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ENDOCRINOLOGY 1980

Endocrinology 1980

Proceedings of the VI International Congress of Endocrinology, Melbourne, Australia, February 10-16, 1980

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1980

ELSEVIER/NORTH-HOLLAND BIOMEDICAL PRESS
AMSTERDAM • NEW YORK • OXFORD

Published by:
The Australian Academy of Science
PO Box 783
CANBERRA CITY ACT 2601.

Sole distributors for the world except Australia:
Elsevier/North Holland Biomedical Press
335 Jan van Galenstraat, 1061 AZ Amsterdam
The Netherlands.

Sole distributors for the USA and Canada:
Elsevier/North Holland, Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017
U.S.A.

ISBN Australian Academy of Science 0 858 47062 4
ISBN Elsevier/North Holland 0 444 80267 3

Printed at Griffin Press Limited, Netley, South Australia.

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HORMONES AND PARTURITION

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The astute deduction of Malpas in 1933 (1) that prolonged pregnancy in human anencephaly was best explained by including the fetal pituitary and adrenals among the endocrine organs providing the hormonal melieu of parturition was supported 30 years later when similar spontaneous malformations in bovine (2,3) and ovine (4) fetuses were shown also to be associated with prolonged pregnancy. The test of the Malpas hypothesis by ablating the fetal pituitary in an experimental animal was successfully accomplished in fetal sheep in 1966 (5). The protracted pregnancies that followed were unequivocal evidence of fetal participation in parturition. When subsequently it was shown that premature parturition was inducible in sheep by stimulating or supplementing fetal adrenocortical secretion (6), the experimentalist had at his disposal a model in which pregnancy length could be reproducibly manipulated in either direction. With concurrent advances in radioimmunoassay, techniques of fetal surgery and the biochemistry and assay of prostaglandins it was inevitable that the major components of the physiological system controlling parturition in sheep were rapidly identified and that similar models were developed. However in many species, notably polytocous mammals and primates, knowledge is sparse and not yet capable of being synthesised into a cohesive hypothesis.

Developments of the past decade have led to the emergence of several novel concepts that are strongly influencing the directions of current research: (1) The control of initiation of parturition is contained within the uterus and involves mainly the fetus, chorion and uterine epithelium. (2) The uterine epithelium has endocrine functions that make major contributions to parturition. (3) Prostaglandins from uterine epithelium mediate many of the effects of other hormones on the uterus in both placenta-dependent and CL-dependent species. (4) Collagenous connective tissue changes in the uterus are as important to the success of parturition as the contractions of smooth muscle.

Progesterone

The patterns of progesterone concentrations in maternal plasma immediately prepartum varies from falling levels (e.g. sheep (7), cow (8), sow (9), rabbit (10), rat (11)) to static levels (e.g. man (12)) to rising levels (e.g. monkey (13), horse (14)) and clearly fails to conform to a unified hypothesis of progesterone withdrawal as a prerequisite to parturition (15). Attempts to explain the discrepancies by postulating reduced myometrial concentrations not reflected in maternal plasma (16) are not supported by recent studies of human myometrium (17). Furthermore, the levels of nuclear progesterone receptors at term are relatively high (18). These reports suggest that myometrial progesterone receptors are saturated at term. As discussed below, myometrium is not the only potential target for progesterone action and the possibility of 'withdrawal' of progesterone from other sites has received little attention. The appearance in human chorio-amnion at term of high affinity binding protein with specificity for progesterone (19) could lead to competition for progesterone with other intracellular binding sites (e.g. lysosomes) and, in effect, withdrawal of progesterone in that tissue although this would not be reflected in tissue concentrations.

The classical view that progesterone acts by raising the membrane potential of smooth muscle cell remains controversial (20, 21) and a number of other sites of action have been suggested. In the smooth muscle, progesterone may stabilise membrane-bound pools of calcium (22) or inhibit formation of gap-junctions (23). In addition, it may act on amnion, chorion and uterine epithelium. Of particular interest is the hypothesis that progesterone inhibits prostaglandin synthesis in the latter tissues by inactivating phospholipase A_2 (24, 25) or, in the case of ovine uterine epithelium, by inhibiting the formation of oxytocin receptors (26). As well as inhibiting prostaglandin synthesis progesterone may antagonise their action; in non-pregnant sheep, progesterone inhibits the oxytocic response to $PGF_{2\alpha}$ injected into the uterine lumen (27).

