Control of parturition in ruminants

C. E. Wood

Department of Physiology, University of Florida College of Medicine, Gainesville, FL 32610–0274, USA

Parturition is a process which, when set into motion, occurs to completion. This review concerns the control of parturition in ruminants. Parturition is an endocrine event, dependent upon the activation of the fetal hypothalamus–pituitary–adrenal (HPA) axis. In sheep and other ruminants, increases in plasma concentrations of cortisol induce the activity of 17-hydroxylase and 17,20 lyase in the placenta, increasing the biosynthesis of oestrogen relative to progesterone. The increase in the so-called E:P ratio increases myometrial activity and culminates in labour and delivery. Much work has been done to identify the mechanism of the endogenous activation of the fetal HPA axis. Recent work suggests that production of prostanoids within the fetal brain influences fetal ACTH secretion, and that induction of prostanoid biosynthesis at the end of gestation might be important in the process of parturition. Oestrogen and androgens, secreted by the placenta at the end of gestation, augment activity of the fetal HPA axis by increasing fetal ACTH secretion and by decreasing negative feedback sensitivity to cortisol. Although significant progress has been made concerning the neuroendocrinology of parturition, many significant questions remain. Is parturition regulated or simply programmed? Is parturition the ultimate result of neuronal maturation within the fetal hypothalamus, or is there a complex interplay between the placenta and fetal hypothalamus? Answers to these and other important questions await further research, but may provide key information which will prove useful in understanding general principles of parturition in many mammalian species.

Introduction

Parturition is an all or none event. It must be timed to match the degree of fetal maturation with the ability of the fetus to survive outside the uterus. Once initiated, the process of parturition is difficult to interrupt or delay. The importance of understanding parturition is understood easily in terms of the consequences of prematurity. The fetus, *in utero*, is on a kind of life-support and that life-support is maintained until the fetus can survive and thrive outside the uterine environment. Premature birth is complicated usually by immature pulmonary function and sometimes by incomplete transition of the cardiovascular system from fetal to neonatal anatomy.

Parturition is an endocrine event: this was first demonstrated by G. Liggins, studying the process of parturition in sheep. He found that electrocoagulation of the fetal pituitary indefinitely delayed parturition in sheep (Liggins *et al.*, 1967) and went on to demonstrate that the endocrine axis that is critical for this process in the sheep is the hypothalamus–pituitary–adrenal axis, because infusion of glucocorticoid (Liggins, 1969) or adrenocorticotropin (ACTH) (Liggins, 1968) initiated premature parturition. This observation, which demonstrated a fundamental endocrine process, would have proven to be of immediate importance in human medicine if not for the observation that the endocrinology of parturition in sheep is not identical to that in humans. These differences, detailed in this review, have confounded the basic premise that the ruminant is a good model of parturition for humans, and have focused many research efforts away from the basic neuroendocrinology of the hypothalamus–pituitary–adrenal axis and towards local effectors in the myometrium and other intrauterine tissues.

The debate concerning the control of parturition can be characterized as focusing on two alternative views. While it is well known that the fetus initiates parturition, it is not clear whether the critical event that initiates the process is a fetal neuroendocrine event or whether the fetal neuroendocrine system is responding to, or otherwise augmented by, external stimuli. Perhaps the development of critical synapses within the fetal paraventricular nuclei of the hypothalamus allows an increase in fetal neuroendocrine function which, in turn, alters fetal oestrogen and progesterone biosynthesis and initiates labour. The second possibility is that, while the presence of the fetus appears to be critical, the augmentation of fetal neuroendocrine activity which ultimately initiates parturition might be the result of other stimuli. These stimuli might be placental hormones or increasing fetal stress (changes in fetal blood gases, glucose, or blood pressure). This review will focus on the endocrinology of parturition, particularly the fetal hypothalamus–pituitary–adrenal (HPA) axis which plays a central role. However, the review will also explore the endocrine and other factors that affect the function of the fetal HPA axis, including the secretion of placental hormones and the responsiveness of the fetus to stress.
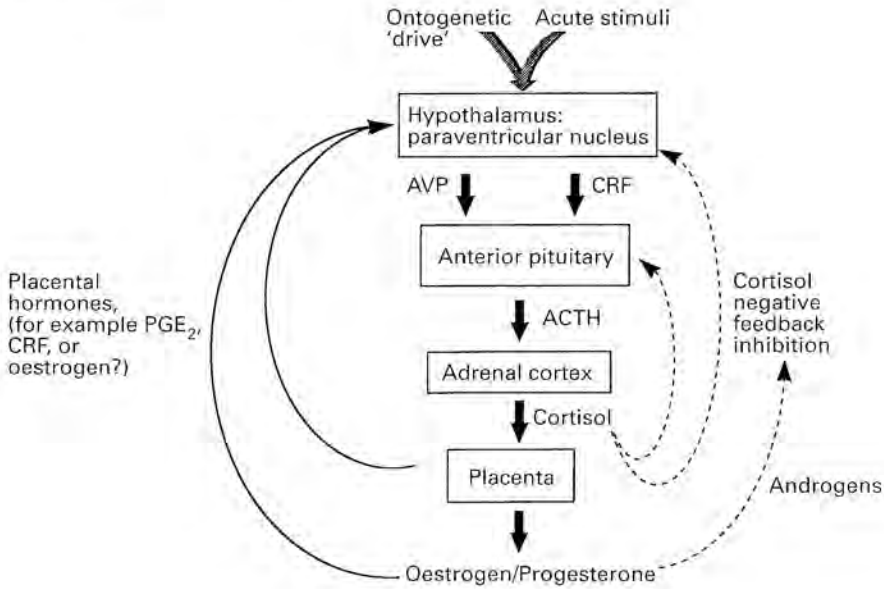
The Fetal Hypothalamus–Pituitary–Adrenal (HPA) Axis

