentration (PRC) had risen significantly by 30 min ($P < 0.02$), reaching a maximum at approximately 90 min. Maternal plasma angiotensin II and aldosterone concentrations both fell initially ($P < 0.05$) but were almost at basal levels by the end of the experiment.

5 Foetal plasma renin, angiotensin II and aldosterone concentrations were unchanged throughout the experiment.

6 Peak values of enaprilic acid, the active principle, were recorded in maternal plasma 65–90 min after administration of 1 mg kg^{-1}, and 25–30 min after the administration of 2 mg kg^{-1}. A trace amount of the active principle was recorded in the foetal plasma of one lamb, whose mother had been given the higher dose. None was recorded in the plasma from three other lambs.

7 Maternal plasma ACE concentrations fell by an average of 84%; in 4 of the 6 ewes in which concentrations were measured they were undetectable after treatment. Foetal plasma ACE concentrations were unchanged throughout.

8 Enalapril at 1 mg kg^{-1} thus exerts a depressor effect on the pregnant ewe which is probably related to its blockade of the renin-angiotensin system. Both direct measurement of the drug and foetal observation suggest that it does not cross the sheep placenta at this dose. This is consistent with its failure to cross the blood-brain barrier. Foetal effects were noted at 2 mg kg^{-1}, and there was an unexpected foetal death in this group.

Introduction

Angiotensin II (AII) is formed when the C-terminal histidyl-leucine dipeptide of the relatively inactive precursor molecule, angiotensin I, is removed by the action of angiotensin converting enzyme (ACE; EC3.4.15.1; kininase II). This enzyme is found on the luminal surface of vascular endothelial cells, and also circulates in blood. It is present in high concentrations in the lungs, which appear to be the primary site of conversion, although human kidneys are also rich in the enzyme (review: Ondetti & Cushman, 1982). ACE activity has also been demonstrated in the human placenta (Litorowicz & Malofiejew, 1978; Warren *et*

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al., 1984) and amniotic fluid (Yasui *et al.*, 1984; Warren *et al.*, 1984).

Captopril (Squibb), the first ACE inhibitor to be widely used as an orally-active antihypertensive agent, contains a sulphydryl group, which binds to the zinc in the converting enzyme. This has been implicated in the causation of some of the side effects reported with long-term captopril therapy (reviews: Atkinson & Robertson, 1979; Heel *et al.*, 1980). A new class of angiotensin-converting enzyme inhibitors, of a totally different structure has now been developed (Patchett *et al.*, 1980). One such drug, enalapril maleate (MK421), is a substituted N-carboxymethyl-dipeptide, which is largely inactive until it has been absorbed and de-esterified in the liver. The active form (enaprilic acid, MK422) is a potent ACE inhibitor, with a long duration of action (Ulm, 1983). The administration of captopril was found to be associated with a marked increase in the perinatal mortality rate in both sheep and rabbits (Broughton Pipkin *et al.*, 1982; Ferris & Weir, 1983). Because of the differences between the two classes of ACE inhibitor, we felt it would be of interest to study the effects of MK421/422 in sheep pregnancy, under the same conditions as those in which we had originally studied captopril (Broughton Pipkin *et al.*, 1982).

The majority of conversion of angiotensin I to II takes place in the lungs in the adult. However, only a small proportion of the foetal right heart output goes through the lungs, and although conversion occurs in the placenta, the overall rate of conversion in the foetal sheep is low (Hebert *et al.*, 1972). We therefore chose not to assess transplacental blockade of ACE activity, as in the ewe, by effects on the pressor response to AI, which would require large doses of AI. Rather, we assessed it simply by measurement of hormone and drug concentrations in the foetal circulation, and by measurement of foetal arterial pressure.

Methods

Nine pregnant crossbred ewes (Clun Forest x Border Leicester or Dorset Horn) of known gestational age were studied. Indwelling cannulae were placed, as previously described, in a foetal carotid artery and jugular vein under general anaesthesia and with strict attention to aseptic technique (Broughton Pipkin & O'Brien, 1978). Cannulae were also placed in the amniotic sac and in a maternal carotid artery and jugular vein. The ewes were given a mixture of streptomycin and dihydrostreptomycin 668 mg (Dimycin, Glaxovet) and procaine penicillin 600 mg (Depocillin, Gist Brocades) intramuscularly for 24 h before, and 72 h after surgery. The foetuses were given ampicillin (Penbritin, Beecham) 125 mg i.v. for the 3 post-operative days.

Ewes were housed throughout in roomy individual pens. They were fed a standard barley/oats/grass pellet diet with additional hay and water *ad libitum*; their sodium intake on this diet was approximately 10 g per day. They also had free access to a mineral lick (98% NaCl) and were thus considered to be sodium replete. Experiments were performed 2–8 days post-operatively, at gestation ages ranging between 120–143 days, median 128 days (term approximately 147 days).

