Role of $\text{PGF}_{2\alpha}$ and oxytocin in parturition in the brushtail possum (*Trichosurus vulpecula*)

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Maturation of the fetal pituitary and adrenal glands allows the secretion of cortisol, which in turn leads to an increase in prostaglandin and mesotocin production. The production of prostaglandin and mesotocin results in an increase in uterine contractions and initiates birth in marsupials. The major metabolite of $\text{PGF}_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (PGFM), has been found in the plasma of the possum at the time of birth and administration of $\text{PGF}_{2\alpha}$ to female possums induced the adoption of the birth position. Evidence that mesotocin is an integral hormone of birth in the tammar wallaby indicates that both $\text{PGF}_{2\alpha}$ and mesotocin or oxytocin are required for marsupial birth. The presence of $\text{PGF}_{2\alpha}$

receptors in the uterus and corpus luteum of the possum, and the *in vitro* uterine responsiveness to $\text{PGF}_{2\alpha}$ or oxytocin, were examined. $\text{PGF}_{2\alpha}$ receptors were not observed in possum uteri and the inability of $\text{PGF}_{2\alpha}$ to cause contractions indicates that $\text{PGF}_{2\alpha}$ is not involved directly in contraction of the uterus at parturition. The presence of oxytocin and mesotocin receptors in the uterus of possums and the ability of oxytocin to induce uterine contraction *in vitro* supports the view that mesotocin is required for expulsion of the young from the uterus. Low numbers of $\text{PGF}_{2\alpha}$ receptors were found in the possum corpus luteum at birth, indicating an involvement of $\text{PGF}_{2\alpha}$ in regression of the corpus luteum.

Introduction

$\text{PGF}_{2\alpha}$ causes regression of the corpus luteum in many eutherian species (Carnahan *et al.*, 1999; Silvia, 1999; Juengel *et al.*, 2000; Niswender *et al.*, 2000). Receptors for $\text{PGF}_{2\alpha}$ are present on large luteal cell membranes (Niswender *et al.*, 2000) in cows (Powell *et al.*, 1975; Rao, 1975), sheep (Powell *et al.*, 1974), horses (Kimball and Wyngarden, 1977), pigs (Gadsby *et al.*, 1990) and rats (Wright *et al.*, 1979). However, in contrast to eutherians, the corpora lutea of marsupials, with the exception of the bandicoots (Marsupialia: Peramelidae), do not persist because of pregnancy, and the plasma concentration of progesterone is declining or has reached basal concentrations at the time of birth (Gemmell *et al.*, 1987).

Contractile $\text{PGF}_{2\alpha}$ receptors have been reported in the myometrium of humans (Wakeling and Wyngarden, 1974; Schillinger and Prior, 1976; Senior *et al.*, 1992, 1993) and in the uterine tissue of rats (Brodth-Eppley and Myatt, 1998), monkeys and hamsters (Wakeling and Wyngarden, 1974). $\text{PGF}_{2\alpha}$ evokes a contractile response in the uterus of rabbits (Hurd *et al.*, 1991; Chen *et al.*, 1998) and baboons (Smith *et al.*, 1998). As in eutherians, 13,14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (PGFM) is a the major metabolite of $\text{PGF}_{2\alpha}$ in marsupials, including the tammar wallaby,

Macropus eugenii (Shaw, 1983a; Tyndale-Biscoe *et al.*, 1983; Lewis *et al.*, 1986), the brushtail possum, *Trichosurus vulpecula* (Gemmell *et al.*, 1987) and the short-nosed bandicoot, *Isodon macrourus* (Gemmell *et al.*, 1980). Plasma PGFM reaches a peak briefly at birth in the tammar wallaby (Tyndale-Biscoe *et al.*, 1983; Lewis *et al.*, 1986) and in the possum (Gemmell *et al.*, 1987). In the bandicoot, PGFM concentrations are high at the time of birth and remain high for several days (Gemmell *et al.*, 1980). These high concentrations of $\text{PGF}_{2\alpha}$ in the bandicoot do not induce regression of the corpus luteum (Gemmell, 1995). In addition, administration of $\text{PGF}_{2\alpha}$ to male and female tammar wallabies (Shaw, 1989, 1990; Hinds *et al.*, 1990), possums and bandicoots (Gemmell *et al.*, 1991) caused the animals to adopt the birth position. A study by Shaw (1983a,b) revealed that injection of a $\text{PGF}_{2\alpha}$ agonist, cloprostenol, during late pregnancy caused uterine contractions in the tammar wallaby. $\text{PGF}_{2\alpha}$ induces strong uterine contractions both *in vitro* and *in vivo* in the tammar wallaby (Renfree *et al.*, 1997).

