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THE EFFECT OF CAPTOPRIL (SQ14,225) UPON MOTHER AND FETUS IN THE CHRONICALLY CANNULATED EWE AND IN THE PREGNANT RABBIT

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SUMMARY

1. An inhibitor of angiotensin-I-converting enzyme activity (D-3-mercapto-2-methylpropanoyl-L-proline, Captopril) was given to five chronically cannulated pregnant ewes and eleven rabbits in late pregnancy.

2. Within 2 min of adminstration to the sheep, Captopril had blocked the maternal conversion of angiotensin I to II, as assessed by the pressor response evoked by the I.V. administration of angiotensin I. Maternal and fetal basal systemic blood pressures had fallen within 10 min of administration. Although maternal systemic blood pressure returned to basal levels within 2 hr, fetal pressures remained low for up to 2 days.

3. All ewes went into spontaneous labour at or near term. One lamb was live-born but very weak and failed to establish suckling. The remaining seven lambs were fresh still-births.

4. Gestation length was significantly prolonged in the treated rabbits by comparison with ten controls. The still-birth rate was 37 % in the treated animals and 6 % in the controls ($P < 0.001$).

5. It is concluded that the administration of Captopril to the two species studied is harmful to the fetus. The observations suggest that the drug rapidly crosses the placenta, and may cross the blood–brain barrier to exert a central effect. It may also interfere with the normal initiation of parturition.

INTRODUCTION

Concentrations of various components of the renin-angiotensin system are increased in pregnancy, both in mother and fetus, and it seems possible that the renin-angiotensin system may be of greater importance in cardiovascular homeostasis in the perinatal period than at any other time. It has also been suggested that an imbalance between angiotensin II and the vasodilator prostanoids may be part of the pathology of pregnancy-induced hypertension. Captopril (SQ14,225; D-mercapto-2-methylpropanoyl-L-proline) is an angiotensin-converting enzyme inhibitor, and a potent hypotensive agent in resistant non-pregnant hypertension (Ferguson, Turini, Brunner, Gavras & McKinstry, 1977). It was therefore felt to be of interest to study the effect of Captopril in late pregnancy.

MATERIAL AND METHODS

Sheep

Experiments were performed on five pregnant Clun cross ewes carrying a total of eight lambs. Each ewe, and one lamb per ewe, had cannulae placed in a carotid artery and jugular vein under general anaesthesia as previously described (Broughton Pipkin & O'Brien, 1978). A cannula was also placed in the amniotic sac. A minimum of 48 hr was allowed to elapse between surgery and any experimental procedure. Ewes were housed singly in roomy individual pens. They received a standard pellet diet of barley, oats and grass with additional hay and water *ad libitum*. Sodium intake on this diet was ~ 10 g/day. They also had free access to a mineral lick (98% NaCl) and were thus considered to be sodium replete.

Experiments were carried out between 119–133 days gestation (term ~ 147 days) with the animals standing quietly in their pens. Maternal, fetal and amniotic fluid pressures were measured with Bell and Howell Type 4-327-L221 pressure transducers connected to a Tarkan Wand W 900 multichannel flat bed recorder. Maternal and fetal heart rates were derived from the arterial pulse. A minimum of 20 min was allowed after the initial setting up of each experiment to permit maternal and fetal blood pressures and heart rates to re-stabilize.

A bolus dose of angiotensin I (AI; Schwarz-Mann, Orangeburg, NJ) (10 or 20 μg; 167–426 ng.kg^{-1}) I.V. was then given to the ewe and flushed in with normal saline. The pressor response evoked by the AI after its conversion to AII was allowed to fade entirely before the bolus was repeated (a minimum of 10 min). Three such boluses were given, together with a control injection in which saline only was administered. Maternal and fetal venous blood samples were then taken, for measurement, of plasma renin concentration, plasma angiotensin II (AII), plasma aldosterone and cortisol concentrations.

When the blood pressure was again stable, Captopril (SQ14,225; Squibb Europe Inc., London) was given I.V. to the ewe in a dose of 2·8–3·5 mg kg^{-1} and flushed in. Bolus injections of AI were repeated 1, 11, 21, 31, 41, 51 and 61 min later. Further blood samples were taken from the ewe at ~ 30 and 60 min after the administration of Captopril and from the fetus at ~ 30 min.