The maternal and foetal blood pressures were allowed to stabilize for 20 min, at which time a maternal arterial blood sample was taken. Maternal angiotensin converting enzyme (ACE) activity was then tested by the i.v. administration of a bolus of 5 or 10 μg angiotensin I washed in with 8 ml normal saline. Following pulmonary conversion to angiotensin II a pressor response was evoked (Figure 1). This was repeated twice more. A control bolus of 8 ml normal saline alone was also given. A second maternal, and a first foetal, blood sample were taken. Following this, a single bolus dose of 1 mg kg^{-1} MK421 (enalapril; Merck Sharp & Dohme Ltd), washed in with 8 ml normal saline was given to 6 ewes; 3 received 2 mg kg^{-1} MK421. The pressor response to angiotensin I was determined at 10–15 min intervals for the next 2–3 h. Further maternal blood samples were taken at 25–35 min, 67–95 min, 121–143 min and 164–193 min after administration of MK421. Foetal samples were taken at 25–40 min and 79–134 min after MK421. Further blood samples were taken up to 14 days after the experiment from 7 of the ewes and 5 foetuses. Maternal samples were usually of 18 ml, and foetal samples of 16 ml size. The volume removed was immediately replaced with i.v. Dextran 70 solution at 37°C.

The blood samples were divided immediately. An aliquot was placed in a mixture of *0*-phenanthrolene (0.025 M) and EDTA (0.125 M). The remainder was placed in lithium heparin. Samples were centrifuged at 2,000 *g* at 4°C for 10 min. The plasma was decanted and stored at −22°C until analysis. Plasma renin and renin substrate, angiotensin II, aldosterone and cortisol concentrations were measured by radioimmunoassay as previously described (Broughton Pipkin *et al.*, 1982). Plasma ACE was measured using a spectrophotometric assay (Warren *et al.*, 1984). Plasma sodium and potassium concentrations were measured by flame photometry. The concentration of MK421 and its active metabolite MK422 were measured by radioimmunossay by Merck, Sharp & Dohme Ltd.

Average maternal and foetal blood pressures were calculated over the control period, and over 30 min epochs after maternal administration of MK421. Average heart rates were similarly calculated.

Arithmetic means ± s.e.mean are quoted throughout unless otherwise stated. A one-way analysis of

variance followed by the modified Student's *t* test was used as appropriate to test for the statistical significance of observed differences between means for blood pressure and heart rate. Wilcoxon's rank test was used for all hormonal comparisons, since the data followed skewed distributions. Linear regression analysis was performed by the method of least squares. Spearman's correlation coefficient (ε) was calculated when the data were not normally-distributed.

Results

All foetuses were known to be alive at the start of the experiment, but for technical reasons satisfactory recordings of blood pressure could not be obtained from one foetus, nor could blood samples be taken.

Blood pressure and heart rate responses

The course of an experiment is illustrated in Figure 1. Eight of the nine ewes were given 3 separate doses of 5 μg AI i.v. to demonstrate ACE activity. The evoked pressor response ranged between 17.3–53.3 mmHg systolic (mean 36.4 ± 5.1) and 14.3–45.7 mmHg diastolic (mean 31.2 ± 4.0). The first ewe was given doses of 10 μg AI i.v.; her mean response was +41.7/+19 mmHg. The bolus administration of normal saline alone had no effect distinguishable from the normal baseline variability. Maternally-administered AI was without effect on foetal blood pressure. There was a brief reflex maternal bradycardia, averaging -20.7 ± 6.7 beats min^{-1} at 1 min after the injection of AI ($P < 0.02$); foetal heart rate was unaltered.

The administration of a bolus dose of MK421 was associated with a pressor response in 4 ewes. This was very pronounced in 1 ewe, given 2 mg kg^{-1} MK421, being a rise of 63 mmHg systolic and 45 mmHg diastolic pressure respectively. In the other ewes the range of increase in systolic and diastolic pressure was 27–38 and 12–16 mmHg respectively. No such response was seen in the foetuses.

This transient pressor response prevented the evaluation of the exact time taken for blockade of the conversion of AI to AII. A bolus dose of AI was given between +3 and +6 min in 4 ewes, and was still associated with a pressor effect, albeit diminished. The pressor response had, however, been abolished by

