Plasma concentrations of progesterone and 13,14-dihydro-15-keto-prostaglandin F-2 α at parturition in the bandicoot, *Isodon macrourus*

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Summary. The concentration of progesterone in maternal plasma of the bandicoot increased from 3.9 to 12.6 ng/ml between 11 and 4 days before parturition and remained at this level for at least 7 days. Concentrations of 13,14-dihydro-15-keto-prostaglandin F-2 α increased from basal levels of 288 pg/ml (4 days before birth) to maximal levels of 2534 pg/ml immediately after parturition, then decreased progressively. It is suggested that PGF-2 α , but not progesterone, influences the initiation of parturition in this species.

Introduction

In recent years a greater understanding of hormonal regulation of parturition has been obtained for eutherian mammals. In sheep, the amount of corticosteroid in the fetal plasma increases as the fetal adrenals develop and this increase causes a decrease in progesterone and a subsequent increase in oestrogen and prostaglandin (PG) F-2 α . These hormonal changes are thought to initiate parturition in the sheep (see review by Thorburn, Challis & Currie, 1977). It is therefore the fetus itself, and not the mother, that appears to exercise the dominant control over the time of parturition in at least some eutherian mammals. In marsupials, information on hormones at the time of parturition is not available although it has been stated that there is considerable development of the fetal adrenal cortex in late gestation in a number of marsupials (Sharman, 1976). The adrenal cortex of the neonatal tammar wallaby at, or even before, birth is capable of secreting corticosteroids (Catling & Vinson, 1976) and the fetal adrenal may thus also be involved in the initiation of parturition in marsupial mammals.

In this study the plasma concentrations of progesterone and 13,14-dihydro-15-keto-prostaglandin F-2 α (PGFM) were determined throughout pregnancy and early lactation in the bandicoot, *Isodon macrourus*, a marsupial about the size of a small rabbit, which bears litters of up to 7 young. It has the shortest known gestation period for a mammal, 12.5 days (Lyne, 1974), and an oestrous cycle length of between 20 days (Lyne, 1976) and 26 days (Hughes, 1962).

Materials and Methods

Animals

Adult female bandicoots (*Isodon macrourus*) were trapped in the environs of Brisbane and transferred to an enclosure (900 m²) which housed a male bandicoot. The bandicoots were examined weekly for signs of mating. Smears were obtained from one of the sacs at the terminal

end of the vagina, using a method described by Lyne (1976). The presence of spermatozoa was taken as evidence of mating and the female was then placed in a cage ($2 \times 2 \times 3$ m) in the Anatomy Department. Blood samples were obtained daily by heart puncture from animals which had been lightly anaesthetized with a halothane (Fluothane: ICI) and oxygen mixture. The blood was collected into heparinized tubes, centrifuged within 15 min of collection and the plasma was frozen until assayed.

Assays

Progesterone. Plasma progesterone concentrations were determined by the radioimmunoassay described previously (Gemmell, 1979) using sheep anti-progesterone-11-hemisuccinate-BSA kindly donated by Dr R. I. Cox. The antiserum is highly specific for progesterone, the only significant cross-reaction being that with 11β -hydroxyprogesterone (11.9%). The limit of sensitivity of the assay was 25 pg/tube and the intra- and inter-assay coefficients of variation were 15.7% ($n = 5$) and 18.1% ($n = 6$) respectively. The assay buffer blank was <0.1 ng/ml and the efficiency of extraction of progesterone from plasma was $86.2\% \pm 1.1$ (s.d., $n = 5$).

PGFM. 13,14-Dihydro-15-keto-prostaglandin F-2 α (PGFM) was determined in plasma samples by a modification of the method of Mitchell, Flint & Turnbull (1976). Goat anti-PGFM-BSA and authentic PGFM were kindly donated by Dr K. T. Kirton (Upjohn Company). Tritiated PGFM (sp. act. 80 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, and its purity was checked by thin-layer chromatography on silica gel in ethyl acetate:formic acid (400:5 v/v). Duplicate plasma samples (0.05–0.2 ml) were extracted with 2 ml redistilled diethyl ether after acidification of the plasma with 50 μ l 2 M-citric acid. The dried extracts were dissolved in 100 μ l 100 mM-Tricine buffer, containing 0.9% (w/v) NaCl, 0.1% NaN₃ and 0.1% gelatin, pH 8.0, and incubated overnight with 100 μ l antibody (1:10 000 initial dilution) and 100 μ l [³H]PGFM (10 000 c.p.m.). Bound and free hormone were separated using dextran-coated charcoal and bound radioactivity counted in POPOP/PPO scintillant containing 5% acetic acid.

The assay was specific for PGFM. The only prostaglandins which significantly cross-reacted in the assay were 15-keto PGF-2 α (12%), PGEM (2.8%) and 15-keto PGE-2 (0.75%); 6,15-dihydro PGF-1 α , 13,14-dihydro-6-keto-PGF-1 α , PGE-1, PGE-2, PGF-2 α , PGF-1 α , PGF-2 β , PGD-1, and PGD-2 all cross-reacted less than 0.2%. Recovery of between 4 and 1000 pg authentic PGFM added to bandicoot plasma yielded a highly significant correlation between observed and expected values ($r = 0.9898$, $P < 0.001$, $n = 9$). The limit of the sensitivity of the assay was 10 pg/tube. Inter-assay coefficient of variation was 14.3%, assay buffer blank was $0.13 \text{ ng} \pm 0.02$ (s.e., $n = 39$), the non-specific binding was $1.54\% \pm 0.15$ (s.e., $n = 27$) and the efficiency of extraction of [³H]PGFM from plasma was $87.6\% \pm 1.2$ (s.e., $n = 39$). Results were corrected for extraction losses.