In the pregnant tammar wallaby, there is an increase in mesotocin receptors 3 days before birth and administration of an oxytocin receptor antagonist (Atobisan) resulted in a delay in birth (Renfree *et al.*, 1996; Sebastian *et al.*, 1998). These results led Renfree and Shaw (1996) to conclude that the key hormones effecting uterine contractions and birth in the tammar wallaby were $\text{PGF}_{2\alpha}$ and mesotocin.

The aim of the present study was to investigate the role of $\text{PGF}_{2\alpha}$ receptors in the uterus and corpus luteum of the

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possum. In addition, the uterine responsiveness to $\text{PGF}_{2\alpha}$ and oxytocin *in vitro* in the possum was examined to determine the possible role of both hormones in the birth process.

Materials and Methods

Animals and treatments

The brushtail possums, *Trichosurus vulpecula*, used in this study were housed at the Native Animal Research Unit, University of Queensland, and were provided with food and water *ad libitum* as described by Gemmell *et al.* (1987). When required, possums were taken from their enclosures in hessian bags and lightly anaesthetized with a halothane (Fluothane; ICI) and oxygen mixture to allow examination of the pouch, obtain a vaginal specimen and determine body weight.

Detection of $\text{PGF}_{2\alpha}$ receptors

Young were removed from the pouches of six possums. Vaginal smears were collected from these possums 5 days after the young had been removed from the pouch until spermatozoa were observed. At day 17 after spermatozoa had been observed, just before the birth of the young, each possum was injected with a lethal dose of pentobarbitone sodium (Nembutal: Rhone Merieux Australia Pty Ltd, Pinkenba, Queensland). The uteri, ovaries and corpora lutea were removed, weighed and frozen immediately at -70°C . Bovine ovaries were collected from Brisbane abattoir and were placed immediately on ice for transportation back to the University. Corpora lutea were dissected from the ovaries and frozen at -70°C . At the time of the assay, each tissue specimen was thawed on ice and homogenized with an Ultra-Turrax in 10 mmol 5% (w/v) Tris-HCl I^{-1} containing 250 mmol sucrose I^{-1} , 1 mmol $\text{CaCl}_2 \text{I}^{-1}$, 1 mmol $\text{MgCl}_2 \text{I}^{-1}$ and 0.02% NaN_3 (pH 7.0). The homogenate was centrifuged at 20 000 *g* for 45 min. The pellet of each tissue specimen was then resuspended to a final dilution of 1:5 (w/v) for bovine corpus luteum and possum gestational and non-gestational uteri, and to 1:6 (w/v) for possum corpus luteum, in incubation buffer (10 mmol Tris-HCl I^{-1} containing 10 mmol $\text{CaCl}_2 \text{I}^{-1}$, 1 mmol $\text{MgCl}_2 \text{I}^{-1}$, 0.02% NaN_3 and 0.01% gelatin, pH 7.0). The diluted tissue suspensions were used immediately in radioreceptor assays. The protein content of the resuspended pellet was measured by the dye-binding method of Bradford (1976) and expressed as mg of protein in the pellet per g wet weight of tissue.