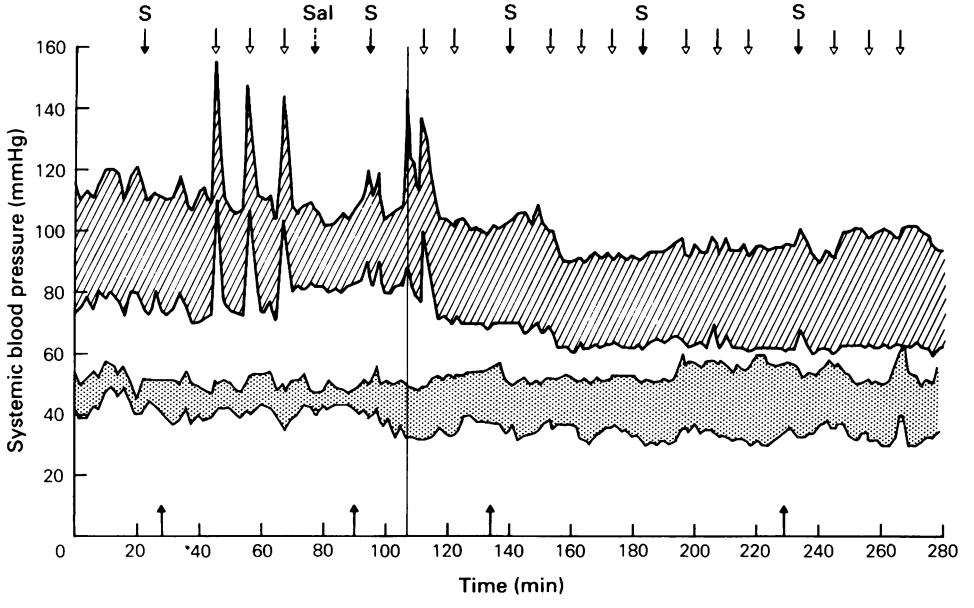

Figure 1 Experimental record from a ewe studied at 128 days gestation. Maternal angiotensin converting enzyme (ACE) activity was demonstrated by the bolus i.v. administration of 5 μg angiotensin I (▽) which was flushed in with saline. A pressor response was evoked after pulmonary conversion to angiotensin II. MK421, 1 mg kg^{-1} i.v., was given at the time indicated by the vertical dividing line. In this ewe, as in 3 others, its administration was accompanied by a marked rise in systolic pressure. A pressor response to angiotensin I was still demonstrable at 5 min after administration, but was abolished thereafter. ▼ S: indicates blood sample taken and ▼ Sal: saline only. The hatched area indicates maternal, and the stippled area foetal systemic blood pressure.

Table 1 The basal foetal and maternal systemic arterial blood pressures and heart rates in ewes before, during and after the maternal administration of MK421

	Time in relation to MK421 administration (min)						
	Before	*+30*	*+60*	*+90*	*+120*	*+150*	*+180*
Maternal blood pressure (mmHg)							
Systolic	100.6 ± 3.7	93.3 ± 4.4	89.5 ± 4.9	89.5 ± 3.7	90.6 ± 3.5	94.3 ± 3.1	91.1 ± 4.0
Diastolic	69.5 ± 3.0	62.3 ± 2.9	60.1 ± 3.5	60.1 ± 2.2	61.8 ± 2.6	62.9 ± 3.9	61.4 ± 5.0
Heart rate (beats min^{-1})	106.4 ± 6.8	115.3 ± 7.8	120.7 ± 8.3	115.6 ± 9.0	119.9 ± 8.3	120.8 ± 11.7	117.6 ± 6.9
n	9	9	9	9	9	7	6
Foetal blood pressure (mmHg)							
Systolic	48.1 ± 2.5	49.8 ± 2.0	50.0 ± 2.3	50.3 ± 3.0	49.8 ± 3.4	48.8 ± 2.8	49.4 ± 3.6
Diastolic	31.8 ± 2.8	32.3 ± 2.5	32.7 ± 2.9	32.0 ± 3.2	31.6 ± 3.6	28.8 ± 4.1	30.6 ± 5.6
Heart rate (beats min^{-1})	167.7 ± 7.9	166.9 ± 7.2	166.1 ± 7.5	167.5 ± 7.1	165.6 ± 7.4	166.3 ± 4.3	165.4 ± 3.1
n	8	8	8	8	8	6	5

Data averaged over 30 min epochs are shown for maternal and foetal systolic and diastolic blood pressure and heart rate in relation to the maternal administration of MK421.