Plasma renin, AII and aldosterone concentrations were measured by radioimmunoassay as previously described (Symonds, Broughton Pipkin & Craven, 1975; Broughton Pipkin & Symonds, 1977; Symonds & Craven, 1977). Plasma cortisol was also measured by radioimmunoassay using an Immophase kit (Corning Medical, MA).

Rabbits

Twenty-one New Zealand white rabbits of known fertility were mated and housed in individual cages. On days 24–28 inclusive (term ~ 31 days) eleven were given 3·3 mg Captopril in 5 ml. saline by mouth; the remaining ten received 5 ml. saline only each day. No other experimental procedure was undertaken. The animals were allowed to litter down spontaneously.

Arithmetical means ±S.E. of means are quoted throughout. Student's unpaired t test has been used as appropriate to assess the statistical significance of differences between means.

RESULTS

Sheep

The indicator dose of AI used resulted in increases of 15–50 mmHg in diastolic, and 24–74 mmHg in systolic, blood pressure (Fig. 1). The response was reasonably consistent in any one animal and was not associated with any significant changes in fetal blood pressure. Saline alone did not evoke a pressor response.

Within 1 min of the maternal administration of Captopril, the diastolic pressor response to AI was reduced to 0–3 mmHg, and the systolic to −9 to 6 mmHg. Basal blood pressure had fallen in all ewes and fetuses by 10 min after Captopril administration (Table 1). The greatest fall was observed in the ewe with the highest pressure. Table 1 also shows the fall in fetal blood pressure over this time; the greatest

proportional fall was seen in the youngest fetus. As Fig. 2 shows, the fall in fetal blood pressure occurred very rapidly, preceding that in the ewe.

Although the pressor response to AI began to reappear by 30–70 min after Captopril administration, in no animal did it return to its pre-blockade value during the period of study (Fig. 1). Maternal blood pressure was returning to pre-blockade values in three of the five ewes by 1 hr after Captropril (Table 1); fetal values however remained low for up to 3 days after the experiment. Maternal basal heart rate rose

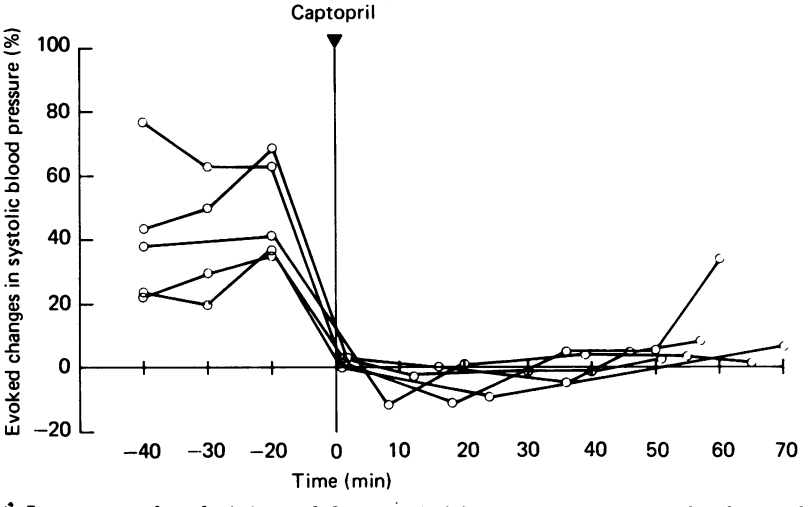

Fig. 1. Intravenously administered Captopril (SQ14,225), 2·8–3·5 mg.kg^{-1} completely abolished the pressor response evoked by angiotensin I in the pregnant ewe.

TABLE 1. Basal blood pressures in ewes and lambs before and after the maternal administration of Captopril. For technical reasons, no blood pressure recording was available from Garbo's lambs

	Time (min)			
	−10	+10	+30	+60
Wanda	128/100	116/88	112/80	108/88
	60/49	55/44	55/43	51/44
Xenoclea	113/97	108/89	105/90	111/93
	38/32	29/24	30/21	26/20
Yvette	90/–	85/–	70/–	74/–
	48/26	46/23	45/24	50/24
Brunnhilde	105/88	99/80	93/74	94/69
	50/41	42/33	42/35	37/29
Garbo	104/69	102/65	85/50	89/54
	—	—	—	—

in four ewes following the administration of Captopril; no consistent change was observed in fetal heart rate.