Results

Plasma progesterone concentrations

Progesterone concentrations began to rise at about 8 days before parturition, reaching a plateau of approximately 12 ng/ml at 4 days before parturition. These values were maintained for at least 7 days and did not alter significantly throughout parturition in the pooled values (Table 1) or in consecutive samples taken from a single animal (Table 2).

PGFM concentrations

As shown in Table 1, PGFM concentrations increased from 4 days before birth to a maximum at 2 h after birth. Values then decreased gradually over the next 3 days. Frequent

sampling of one animal at the time of birth indicated that PGFM levels dramatically increased at this time (Table 2).

Table 1. Concentrations of progesterone and PGFM in the plasma of bandicoots before, at and after parturition

		Before parturition (days)												After parturition				
		12	11	10	9	8	7	6	5	4	3	2	1	2 h	2 h	1 day	2 days	3 days
Progesterone (ng/ml)	Mean	3.6	3.9	4.7	4.0	6.2	6.1	8.3	10.9	12.6	13.7	11.3	13.4	12.5	10.0	13.0	11.4	14.4
	s.d.		1.0	0.8	1.8	4.0	2.8	2.0	5.0	4.1	5.4	4.3	7.7	4.8	2.7	3.5	4.6	7.5
	<i>n</i>	1	3	3	4	7	6	5	8	9	12	11	12	3	6	14	10	7
PGFM (pg/ml)	Mean								172	288	323	306	1092	2301	2534	2039	1109	440
	s.d.								159	218	189	157	1477	2342	947	502	1692	387
	<i>n</i>								2	6	11	10	11	3	5	7	9	10

Table 2. Concentrations of progesterone and PGFM in the plasma of a bandicoot before, at and after parturition

		Before parturition					After parturition				
		3 days	2 days	29 h	21 h	5 h	0 h	3 h	7 h	2 days	3 days
Progesterone (ng/ml)		8.0	7.4	7.4	8.4	10.8	11.8	9.6	10.4	12.1	7.4
PGFM (pg/ml)		465	258	401	427	4960	>10 000	3425	2070	1229	1326

Discussion

It has been suggested that, for parturition to take place in the eutherian mammal, a decrease in the concentration of progesterone is required. This withdrawal of progesterone converts the suppressed and refractory pregnant uterus into an active organ (Csapo, 1956, 1977; Csapo & Takeda, 1965). This may be so in domestic animals (sheep, cows and goats, see review by Thorburn *et al.*, 1977), and in women (Turnbull *et al.*, 1974), but such withdrawal would not appear to be necessary for parturition in the bandicoot since there were no appreciable changes in progesterone concentration immediately before, during or after parturition. There was a marked change in PGFM concentrations which increased from 1 day before parturition to maximum levels at the time of birth. PGFM concentrations remained elevated for several days after birth.

Although the actual role of PGF-2 α during parturition still has to be elucidated, it is now clear that prostaglandin production is a prerequisite for normal birth in a wide range of eutherian mammals. Since PGFM is the major metabolite of PGF-2 α , and reflects the production rate of the primary prostaglandin (Cornette, Harrison & Kirton, 1974; Green, Bygdeman, Topozada & Wqvist, 1974), increased PGF concentrations may also be involved in parturition in the bandicoot.

Little is known about the mechanisms controlling birth in marsupials. Sharman (1976) suggested that the control of gestation length in marsupials is determined by the fetus. This statement was based on the results of Poole (1975) for gestation periods in Eastern grey kangaroos carrying embryos sired by Western grey kangaroos. An immunological involvement

has also been suggested for parturition in marsupials (Moors, 1974). The marsupial blastocyst is completely enclosed within a shell membrane (Hill, 1910; Sharman, 1961), a structure which is absent during eutherian embryogenesis (Sharman, 1961). This shell membrane prevents intimate contact between fetal and maternal tissues until implantation when the shell membrane breaks down. Parturition occurs 7–10 days after implantation (Hughes, 1974). Moors (1974) suggested that the persistence of the shell membrane protected the blastocyst from immunological recognition by the mother, and that, after implantation, the maternal lymphocytes recognize the 'foreign' fetus and a homograft reaction is initiated. If this form of reaction was responsible for parturition in the bandicoot it would be expected that the placenta, containing fetal tissue, would be expelled with the fetuses. However, this does not occur, the placenta being retained and absorbed in approximately 8 days (Hill, 1897).

Although many marsupials have a yolk sac placenta, bandicoots also have a chorio-allantoic placenta, the trophoblast of which is present until approximately 1 day before birth (Padykula & Taylor, 1976). It is possible that this loss of trophoblast in some way initiates parturition.

This study has provided preliminary information on the hormonal control of parturition in the marsupial. Further investigations will examine whether the raised levels of PGFM are involved in initiating uterine contraction, despite raised progesterone concentrations, or if they play some other role in the initiation of parturition. The changes in concentrations of other hormones, e.g. oestradiol and fetal cortisol, also need to be measured.

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