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Comparison of the transplacental transfer of enalapril, captopril and losartan in sheep

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1 The transplacental transfers of three drugs (enalapril, captopril and losartan) which block the renin angiotensin system and have different lipophilicities were studied in chronically catheterized foetal sheep (125–139 days gestation).

2 The ability of the foeto-placental unit to convert enalapril to enalaprilat was studied in two chronically catheterized foetuses. Enalapril (3 mg kg^{-1}, 7.9 μmol kg^{-1}) given i.v. to the foetuses abolished the foetal pressor response to 5 μg angiotensin I (AI) in one foetus and attenuated the pressor response in the other.

3 Enalapril (100 mg, 5.7 μmol kg^{-1}) given i.v. to the ewe ($n = 5$) abolished the maternal pressor response to 2.5 μg AI ($n = 1$) and attenuated the maternal pressor response to 5 μg AI ($n = 5$, $P < 0.001$). The foetal pressor response to 5 μg AI ($n = 2$) and 10 μg AI ($n = 3$) did not change. The maternal and foetal pressor responses to angiotensin II (AII; $n = 5$) did not change.

4 Foetal pressor responses to 5 μg AI ($n = 1$) and 10 μg AI ($n = 2$) were attenuated within 11 min of their mothers ($n = 3$) being given i.v. captopril (15 mg, 1.5 μmol kg^{-1}). Foetal pressor responses to 5 μg AII ($n = 1$) and to 10 μg AII ($n = 2$) did not change.

5 Losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}) given i.v. to the foetus ($n = 9$) attenuated the foetal pressor response to 5 μg AII ($P < 0.001$) but the maternal pressor response to 5 μg AII did not change.

6 Losartan (100 mg, 21.7 μmol kg^{-1}) given i.v. to the ewe ($n = 5$) attenuated the maternal pressor response to 5 μg AII ($P < 0.002$) but the foetal pressor response to 5 μg AII did not change.

7 It is concluded that the foeto-placental unit of the sheep can metabolize enalapril to enalaprilat. Captopril readily crosses the sheep placenta but enalapril and losartan do not. Thus, the transplacental transfer of these drugs does not parallel their lipid solubilities. Furthermore the results show that AT_1 receptors are important in mediating the vasoconstrictor effects of AII in the foetus.

Keywords: Enalapril; captopril; losartan; transplacental transfer; foetus; foetal sheep

Introduction

The transport of substances across the placenta may occur by the following mechanisms: ultrafiltration, simple and facilitated diffusion, active transport, pinocytosis and breaks in placental villi (Parke, 1984). For transport of drugs, simple diffusion is usually the most important mechanism (Goldstein *et al.*, 1974; Parke, 1984; Benet & Sheiner, 1985) and the lipid solubility of a drug is considered a good index of its ability to cross the placenta, i.e. the more lipid soluble the drug the more readily it should cross the placenta (Mirkin, 1973; Parke, 1984; Benet & Sheiner, 1985; Wang *et al.*, 1985). The lipid solubility of a drug is indicated by its octanol/water partition coefficient (Kp), where:

$$Kp = \frac{\text{drug concentration in organic phase}}{\text{drug concentration in aqueous phase}}$$

Kp varies with the degree to which the drug is ionized and is therefore dependent on the pH and the p*K*a of the ionizable groups present within the molecule. Oh & Mirkin (1971) showed that sodium salicylate, which is completely ionized at physiological pH, crossed placental membranes more readily than it penetrated the central nervous system. Hence, although lipoprotein in nature, the placental barrier cannot be assumed to have the same properties as other biological membranes.

Drugs that block the renin angiotensin system (RAS) are amongst the most effective antihypertensive agents available. Enalapril (its active metabolite, enalaprilat) and captopril are nonpeptidic angiotensin converting enzyme (ACE) inhibitors. They have very low Kp values and low protein binding (Table 1). Enalapril is more potent and longer acting than captopril (Biollaz *et al.*, 1981; Gavras *et al.*, 1981; Sweet *et al.*, 1981). Cushman *et al.* (1989) compared the relative potencies of enalapril maleate and captopril in spontaneously hypertensive rats and found that in *ex vivo* studies, an oral dose of 20 mg kg^{-1} (41 μmol kg^{-1}) enalapril maleate produced the same ACE-inhibitory effect as 30 mg kg^{-1} (138 μmol kg^{-1}) captopril and in *in vitro* studies enalapril had an I_{50} of 2.8 nmol l^{-1} while captopril had an I_{50} of 9.7 nmol l^{-1}.

