Maturation of cytokine receptors in preparation for birth
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Abstract
The elucidation of the tissue-specific profile of expression of the prolactin (PRL) and growth hormone (GH) receptors during embryonic and fetal development in a range of species has provided a new impetus for the delineation of the specific roles of the hormone ligands for these receptors in development. During late gestation, there is a requirement to shift from a phase of predominant cellular proliferation, where placental nutrient supply is a dominant influence on organ and body growth, to one of functional differentiation, which is required for independent homoeostasis after birth. In this review we discuss the interactions between the pre-partum increases in cortisol and thyroid hormones and the synthesis, secretion and actions of fetal PRL and GH. We also review the changes that occur in the tissue-specific expression of the PRL and GH receptors before birth which may play an important role in precocial species in the successful transition of the fetus to extra-uterine life.

Introduction
During the past decade, an important new family of receptors has been identified, the class 1 cytokine receptors, which are single-pass transmembrane chains containing sequences of highly conserved amino acids in both the extracellular and the intracellular domains; members of this family share several structural and functional characteristics [1]. Members of this superfamily of receptors include receptors for granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, erythropoietin, thrombopoietin, several of the interleukins, prolactin (PRL), growth hormone (GH) and leptin [1,2]. These receptors mediate a myriad of endocrine, paracrine and autocrine events, and their stimulation has been implicated in a range of developmental processes. These include the successful implantation of the fertilized ovum in a receptive uterus, the differentiation and development of embryonic organs and tissues, and the successful transition of the fetus to extra-uterine life [2,3]. As the mechanisms underlying receptor activation and intracellular signalling have been elucidated, and as target genes have been characterized in specific tissues, new light has been thrown on the potential role of the ligands for these receptors in development.

While it has been well established that the fetal pituitary gland in many species secretes PRL and GH, the physiological roles and/or target sites of action for these hormones in fetal life have remained unclear. The elucidation of the tissue-specific profile of expression of PRL receptors (PRLRs) and GH receptors (GHRs) during embryonic and fetal development has provided a new impetus for the delineation of the specific roles of PRL and GH in development [3]. This review aims to integrate current information on the

Key words: fetus, growth hormone, leptin, prolactin, prolactin receptor.
Abbreviations used: GH, growth hormone; GHR, growth hormone receptor; IGF, insulin-like growth factor; PRL, prolactin; PRLR, prolactin receptor; T₃, tri-iodothyronine.
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physiological regulation of the synthesis and secretion of PRL and GH with findings on the expression and activity of PRLRs and GHRs in fetal tissues. The role of leptin and the activation of the leptin receptor in fetal life is also briefly discussed.

Regulation of PRL synthesis and secretion before birth

It is well established in several species, including the sheep, that PRL does not cross the placenta, and that the source of PRL in the fetal circulation is the fetal pituitary [4,5]. During late gestation in the sheep (term is 147 ± 3 days of gestation), PRL secretion is pulsatile, with a mean pulse frequency of around one pulse every 3–4 h [6]. In contrast with that in the adult, fetal PRL synthesis and secretion are maintained by a tonic stimulatory, rather than inhibitory, drive from the hypothalamus [7,8]. There is an increase in PRL synthesis and secretion in fetal sheep at around 135 days’ gestation, which is dependent on the presence of an intact and functional fetal hypothalamus [8]. It has been suggested that the increase in PRL synthesis and secretion in the late-gestation sheep fetus may be related to the concomitant increase in circulating oestrogens at this time [4,5]. Interestingly, it has been demonstrated in the sheep that fetal PRL is actively regulated in late gestation by the placental nutrient supply and by the prevailing external photoperiod [7–9].

Fetal PRL and placental nutrient supply

We have shown recently that fetal PRL synthesis and secretion are markedly decreased in sheep after experimental restriction of placental, and hence fetal, growth [9]. We found that PRL mRNA expression was suppressed in the anterior pituitary of placentally restricted fetuses, and that pituitary PRL mRNA levels were directly related to placental and fetal weight [9]. Furthermore, PRL mRNA levels in the fetal anterior pituitary were directly related to fetal arterial partial pressure of oxygen and to plasma glucose concentrations, and these effects appeared to be independent. Plasma PRL concentrations were also significantly reduced in the growth-restricted fetuses, although the normal gestational age-related increase in circulating PRL was maintained (Figure 1).