Fig. 3 illustrates the observed changes in maternal plasma renin and aldosterone concentrations following the administration of Captopril. Plasma renin had risen in all ewes by 60 min after Captopril. Plasma aldosterone and AII fell in four of the five ewes over the first 30 min; in three ewes both had started to recover by 60 min. There was a statistically significant relationship between the proportional fall in maternal

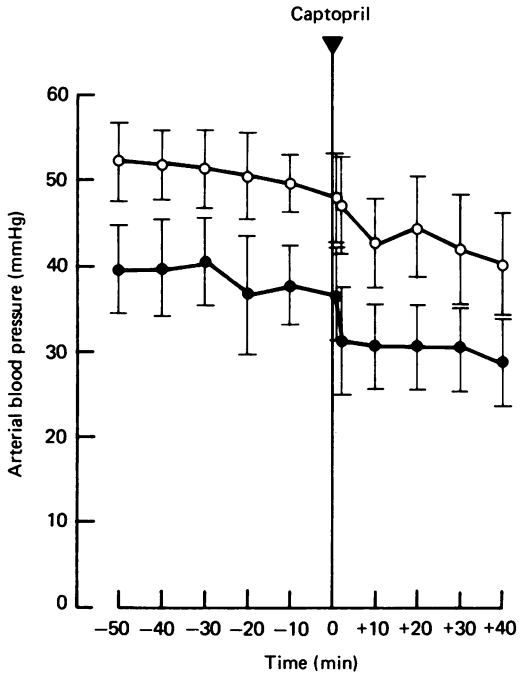

Fig. 2. The administration of Captopril (SQ14, 225) to the ewe resulted in an immediate, marked drop in fetal basal diastolic blood pressure (●), and a somewhat slower fall in fetal systolic (○) blood pressure. Mean values ± S.E. of means.

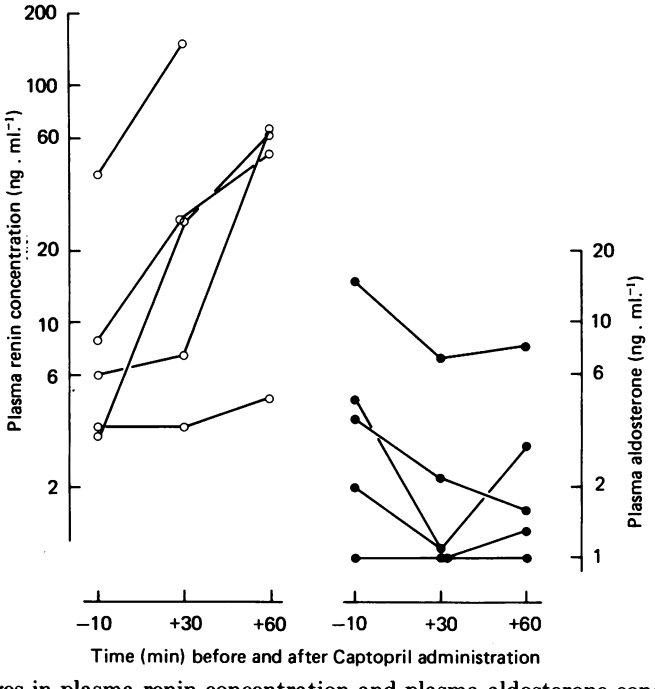

Fig. 3. Changes in plasma renin concentration and plasma aldosterone concentration in five ewes before and after the administration of Captopril. The chages are those which would be expected following blockade of angiotensin II production.

diastolic blood pressure 30 min after Captopril and the fall in angiotensin II concentration ($P < 0{\cdot}02$) and a similar relationship at 1 hr ($P < 0{\cdot}05$). Plasma cortisol fell over the first half hour in all animals, but rose again subsequently in four of the five ewes.