However, captopril given i.v. to the pregnant ewe has been shown to rapidly cross the placenta and block the foetal pressor response to angiotensin I (AI) (Kingsford &

Table 1 Comparison of molecular weights (mol. wt.), octanol-water partition coefficients (Kp) at pH 7 and serum protein binding of enalapril, enalaprilat, captopril and losartan

Compound	*Mol. wt.*	*Kp* (pH 7)	*Serum protein binding* (%)
Enalapril	376.46	0.071 (Randive *et al.*, 1992)	NA
Enalaprilat	348.4	<0.001 (Randive *et al.*, 1992)	40% (Lin *et al.*, 1988)
Captopril	217.9	0.004 (Randive *et al.*, 1992)	25–30% (Kubo & Cody, 1985)
Losartan	461.01	15 ± 1 (MSD)	97–99% (MSD)

NA = not available; MSD = data supplied by Merck Sharp & Dohme.

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Lumbers, 1989; Lumbers *et al.*, 1992). Broughton Pipkin & Wallace (1986) reported that enalapril given i.v. to the pregnant ewe did not appear to cross the sheep placenta. They tested maternal ACE activity by giving bolus injections of 5 or 10 μg AI and by measuring ACE levels. However, although foetal ACE levels were measured, foetal pressor responses to AI were not tested. Enalapril is a prodrug with little or no direct biological activity (Gross *et al.*, 1981). In the adult, it is desterified in the liver to the very potent and long-lasting ACE inhibitor, enalaprilat (Ulm, 1983). Thus it is important to establish whether or not the foeto-placental unit is capable of converting enalapril to enalaprilat.

ACE inhibitors not only block the production of angiotensin II (AII) but also increase endogenous bradykinin levels (Yang *et al.*, 1970; 1971; Das & Soffer, 1975; Ondetti *et al.*, 1977; Swartz *et al.*, 1979) and prostaglandin release (McGiff *et al.*, 1972; Murthy *et al.*, 1978; Swartz *et al.*, 1980; Moore *et al.*, 1981). Therefore if studies are designed to investigate the role of the RAS, a more specific method would be to inhibit the actions of AII by receptor blockade. Losartan is a nonpeptide, AT_1 receptor antagonist with a high Kp (Table 1) that lacks agonist, kinin and prostaglandin inducing properties (Wong *et al.*, 1990a,b). Losartan is metabolized from a competitive AT_1 receptor antagonist to a primary metabolite, EXP3174, which is a non-competitive receptor antagonist (Smith *et al.*, 1992). EXP3174 has been shown to be partly responsible for the hypotensive effect of losartan in rats (Wong *et al.*, 1990c) and man (Ohtowa *et al.*, 1993) but not dogs (Wong *et al.*, 1991). There is no information on the metabolism of losartan to EXP3174 in sheep. There are no reports in the current literature on the transplacental transfer of losartan.

Although a mixture of both AT_1 and AT_2 receptor populations have been reported in the foetus, the majority of receptors appear to be AT_2 (Tsutsumi *et al.*, 1991a,b; Grady *et al.*, 1991; Viswanathan *et al.*, 1991; Grone *et al.*, 1992). All known biological actions of AII appear to be mediated via AT_1 receptors and currently no physiological functions have been definitely associated with the AT_2 receptor. Therefore, before assessing the transplacental transfer of losartan we established that i.v. administration of losartan to the foetus attenuated the foetal pressor response to AII, i.e. the ovine foetus had functional AT_1 receptors.

Because drugs which block the RAS are potentially very effective antihypertensive agents and because the lipid solubility of a drug is generally accepted as a good index of its ability to cross the placenta, we assessed the transplacental transfer of three drugs which inhibit the RAS and which have different Kps. These drugs were the ACE inhibitors, captopril and enalapril and the AT_1 receptor antagonist, losartan. As the foetal RAS is important in the maintenance of normal foetal renal function (Lumbers & Stevens, 1987; Lumbers *et al.*, 1993) it can be inferred that adverse foetal renal effects will be applicable to all inhibitors of the RAS that cross the placenta.

In these experiments the ability of the ovine foetus to convert enalapril to enalaprilat was assessed by testing foetal pressor responses to AI before and after i.v. administration of enalapril to the foetus. The effects of enalapril and captopril on the transplacental blockade of ACE activity were assessed by testing maternal and foetal pressor responses to AI before and after i.v. administration of these ACE inhibitors to the ewe. The transplacental transfer of losartan was assessed by examining maternal and foetal pressor responses to AII before and after i.v. administration of losartan to the ewe.