It has been demonstrated previously that restriction of placental growth in the sheep reduces the synthesis of insulin-like growth factor-I (IGF-I) in a range of fetal tissues and decreases circulating IGF-I concentrations [10]. Interestingly, IGF-I has been shown to stimulate PRL gene expression and secretion in pituitary cells in vitro, and pituitary PRL mRNA levels in vivo were significantly lowered in a transgenic mouse model with disrupted IGF-I expression [11,12]. Thus there may be an interaction between circulating IGF-I and PRL which contributes to the suppression of PRL synthesis and secretion in the placentally restricted fetus. While PRL concentrations are low in the chronically hypoxaemic, hypoglycaemic sheep fetus, there is evidence from in utero cordocentesis studies, and studies in which cord blood samples were collected at birth, that circulating PRL levels may be either normal or increased in the growth-restricted human fetus and newborn [13,14]. Clearly, further work is needed to elucidate the nature of the interactions between fetal glucose, oxygen and IGF-I and the PRL synthetic and secretory capacity of the fetal pituitary.

The direct relationship between PRL mRNA expression and growth in the placentally restricted sheep fetus suggests that, under suboptimal intrauterine conditions in this species, the synthesis and secretion of PRL may be regulated to limit its action at potential fetal target tissues. It has been
reported that fetal plasma concentrations of the placental hormone placental lactogen are also reduced in placentally restricted fetuses [15]. Placental lactogen shares structural identity with GH and PRL, and there is evidence that placental lactogen can form a 1:2 complex with the PRLR [16]. However, the lack of an arteriovenous difference in placental lactogen concentration across the placenta in either normal or placentally restricted fetal sheep [15] does not appear to support a direct role for this hormone in the processes underlying the regulation of fetal growth.

**Fetal PRL and photoperiod**

Although fetal sheep are not exposed directly to light in utero, fetal PRL concentrations change with the length of the prevailing photoperiod [7,17]. As in the adult sheep, fetal PRL concentrations are higher when ewes are maintained in long compared with short photoperiods (60–80 ng/ml and 5–10 ng/ml respectively) [7,17]. Evidence from our own and other laboratories suggests that the increase in maternal melatonin concentration during the dark phase is important in the transduction of information about the length of the photoperiod to the sheep fetus [7,17]. We have shown in the fetal sheep [7], and Lincoln and colleagues [18] have demonstrated in the adult ram, that plasma PRL concentrations were higher under long compared with short photoperiods in animals in which the hypothalamo–pituitary axis was disconnected surgically. We have speculated that melatonin may act at the pars tuberalis of the fetal pituitary to influence PRL synthesis and secretion from the pars distalis [7]. Finally, we demonstrated in fetal sheep in which the hypothalamo–pituitary axis had been disconnected surgically that the fetal PRL response to an intermediate (12 h) photoperiod was altered by prior exposure to a long or short photoperiod, and concluded that the sheep fetus can construct a photoperiodic ‘history’ in utero [19].

Interestingly, it also appears that the effect of photoperiodic history on PRL secretion in intact fetal sheep is either masked or suppressed by the stimulatory factors associated with an increase in gestational age, acting at the fetal hypothalamus [19]. It appears from this study that, although the sheep fetus has the neuroendocrine capacity to construct a photoperiodic history, the prevailing photoperiod and gestational age of the fetus exert relatively greater influences on fetal PRL secretion than previous photoperiod [19]. The effects of increased plasma concentrations of PRL in the sheep fetus during a long photoperiod (i.e. through a summer gestation) are unknown, although there are reports of seasonal differences in fetal growth profiles in sheep that are independent of differences in maternal nutrition [20]. Because PRL synthesis and secretion are responsive to changes in the external photoperiod, it has been speculated that the PRL axis may play a role in the transduction of signals about the external environment to the developing fetus in preparation for extra-uterine life. In this context, there has been significant interest in the ontogenic regulation of the expression of the PRLR in the tissues of the developing fetus before birth.