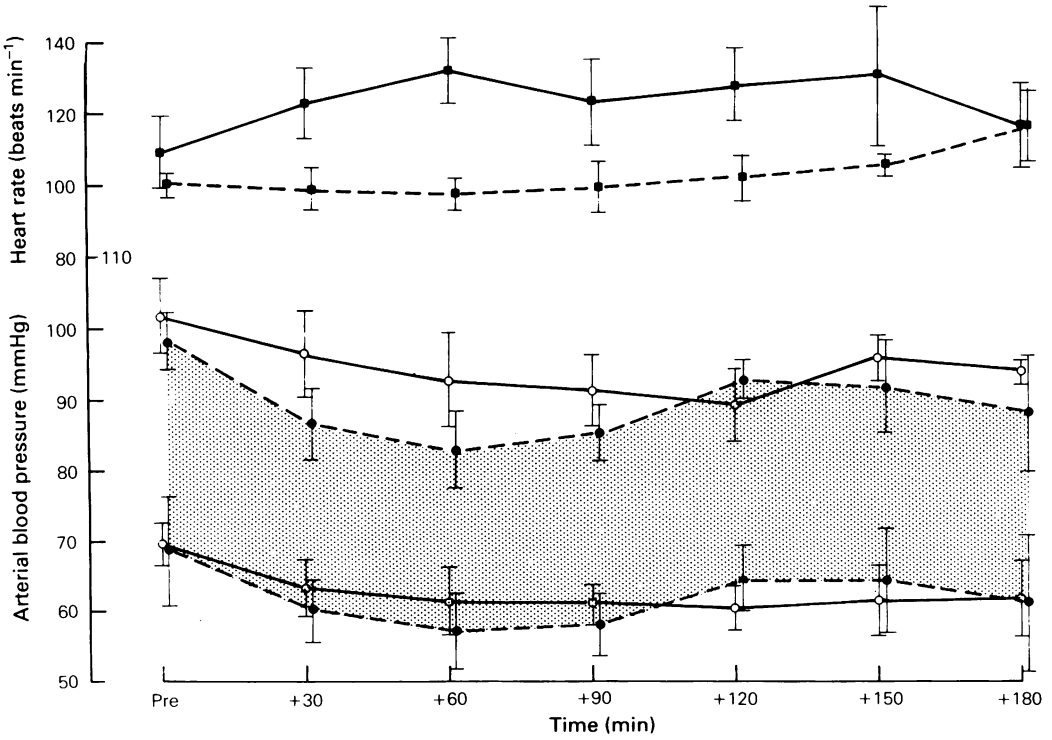

Figure 2 Mean values, over 30 min epochs, for maternal blood pressure and heart rate following the maternal administration of 1 (——) or 2 (---) mg kg^{-1} MK421. The systemic blood pressure had fallen in all ewes by 30 min after administration of MK421, reaching a nadir of 89.4 ± 5.2/60.4 ± 3.3 mmHg at 120 min in the low dose group, and 85.5 ± 3.9/58.1 ± 4.3 mmHg at 90 min in the high dose group. These values are very similar, although the rate of fall may have been slightly faster in the high dose group. Vertical lines indicate s.e.mean.

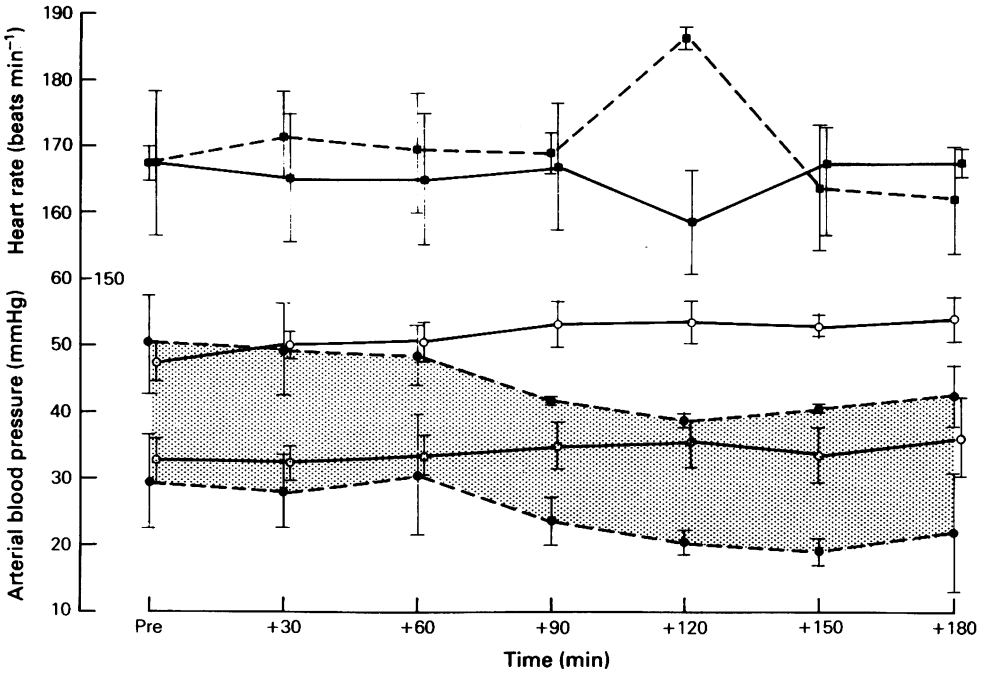

Figure 3 Mean values, over 30 min epochs, for foetal blood pressure and heart rate following the maternal administration of 1 (——) or 2 (---) mg kg^{-1} MK421. Mean foetal blood pressure rose slightly over the course of the experiment in the low dose group. However, foetuses whose dams had received the higher dose showed a marked fall in systemic blood pressure at 90 min. The blood pressure appeared to be recovering by the end of the experiment. Vertical lines indicate s.e.mean.