Methods

Surgery

Pregnant ewes were anaesthetized with 1.5 g sodium thiopentone, i.v. Anaesthesia was maintained with 1–3% halothane in oxygen; the foetus was also anaesthetized by these agents. Under aseptic conditions, polyvinyl catheters (1.5 mm O.D., 1.0 mm I.D.) were inserted into a foetal femoral artery and both recurrent tarsal veins. Polyvinyl catheters (2.7 mm O.D., 1.5 mm I.D.) were inserted into a maternal femoral vein and femoral artery and into the amniotic cavity. At the end of surgery and for the next 2 days, 3 ml of a mixture of procaine penicillin (600 mg) and dihydrostreptomycin sulphate (750 mg) were given i.m. to the ewe and intra-amniotically to the foetus. The ewes were housed in metabolic cages in a laboratory maintained at 18–20°C. They had free access to water and were given a diet of lucerne chaff (up to 2 kg day^{-1}), oats (140 g day^{-1}) and 6 g NaCl. These experiments were approved by the Animal Care and Ethics committee of the University of New South Wales.

Experimental protocol

Experiments were carried out in 12 chronically catheterized pregnant ewes and their foetuses (gestation age 125–139 days). Intra-amniotic and maternal and foetal arterial pressures were monitored continuously with Bell and Howell pressure transducers connected to a Grass Polygraph recorder. Pressor responses to i.v. bolus doses of AI and AII in ewes and their foetuses were staggered in time and were tested at least in duplicate. When AI and AII were given to the ewe, foetal arterial pressure was unaffected; likewise when AI and AII were given to the foetus, maternal arterial pressure did not change.

Enalapril

The ability of the foeto-placental unit of the sheep to convert enalapril to enalaprilat was tested in two chronically catheterized foetal sheep (135 days gestation). Foetal pressor responses to 5 μg AI and 5 μg AII were measured before and for up to 30 min after the i.v. administration of enalapril to the foetus (3 mg kg^{-1}, 7.9 μmol kg^{-1}).

Placental transfer of enalapril was tested in 5 sheep aged 126–139 days gestation (mean ± s.e.mean: 133 ± 2 days). Maternal and foetal pressor responses to AI and AII were measured before and after the ewes were given i.v. enalapril (100 mg, 5.7 μmol kg^{-1}). Maternal ACE activity was assessed by measuring the maternal pressor response to 2.5 μg AI ($n = 1$) and 5 μg AI ($n = 5$). Foetal ACE activity was assessed by measuring the foetal pressor responses to 5 μg AI ($n = 2$) and 10 μg AI ($n = 3$). Maternal pressor responses to 5 μg AII ($n = 5$) and foetal pressor responses to 5 μg AII ($n = 2$) or 10 μg AII ($n = 3$) were also measured. Pressor responses were tested for up to 121 min (mean ± s.e.mean: 49 ± 18 min) following the administration of enalapril to the ewe.

Captopril

Three of the ewes (133, 134 and 139 days gestation) were then given an i.v. bolus of captopril (15 mg, 1.46 μmol kg^{-1}), 36 ± 3 min after enalapril, and maternal pressor responses to 5 μg AI ($n = 3$) and 5 μg AII ($n = 3$) were measured. In two of these sheep, maternal pressor responses to 5 μg AI and 5 μg AII and foetal pressor responses to 10 μg AI and 10 μg AII were measured for up to 38 min. In the other sheep, maternal and foetal pressor responses to 5 μg AI and 5 μg AII were measured for 33 min.

Losartan

In nine sheep aged 125–132 days gestation (130 ± 1 days) maternal and foetal pressor responses to 5 μg AII were measured before and 1 h after foetuses were given an i.v. bolus of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}). These foetuses were given a second i.v. bolus of losartan

(10 mg kg^{-1}, 21.7 μmol kg^{-1}) and again, after 1 h, maternal and foetal pressor responses to 5 μg AII were measured.

In five sheep aged 126–135 days gestation (131 ± 2 days) maternal and foetal pressor responses to 5 μg AII were measured before and after ewes were given an i.v. bolus of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}). Pressor responses were measured for up to 97 min (60 ± 14 min) after administration of losartan to the ewe.