The fetal HPA axis is functionally similar in most respects to the adult HPA axis (Fig. 1). However, there are some important differences: (1) the fetal brain is still developing late in gestation; and (2) the fetal HPA axis communicates with the maternal HPA axis via the placenta. However, the fetal axis functions in a manner similar to that of the adult axis in that the fetal HPA axis responds to various stimuli (which have been termed 'stresses'). However, the activity of the fetal HPA axis can be altered via 'non-stressful' inputs (for example ontogenetic changes in the activity of the HPA axis).

Several 'stresses' have been investigated in terms of their effects on fetal ACTH secretion. These stimuli include haemorrhage (Wood *et al.*, 1989a), arterial hypotension (Wood *et al.*, 1982; Tong *et al.*, in press), hypoxia, hypercapnia, or asphyxia, and acidaemia (Cudd and Wood, 1996). The ACTH responses to these stimuli are probably considered as the efferent limbs of reflex loops which are subserved by identifiable receptors. For example, ACTH response to hypotension is, in part, mediated by baroreceptors in the fetal carotid sinus or aortic arch (Wood, 1989b) (Fig. 2). The reflexes mediating fetal ACTH responses to the various stimuli, or 'stresses', are only partially identified, making one of the challenges of the future the provision of a more complete understanding of the neuroendocrinology of fetal stress. It has been proposed that repeated fetal hypoxia might alter the timing of parturition secondary to increases in fetal ACTH and cortisol concentrations in plasma. It may be assumed that the ACTH response to hypoxia is mediated by the arterial chemoreceptors, in a similar way to the control of fetal heart rate by these receptors. However, denervation of the carotid sinus chemoreceptors has no measurable effect on fetal ACTH secretion (Giussani *et al.*, 1996). It is possible that the ACTH response to hypoxia could be mediated by the generation of a paracrine or autocrine mediator in the brain of the fetus: it is known that the vasopressin response to hypoxia is blocked by the administration of an adenosine receptor blocker (Koos *et al.*, 1994), and also by pretreatment with indomethacin to block the biosynthesis of prostanoids (Tong *et al.*, 1998).

In adult animals of many species, plasma ACTH concentrations change spontaneously in a 24 h pattern, secondary to a so-called 'circadian' rhythm. This rhythm in rodents and in humans is 'entrained' or reset each day by the timing of exposure to light (the 'light-cycle'). Other factors, such as feeding, can also set a diurnal rhythm in animals maintained in constant light. These changes in ACTH secretion rate are not considered to reflect stress or response to any noxious stimulus in the environment. In fact, responses to 'stressors' have been defined as the increase in activity of the HPA axis above the activity that would otherwise be expected at that time of day. However, fetal ruminants are different, in that there is no endogenous circadian rhythm in ACTH secretion (Bell and Wood, 1991; Simonetta *et al.*, 1991). Although it is sometimes assumed that the axis exhibits circadian activity, it is clear that sheep lack circadian variation in plasma ACTH concentrations, even in adult life (Bell and Wood, 1991). Apparent 24 h rhythms in plasma concentrations of ACTH in the

(a) The hypothalamus–pituitary–adrenal (HPA) axis

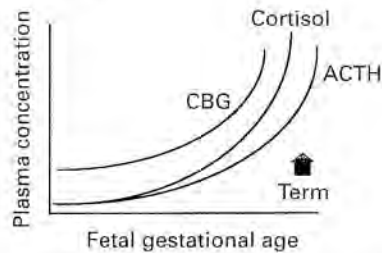

(b) The ontogeny of the fetal HPA axis

Fig. 1. (a) Schematic diagram of the ovine fetal hypothalamus–pituitary–adrenal (HPA) axis. Solid arrows (left) represent stimulatory interactions and dashed arrows (right) represent inhibitory interactions. Note that cortisol from the fetal adrenal inhibits fetal ACTH secretion by an inhibitory action both at the fetal hypothalamus and at the fetal pituitary, and that androgen from the placenta interrupts the cortisol negative feedback. (b) Schematic representation of the ontogeny of the fetal hypothalamus–pituitary–adrenal axis.

fetus have been secondary to feeding regimens other than *ad libitum* (Simonetta *et al.*, 1991) and, presumably, fluctuations in blood concentrations of metabolic substrates.