+ 10 min and had not started to recover by the end of the period of observation.

The mean basal foetal and maternal systemic arterial blood pressures and heart rates in all ewes before, and during 30 min epochs after, the maternal administration of MK421, are summarized in Table 1. Although the fall in maternal blood pressure was initially somewhat greater at the higher dose of MK421 (Figure 2), there was considerable overlap of data. Both treatment groups have therefore been considered together. The fall in basal maternal blood pressure was significant over the first 30 min ($P < 0.02$, $P < 0.05$, systolic and diastolic respectively) and reached a mean maximum by 90 min after administration of MK421. The mean falls in maternal systolic and diastolic pressure at this time were 11.1 ± 1.4 ($P < 0.001$) and 9.4 ± 1.9 ($P < 0.002$) mm Hg respectively. The changes in maternal heart rate were very variable. The mean maximum increase (14.3 ± 5.6 beats min^{-1}; $P < 0.05$) occurred 60 min after the administration of MK421.

Foetal blood pressure in the low dose group was very stable (Figure 3). Foetal blood pressure fell to a greater extent in the lambs of mothers given 2 mg kg^{-1} MK421 (Figure 3) but since the number of lambs in this group was small, statistical analysis is not appropriate. Foetal heart rate was stable over this time.

It can be seen from Figure 4 that the change in diastolic blood pressure of the ewes 90 min after administration of MK421 appeared to be inversely associated with the initial plasma angiotensin II concentrations ($r = -.823$, $P < 0.01$). There was also a loose inverse association with the initial evoked response to 5 μg AI ($P < 0.05$).

One ewe was also given 1 mg kg^{-1} MK421 i.v. 8 days after delivery. The magnitude and duration of the depressor response were within the range seen in the pregnant animals.

Hormonal responses

Two blood samples were taken in the control period. The first, taken shortly after the start of the experiment, had significantly higher concentrations of renin (4.0 ± 1.1 compared with 3.4 ± 1.0 ng ml^{-1} h^{-1}) and aldosterone showed a similar trend (7.3 ± 2.5 compared with 5.4 ± 1.3 ng dl^{-1}). This presumably

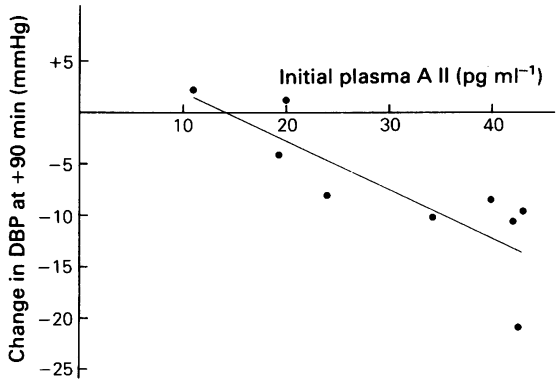

Figure 4 The effect of maternally-administered MK421 on maternal diastolic blood pressure (DBP) in relation to initial plasma angiotenin II (AII) concentration is shown. There was a statistically significant inverse association between the change in DBP at 90 min and basal plasma AII concentrations ($P < 0.01$). This supports the hypothesis of a role for AII in maintaining the systemic blood pressure in ovine pregnancy.

reflects a degree of disturbance while the experiment was being set up. Paired comparisons of hormone concentrations before and after the administration of MK421 have thus been made with the second sample as baseline. A significant association was found between plasma aldosterone and cortisol concentrations in all samples obtained during the control period ($r = 0.7683$, $P < 0.001$). There was no evidence for an association between either plasma renin concentration (PRC) or AII and aldosterone concentrations in the maternal samples.

Maternal plasma renin concentrations increased sharply following the administration of MK421 (Figure 5). This rise was statistically significant at the time of the first sample after MK421 (~30 min after MK421) when PRC had risen from 3.4 ± 1.0 to 15.4 ± 5.7 ng ml^{-1} h^{-1} ($P < 0.02$). The maximal mean concentration (24.4 ± 7.6 ng ml^{-1} h^{-1}) was reached some 90 min after the administration of the drug, but concentrations were still significantly higher than basal at the end of the experiment ($P < 0.05$). Plasma AII concentrations were lower in all samples save one at 30 min (16.9 ± 6.7 compared with 30.2 ± 12.0 pg ml^{-1}). However, since AII concentrations increased subsequently in all ewes except one, the nadir presumably occurred before the sample at 30 min. Concentrations of AII were approaching basal values by the end of the experiment (24.5 ± 7.4 pg ml^{-1}). The mean maternal aldosterone concentration also fell in the first 30 min after MK421 (5.4 ± 1.3 to 2.8 ± 0.9 ng dl^{-1}; $P < 0.05$), and rose steadily thereafter to 4.7 ± 1.7 ng dl^{-1} at the end of the experiment.