In another sheep, maternal and foetal pressor responses to 2.5 μg AII and 5 μg AII were measured before and for 1 h after the ewe was given an i.v. bolus of losartan (200 mg, 9.2 μmol kg^{-1}). The foetus was given an i.v. bolus of losartan (200 mg, 144.6 μmol kg^{-1}), 103 min after losartan had been given to the ewe, and the maternal pressor responses to 2.5 μg AII and 5 μg AII and the foetal pressor responses to 2.5 μg AII, 5 μg AII and 100 μg AII were tested. In a second sheep maternal and foetal pressor responses to 2.5 μg AII, 5 μg AII and 10 μg AII were measured before the ewe was given an i.v. bolus of losartan (300 mg, 13.8 μmol kg^{-1}). Then the maternal pressor responses to 10 μg AII and the foetal pressor responses to 2.5 μg AII, 5 μg AII and 10 μg AII were tested. The foetus was given an i.v. bolus of losartan (18.9 mg, 13.7 μmol kg^{-1}), 40 min after losartan was given to the ewe, and the maternal pressor responses to 10 μg AII and the foetal pressor responses to 2.5 μg AII, 5 μg AII and 10 μg AII were tested.

Foetal body weight (FBW) at the time of the experiment was calculated from the formula:

$$FBW = e^{\ln(\text{gestational age, days}) \times 2.94182 - 6.27298}$$

(Gibson & Lumbers, 1993)

Analysis of data

Values are for systolic and diastolic pressures (unless otherwise stated) and are expressed as mean ± standard error of the mean (s.e.mean). Foetal systolic and diastolic pressures were corrected for IAP prior to determining the pressor responses. Data were analysed with an IBM compatible PC and the software package SPSS/PC (Nie *et al.*, 1982). Student's paired *t* test was used to test for statistical significance before and after treatment. As only two foetuses were given enalapril, individual results for these experiments are given. In the study in which foetuses were given two doses of losartan, two-way analysis of variance was used to analyse data and Newman-Keuls test was used to find the means which differed (Zar, 1984).

Drugs

Sodium thiopentone (Pentothal, Abbott Australasia Pty. Ltd.; Sydney, N.S.W., Australia) and halothane (Fluothane, ICI; Macclesfield, Cheshire, England) were used as anaesthetic agents. Procaine penicillin and dihydrostreptomycin mixture (Hydropen, Bomac Laboratories; Asquith, N.S.W., Australia) was administered post-operatively. Enalapril (pure substance; Merck Sharp & Dohme; South Granville, N.S.W., Australia), captopril (Squibb; Noble Park, Victoria, Australia) and losartan (potassium salt; The Du Pont Merck Pharmaceutical Company; Wilmington, DE, U.S.A.), angiotensin I (Boehringer Mannheim Australia Pty. Ltd.; Sydney, N.S.W., Australia) and angiotensin II (Hypertensin, Ciba Geigy; Basle, Switzerland) were dissolved in 0.15 M NaCl on the day of use. Sodium pentobarbitone i.v. (Lethobarb, Arnolds of Reading; Victoria, Australia) was used to kill the ewe and foetus.

Results

Enalapril

The ability of the foeto-placental unit to convert enalapril to enalaprilat was tested in two foetuses. In one foetus the pressor response to 5 μg AI decreased from 11.9/7.0 mmHg during control to 4.2/6.3 mmHg 6 min after enalapril and was abolished 8 min after enalapril was given to the foetus. The pressor response to 5 μg AII did not change from control values of 18.3/13.3 mmHg. In the other foetus, the pressor response to 5 μg AI fell from 18.2/15.2 to 8.2/5.9 after 10 min and was 5.4/4.1 mmHg after 30 min. The foetal pressor response to 5 μg AII did not change from control values of 19.1/14.7 mmHg.

The i.v. administration of enalapril to pregnant ewes completely blocked the maternal pressor response to 2.5 μg AI ($n = 1$) and attenuated the maternal pressor responses to 5 μg AI ($n = 5$, $P < 0.001$; Figure 1a). The foetal pressor response to 5 μg AI ($n = 2$) and 10 μg AI ($n = 3$) did not change (Figure 1b). In one of these sheep the maternal pressor response to 2.5 μg AI (27.5/30.0 mmHg) was abolished within 6 min of administration of enalapril (Figure 1a) and the maternal pressor response to 5 μg AI was markedly attenuated by 12 min (from 41.9/41.3 to 10.0/6.7 mmHg) and by 78 min it was 8.5/6.0 mmHg. The foetal pressor response to 10 μg AI was unchanged throughout this period; before enalapril was given to the ewe it was 14.6/9.6 and after 68 min it was 13.25/8.25 mmHg. The maternal pressor response to 5 μg AII ($n = 5$) and the foetal pressor responses to 5 μg AII ($n = 2$) and 10 μg AII ($n = 3$) did not change (Figure 2).