In those species in which progesterone levels fall before parturition, three distinct

mechanisms causing reduced progesterone secretion have been identified. When the placenta is the source of progesterone as in sheep, activation by fetal cortisol of 17α-hydroxylase and C-17,20 lyase promotes oestrogen and androgen synthesis at the expense of secreted progesterone (28). When the corpus luteum is the source, luteolysis leads either to diminished activity of the pathway leading to the synthesis of progesterone and its metabolites as in the goat (29) or to diversion of the transformation of pregnenalone to progesterone into a pathway leading to the formation of 20α-dihydroprogesterone as in the rat (30).

Oestrogen

Regardless of whether the corpus luteum or the placenta is the major source of progesterone, the placentas of many species contain aromatase activity (31) and synthesise oestrogen in late pregnancy. Placentas vary, however, in their ability to utilise C-21 steroids as substrates for oestrogen synthesis depending on the presence or absence of 17α-hydroxylase activity. Man (32) and possibly subhuman primates (33,34) and mares lack placental 17α-hydroxylase and are dependent on extraplacental sources of C-19 steroids. Primate placentas utilise androgens of adrenal (fetal and maternal) origin whereas the equine placenta is dependent on androgen (DHAS) from the fetal gonads (35). In those placentas containing 17α-hydroxylase there is probably variability both in the pattern of activity throughout gestation and in the degree of activation of the enzyme by fetal cortisol. In sheep, for example, activity of the enzyme (and, consequently, oestrogen synthesis) is low until near term when it increases rapidly in response to a surge of fetal cortisol (36). Similar activation of 17α-hydroxylase occurs in the goat placenta but is not reflected in a marked elevation of oestrogen synthesis, probably because formation of androgens remains rate limited by C-17, 20 lyase (37) unlike the sheep in which activation of C-17,20 lyase is associated with the marked reduction in intraplacental concentration of progesterone (38).

As with progesterone, the patterns of plasma oestrogen concentrations immediately prepartum differ widely between species, varying from sharply rising (e.g. sheep (39)) to static or very slowly rising (e.g. Man (40); guinea pig (41)) to falling levels (e.g. horse (35)). The varying patterns may be explained to a large extent on the basis of the source of substrate for oestrogen synthesis and whether or not placental 17α-hydroxylase activity is readily induced by fetal cortisol; when substrate comes mainly from the fetal adrenal, the pattern of oestrogen synthesis follows that of the secretory activity of the fetal zone of the fetal adrenal and rises only slowly, if at all, near term; when the gonad provides the substrate, synthesis reaches a mid-gestation peak at the time of maximal gonadal activity and falls towards term; when the substrate is of placental origin, oestrogen levels rise sharply to a greater or lesser extent according to the degree of activation of 17α-hydroxylase and C-17,20 lyase. The response in maternal oestrogen levels to administration of corticosteroids is predictable on the same basis; levels may fall, rise or remain static according to whether the adrenal, placenta or gonads respectively is the source of substrate. These conclusions assume that substrate availability rather than placental aromatase activity is rate-limiting in oestrogen synthesis. This is likely to be generally true since infusion of androstenedione or DHA increases oestrogen synthesis in several species.

The administration of oestrogen near term induces parturition in some species but not others. Success or failure appears not to depend on whether maintenance of pregnancy is placenta-dependent or CL-dependent but rather on whether parturition is normally preceded by elevated levels of oestrogen. For example, sheep and goats are similar in showing prepartum oestrogen rises and in being inducible by oestrogen administration but differ in the source of progesterone. On the other hand in species with static or falling oestrogen levels before parturition (e.g. primates, guinea pigs, horses), oestrogen fails to induce parturition. This suggests that the prepartum oestrogen rise present in some species plays a direct part in the initiation of parturition.

Fetal Glucocorticoid

The concentration of unbound cortisol or corticosterone in fetal plasma increases at a variable rate towards term in every species investigated to date (42). In the human fetus, single assays of total corticosteroids in cord blood of infants born before term (43) or serial assays of either amniotic fluid cortisol (44, 45) or urinary corticosteroid sulphates (46) suggest a 1.5-2 fold increase in the last four weeks of pregnancy. By contrast, fetal plasma cortisol increases up to 10 fold in the sheep during the last week of pregnancy (47). Until recently it was thought that the rat fetus was an exception to the general rule in

that total corticosteroid concentration falls in the three days before parturition. However, levels of free corticosteroid rise until the day before parturition and the fall in total corticosteroids is attributable to a simultaneous fall in levels of CBG (48).