**The PRLR**

Multiple isoforms of the PRLR have been identified, which arise from alternative splicing of the primary PRLR transcript and which differ in the length and composition of the cytoplasmic tail [21]. It is clear from a range of studies that mRNA transcripts encoding long and short forms of PRLR are distributed widely in tissues derived from all three germ layers in the fetal rat and mouse [22–25], and that immunoreactive PRLR is present in tissues derived from mesoderm and endoderm in the early human fetus [26]. We have utilized the advantages of the sheep as a large animal model for fetal development to investigate the ontogenic profile and factors that regulate the profile of expression of the PRLR in a range of fetal tissues. There are two isoforms of PRLR in the sheep – the cDNA sequence of the shorter form (PRLR2) is identical with that of the longer form (PRLR1) until nucleotide 420, at this point there is an insertion of 39 bases which encode 11 amino acids followed by two stop codons [27]. Translation of the PRLR2 mRNA therefore generates the short form of the PRLR [27]. While the long form of the PRLR can transmit a full intracellular signal, the function of the short form is not clear. There are tissue-, age- and gender-specific patterns of expression of the long and short forms of the PRLR, and it has been postulated that an excess of the short form acts via a ‘dominant negative’ effect to limit homodimerization of the long form, and hence intracellular signalling [2].

We have demonstrated expression of the mRNAs encoding the long and short forms of PRLR in a range of tissues in the late-gestation sheep fetus, including the kidney, adrenal cortex, perirenal adipose tissue and liver [28–30]. We have shown that the levels of mRNAs encoding the long
and short forms of the hepatic PRLR are similar in the late-gestation sheep fetus, whereas the short form of the PRLR predominates in the liver of the adult rat [31]. These differences could be species-dependent, and may arise from divergence in the structural organization of the ovine and rat PRLR genes [20]. Alternatively, there may be differences in the requirements for PRL activity in the fetal liver during development compared with the adult liver during pregnancy. We have also reported that there was an increase in the expression of both forms of PRLR in the fetal sheep liver after 130 days' gestation, coincident with the timing of the pre-partum cortisol surge [28].

In a series of studies, we found that expression of PRLR1 and PRLR2 mRNAs in the fetal sheep liver was stimulated co-ordinately by cortisol, reduced after disconnection of the hypothalamus from the pituitary (HPD) and restored after subsequent cortisol replacement in the HPD fetus (Figure 2) [28,30]. Interestingly, the expression of PRLR1 appeared marginally more sensitive to the stimulatory actions of cortisol than the expression of PRLR2 [28,30]. In contrast, there was no effect of varying the length of the external photoperiod [30] or of restriction of the placental nutrient supply on expression of either the PRLR1 or PRLR2 mRNA in the fetal liver during late gestation [9]. It is not yet known whether cortisol acts directly or indirectly to regulate hepatic PRLR mRNA levels in the fetal sheep. Although promoter regions of the rat PRLR gene have been identified, none of these contain a glucocorticoid response element [32], and there is no current information that defines the regulatory region of the sheep PRLR gene. It is also possible that glucocorticoids may regulate PRLR transcription indirectly by modulating the transcriptional activity of gene(s) encoding proteins which, in turn, regulate PRL gene transcription. Furthermore, although infusion of cortisol does not stimulate PRL synthesis and secretion [8], cortisol can stimulate other hormones, including an increase in the plasma tri-iodothyronine (T3) concentration, which may then stimulate hepatic PRLR expression.

It has also been demonstrated that expression of the GHR and IGF-1 mRNAs increases between 130–140 days of gestation in the fetal sheep liver, and that cortisol infusion before 130 days also stimulates GHR and IGF-1 gene expression [33,34]. In the following section we review the evidence that regulation of PRLR, GHR and IGF-1 gene expression either directly or indirectly by cortisol plays an important role in the changes that occur in hepatic metabolism and somatotropic regulation before and after birth.