Plasma cortisol concentrations did not change significantly over the course of the experiment, nor did serum sodium concentrations. There was a small, statistically significant, fall in serum potassium concentration over the first 30 min after MK421 (3.5 ± 0.1 compared with 3.7 ± 0.1 mmol l^{-1}, $P < 0.05$), returning to 3.6 ± 0.2 mmol l^{-1} by the end of the experiment.

The association between plasma aldosterone and cortisol concentrations observed before MK421 was given (see above) was maintained in the samples after its administration ($r = 0.5774$, $P < 0.002$). However, the slope of the calculated line relating aldosterone to cortisol concentrations was significantly less during the experimental period (b = 0.01 compared with 0.03; $P < 0.005$), implying a blunting of the association. The calculated zero intercepts were very similar.

Foetal plasma AII concentrations were highly significantly correlated with PRC overall ($r = 0.772$, $P < 0.002$). Foetal plasma aldosterone and renin

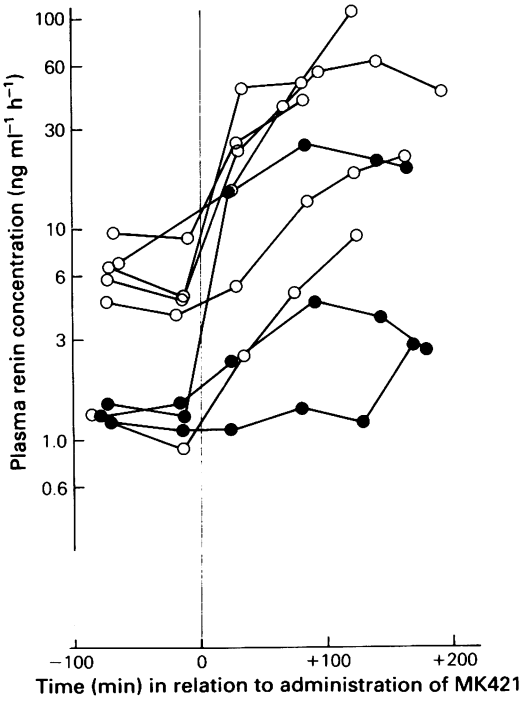

Figure 5 Maternal plasma renin concentration increased in all ewes following the administration of MK421 at either 1 mg kg^{-1} (○) or 2 mg kg^{-1} (●). The mean rise was statistically significant at the time of the first blood sample after administration ($P < 0.02$) and peaked at approximately 90 min, which was also the time at which the lowest mean maternal blood pressure were recorded.

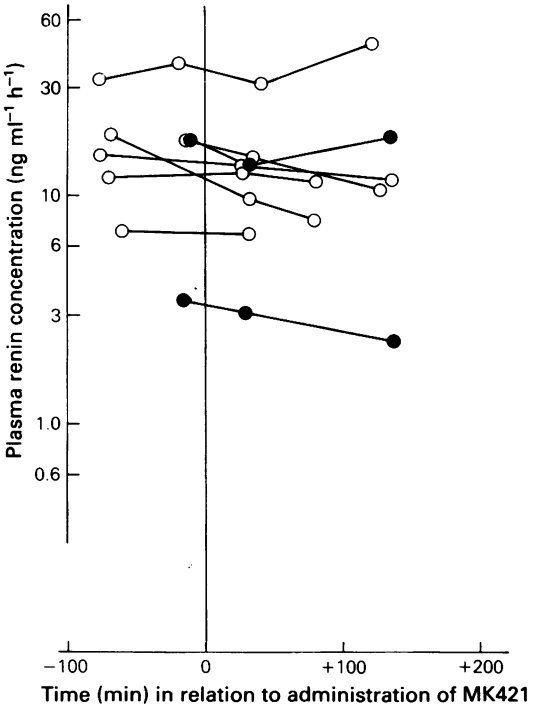

Figure 6 Foetal plasma renin concentrations were unchanged following the maternal administration of either 1 (○) or 2 (●) mg kg^{-1} MK421.

concentrations were also strongly correlated ($r = 0.768$, $P < 0.002$). There was a weaker, but still significant, association between plasma AII and aldosterone concentrations ($r = 0.798$, $P < 0.02$) but no evidence for an association between foetal cortisol and aldosterone concentrations. No change was observed in any of the foetal hormones measured over the course of the experiment (e.g. Figure 6).

Blood samples were taken from mother and foetus for as long as cannula patency allowed until term. Data were compared with samples taken serially from other animals, not given MK421, over comparable periods. Both maternal and foetal PRC were within the normal range by one day after the experiment, and showed only fluctuations within the normal range thereafter.