A rising level of corticosteroid in fetal plasma has as its function the induction of a variety of enzymes concerned with fetal maturation and enhanced ability to survive after birth (49). The relationship between cortisol concentrations and fetal maturation is particularly close (50). In certain species, notably sheep, goats, cattle and pigs there is good evidence from the response to fetal hypophysectomy (5, 51), adrenalectomy (51) or fetal infusions of corticotrophin (ACTH) or corticosteroids (53) that the fetal control of parturition is mediated by cortisol. The only mechanism by which cortisol is known to stimulate parturition is by activation of placental 17α-hydroxylase. Species that are dependent for placental oestrogen synthesis on C-19 substrates from adrenals or gonads (e.g. Man, subhuman primates, horse) are not inducible by massive corticosteroid treatment even near term (54, 55).

Despite extensive investigation, particularly in sheep, the factors regulating cortisol secretion by the fetal adrenal remain obscure. The prepartum rise in cortisol secretion in fetal sheep is followed rather than preceded by elevated plasma levels of ACTH (56) although infrequent sampling allows the possibility of pulsatile release of ACTH that escaped detection. Between 90 and 135 days (57) the fetal sheep adrenal is relatively refractory to short-term elevations of ACTH levels elicited by infusions of synthetic $ACTH_{1-24}$ (58), by an hypoxaemic stimulus (59) or by fetal haemorrhage (60). Thus, the timing of parturition in this species seems to be determined by the factors responsible for the heightened responsiveness of the fetal adrenal. To which, if any, of the various possible factors the change in responsiveness should be attributed is as yet unclear. In part, it is due to increased cortical mass since it has been calculated that this increases 6 times in the last 8 days of intrauterine life (61). The stimulus to accelerated cortical growth is unknown but it may be a further reflection of greater sensitivity to ACTH. Inhibition by ACTH-related peptides including α-MSH and CLIP identified in fetal pars intermedia has been suggested (62, 63). Responsiveness is enhanced by fetal infusions of dexamethasone (57) but not by prostaglandin E_2 (unpublished observations) or prolactin (64). An adrenocortical cell with distinctive morphological characteristics which makes its appearance at the time of heightened responsiveness might respond differently to ACTH (65). An additional contribution to the surge of cortisol preceding parturition in sheep is made by the continuing rise in plasma levels of ACTH in the face of markedly elevated levels of cortisol. There is some evidence that this upward shift in the threshold of negative feedback of cortisol on ACTH secretion is attributable to a positive feedback effect of cortisol on the threshold. During infusion of $ACTH_{1-24}$ into intact fetal sheep levels of total immunoreactive ACTH become markedly elevated compared to values of hypophysectomised fetuses (66). Furthermore, after a single intrafetal injection of dexamethasone cortisol levels are depressed for less than 24 hr and then become elevated (67).

Prostaglandins

The bewildering species variation observed in the relationships of progesterone, oestrogen and corticosteroids in parturition seems to apply to a much lesser degree to prostaglandins. Prostaglandin inhibitors prolong pregnancy in all species studied to date and, with the notable exception of the ferret (68), administration of prostaglandins invariably induces abortion or parturition if given in adequate doses. This circumstantial evidence supporting the idea that prostaglandins form a pathway shared by most mammals is reinforced by the observation that release of prostaglandins into the uterine vein and amniotic fluid during parturition has been observed in a wide variety of species. However, only in ruminants has an increase in circulating levels of prostaglandins been observed prior to (rather than during) parturition (69, 70, 71).

The source of uterine prostaglandins includes myometrium, uterine epithelium, amnion, chorion, placenta and cervix but there is a paucity of systematic studies from which to determine either the quantitative relationships of the various tissues or the spectrum of prostaglandins formed in each. In sheep, the major site of synthesis is the placenta (72) within which the component derived from the uterine epithelium releases mainly $PGF_{2\alpha}$ into the maternal circulation and the component derived from the conceptus releases mainly PGE_2 into the fetal circulation (73); the myometrium is less important as a source of prostaglandins. In Man, concentrations of PGE_2 in uterine tissues during pregnancy exceed those of $PGF_{2\alpha}$ (74) and in monkeys, the rate of production of PGE in superfused tissues is

greater than of PGF (75). In both species, the uterine epithelium appears to be a major site of synthesis of prostaglandins. Near term, synthesis changes towards a predominance of PGF (75). However, the need for cautious interpretation is suggested by discrepancies between in vitro and in vivo studies. For example, PGE is undetectable in amniotic fluid throughout most of the latter part of pregnancy (76) despite abundant production by superfused amnion (75).