**GH and IGF-I**

After birth, GH and IGF-I are two key factors in the endocrine control of growth in postnatal life. Postnatally, GH binds to specific cell surface receptors (GHRs) in the liver and other target tissues, and stimulates the production of IGF-I which, in turn, stimulates cell proliferation in a variety of tissues. Interestingly, while plasma GH concentrations are high in fetal life, GH appears to have a limited role at most in the control of prenatal growth. There is a transition from the GH-independent synthesis of IGF in fetal tissues to the GH-dependent synthesis of hepatic IGF-I in the postnatal period [37]. In the sheep, there is an increase in the abundance of the hepatic GHR mRNA and in the expression of the adult liver-specific GHR mRNA transcript, which contains exon 1A [33,34]. Evidence from a series of *in vitro* cortisol infusion and fetal adrenalectomy experiments suggests that the pre-partum rise in fetal
cortisol stimulates the rise in hepatic total GHR mRNA abundance, activates exon IA expression and increases hepatic IGF-I expression before delivery [34]. Cortisol also suppresses IGF-II gene expression and enhances glucogenic enzyme activities in the fetal liver in the pre-partum period. Interestingly, it has been shown recently that $T_3$ modifies the developmental shift in hepatic IGF synthesis and may mediate, in part, the maturational actions of cortisol on the hepatic somatotrophic axis [35]. Activation of the switch in hepatic IGF gene expression may therefore be impaired or delayed in infants delivered prematurely, i.e. before the normal pre-partum increases in fetal cortisol and $T_3$.

One potential explanation for the lack of GH responsiveness in the prenatal period may be the antagonistic actions of the placental hormone placental lactogen at the ovine GHR. A recent study demonstrated that ruminant placental lactogens antagonize the actions of ovine GH in homologous systems because they cannot homodimerize ovine GHs [36]. The hepatic GHR and plasma IGF-I responses to exogenous GH are lower, however, in lambs up to 2 months of age compared with those measured in 1-year-old animals [37]. There are reports in the literature that describe a stimulatory effect of PRL on development and growth-related genes in the liver, including ornithine decarboxylase, c-myc and IGF-I [2]. Administration of PRL to the hypophysectomized rat had significant effects on body weight gain, serum IGF-I and hepatic IGF-I expression [38]. Interestingly, in these studies, the potency of PRL compared with GH was more marked in acute experiments compared with the effects seen after chronic administration. It is clear that further work is required to determine the precise role of PRL during the period in fetal and early postnatal life when the animal is relatively non-responsive to the actions of GH.

Other cytokine receptors and fetal development

There are also ligands for other cytokine receptors, such as the polypeptide hormone leptin, that have not been identified previously as playing a major role before birth. Plasma leptin concentrations in term are positively correlated with placental and infant weight, and levels decrease rapidly after birth [39–41]. It has been demonstrated that leptin mRNA is expressed in perirenal adipose tissue in the sheep fetus, and that there is a direct relationship between the expression of leptin and fetal body weight in late gestation [42]. Furthermore, leptin receptors are expressed in the fetal hypothalamus during late gestation [43]. It is therefore possible that activation of the leptin receptor plays a role in the regulation of energy balance before birth.

Conclusion

In summary, during late gestation there is a requirement to shift from a phase of predominant cellular proliferation, where placental nutrient supply is a dominant influence on organ and body growth, to the differentiation of function required for the maintenance of independent homoeostasis after birth. It appears likely that the prepartum changes in the synthesis, secretion and actions of fetal PRL, GH and leptin at their specific receptors may play a key role in this transition period. Further elucidation of the intracellular consequences of activation of the cytokine receptors in key fetal organ systems in preococial species will be essential in order to understand the mechanisms that prepare the fetus for life after birth.

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References

Leptin and its role in pregnancy and fetal development – an overview

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Abstract

Leptin is a hormone that is secreted by adipose cells in proportion to adipose mass, and therefore a low leptin level signifies depletion of energy stores. It has been proposed that leptin is one of the signals controlling sexual maturation. For example, humans and rodents lacking leptin fail to undergo complete puberty, while overexpression of leptin in mice causes early puberty. The placenta also produces leptin in human pregnancy, increasing the amount in the maternal circulation. The effects of the increased leptin levels during pregnancy are not clear. In contrast, the mouse placenta does not produce endocrinologically significant amounts of leptin. The mouse placenta does secrete a leptin-binding protein, the production of which correlates with a large increase in maternal leptin levels. The physiology of leptin during pregnancy and fetal development differs significantly between species, and is not well understood in any.

Introduction to leptin biology

Leptin is a hormone that is produced predominantly by adipose cells [1,2]. Circulating leptin levels are proportional to adipose tissue mass. Thus leptin levels can be thought of as a signal to the body of its energy reserves. Although often discussed in terms of elevated leptin levels in