Captopril

Pressor responses to AI were attenuated in each of the foetuses whose mothers were given captopril. In one foetus, the pressor responses to 5 μg AI had fallen from 14.4/9.4 to 4.6/3.8 mmHg, 11 min after captopril was given i.v. to the ewe but the foetal pressor response to 5 μg AII was

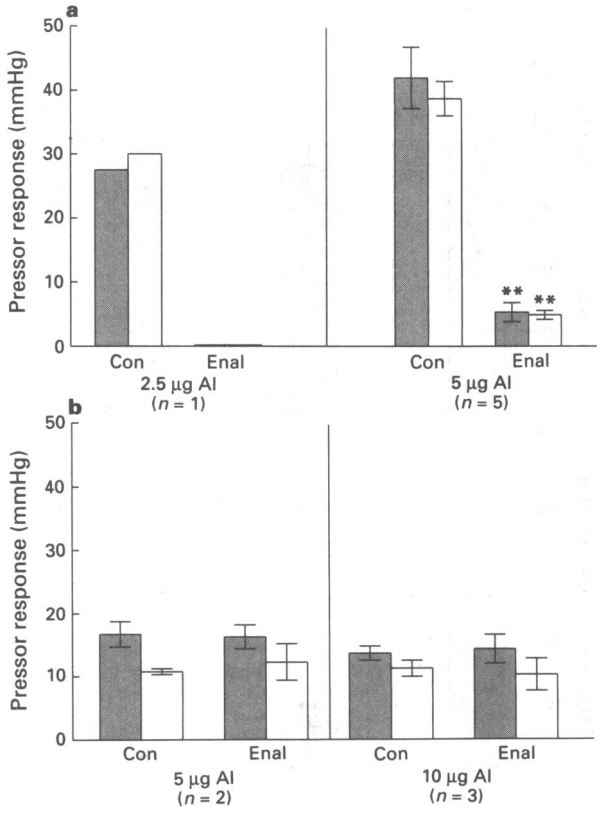

Figure 1 Mean ± s.e.mean for systolic (hatched columns) and diastolic (open columns) pressor responses of (a) the ewe to 2.5 μg angiotensin I (AI) and 5 μg AI and (b) the foetus to 5 μg AI and 10 μg AI before (Con) and after (Enal) the ewe was given i.v. enalapril (100 mg, 5.7 μmol kg^{-1}). **$P < 0.001$ when compared with control.

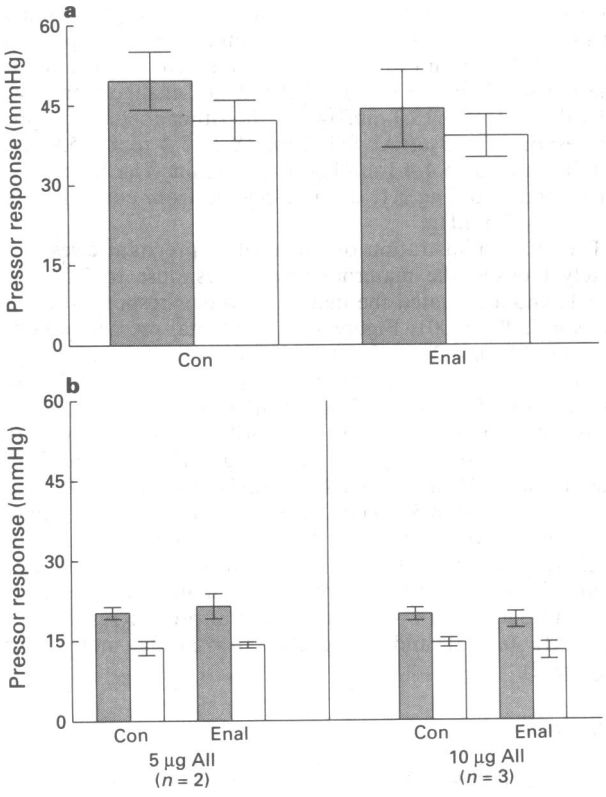

Figure 2 Mean ± s.e.mean for systolic (hatched columns) and diastolic (open columns) pressor responses of (a) the ewe to 5 μg angiotensin II (AII) ($n = 5$) and (b) the foetus to 5 and 10 μg AII before (Con) and after (Enal) the ewe was given i.v. enalapril (100 mg, 5.7 μmol kg^{-1}).

