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The growth hormone/prolactin gene family in ruminant placentae

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Ruminant placentae produce at least two distinct subclasses of the growth hormone/prolactin gene family, the placental lactogens and prolactin-related proteins. Placental lactogens have been purified from cattle, goat and sheep placentae, and the amino acid sequences of bovine and ovine placental lactogen are known. Bovine and ovine placental lactogens are structurally more similar to prolactin than they are to growth hormone. In addition, six unique mRNAs have been described in cattle that encode prolactin-related proteins that are structurally distinct from ruminant placental lactogens. All characterized ruminant placental lactogens and prolactin-related proteins are products of chorionic binucleate cells, but specific biological functions of these placental hormones have not been elucidated. Ovine placental lactogen may modify maternal and fetal intermediary metabolism to provide energy substrates to the fetus. Bovine placental lactogen has been implicated as a luteotropic agent, and is also capable of stimulating mammogenesis and lactogenesis. No ruminant placental lactogen receptor has been structurally characterized, although they are presumed to be similar to either the growth hormone or prolactin receptor. Available technologies will allow many of the questions regarding the regulation, mechanism of action and function of these placental hormones to be addressed.

Introduction

In eutherian mammals, the placenta is responsible for directly mediating or modulating the maternal environment required for maintenance of normal fetal growth and development. The placenta is the site of nutrient and waste transfer between the mother and fetus, serves as a barrier against pathogens and the maternal immune system, and functions as an active endocrine organ. Among the hormones synthesized and secreted by the placenta are members of the growth hormone (GH)/prolactin gene family, including the placental lactogens (PLs), prolactin-related proteins (PRPs) and placental growth hormone (GH-V; Roberts and Anthony, 1994). Most species that have been examined secrete one or more of the placental members of this family; horses, pigs and rabbits are exceptions. Primates secrete both PL and GH-V, whereas non-primate species produce one or more PLs and, at least in rodents and ruminants, one or more PRPs.

Although ruminant PLs have been known to exist for approximately 20 years, and the PLs expressed by bovine and ovine placentae were amongst the earliest purified to homogeneity, we still cannot draw firm conclusions about their regulation, their exact mechanism of action nor their specific biological functions. Use of recombinant DNA methods led to the discovery of mRNA encoding PRPs within the

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	oPL	bPL	bPRP-I	bPRP-II	bPRP-III	bPRP-IV	bPRP-V	bPRP-VI	bPRL	bGH
oPL		65.7	32.3	32.3	31.8	32.3	31.8	33.8	47.5	24.2
bPL	83.8		30.0	29.5	31.5	29.5	30.0	32.5	50.2	23.7
bPRP-I	63.6	62.4		75.2	59.9	75.2	51.0	53.7	44.7	21.0
bPRP-II	59.2	61.0	86.2		56.9	99.5	44.1	51.2	43.7	24.2
bPRP-III	59.7	62.8	80.0	78.2		56.9	56.9	67.2	43.2	20.2
bPRP-IV	61.0	61.2	89.5	95.8	78.4		44.1	51.2	43.7	24.2
bPRP-V	62.4	63.2	76.8	71.5	77.6	74.9		56.2	42.2	18.4
bPRP-VI	62.2	62.2	78.8	75.9	83.8	78.4	79.6		45.2	17.9
bPRL	67.8	69.9	66.5	68.1	68.4	68.1	68.7	68.1		27.9
bGH	53.6	52.9	56.7	58.1	60.0	55.0	53.11	52.3	52.8	

Fig. 1. Nucleotide and amino acid sequence identity among the members of the ruminant growth hormone/prolactin gene family. The sequence comparisons were generated using NALIGN (nucleotide comparisons) and PALIGN (amino acid comparisons) programs with PC/GENE™ (IntelliGenetics, Inc., Mountain View, CA). Only the protein coding regions were used for the nucleotide sequence comparisons, and the mature (minus the leader peptide) amino acid sequences were used in the amino acid comparisons. (□) percentage nucleotide sequence identity; (□) percentage amino acid sequence identity. PL: placental lactogen; PRP: prolactin-related proteins; PRL: prolactin; GH: growth hormone.

bovine placenta (Schuler *et al.*, 1991). However, their existence in other ruminant placentae has yet to be reported, and we know even less about the regulation and function of the ruminant PRPs than we do about the PLs. This review will focus on our current knowledge of structure, regulation, mechanism of action and proposed functions of the ruminant PLs and PRPs.