Although the fetal HPA axis does not exhibit true circadian rhythmicity, it does follow an ontogenetic pattern of activity. At the end of gestation, fetal ACTH and cortisol secretion rates increase in a semilogarithmic pattern (Wood., 1989a) (Fig. 1). This spontaneous increase in activity might be considered as analogous to the circadian changes in axis activity in other species, since it might reflect an endogenous 'programme' within the fetal hypothalamus and not a response to external or even internal 'stresses'. By analogy to the adult rat or human, therefore, it is possible to define fetal 'stress' as an increase in fetal HPA axis activity above the level that would otherwise be expected at that time in gestation (Wood, 1989a). An alternate view of the ontogenetic increase in
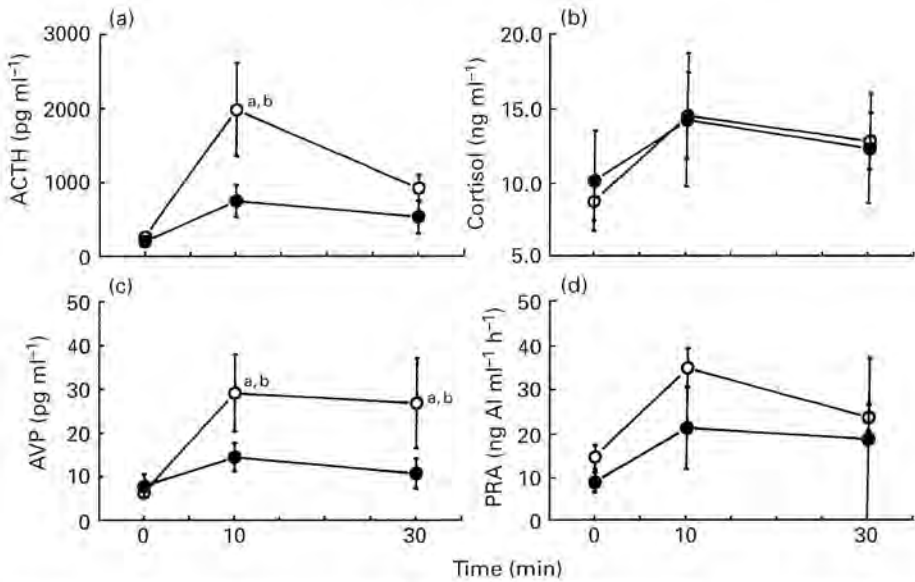
Fig. 2. Fetal plasma (a) ACTH, (b) cortisol, (c) arginine vasopressin (AVP), and (d) plasma renin activity (PRA) responses to hypotension (reduction of fetal blood pressure approximately 50% by vena caval obstruction from 0 to 10 min) in intact fetuses (open symbols) and in sinoaortic denervated fetuses (filled symbols). Sinoaortic denervation significantly reduced the ACTH, AVP, and PRA responses to hypotension, demonstrating the importance of arterial baroreceptors or chemoreceptors in mediating the hormonal responses to hypotension in the fetus. (Reprinted from Wood, 1989b, with permission.)

activity of the fetal HPA axis is that it is the result of increasing sensitivity to fetal stressors (McMillen *et al.*, 1995). According to this hypothesis, the fetus at term recognizes that factors such as the 'fetal' concentrations of glucose and oxygen, and blood pressure are low compared with the values that would be observed in adult animals. It is as though the fetus begins to respond to these variables as it would after birth, driving the activity of the HPA axis higher until parturition is initiated.

Biosynthesis of Progesterone, Oestrogen and the Initiation of Parturition

Parturition could not proceed without uterine contraction. Indeed, it is the contraction of the uterus which ultimately defines labour and results in delivery of the fetus to the extrauterine environment. The ability of the uterine smooth muscle to contract depends on the membrane potential of the smooth muscle cells and the ability of the cells to communicate. A unifying hypothesis that addresses the mechanism of the 'final common pathway' involved in parturition involves the spontaneous changes in the secretion of oestrogen and progesterone at the end of gestation (reviewed in Wood, 1989a). According to this hypothesis, the activity of the uterine myometrium is influenced by the placental production of the steroid hormones progesterone and oestrogen. Throughout much of pregnancy, the placenta synthesizes and secretes large amounts of progesterone. The resulting high concentrations of progesterone maintain uterine quiescence, mainly by hyperpolarizing the myometrial cells. At the end of gestation in many species, there is an increase in the rate of production of oestrogen relative to the rate of production of progesterone. The oestrogen produces a relative depolarization of the uterine myometrial cells, tending to augment their activity. The changes in plasma and tissue concentrations of oestrogen and progesterone also stimulate the formation of gap junctions (Ou *et al.*, 1997). Smooth muscle activation is associated with the synthesis and release prostaglandin $F_{2\alpha}$, which acts in an autocrine and paracrine manner to

augment the force of contraction. Indeed, knockout studies in mice have demonstrated that the knockout of the receptor for prostaglandin $F_{2\alpha}$ completely abolishes the process of labour and delivery (Sugimoto *et al.*, 1997).