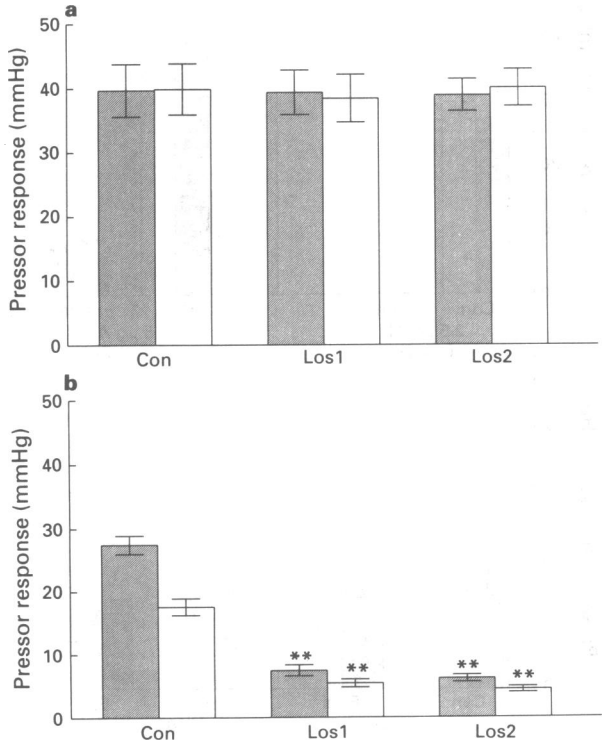

Figure 3 Mean ± s.e.mean for systolic (hatched columns) and diastolic (open columns) pressor responses of (a) the ewe and (b) the foetus to 5 μg angiotensin II (AII) before (Con), 1 h (Los1) and 2 h (Los2) after the i.v. administration of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}) to the foetus. $^{**}P < 0.001$ when compared with control ($n = 9$).

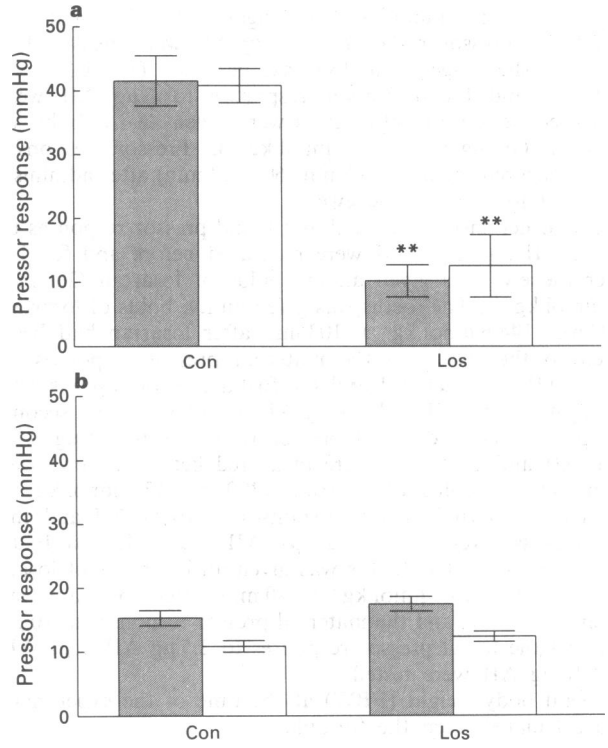

Figure 4 Mean ± s.e.mean for systolic (hatched columns) and diastolic (open columns) pressor responses of (a) the ewe and (b) the foetus to 5 μg angiotensin II (AII) before (Con) after the i.v. administration of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}) to the ewe. $^{**}P < 0.002$ when compared with control ($n = 5$).

unchanged from control values of 23.8/13.6 mmHg. In another foetus, the pressor response to 10 μg AI fell from 17.0/14.5 to 8.8/10.0 mmHg, 7 min after captopril was given i.v. to the ewe and the pressor response to 10 μg AII was unchanged from control values of 19.9/15.6 mmHg. In the third foetus, the pressor response to 10 μg AI fell from 11.7/11.1 to 6.9/5.6 mmHg, 8 min after captopril was i.v. given to the ewe and the foetal pressor response to 10 μg AII was unchanged from control values of 15.7/10.1 mmHg.