Structure and Regulation of Ruminant Placental Lactogens and Prolactin-related Proteins

Primary structure

Placental lactogens have been purified from the placentae of cattle (Murthy *et al.*, 1982), goats (Currie *et al.*, 1990) and sheep (Warren *et al.*, 1990b), and the primary structures of bovine PL (bPL: Schuler *et al.*, 1988) and ovine PL (oPL: Warren *et al.*, 1990b) have been determined by a combination of amino acid and cDNA sequencing. Both bPL and oPL (Fig. 1) are structurally more similar to prolactin than they are to GH in both nucleotide and amino acid sequence. Although bPL and oPL are more similar to each other than they are to other members of this gene family, the interspecific divergence in the primary sequence of the ruminant PLs is greater than the interspecific divergence of either prolactin or GH. Analysis of ruminant PL sequence similarities (Wallis, 1993) indicated that the rate of nonsynonymous substitution was greater than the rate of synonymous substitution, leading to the suggestion that the more rapid rate of evolution between bPL and oPL resulted from adaptive rather than from neutral mutations.

Ovine PL is a nonglycosylated polypeptide with an apparent M_r of 22 000 (Warren *et al.*, 1990a), which appears to undergo little post-translational processing other than removal of a 38 amino acid leader peptide (Warren *et al.*, 1990b). Caprine PL (Currie *et al.*, 1990) has similar properties ($M_r \approx 22\,500$; pI ≈ 8.35) to oPL (Warren *et al.*, 1990a), and although not proven, it is unlikely that caprine PL is glycosylated. In contrast, bPL is secreted as multiple isoforms (32–34 000 M_r; Murthy *et al.*, 1982) that contain both Asn-linked and *O*-linked oligosaccharide chains (Byatt *et al.*, 1990).

Removal of the Asn-linked oligosaccharides increases (1.2–2.3 fold) the binding of bPL to the GH receptor but not to the Nb2 cell prolactin receptor (Byatt *et al.*, 1990), whereas removal of *O*-linked oligosaccharides has no effect on receptor binding. None of the ruminant PLs has been crystallized, so it remains unclear whether the differences in their primary sequence and occurrence of glycosylation result in overall structural divergence sufficiently significant to alter function.

In cattle, the placenta can produce at least six prolactin-related proteins (Schuler *et al.*, 1991) that are structurally distinct from PL (Fig. 1). All bovine prolactin-related proteins (bPRPs) were identified by screening placental cDNA libraries, and are predicted to be glycoproteins containing Asn-linked oligosaccharide chains. The six bPRPs, as a group, are structurally more similar to each other than they are to PL, prolactin or GH, but there is considerable diversity among the bPRPs in their primary amino acid sequences (Fig. 1). Translation of bPRP-I has been demonstrated (Zieler *et al.*, 1990), and it exists as two M_r forms ($\approx$34 000 and 35 000) each containing Asn-linked oligosaccharide chains. Identification of prolactin-related proteins or their mRNA in other ruminant placentae has not been reported. However, we (E. P. Kayl and R. V. Anthony, unpublished) have preliminary evidence from reverse transcriptase–polymerase chain reaction of ovine placental mRNA that there are at least three analogous prolactin-related proteins in sheep.

Gene structure and expression

Structural genes encoding bPL, bPRP-1 and oPL have been isolated and characterized (Ebbitt *et al.*, 1989; Kessler and Schuler, 1991; Liang and Anthony, 1993). All three genes comprise five exons and four introns, and the exons of each gene span 10–12 kilobases (kb) of DNA. At least in cattle, all placental members of this gene family are linked on chromosome 23 with the bovine PRL gene (Dietz *et al.*, 1992). Consensus sequences for activator protein-1 and -2 response elements reside within the immediate 5′ flanking regions of the bPL and oPL genes, which may be involved in basal promotor activity. As yet, neither these response elements nor other putative response elements identified by sequence analysis have been tested for functionality. The lack of ruminant-derived chorionic cell lines has hindered a thorough characterization of the transcriptional control of these genes. Gene copy number was not determined for the bPL or bPRP-1 genes, but we have evidence that oPL is encoded by a single copy gene (R. Liang and R. V. Anthony, unpublished). However, evidence for differential splicing of the bPL transcript and allelic variants of the bPL gene has been reported by Yamakawa *et al.* (1990) and Kessler and Schuler (1991).