When comparing most mammalian species, the changes in plasma oestrogen and progesterone are not very uniform (Holtan *et al.*, 1991), and this has led some to question the fundamental importance of this construct. Nevertheless, it is clear that in all species studied to date, the maintenance of pregnancy depends on the continued presence of progesterone, and it is therefore possible that there are significant differences among species in the biochemical and molecular event(s) within the myometrium which alter the responsiveness of the tissue to oestrogen and progesterone.

If the notion that spontaneous changes in oestrogen and progesterone are integral to the final common pathway of parturition is accepted, it is important to understand the endocrine mechanisms which alter placental steroidogenesis. In sheep and other ruminants, before the final stages of pregnancy, the placenta contains the enzymatic machinery for the biosynthesis of progesterone, but lacks the critical enzyme needed for 17α-hydroxylase activity (cytochrome $P450_{c17}$) (reviewed in Wood, 1989a). Therefore, in the preterm sheep fetus, the placenta secretes copious amounts of progesterone, but very little oestrogen. At the end of gestation, increases in plasma cortisol concentration induce cytochrome $P450_{c17}$ in the placenta, allowing an increase in the rate of synthesis of oestrogen at the expense of the rate of synthesis of progesterone. For this reason, the induction of parturition in sheep and in other ruminants depends on the activation of the fetal HPA axis which occurs ontogenetically at the end of gestation.

The increase in oestrogen biosynthesis at the end of gestation is a common theme among mammalian species. However, the strategy for producing this increase varies. In humans and other primates, cytochrome $P450_{c17}$ is not inducible (by cortisol or by any other circulating hormones) (Challis *et al.*, 1974). In the fetuses of these species, the adrenal cortices contain an identifiable pattern of zonation. The adrenal cortex contains a so-called 'fetal' zone and a so-called 'adult' or 'definitive' zone (reviewed in Wood, 1989a). The fetal zone contains the enzymes necessary to synthesize dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHAS), but cannot synthesize cortisol, corticosterone, aldosterone, testosterone, or oestradiol because it lacks 3β-hydroxysteroid dehydrogenase (3β-HSD). However, the adult zone of the fetal adrenal contains all of the enzymes necessary to produce cortisol, corticosterone and aldosterone, the major products of the adrenal gland of the postnatal animal. Both the fetal and the adult zones are under the trophic control of ACTH from the fetal pituitary. When fetal ACTH secretion is increased, the fetal zone increases its production of DHEA and DHAS, and the adult or definitive zone increases its production of cortisol and corticosterone. The mass of the fetal zone greatly exceeds the mass of the adult zone; the mass increases throughout the last half of gestation, and then decreases after birth. The DHEA and DHAS produced by the fetal zone during fetal life is important as a supply of substrate to the placenta for biosynthesis of oestrogens. The placenta, because it lacks cytochrome $P450_{c17}$, cannot synthesize oestrogen from cholesterol. However, the placenta overcomes this deficiency through the use of fetal (17α-hydroxylated) steroidogenic precursors as substrate for the production of oestrogen. For this reason, an increase in the rate of secretion of fetal ACTH increases the placental production of oestrogen, and decreases in the rate of ACTH secretion decrease the rate of secretion of oestrogen in these species.

The horse fetus provides an interesting comparison to those of sheep and primates. If the contention that the final common pathway for increasing uterine contractility is an increase in the oestrogen-to-progesterone ratio is accepted, it becomes apparent that the variants in schema for oestrogen biosynthesis all result in the same final endpoint. The horse is a species without an inducible placental cytochrome $P450_{c17}$ (Mason *et al.*, 1993). Therefore, oestrogen biosynthesis occurs using a fetoplacental unit, a strategy which is in general similar to that of primates. In the horse, the gonads synthesize and secrete large amounts of DHEA, and the initiation of parturition is interrupted by the process of fetal gonadectomy (Pashen and Allen, 1979). This finding suggests that an increase in the activity of the fetal gonad at the end of gestation initiates parturition in this species and that the gonads of the horse are under trophic control by the fetal pituitary.
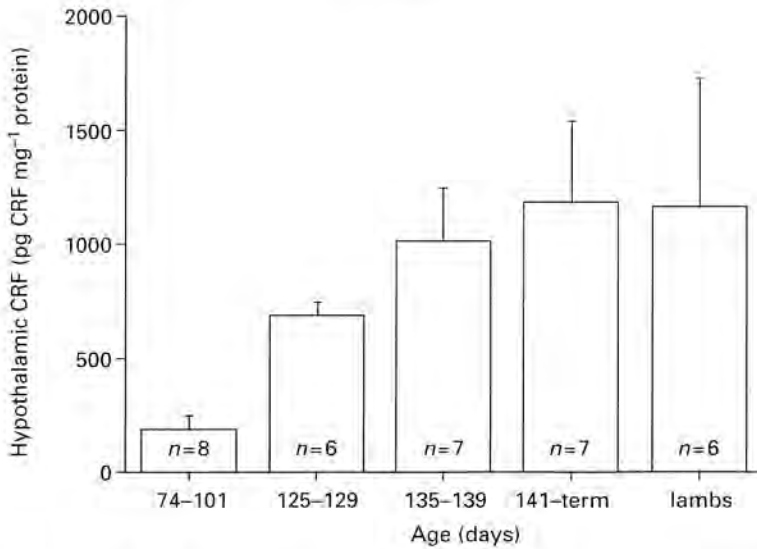
Fig. 3. Hypothalamic content of immunoreactive corticotrophin-releasing hormone (CRF). The tissue concentrations were significantly increased as a function of age, as tested by Dunn's test. The numbers of animals per group are shown within each bar. Values are means ± SEM. (Modified from Saoud and Wood (1996a) with permission from Elsevier Science).