The production by uterine tissues in vitro of both thromboxane A_2 (assayed as the metabolite, TxB_2) (77) and prostacyclin (assayed as the metabolite, 6-keto-$PGF_{1\alpha}$) (78) is low in monkeys and stems mainly from the myometrium whereas in the rat prostacyclin (PGI_2) is the predominant prostaglandin released by the pregnant uterus (79). Studies in sheep (80) do not encourage the belief that either TxB_2 or PGI_2 plays a significant part in parturition. In particular there is no support for the suggestion (81) that prostaglandin synthesis is diverted to formation of PGI_2 throughout pregnancy until term when it is switched to $PGF_{2\alpha}$.

The role of the fetal membranes in uterine prostaglandin synthesis is considered to be complementary to the uterine epithelium with which they are in intimate contact. The human amnion and chorion are rich in arachidonic acid stored mainly as phospholipids (82) and contain phospholipase A_2, an enzyme that catalyses the hydrolysis of fatty acid in the sn-2 position of glycerophospholipids. The prevailing view is that the human fetal membranes may supplement the supply of free arachidonic acid to the adjacent uterine epithelium as well as synthesising and metabolising prostaglandins (83, 24).

The rate of synthesis of prostaglandins by uterine tissues represents a balance between stimulatory and inhibitory factors. Since control of prostaglandin synthesis is considered to be mainly in the rate of deacylation-reacylation of arachidonate-containing lipids, it is likely that such factors influence the activity of phospholipase A_2 rather than the activity of cyclooxygenase. Deacylation of uterine phospholipids is usually ascribed to the phospholipase A_2 of lysosomal origin (84) but this remains unproven.

Oestrogen stimulates uterine synthesis of $PGF_{2\alpha}$ in pregnant sheep (85) and various ovariectomised rodents (86). The response to oestrogen is enhanced by prior treatment with progesterone (86) but inhibited by simultaneous treatment (85). The rate of synthesis of $PGF_{2\alpha}$ is very high in proliferative human endometrium compared to early proliferative and secretory endometrium (87) but the effects of oestrogen on pregnant human endometrium have not been reported. The above evidence has led to the proposal that oestrogen plays a large part in the release of prostaglandins at the start of parturition (81). While this hypothesis has much to commend it in species in which circulating oestrogen levels rise sharply at parturition (e.g. sheep) or in which oestrogen treatment causes premature parturition (e.g. goat), it is difficult to reconcile with the absence of change in oestrogen levels at term in species such as primates.

Oxytocin-induced prostaglandin production by uterine tissues has been demonstrated in several species including rats (88), rabbits (89) and sheep (90). The major site of increased production appears to be uterine epithelium which remains responsive to oxytocin even when isolated from the myometrium (91, 92). Ovine uterine epithelium contains high affinity oxytocin receptors which increase in concentration when exposed to oestrogen (91). Release of prostaglandins only partially mediates the effect of oxytocin in uterine contractability since in both pregnant rats (92) and sheep (93) the contractile response to oxytocin is not blocked by indomethacin. While oxytocin-induced production of $PGF_{2\alpha}$ may contribute to an increase in the power of contractions in the expulsive phase of labour when oxytocin levels are elevated, it is less likely to be of importance to the initiation of parturition when circulating levels of oxytocin are low (see below).

Kinins including bradykinin (94) and kallikrein-kinin (95) stimulate prostaglandin production and uterine contractions. The kallikrein inhibitor, aprotinin, prolongs parturition in the rat but has no effect on luteolysis (95).

Strong circumstantial evidence exists for the presence during pregnancy of endogenous inhibitors of uterine prostaglandin synthesis other than progesterone. The pulsatile release of $PGF_{2\alpha}$ preceding luteolysis in the non-pregnant cycle is suppressed when conception occurs (96, 97) although the circulating concentrations of oestradiol-17β and progesterone do not differ. Homogenates of 14-16 day but not 21-23 day ovine conceptuses inhibit luteolysis when injected into the uterus (98). The active material appears to be a thermolabile, soluble protein that acts locally, probably by inhibiting release of $PGF_{2\alpha}$.