Bovine PL, bPRP-1 and oPL are products of chorionic binucleate cells (Milosavljevic *et al.*, 1989; Kappes *et al.*, 1992; Fig. 2), which deliver their products to the maternal circulation by migrating to and fusing with the feto–maternal syncytium (Wooding, 1992). Expression of bPL and bPRP-I mRNA has been detected as early as day 17 of gestation (Kessler *et al.*, 1991), and their expression appears to increase at least until day 140 of gestation. Expression of oPL mRNA increases from day 60 to day 120 of gestation (Kappes *et al.*, 1992) when it appears to reach a plateau (Fig. 3). However, if the concentration of oPL mRNA is expressed on a per cell basis, expression does not change from day 60 to day 135 of gestation (Kappes *et al.*, 1992). The percentage of chorionic epithelial cells that are binucleate remains constant during this time (Wooding, 1982), but as gestation progresses there is an increase in the total amount of chorionic epithelium within the placentome. Taken together, these results suggest that the increase with gestational age in concentrations of oPL mRNA was a result of increased total chorionic epithelial cells, including a proportional increase in total chorionic binucleate cells (Kappes *et al.*, 1992). This raises the question of whether or not the primary regulator of oPL gene transcription is the progression of differentiation of chorionic primary cells into binucleate cells (i.e. constitutive expression). Until a system is developed to examine binucleate cell differentiation, this hypothesis will be difficult to test thoroughly.

Secretion

The interspecific divergence of ruminant PLs is not only observed with their primary amino acid sequences, but is also apparent in the amount of PL that reaches the maternal circulation. Ovine PL

hypoglycaemia in either the mother or fetus (Brinsmead *et al.*, 1981), which leaves the identity of the stimulus in question.

Mechanism of Action of Ruminant Placental Lactogens

Cattle

An issue that remains to be resolved is the mechanism of action by which ruminant PLs and PRPs exert their effects. More specifically, the question relates to whether there are unique PL or PRP receptors. Specific binding sites for bPL have been identified in endometrium (Kessler *et al.*, 1991) and luteal tissue (Lucy *et al.*, 1994) which have little affinity for bGH or bPRL. However, Staten *et al.* (1993) demonstrated that bPL binds the bovine growth hormone (bGH) receptor in a 1:1 stoichiometry rather than the 1:2 stoichiometry observed with bGH. In addition, a monoclonal antibody that competes with bGH for its binding site (Staten *et al.*, 1993) does not compete with bPL binding to the bGH receptor or the recombinant bGH binding protein (bGHBP; extracellular domain of bGH receptor). Scott *et al.* (1992) demonstrated that bovine endometrium, corpus luteum and fetal liver all contain mRNA encoding the bGH receptor, and Lucy *et al.* (1993) provided evidence that the corpus luteum contains protein that is immunoreactive with antisera raised against the bGHBP. These data raise the possibility that bPL is acting through the bGH receptor by binding to a different site on the receptor. However, if this is true, the question must be raised as to why specific bGH binding cannot be demonstrated with endometrial or luteal microsomal membranes (Kessler *et al.*, 1991; Lucy *et al.*, 1994). Lucy *et al.* (1993) observed two types of bGH receptor mRNA (4.4 and 4.7 kb) from luteal tissue and a single type (4.4 kb) from liver. It is possible that the 4.7 kb transcript encodes a variant form of the bGH receptor that retains its binding affinity for bPL, but has significantly reduced affinity for bGH owing to conformational changes in the receptor.

Scott *et al.* (1992) reported that the bovine prolactin (bPRL) receptor is also expressed by the corpus luteum, endometrium and fetal liver. When the bPRL receptor was expressed in COS cells (Scott *et al.*, 1992), bPL and bPRL exhibited similar affinity for this receptor (0.20 versus 0.31 nmol l^{-1}; respectively), raising the possibility that in some tissues, bPL may be exerting its action through the bPRL receptor. Recent evidence from our laboratory (Anthony *et al.*, 1994) demonstrates that there are two forms of ovine prolactin (oPRL) receptor mRNA in adult ovary and fetal liver. One oPRL receptor mRNA (oPRLR-1) is predicted to encode a receptor analogous to the reported bPRL receptor (Scott *et al.*, 1992; Fig. 4), and one encodes a truncated form of the oPRL receptor (oPRLR-2). Termination of translation of oPRLR-2 (Anthony *et al.*, 1994) is predicted to occur between Homology box 1 and 2 (DaSilva *et al.*, 1994), at a site analogous to that of the short-form prolactin receptors expressed in mice (Davis and Linzer, 1989) and rats (Boutin *et al.*, 1988). Truncation of the rat PRL receptor between Homology box 1 and 2 disrupts the phosphorylation of and the association between JAK2 tyrosine kinase and the receptor (DaSilva *et al.*, 1994), although the affinity of prolactin is equivalent to that exhibited by the full-length receptor. If an analogous 'short' prolactin receptor is expressed in ruminants, it may bind PL or bPRP, possibly acting through a signal transducer other than JAK2.