The Ontogeny of the Fetal HPA Axis and the Initiation of Parturition

In sheep, parturition is initiated by a spontaneous increase in activity of the fetal HPA axis. From both a practical and theoretical viewpoint, it is important to understand the mechanism of the increase in fetal ACTH secretion. For example, is the increase in fetal ACTH secretion the result of neuronal maturation within the fetal hypothalamus, or is the increase in fetal ACTH secretion the result of stimulation by another hormone (for example, from the placenta)? Although the mechanism of the increase in fetal ACTH secretion is not understood completely, there are several important facts that have provided a key understanding of several control mechanisms that stimulate or allow increases in cortisol concentration of fetal plasma. These mechanisms will be addressed individually.

The initiation of parturition in sheep requires an intact HPA axis. As mentioned previously, hypophysectomy or bilateral adrenalectomy interrupts the process of parturition. However, bilateral ablation of the paraventricular nuclei or implantation of dexamethasone crystals in the paraventricular nuclei bilaterally also interrupts the process of parturition (Myers *et al.*, 1992). In the final stages of fetal development, the activity of the fetal HPA axis increases spontaneously. Changes at every level of the axis can be demonstrated during the preparturient increase in activity of the HPA axis.

In the hypothalamus, there is an increase in the content of CRH (Saoud and Wood, 1996b) as well as an abundance of mRNA for CRH (Myers *et al.*, 1993) (Fig. 3). Some studies have identified an increase in hypothalamic content of arginine vasopressin (AVP) mRNA (Matthews and Challis, 1995). However, the major increase in hypothalamic AVP content appears to occur after birth (Saoud and Wood, 1996b). In the anterior pituitary, there is an increase in the concentration of ACTH, as well as an augmentation of pituitary post-translational processing of pro-opiomelanocortin (POMC) (Saoud and Wood, 1996c). An increase in the abundance of mRNA for POMC has been demonstrated (McMillen *et al.*, 1990). Histologically, the corticotropes of the anterior pituitary undergo developmental changes. Before day 120 of gestation, the corticotropes in the ovine fetal anterior pituitary appear to be larger than the corticotropes in late gestation (Antolovich *et al.*, 1989). The

apparent development of corticotropes in late gestation has been referred to as a change from 'fetal'- to 'adult'-type corticotropes. It is not known whether the function of these morphologically different corticotropes are different from each other. However, it is possible that at least some of the maturation of the anterior pituitary content of POMC and its response to releasing hormones might be explained by the maturation of the corticotrope itself.

Throughout the last 2–3 weeks of development *in utero*, the fetal adrenal gland increases in size relative to fetal body weight, and the cellular sensitivity to ACTH increases (reviewed in Wood, 1989). The increase in sensitivity to ACTH is partially the result of increased adrenal cortical mass and partially the result of increased cellular responsiveness to ACTH. In the adrenal cortical cells, there is an increase in the density of ACTH receptors with a resultant increase in the adrenal cAMP response to ACTH (Durand *et al.*, 1981). This increase in the size and sensitivity of the fetal adrenal, combined with the increased circulating concentrations of ACTH, account for the increase in cortisol secretion rate which ultimately triggers parturition.