Measurements of both MK421 (precursor) and MK422 (active form) were available in plasma from six ewes and four foetuses. Three ewes had received 1, and three 2 mg kg^{-1} MK421. The experiments were not designed primarily to study the pharmacokinetics of the drugs, and the timing of samples was thus not optimum for this purpose. Peak values of the active principle in the samples taken were recorded 65–90 min after administration of 1 mg kg^{-1}, and 25–30 min after administration of 2 mg kg^{-1} MK421. Mean peak values recorded were 146.0 ± 46.6 ng ml^{-1} in the lower dose group and 159.7 ± 32.2 ng ml^{-1} in the higher dose group. A higher proportion of the total drug measured was in the active form at all sampling times in the ewes receiving the higher dose. The precursor form had been cleared from the maternal circulation in 2 h in four ewes, and in 3 h in the remaining 2.

At the time of peak maternal plasma concentrations of precursor, foetal plasma concentrations were between 0–9.0% maternal. Foetal plasma concentrations of the active principle were below the limit of sensitivity of the assay in 3 foetuses, and were 0.1 ng ml^{-1} in the fourth, whose mother had been given 2 mg kg^{-1} MK421.

Plasma ACE was measured in samples from six ewes and four foetuses. Maternal plasma ACE fell steadily from a mean value of 7.7 ± 1.4 u l^{-1} before MK421 administration to a nadir of 1.2 ± 0.8 u l^{-1} two hours after administration ($P < 0.01$). Undetectable values were recorded after treatment in one or more samples from 4 of the ewes. Foetal plasma ACE concentrations were unchanged from an initial value of 10.8 ± 1.1 u l^{-1}. Only a small number of samples could be assayed, and there was no obvious association between a fall in plasma ACE and that in systemic blood pressure.

Foetal outcome

There were three unexpected foetal deaths in this series, two to ewes who had received 2 mg kg^{-1} MK421. In two foetuses, features were present which may have contributed to their demise. One was subsequently found to have skeletal abnormalities, which must have developed in early pregnancy. The other was noted at surgery to be somewhat polycythaemic (Hct 56%) and was still so at the time of experiment (Hct 58%). The third was an apparently normal full term lamb.

Discussion

Studies using the ACE inhibitors captopril (MacGregor *et al.*, 1981b) and MK421 (MacGregor *et al.*, 1981a) suggest that even in normotensive non-pregnant subjects receiving a normal salt intake, the renin-angiotensin system is partly responsible for the maintenance of blood pressure. The mechanisms which maintain blood pressure in the normotensive pregnant women differ from those in the non-pregnant state. Experiments performed 30 years ago (Assali *et al.*, 1952) suggested that the autonomic nervous system was of relatively less importance in the maintenance of

normal blood pressure during pregnancy. If this is so, hormonal influences presumably play a relatively more important role. There is marked activation of the renin-angiotensin system in pregnancy in both humans (Weir *et al.*, 1975) and sheep (Fleischman *et al.*, 1975; Carver & Mott, 1978). Blockade of the maternal renin-angiotensin system in the conscious pregnant sheep, whether with the receptor blocker, Saralasin (Broughton Pipkin & O'Brien, 1978) or captopril (Broughton Pipkin *et al.*, 1982) results in a fall of maternal blood pressure. However, whereas the effects of Saralasin are short-lived, and apparently not transmitted to the foetus, the maternal administration of captopril is associated with a rapid foetal hypotension, and a much increased stillbirth rate in both sheep and rabbits (Broughton Pipkin *et al.*, 1982; Ferris & Weir, 1983). Its administration to pregnant guinea-pigs was associated with a marked fall in foeto-placental ACE activity (Davidson *et al.*, 1981). It was not possible to measure plasma concentrations of captopril at the time of these studies, but the assumption must be that it can readily cross the placenta, in these three species. It is also known to cross the blood-brain barrier in the rat (Cohen & Kurz, 1983).

The results of the experiments presented here contrast with this. The directly measured concentration of MK421 in foetal blood never exceeded 9.0% of maternal concentrations, while the active principle itself was only detected in one foetus. This minimal transfer is confirmed by the unchanged foetal hormone concentrations and the stability of foetal blood pressure, at least in the foetuses whose mothers received the lower dose. It is of interest that MK421 has also been reported not to cross the blood-brain barrier (Cohen & Kurz, 1983). Lipid solubility is an important factor in determining the transfer of a drug across both the blood-brain and placental barriers, and MK421 is known to be of extremely low lipid solubility.

This failure of placental transfer is of considerable theoretical interest. The drugs commonly used in the treatment of hypertension in pregnancy all exert effects across the placenta, and can be present in human foetal plasma at birth in concentrations equivalent to those in the maternal circulation (labetalol, Michael, 1979; propranolol and sotalol, Erkkola *et al.*, 1982; atenolol, Woods & Morrell, 1982). However, the sheep placenta is syndesmochorial, by comparison with the haemochorial placenta of man (see Dawes, 1968) and any attempt at cross-species extrapolation of the data here presented would thus be more than usually hazardous.