Losartan

The i.v. administration of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}) to foetuses attenuated the foetal pressor response to 5 μg AII. The foetal pressor response fell from 27.4 ± 1.5/17.5 ± 1.3 to 7.4 ± 0.9/5.4 ± 0.6 mmHg ($P < 0.001$) 1 h after the first dose of losartan and was 6.1 ± 0.6/4.4 ± 0.5 mmHg ($P < 0.001$, compared with control) 1 h after the second dose of losartan (Figure 3b). There was no difference between the foetal pressor responses at 1 h and 2 h. Maternal pressor responses to 5 μg AII were not changed (Figure 3a).

The i.v. administration of losartan (10 mg kg^{-1}, 21.7 μmol kg^{-1}) to pregnant ewes attenuated the maternal pressor responses to 5 μg AII ($P < 0.002$; Figure 4a); the foetal pressor responses to 5 μg AII did not change (Figure 4b).

In two other sheep, pressor responses to a number of doses of AII were established. In one sheep an i.v. bolus of losartan (200 mg, 9.2 μmol kg^{-1}) to the ewe attenuated the maternal pressor response to 2.5 μg AII (from 50.0/30.0 to 10.0/15.0 mmHg) and to 5 μg AII (from 55.0/33.0 to 35.0/25.0 mmHg); but did not change the foetal pressor responses to 2.5 μg AII (from 22.5/12.5 to 25.0/12.5 mmHg) and to 5 μg AII (from 35.0/20.0 to 35.0/22.0 mmHg). In the same animal, an i.v. bolus of losartan (200 mg, 144.6 μmol kg^{-1}) to the foetus abolished the foetal pressor response to 2.5 μg

AII and to 5 μg AII. The foetal pressor response to 100 μg AII was only 10.0/10.0 mmHg, 17 min after administration of losartan to the foetus. The maternal pressor responses to AII were not attenuated by losartan administration to the foetus (2.5 μg AII: from 25.0/20.0 to 30.0/30.0 mmHg; 5 μg AII: from 35.0/25.0 to 45.0/35.0 mmHg).

In the second sheep administration of an i.v. bolus of losartan (300 mg, 13.8 μmol kg^{-1}) to the ewe abolished the maternal pressor response to 10 μg AII. The foetal pressor responses to 2.5 μg, 5 μg and 10 μg AII were not changed from control values of 23.0/14.0, 25.0/16.3 and 46.5/29.4 mmHg, respectively (after losartan they were 22.5/15.0, 25.0/17.5 and 50.0/26.0 mmHg, respectively). The maternal pressor response to 10 μg AII was still completely blocked 48 min after losartan had been administered to the ewe. The i.v. administration of losartan (18.9 mg, 13.7 μmol kg^{-1}) to the foetus completely blocked the foetal pressor response to 2.5 μg AII and 5 μg AII and markedly attenuated the foetal pressor response to 10 μg AII from 46.5/29.4 to 13.5/8.5 mmHg.

Discussion

In these experiments the transplacental transfers of enalapril, captopril and losartan were determined by direct bioassay. Direct bioassay not only provides an assessment of the transplacental transfer of a drug but also indicates the biological impact of a particular dose of a drug given to the mother on both mother and foetus.

Because enalapril is a prodrug with little or no direct biological activity (Gross *et al.*, 1981) it was necessary to establish whether or not the foeto-placental unit of the sheep was capable of converting enalapril to enalaprilat. The results show that the foeto-placental unit of the sheep is capable of this conversion.

Administration of 100 mg (5.7 μmol kg^{-1}) enalapril i.v. to the pregnant ewe inhibited the maternal but not the foetal pressor response to AI (Figure 1). These results are in agreement with the findings of Broughton Pipkin & Wallace (1986) and indicate that enalapril does not cross the sheep placenta.

Although enalapril given i.v. to the ewe did not inhibit the foetal pressor response to AI, captopril given i.v. to the ewe attenuated, within 11 min, the foetal pressor response to AI in all three foetuses in which it was studied. Captopril was given to the ewes 36 ± 3 min following enalapril and pressor responses tested for up to 38 min. Enalapril was shown to have no effect on foetal pressor responses to AI within this time period. Therefore the inhibition of the foetal pressor response to AI following administration of captopril to the ewe are due to the effects of captopril alone.