Increases in adrenal sensitivity to ACTH at the end of gestation are also, in part, the result of accelerated processing of ACTH from POMC in the anterior pituitary corticotropes. The increase in POMC processing is reflected in an increase in the plasma concentration of fully processed ACTH relative to larger molecular weight forms of immunoreactive ACTH (iACTH) (Thorburn *et al.*, 1991). Measurements of plasma concentrations of iACTH are made using radioimmunoassay, a technique which is dependent upon the binding characteristics of the antiserum used in the assay. An antiserum that binds to an epitope within $ACTH_{1-39}$ would be expected to crossreact with precursor forms of ACTH (such as POMC) or partially processed forms of the precursor (such as the 22 kDa fragment of POMC which is known as 'pro-ACTH') (Eipper and Mains, 1980). In all studies of ACTH that can be immunoassayed in unchromatographed plasma, the value obtained from the radioimmunoassay represents a mixture of unprocessed and partially processed POMC and fully processed ACTH. Thorburn and colleagues (1991) demonstrated that the concentration of fully processed ACTH in plasma increases in late gestation, and that the relative proportion of fully processed to larger molecular weight forms increases. In agreement with this observation was the report by Castro *et al.* (1993) that the ratio of biologically active to immunologically reactive ACTH was increased in fetal plasma in late gestation. The study of adrenal responsiveness to plasma ACTH or ACTH-like peptides using dispersed adrenal cells has provided an understanding of how changes in POMC processing might increase adrenal sensitivity at the end of gestation (Schwartz *et al.*, 1995). In adrenal cells from adult sheep, only fully processed ACTH is biologically active. POMC and pro-ACTH are not active either as agonists or antagonists in the adult cells. In fetal cells, however, these larger peptides act as competitive antagonists at the adrenal gland. In fact, pro-ACTH inhibits ACTH action in nearly equimolar concentrations (Schwartz *et al.*, 1995). Because of the action of ACTH as stimulator of adrenal secretion and the action of POMC and pro-ACTH as inhibitors of adrenal secretion, an increase in the ratio of ACTH:POMC or ACTH:pro-ACTH, as occurs spontaneously in late gestation, will augment adrenal sensitivity to ACTH.

The fetal adrenal requires ACTH for maintenance of its mass, and increases in ACTH concentrations stimulate adrenal growth. It is possible that the sensitivity of the fetal adrenal is dynamically regulated. In sheep fetuses, changes in plasma ACTH and cortisol concentrations sometimes appear to be dissociated from each other. A good example of this dissociation in relation to chronic changes is that the ontogenetic increase in fetal plasma cortisol at the end of gestation has been observed to increase before the increases in fetal plasma ACTH concentration (reviewed in Wood, 1989). A good example in relation to acute changes is that the increase in cortisol concentration of fetal plasma in response to hypotension (Wood *et al.*, 1982) and hypoxia (Giussani *et al.*, 1994) does not always mirror changes in fetal plasma ACTH concentration. Particularly interesting is the observation that carotid sinus denervation blunts the cortisol, but not the iACTH, response to acute hypoxia in sheep fetuses (Giussani *et al.*, 1994). This finding suggests that a component of the HPA axis response to acute stimulation in the sheep fetus involves a dynamic change in adrenal sensitivity to ACTH. In support of this notion is the observation that bilateral splanchnic nerve resection decreased the magnitude of the fetal plasma cortisol response to acute hypoxia (Myers *et al.*, 1990). The proposal that splanchnic nerves alter adrenal responsiveness or
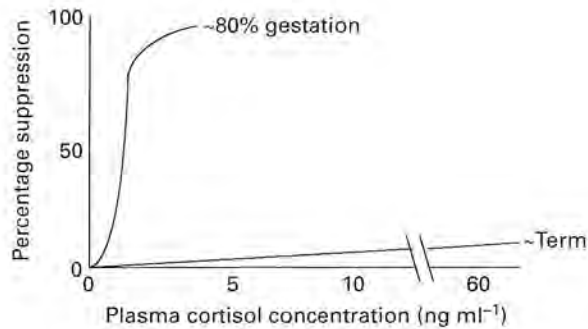
Fig. 4. Schematic representation of the reduction in sensitivity of cortisol negative inhibition of fetal ACTH secretion. In fetuses of between 117 and 131 days of gestation, cortisol effectively inhibits the ACTH response to acute hypotension. In term fetuses, supraphysiological increases in plasma cortisol concentration do not inhibit ACTH responses to hypotension. Data are redrawn and adapted from Wood (1986, 1988).

sensitivity to ACTH is not new and is not restricted to the sheep fetus. Clearly, a better understanding of dynamic changes in adrenal sensitivity in sheep fetuses is needed, because it might be an important part of the puzzle of the mechanism of parturition in this species.

Accompanying the increase in cortisol secretion rate is an increase in the circulating concentrations of corticosteroid-binding globulin (CBG: transcortin). This increase in plasma concentrations of CBG masks the biological activity of the circulating cortisol. However, the binding activity of CBG in sheep fetuses *in vivo* is relatively weak: approximately 25% of the circulating cortisol is unbound, and therefore biologically active (Wood, 1988; Wood, 1986). This is because the concentration of CBG in fetal sheep is low compared with comparable concentrations in the blood of adult rats or humans, and probably because of the large number of steroids circulating in fetal plasma which also bind to (and displace cortisol from) CBG. CBG has been shown to decrease cortisol action. For example, CBG decreases the negative feedback action of corticosterone in adult rats (Kawai and Yates, 1966). It has been proposed that increasing concentrations of CBG significantly interfere with cortisol action (especially in terms of cortisol negative feedback control of ACTH secretion) (Challis and Brooks, 1989). Although this is theoretically possible, the binding efficiency of ovine fetal CBG for cortisol *in vivo* is likely to be too low for dynamic changes in CBG concentration to play a significant role in adjusting cortisol action at target tissues (Wood, 1986).

What Drives Activity of the Preparturient Fetal HPA Axis?