The late fall in blood pressure in the lambs whose mothers had been exposed to the higher dose may have been a response to changes in their utero-placental blood supply, rather than to any direct effect of the drug. The administration of captopril to pregnant rabbits resulted in a fall of more than one third in uterine blood flow although blood pressure only fell by 18% (Ferris & Weir, 1983). The maximum fall in maternal blood pressure in ewes given $2\,mg\,kg^{-1}$ MK421 approached this value by approximately 90 min, and it seems probable that this was also accompanied by a fall in uteroplacental blood flow, evoking the reduction in foetal blood pressure seen at this time (Figure 3).

MK421 is given by mouth in clinical practice (Gomez *et al.*, 1983). Drenching animals provides a way of administering drugs orally, but accurate dose administration becomes impossible and since animals normally resent the procedure, stability of cardiovascular parameters is lost. We thus gave the MK421 intravenously, which accounts for its more rapid onset of action (cf. Ulm, 1983). Cohen & Kurz (1983) observed maximal inhibition of serum ACE within 3 min of the i.v. administration of $1\,mg\,kg^{-1}$ MK421 to rats. Our data suggest that the onset of action is slightly slower in pregnant ewes, which could be a species difference or one related to the effects of pregnancy *per se* on the liver.

The fall of some 44% in plasma AII which we observed is of the same order of magnitude as that found by other groups studying salt-replete human volunteers (MacGregor *et al.*, 1981a; Brunner *et al.*, 1981; Shoback *et al.*, 1983; Hodsman *et al.*, 1984). Thus, even though the pressor response to AI is completely abolished, the production of AII appears not to be. In part this may be artefactual, in that the increase in PRC evokes a marked rise in AI (Biollaz *et al.*, 1982; Hodsman *et al.*, 1984), which will cross-react to a very small extent with the antisera used in the AII radioimmunoassay. It is also possible that differences between plasma and tissue ACE activity may permit a degree of residual activity. Brunner *et al.* (1981) showed indeed that AII and aldosterone concentrations fall only when plasma ACE is reduced to very low levels. However, it is noticeable from other studies that MK421 continues to evoke an effect on the blood pressure after plasma AII concentrations have returned to near basal (Hodsman *et al.*, 1984; Given *et al.*, 1984). Early experiments suggested that blockade of AII production was not the sole mechanism by which another ACE inhibitor, captopril, exerted its effect (Thurston & Swales, 1978; Fagard *et al.*, 1979). Both increased production of E series prostaglandins and accumulated bradykinin were suggested as mechanisms (Swartz *et al.*, 1980). However, MK421 appears to differ in this respect also, its administration not being associated with changes in plasma bradykinin or prostaglandin E_2 metabolites in man (Shoback *et al.*, 1983), or rats (Schiffrin *et al.*, 1984). It is possible that blockade of the effects of AII on the facilitation of noradrenergic neurotransmission or withdrawal of vagal inhibition may alter baroreflex sensitivity over a

slightly longer time scale, but this possibility has not been formally tested.

AII is a potent stimulus to aldosterone release, so that the decreased plasma aldosterone concentration was expected. The blunting of the association between plasma cortisol and aldosterone concentrations during ACE inhibition is in accordance with the suggestion that AII acts both early and late in the corticosynthetic chain (see Fraser *et al.*, 1979) and may have a general potentiating effect in the early part of the chain.

There was one unexplained fresh stillbirth in this series, to a ewe who had received MK421 2 mg kg^{-1} 11 days earlier. Histological examination of lung sections suggested that the foetus had made strong respiratory efforts but without adequate lung expansion, indicative of severe intrapartum asphyxia, and/or lungs possibly deficient in surfactant. Labour had been noted to be prolonged in this ewe. This was also so in ewes who had received captorpil, and gestation length *per se* was prolonged in treated rabbits (Broughton Pipkin *et al.*, 1982). The initiation of parturition requires a cascade of events, and it is possible that powerful enzyme blockers such as ACE inhibitors interferes with the integration of such a cascade.

In conclusion, we have shown that a fixed i.v. dose of MK421 1 mg kg^{-1} is associated with blockade of the maternal renin-angiotenin system and a depressor effect, the magnitude of which is proportional both to the initial AII and to the initial pressor response to AI. It is without apparent effect on either the renin-angiotensin system or basal blood pressure or heart rate of the foetus. At 2 mg kg^{-1}, some 7 times higher than the clinically recommended dose, a somewhat greater depressor response was noted in the ewes. Furthermore, although neither precursor nor active principle crossed the placenta in other than trace amounts, foetal blood pressure also fell at this dose and there was one inexplicable foetal death. The role of the renin-angiotensin system in mother and foetus remains to be fully clarified. These data support the hypothesis of an important role for the system in the maintenance of cardiovascular homeostasis in pregnancy.

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