The rapid inhibition of the foetal pressor response to AI following administration of captopril to the ewe is similar to results previously reported (Kingsford & Lumbers, 1989; Lumbers *et al.*, 1992). It has also been shown that captopril given i.v. to the chronically catheterized pregnant ewe (15 mg i.v. bolus followed by a continuous infusion at 6 mg h^{-1}) not only crosses the placenta and inhibits the foetal pressor response to 5 μg AI but also causes a fall in foetal mean arterial pressure (Lumbers *et al.*, 1992; 1993). Broughton Pipkin *et al.* (1982) also showed that captopril (2.8–3.5 mg kg^{-1}) given as an i.v. bolus to the pregnant ewe caused a fall in foetal arterial pressure within 10 min of administration.

Since losartan given i.v. to the foetus inhibited the foetal pressor response to AII, AT_1 receptors must be important in mediating the pressor effect of AII in the foetus. Thus it is valid to test for the transplacental transfer of losartan by measuring pressor responses to AII. There was no evidence that losartan crossed the sheep placenta.

Enalapril, enalaprilat and captopril have very low lipid solubilities whereas losartan is very lipid soluble (Table 1). Both enalaprilat and captopril exhibit similarily low levels of protein binding (Table 1). Enalapril, enalaprilat, captopril and losartan all have molecular weights <600 (Table 1) and such molecules should cross the placenta more readily if sufficiently lipid soluble (Parke, 1984). Since the lipophilicities and protein bindings of enalaprilat and captopril are similar, the more rapid rate of transfer of captopril (in sufficient amounts to be biologically effective) compared with enalapril cannot be explained on the basis of different lipophilicity or protein binding. The molecular weight of captopril is about half that of losartan but captopril is also several orders of magnitude less lipid soluble than losartan (Table 1). It is unlikely that difference in molecular weight alone could explain the rapid transplacental transfer of captopril. It is possible that the rapid inhibition of the foetal RAS following captopril administration to the ewe is due to active transport of captopril across the placenta via an amino acid carrier-mediated system. There is some evidence that the rapid rate of absorption of captopril from the gastrointestinal tract (despite its low lipid solubility) is due to an amino acid carrier-mediated system in rats (Hu & Amidon, 1988). A variety of transport systems for individual amino acids exist in the placenta (Verhaeghe & Assche, 1992). Finally, metabolism of enalapril, enalaprilat and losartan by the placenta into inactive metabolites cannot be excluded.

The sheep placenta is an epitheliochorial placenta whereas the placenta of man is haemomonochorial (Faber & Thornburg, 1983). Thus it should not be assumed that these results obtained in the sheep would be the same in man. However, the pregnant ewe and her foetus is one of the most popular models in developmental biology and pharmacology. In 1982 Broughton Pipkin *et al.* reported that captopril given to pregnant ewes and rabbits increased the rate of still-births in both species. The clinical use of captopril to treat pregnancy associated hypertension has been associated with foetal (Knott *et al.*, 1989) and neonatal anuria (Guignard *et al.*, 1981; Rothberg & Lorenz, 1984). Studies by Lumbers *et al.* (1993) showed that captopril given to the pregnant ewe rapidly crossed the placenta and disrupted foetal renal function and explained why the use of captopril in human pregnancy is associated with anuria. Schubiger *et al.* (1988) reported acute renal failure in a neonate whose mother was treated with enalapril for pregnancy-associated hypertension suggesting that enalapril, like captopril, crosses the human placenta. However, Broughton Pipkin & Wallace (1986) found that enalapril given i.v. to the ewe (1 mg kg^{-1}) did not cross the sheep placenta. Our results also show that captopril, but not enalapril or losartan, rapidly crosses the sheep placenta. Because the lipid solubility of a drug is so widely accepted as a good clinical index of its ability to cross the placenta (Mirkin, 1973; Goldstein *et al.*, 1974; Parke, 1984; Wang *et al.*, 1985; Benet & Sheiner, 1985) the results described above are of great interest because captopril, enalapril and losartan did not cross the sheep placenta as would be predicted from their lipid solubilities.

Thus, it is concluded that the foetal sheep can metabolize enalapril to enalaprilat and that AT_1 receptors are important in mediating the vasoconstrictor effects of AII in the foetus. Captopril with a very low Kp crosses the epitheliochorial placenta of the sheep whereas enalapril, which also has a low Kp, and losartan which has a high Kp do not cross. Thus Kp cannot be used as a measure of the ease with which these drugs cross the sheep placenta.

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