Radioreceptor assay

The radioreceptor assay mixture consisted of 0.1 ml of tissue suspension, 0.1 ml [^3H]prostaglandin $\text{F}_{2\alpha}$ (2×10^5 c.p.m. ml^{-1} ; 209 Ci mmol^{-1} ; Amersham International, Little Chalfont), and 0.1 ml of incubation buffer, containing 0, 1, 2, 4, 8, 16, 32, 64, 100 and 1000 nmol $\text{PGF}_{2\alpha}$ (ICN Biomedicals, Seven Hills, Sydney, NSW). The samples were incubated at room temperature for 90 min, and then bound

$\text{PGF}_{2\alpha}$ was precipitated by the addition of 0.5 ml γ -globulin (4 mg ml^{-1}) and 1.5 ml of 16% (w/v) polyethyleneglycol (PEG) followed by centrifugation at 1500 *g* for 10 min. The precipitate was dissolved in 1 ml water, added to 4 ml of scintillation fluid (Starscint, Packard, Canberra) and counted for 10 min with an efficiency of 35% in a Packard model 1900CA liquid scintillation counter (Packard). Binding data were analysed using the LIGAND computer program (Munson and Rodbard, 1980). In all cases a two-site model was not significantly better than a one-site model, so data for the one-site fit are presented. B_{max} values are presented as nmol per mg protein. The ligand specificity of the receptor was determined by performing competitive binding radioreceptor assays on bovine corpus luteum with prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), 13,14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (PGFM ; the major metabolite of $\text{PGF}_{2\alpha}$) and arachidonic acid (AA; the precursor to prostaglandin synthesis). All ligands were purchased from ICN Biomedicals. The interaction of each ligand with the $\text{PGF}_{2\alpha}$ receptor was expressed as a semi-log competitive displacement curve.

Conditions for the receptor assay

The composition of the buffers used for the radioreceptor assays were based on those used by Wiepz *et al.* (1992). These workers found that the optimal pH for maximal binding of [^3H] $\text{PGF}_{2\alpha}$ was 5.5–6.0. A test for optimal binding to rat ovarian tissue at pH 6.0 and 7.0 was performed and there was no significant difference between the two conditions. Therefore, all assays were performed at pH 7.0, under conditions closer to physiological pH.

Tissue preparation (organ bath)

Vaginal smears were collected from day 5 after removal of pouch young, until spermatozoa were observed. Two possums at day 13 of gestation (13 days after the observation of spermatozoa) and three non-pregnant possums (18 days from removal of pouch young) were injected with a lethal dose of pentobarbitone sodium. Corpora lutea were not present on the ovaries of two of the anoestrous possums, whereas a corpus luteum was observed in the third possum. The uteri collected were placed immediately into Tyrode's solution at 35°C , bubbled with 95% O_2 and 5% CO_2 , and cut into longitudinal sections. The sections were suspended with sutures in 25 or 30 ml organ baths containing Tyrode's solution bubbled with 95% O_2 and 5% CO_2 and were stimulated at 1 Hz at 35°C . The baths contained examples of either gravid or non-gravid uteri (from the pregnant possums), uteri from the contralateral and ipsilateral side containing the corpora lutea (from the possum undergoing an oestrous cycle) or non-gravid uteri (from possums in anoestrus). Four strips of uterus, 2–3 cm in length and about 5 mm in width, two from the gravid and two from the non-gravid uteri from each of the two pregnant and three non-pregnant possums, were tested. The order of drug administration to this tissue was

acetylcholine (concentration range from 1×10^{-6} mol l $^{-1}$ to 1×10^{-4} mol l $^{-1}$), and after washing the uterine tissue, PGF $_{2\alpha}$ (concentration range from 1×10^{-10} mol l $^{-1}$ to 3×10^{-6} mol l $^{-1}$) and oxytocin (0.1 iu).

Experimental procedure (organ bath)

Uterine sections were suspended in the organ baths at a resting tension of 10 mN adjusted to give the maximal twitch response. During the 45 min equilibration period, the tissues were washed at least five times with Tyrode's solution. The tissues were contracted twice using a synthetic oxytocin (Syntocinon 10 iu ml $^{-1}$; Novartis Pharmaceuticals Australia Pty Ltd, NSW). Cumulative dose-response curves were measured for acetylcholine (from 10 nmol l $^{-1}$ to 0.3 mmol l $^{-1}$) and PGF $_{2\alpha}$ (from 0.1 nmol l $^{-1}$ to 3 μ mol l $^{-1}$). Tissues were washed at least four times with Tyrode's solution after each compound dose range was completed.