There was no consistent pattern of hormone response in the lambs. Plasma AII fell in the two oldest (132 and 133 days). Plasma renin concentration however only

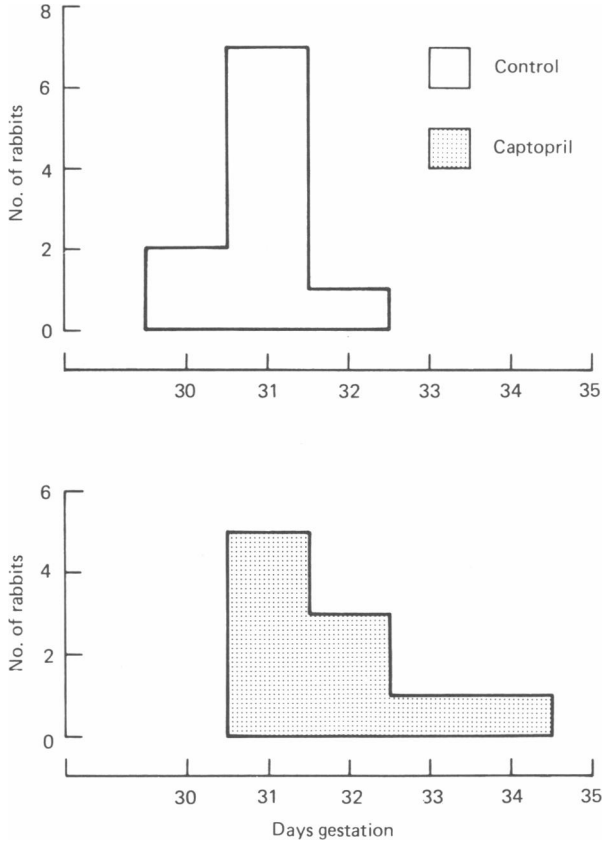

Fig. 4. Frequency histograms for gestation age at delivery in ten rabbits given oral Captopril between day 24 and day 28 of pregnancy, and in ten control rabbits.

rose in one of these and actually fell in three lambs. Plasma aldosterone was, and remained, below the limit of detection (1 ng . 100 ml.$^{-1}$) in the three youngest lambs; in the two remaining lambs it rose in one and fell in the other.

All ewes went into spontaneous labour 4–23 days later, near term. One lamb, a singleton, was live-born, but, although of a good size, was very weak, required oxygen in the immediate neonatal period and failed to establish suckling. All of the remaining lambs were fresh still-births, one delivering by the breech. A mild uterine infection was present in one ewe. The fetuses appeared to have developed normally since surgery. No gross fetal abnormalities were detected at autopsy.

Rabbits

One doe in the treated group died on day 31; she was not in labour at the time and the cause of death could not be established. This animal has been excluded from all statistical analysis.

The gestation age at delivery in the other treated and untreated rabbits is summarized as a frequency histogram in Fig. 4. Gestation length was significantly greater in the treated group ($P < 0{\cdot}05$). The control rabbits were delivered of eighty-six babies, of which eighty-one were live-born (94 %). The rabbits treated with Captopril gave birth to seventy-five babies, of which forty-seven were live born. This different is statistically highly significant (χ^2 test with Yates' correction = 26·4; $P < 0{\cdot}001$). The dead fetuses were delivered to eight of the treated, and four of the untreated does. Four fetuses (three Captopril, one control) were macerated; the remainder appeared to have died shortly before delivery. The frequency histogram for the birth weights of the live-born rabbits shows a slight shift to the right for the treated group, consistent with the longer duration of gestation.

DISCUSSION

Captopril is a powerful competitive inhibitor of angiotensin converting-enzyme (Ferguson *et al.* 1977). It thus prevents the cleavage of the C-terminal His–Leu dipeptide from angiotensin I and blocks the formation of both angiotensin II and [des-Asp^1] angiotensin II (angiotensin III). The observed complete blockade of pressor response to I.V. AI, together with an increase in plasma renin concentration and fall in AII concentration suggest strongly that the converting enzyme had been completely inhibited. Although AII concentrations did not fall to zero, this may well have been an artifact, since high concentrations of angiotensin I are likely to accumulate in the plasma during the administration of Captopril and may have cross-reacted with the antiserum used in the assay of angiotensin II (Morton, Casals-Stenzel, Lever, Millar, Riegger & Tree, 1979).

There exists in late pregnancy in both the sheep and the human, a statistically highly significant relationship between diastolic blood pressure and simultaneously measured venous angiotensin II concentrations (Broughton Pipkin & O'Brien, 1978; Symonds & Broughton Pipkin, 1978). This relationship may be causal, in that administration of the angiotensin receptor blocker, [Sar^1] [Ala^8] angiotensin II (Saralasin), resulted, in the sheep, in a dose-dependent fall in diastolic blood pressure, the magnitude of which was directly proportional to the initial concentration of angiotensin II (Broughton Pipkin & O'Brien, 1978). The suggestion of causality receives further support from the experiments reported here, since blockade of the renin–angiotensin system by a totally different method again resulted in a fall in maternal blood pressure, the magnitude of which was proportional to the change in circulating concentrations of angiotensin II.