Perhaps the most important question that is critical to our understanding of ovine parturition is the mechanism of the increased activity of the fetal HPA axis. Functionally, the increase in activity of the fetal HPA axis at the end of gestation appears to be different from the increase in activity of the fetal HPA axis in response to a specific stimulus. It is unlikely that the preparturient increase in activity represents the response to a chronic stress, since chronic stimuli often result in an attenuation of ACTH secretion after the initial response (Harvey *et al.*, 1993). For example, acute hypoxia stimulates vigorous ACTH responses in sheep fetuses(Giussani *et al.*, 1994), but long-term hypoxia produced by high altitude residence produces an adaptation of the HPA axis, so that long-term changes in fetal plasma cortisol and ACTH are not measurable (Harvey *et al.*, 1993). Preparturient increases in fetal plasma ACTH and cortisol concentrations, which become progressively pronounced in the last 2–3

weeks of ovine fetal life, are more likely to be caused by a 'programmed' maturation in the fetal hypothalamus or elsewhere in the fetal central nervous system, or by an external influence on the fetal hypothalamus or pituitary, such as the progressive increase in the secretion of a placental hormone.

One important link in the chain of events that initiates parturition in sheep is the interruption of cortisol negative feedback inhibition of fetal ACTH secretion (Fig. 4). This reduction in negative feedback sensitivity occurs in the last few days of fetal life, and functions to allow simultaneous increases in fetal ACTH and cortisol secretion (Wood, 1988). As important as this process might be, it cannot fully explain the process of parturition by itself. It is logical to assume that the preparturient increases in fetal ACTH and cortisol secretion require some stimulation of the axis, as well as interruption of negative feedback.

Recent evidence suggests that at least some of the increase in the activity of three fetal HPA axis at the end of gestation is the result of positive feedback cycle involving oestrogen secreted by the placenta. Physiological, chronic increases in oestradiol concentrations in fetal plasma greatly augment ACTH concentrations in fetal plasma, in unstimulated and stimulated conditions (Saoud and Wood, 1997). The increases in oestradiol concentrations in fetal plasma in that study were within the range of plasma concentrations that are measured endogenously at the end of gestation. We have also reported that chronic physiological increases in androstenedione concentrations in fetal plasma interrupt cortisol negative feedback inhibition of fetal ACTH secretion (Saoud and Wood, 1997). Chronic increases in both oestradiol and androstenedione significantly advance the day of spontaneous parturition (that is, promote premature parturition), indicating that the changes in placental secretion of these two hormones might be an integral part of the process of parturition in sheep. This apparent positive feedback loop might be a feature of the endocrinology of parturition in several species, because simultaneous treatment of pregnant baboons with androstenedione promotes premature parturition (Farber *et al.*, 1997).

Another hypothesis that has been advanced to explain the increase in activity of the fetal HPA axis is that prostaglandin E_2 (PGE_2), secreted by the placenta, circulates in fetal plasma and acts as a hormone to stimulate fetal ACTH secretion (Thorburn *et al.*, 1991). What makes this idea particularly attractive is the fact that circulating concentrations of PGE_2 increase in fetal plasma at the end of gestation, apparently mirroring the changes in ACTH concentration in fetal plasma (Thorburn *et al.*, 1991). The activity of prostaglandin endoperoxide synthase (PGHS) in the cotyledons increases at the end of gestation (Rice *et al.*, 1990), and intravenous infusions of PGE_2 into fetal sheep increase circulating concentrations of iACTH (Thorburn *et al.*, 1991). However, the observation that intracarotid arterial infusions of PGE_2, large enough to increase predicted carotid arterial plasma concentrations of PGE_2 well above the physiological range, are not sufficient to increase fetal ACTH secretion casts some doubt on this hypothesis (Cudd and Wood, 1992). Although it is unlikely that PGE_2 from a placental source stimulates preparturient increases in fetal ACTH secretion, it is still possible that PGE_2 has an important influence on the neuroendocrine mechanisms governing ACTH release in the fetus. The fetal central nervous system contains significant amounts of PGHS, and immunoreactive PGHS has been localized within hypothalamic regions known to be important for controlling activity of the HPA axis (Breder *et al.*, 1992). Recent evidence suggests that the abundance of immunoreactive enzyme increases within these areas before the normal time of parturition (Deauseault and Wood, 1998). It is therefore conceivable that endogenous production of PGE_2 within the brain significantly augments the activity of the HPA axis before birth. It is well known that inhibition of prostaglandin biosynthesis can delay parturition. This is thought to be the result of a reduction in prostaglandin biosynthesis within the myometrium, particularly the result of a reduction in $PGF_{2\alpha}$ production. However, it is possible that general treatment with prostaglandin synthesis inhibitors might also functionally impair the augmentation of the fetal HPA axis which is critical to the initiation of parturition.