Data analysis (organ bath)

Data are given as the mean $\pm$ SEM of the experiments measuring the increase in force of contraction in mN.

Results

Affinity and specificity of the PGF receptor

Binding analysis of corpus luteum membranes in cows revealed a binding site with a K_a of 2.7×10^8 mol l $^{-1}$ and a $B_{\max}$ of 160 pmol mg $^{-1}$ protein (Fig. 1a). The binding was specific for PGF $_{2\alpha}$ (Fig. 1b). The ligand affinity was found to be in the following order of decreasing affinity: PGF $_{2\alpha}$ > PGD $_2$ > PGE $_2$ > PGFM > arachidonic acid. The higher affinity for PGF $_{2\alpha}$ than for PGD $_2$ or PGE $_2$ distinguishes these receptors from other prostaglandin receptors. Arachidonic acid (AA) did not displace PGF $_{2\alpha}$ binding.

PGF receptors in the corpus luteum and uterus of the possum

The bovine corpus luteum was used as a positive control for all receptor assays performed on possum uterine and luteal tissue. PGF $_{2\alpha}$ receptors were not present in possum uterus. The specific binding of possum corpus luteum collected on the day of birth was low, as shown in the Scatchard plot (Fig. 1c). The affinity of PGF $_{2\alpha}$ receptors of possum corpus luteum was estimated to be 2.8×10^8 mol l $^{-1}$ and the receptor density ($B_{\max}$) was 25 pmol l $^{-1}$. Possum corpora lutea collected on day 25 after removal of pouch young (equivalent to between day 14 and day 16 of the 17.5 day gestation period) did not contain receptors for PGF $_{2\alpha}$. A comparison of the PGF $_{2\alpha}$ receptor concentration, normalized for protein content, in the mid-cycle bovine corpus luteum and the possum corpus luteum at birth (Fig. 2) shows a $B_{\max}$ of only 5 pmol mg $^{-1}$ for the possum compared with 48.9 ± 12 pmol mg $^{-1}$ protein ($n = 7$) for bovine corpus luteum.

Fig. 1. (a) A representative Scatchard plot of PGF $_{2\alpha}$ binding to bovine corpus luteum. (b) The binding specificity of bovine luteal PGF $_{2\alpha}$ receptors showing the following order of decreasing affinity: PGF $_{2\alpha}$ (■) > PGD $_2$ (□) > PGE $_2$ (▼) > PGFM (◆) > AA (▲). AA: arachidonic acid; PG: prostaglandin. (c) A representative Scatchard plot of PGF $_{2\alpha}$ binding to possum corpus luteum.

Organ bath

In all strips of uterus from the two pregnant and three non-pregnant possums tested, 0.1 iu oxytocin added to the 25 ml bath gave a rapid contraction of 15–46 mN, although this may not be a maximal response as only one concentration was tested. After washout, a dose-response curve to

Fig. 2. A comparison of the PGF_{2α} receptor density in the mid-cycle bovine corpus luteum (CL; $n = 7$) and the possum corpus luteum at birth ($n = 1$ from a pool of ten corpora lutea).

acetylcholine in all uterine strips gave contraction over a concentration range from 10 nmol to 0.3 mmol l⁻¹ with maximal responses between 50 and 100% of that of oxytocin. There did not appear to be any differences between gravid and non-gravid or non-pregnant uteri. PGF_{2α} did not produce a response in any possum uterus at concentrations from 0.1 nmol to 3 μmol l⁻¹ (Fig. 3). Rat uterus, which was used as a positive control for PGF_{2α}, did give a positive response.