There is a marked difference between the fetal effects of maternal administration of Captopril and of Saralasin in that Captopril appears to cross the placenta extremely rapidly, while Saralasin, a molecule some five times larger, does not appear to cross the placenta intact. Thus maternally administered Saralasin has no effect on fetal blood pressure, although fetally administered Saralasin does, in the majority of

fetuses (Broughton Pipkin & O'Brien, 1978; Iwamoto & Rudolph, 1979). Captopril, by contrast, exerted a marked and prolonged depressor effect on the fetal lambs here described.

Captopril 500 μg, given into the lateral brain ventricles of adult, spontaneously hypertensive rats, evoked a prolonged decrease in blood pressure, and blockade of the effects of centrally administered AI for up to 48 hr (Unger, Kaufmann-Buhler, Scholkens & Ganten, 1981). The blood–brain barrier may not be functionally tight even in the mature fetus (Backay, 1956). Furthermore, a recent report (Evered, Robinson & Richardson, 1980) suggests that, even in the adult rat, Captopril can cross the blood–brain barrier. It is therefore possible that Captopril, having crossed the placenta, crossed the blood–brain barrier and was exerting a central effect on fetal blood pressure.

Captopril has also been reported to increase plasma concentrations of 13,14-dihydro-15-keto-prostaglandin E_2 (PGE_2 M) in normal man (Swartz, Williams, Hollenberg, Levine, Dluhy & Moore, 1980). The same authors reported that the depressor response to Captopril correlated well with changes in PGE_2 M. Captopril also appears to lower peripheral resistance (Antonaccio, Rubin & Horovitz, 1980). If it were to exert a similar effect in the pregnant sheep and its fetus, increasing PGE_2 and blocking AII production, then placental perfusion could be dangerously jeopardized and fetal tissue perfusion fall to a low level.

In spite of the fetal hypotension associated with the maternal administration of Captopril, the fetuses failed to respond by an increase in heart rate. A similar lack of effect on heart rate was noted in humans following the chronic administration of Captopril (Fagard, Amery, Lijnen & Reybrouck, 1979). Alterations in cardiac output in the fetal lamb are almost entirely mediated through changes in heart rate, since alterations in stroke volume are only possible in late gestation (Rudoph & Heymann, 1973). This again points to the probability of diminished tissue perfusion in these fetuses.

Captopril is mainly cleared by the kidneys. Since renal function *in utero* is very low by adult standards (Barnes, 1976), this may be another reason for the prolonged effect of Captopril in the fetus. Captopril has been given to a pregnant woman with severe pregnancy-induced hypertension at 28 weeks gestation (Dr J. P. Guignard, personal communication). The maternal blood pressure fell dramatically. When the fetus was subsequently delivered by Casearean section, its own blood pressure was extremely low, it was in renal failure and it died at eight days of age. At autopsy, renal development was grossly normal. It therefore seems likely that Captopril was exerting a similar effect in the human as in the other animal species.

Gestation length was significantly prolonged in the rabbits and it was a clinical impression that labour was prolonged and unco-ordinated in the sheep. It is therefore possible that the high perinatal mortality in both species was a simple consequence of delayed delivery upon fetuses already under stress, and that delivery by Caesarean section would have minimized the mortality rate.

There are however other possibilities. The fetal lamb appears to play a major role in the initiation of parturition through a surge in cortisol production (Liggins, Fairclough, Grieves, Kendall & Knox, 1973). Fetal cortisol concentrations were not measured in these experiments, but maternal cortisol fell in all animals following Captopril and a similar effect may have occurred in the fetus. A final speculation is

provoked by the observation that angiotensin II administered into the lateral cerebral ventricle of the conscious rat produces an 8-fold increase in plasma oxytocin concentration within one minute (Lang, Rascher, Heil, Unger, Wiedmann & Ganten, 1981). Blockade of such an effect, if it exists in the sheep, might again interfere with the cascade of events necessary for a synchronized and successful labour and delivery.

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