It has been proposed that the release of ACTH, CRH or both hormones into the fetal blood is a physiologically important mechanism by which the placenta affects the timing of parturition in primates (Keller-Wood and Wood, 1991a). Ovine placenta contains measurable amounts of immunoreactive ACTH, but little CRH (Keller-Wood and Wood, 1991a,b, 1991b). There are no
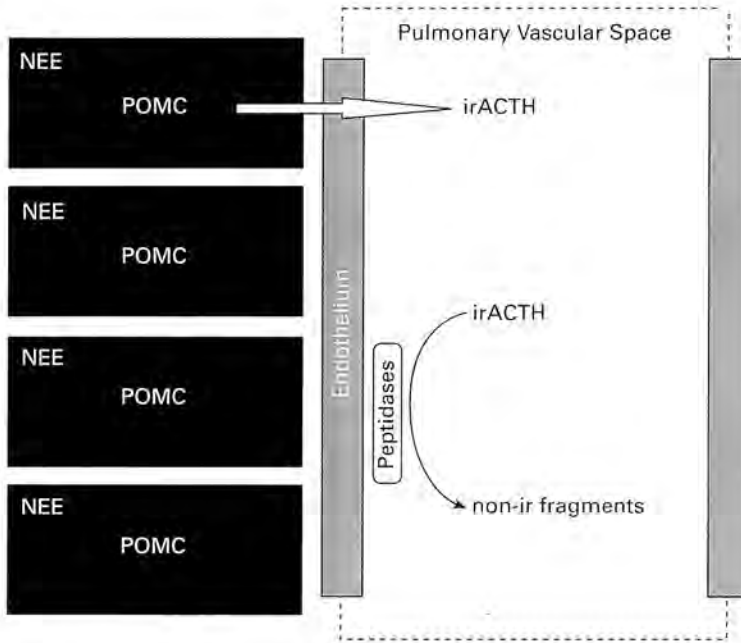
Fig. 5. Schematic representation of a conceptual model of the process of uptake and secretion of immunoreactive ACTH by the fetal lung. Proopiomelanocortin (POMC) is the precursor molecule for ACTH, and is synthesized in the fetal pulmonary neuroendocrine cells (NEE). This model assumes simultaneous secretion of immunoreactive ACTH from lung and clearance of approximately 48% of immunoreactive ACTH in pulmonary plasma.

arteriovenous differences across the ovine placenta (either in the fetal circulation or in the maternal circulation) for either peptide (Keller-Wood and Wood, 1991a, 1991b), suggesting that there is no net release of either peptide.

Although the placenta is not a source of either ACTH or CRH in the sheep fetus, the fetal lung contains significant amounts of immunoreactive ACTH (Cudd et al., 1993). The concentration of the peptide in pulmonary tissue, when expressed as ng iACTH per mg protein, is highest in fetuses at mid-gestation and decreases as the fetus matures (Cudd et al., 1993). The peptide is released from the lung into the fetal bloodstream under basal conditions and during acute surgical stress (Cudd et al., 1993; Cudd and Wood, 1995). Indeed, under basal conditions, the lung appears to both secrete iACTH into the bloodstream and metabolize iACTH which reaches the lung via the arterial blood (Cudd and Wood, 1995) (Fig. 5). We have recently demonstrated that the cells within the lung that contain iACTH are pulmonary neuroendocrine cells, both in the form of neuroendocrine epithelial cells (NEEs) and in the form of neuroendocrine bodies (NEBs) (Wood et al., 1998). The decrease in concentration of iACTH in pulmonary tissue coincides with the reported decrease in density of NEEs and NEBs within the lung throughout development (Scheuermann, 1991). The release of iACTH from these cells during acute surgical stress is consistent with the presumed function of these cells as chemosensitive tisssue (Adriaensen and Scheuermann, 1993). Immunoblot analysis of the pulmonary iACTH demonstrates the presence of a form with a molecular weight which is consistent with POMC, and processing products that are different from those identified in fetal pituitary (Saoud and Wood, 1996c). The products of post-translational processing within the lung appear to be consistent with the processing of POMC in adrenal chromaffin tissue (Wood et al., 1998).

Conclusions

Much progress has been made since the original observations by G. C. Liggins concerning the effects of interruption of the HPA axis on the process of parturition in sheep. Yet, it is also undeniable that the answers to the larger questions still elude us. We still do not know, for example, why the activity of the HPA axis increases at the end of gestation. Is this a function of neuronal maturation within the paraventricular nuclei, or is this a function of changing responsiveness to stimuli or changing circulating concentrations of placental hormones, or is this some combination of these factors? Is parturition regulated, and if so, how? Is the process simply programmed, or is there sensation of one or more variables which signal a readiness for birth? Providing the answers to these questions will require answers to more specific questions. For example, how important are dynamic changes in adrenal sensitivity to ACTH? If these changes are important, what causes them: changes in neural efferent activity to the adrenal, or changes in ACTH-like peptides from the pituitary and extrapituitary sites? How important is the positive feedback cycle between oestrogens, androgens, and ACTH? If this is important, we must determine whether conjugated oestrogens (the most abundant form of oestrogen in fetal plasma) are biologically active at the pituitary and hypothalamus.

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