Discussion

The administration of PGF_{2α} to male and female tammar wallabies (Shaw, 1989, 1990), possums and bandicoots (Gemmell *et al.*, 1991) induced the animals to adopt the birth position. PGF_{2α} is therefore involved in a behavioural response at birth in marsupials, and may exert its effect directly on the brain, rather than indirectly by stimulation of uterine or vaginal contractions. PGF_{2α} also plays an important role in uterine contraction in those eutherian species studied (Hurd *et al.*, 1991; Chen *et al.*, 1998; Smith *et al.*, 1998), and has been shown to stimulate uterine activity in the tammar wallaby (Young, 1978; Shaw, 1983b). In the present study, PGF_{2α} receptors were not observed in the possum uterus on the day of birth and PGF_{2α} did not cause contractions in possum uteri *in vitro*. Therefore, PGF_{2α} does not appear to be involved in uterine contractions in the possum. This is in contrast to findings in the tammar wallaby, in which the injection of PGF_{2α} or its analogue caused uterine contractions in late pregnancy (Young, 1978; Shaw, 1983b). It is possible that PGF_{2α} causes uterine contractions indirectly in the tammar wallaby, by acting on receptors in the brain. The fact that PGF_{2α} also causes male and female tammar to adopt the birth position indicates that the hormone is not acting directly upon uterine or vaginal tissues to cause this behaviour, but rather its action on the brain initiates a

Fig. 3. A dose-response curve of possum uterus to acetylcholine (from 1×10^{-6} mol l⁻¹ to 1×10^{-4} mol l⁻¹), and after washing, to PGF_{2α} (1×10^{-10} mol l⁻¹ up to 3×10^{-6} mol l⁻¹) and oxytocin (0.1 iu). A response was observed with acetylcholine and oxytocin, but not with PGF_{2α}. The movement of the chart was 1 cm in 10 min.

behavioural response and perhaps also initiates uterine contractions. PGF_{2α} may not be directly involved in uterine contractions. In response to PGF_{2α} the brain may release mesotocin, the oxytocin-like peptide secreted in these marsupial species (Chauvet *et al.*, 1981). Oxytocin receptors have been found in the uterus of the possum (Sernia *et al.*, 1990, 1991), the tammar wallaby (Shaw, 1983a; Tyndale-Biscoe and Renfree, 1987; Sebastian *et al.*, 1998) and the quokka, *Setonix brachyurus* (Heller, 1973). These receptors have also been shown to be responsive to both mesotocin and oxytocin.

In eutherian mammals, PGF_{2α} is a well-known luteolytic agent (Carnahan *et al.*, 1999; Silvia, 1999; Juengel *et al.*, 2000; Niswender *et al.*, 2000) and receptors for PGF_{2α} have been demonstrated on luteal cell membranes of various eutherian species (Powell *et al.*, 1974, 1975; Rao, 1975; Kimball and Wyngarden, 1977; Wright *et al.*, 1979; Gadsby *et al.*, 1990; Niswender *et al.*, 2000). In the possum, a brief peak of PGFM occurs at the time of birth (Gemmell *et al.*, 1987). This peak has been suggested to relate to parturient behaviour observed upon injection of PGF_{2α} (Gemmell *et al.*, 1991). However, the corpus luteum of the possum regresses at birth as shown by decreasing or basal concentrations of progesterone at this time (Gemmell *et al.*, 1987). In the present study, possum corpus luteum showed low concentrations of specific binding of PGF_{2α} to receptors, indicating that PGF_{2α} may also be involved in the regression of the corpus luteum at the time of birth. This would also indicate similarities in the mechanisms of luteolysis between eutherians and marsupials.

In conclusion, PGF_{2α} receptors are not present in possum uteri, indicating that PGF_{2α} is not involved directly in contraction of the uterus at parturition. The inability of PGF_{2α} to cause contractions in possum uterus *in vitro* supports this view. The presence of oxytocin receptors in the possum uterus (Sernia *et al.*, 1990, 1991) and the ability

of oxytocin to cause uterine contraction *in vitro* supports the view that mesotocin is required for expulsion of the young from the uterus. Low numbers of $\text{PGF}_{2\alpha}$ receptors were found in the possum corpus luteum at birth, indicating an additional involvement of $\text{PGF}_{2\alpha}$ in the regression of the corpus luteum as well as in initiation of parturient behaviour.

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