Sheep

Binding of oPL has been examined most extensively with maternal and fetal liver microsomes (Freemark *et al.*, 1986; Freemark and Comer, 1989). Ovine PL expresses specific high-affinity binding ($K_d \approx 0.12$–0.5 nmol l^{-1}; Freemark and Comer, 1989; Pratt *et al.*, 1991) to fetal liver microsomes, whereas ovine GH (oGH) or oPRL exhibits little to no specific binding to these membranes (Freemark *et al.*, 1986). Saturation analyses of oPL binding sites in fetal liver (Pratt *et al.*, 1991) indicate that the concentration of the binding site does not change with increasing gestational age (Table 1), but the concentration does increase when the data are expressed per milligram of DNA, suggesting increased numbers of receptors per cell. Furthermore, saturation analyses using oPL, oGH or oPRL as the radiolabelled ligand (Pratt *et al.*, 1991) and fetal liver microsomes demonstrated saturable binding with

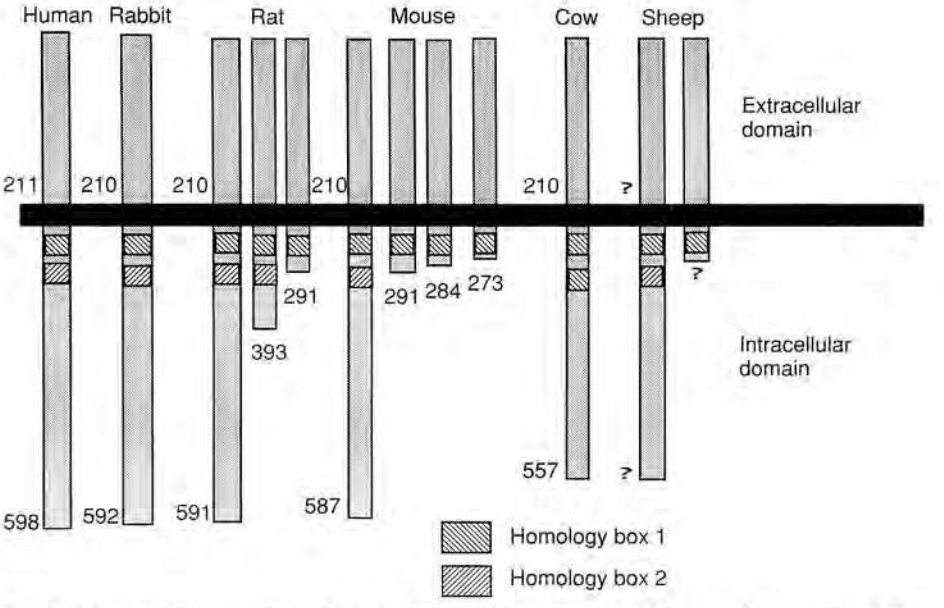

Fig. 4. Schematic representation of the various prolactin receptors that have been reported. The solid black bar represents the cellular membrane. Homology boxes 1 and 2 are as described by DaSilva *et al.* (1994). The numbers presented above the solid black bar represent the number of amino acids in the extracellular domain of the receptors, and the numbers below the bar represent the total number of amino acids in the mature receptor protein. Note that the mouse, sheep and rat all have mRNA which encode a receptor that is truncated between Homology boxes 1 and 2.

Table 1. Concentrations of ovine placental lactogen binding sites in fetal liver from mid- to late gestation

Gestational age (days)	K_d (pmol l^{-1})	fmol mg^{-1} protein	fmol mg^{-1} DNA
60	120.0	14.0	14.4
90	123.0	8.9	14.5
105	148.0	9.6	21.0
120	123.0	9.1	35.2
135	96.5	7.4	37.5

Data derived from Pratt *et al.* (1991).

oPL, but no specific binding of oGH or oPRL. Freemark and Comer (1989) reported the partial purification of the maternal and fetal liver oPL binding site. The apparent M_r of this receptor was $\approx$44 000, with a 30–50 times greater affinity for oPL than for oGH (Freemark and Comer, 1989), but no amino acid sequence information was reported.

Recent results from cross-linking and immunoprecipitation studies (Breier *et al.*, 1994b) indicate that oPL and oGH can bind an identical or very similar receptor in fetal liver microsomes obtained at 125–135 days of gestation. The amount of specific binding of oGH ($1.2 \pm 0.4\%$) is much less than that of oPL ($7.6 \pm 2.4\%$), but the combination of these results led Breier *et al.* (1994b) to suggest that oGH and oPL bind to a common or a related receptor protein. This suggestion is corroborated by demonstrations that there is oGH receptor mRNA in fetal liver (Klempt *et al.*, 1993; Pratt and Anthony, 1993). However, when oGH receptor cDNA is expressed in CHO cells, GH exhibits greater affinity (K_d

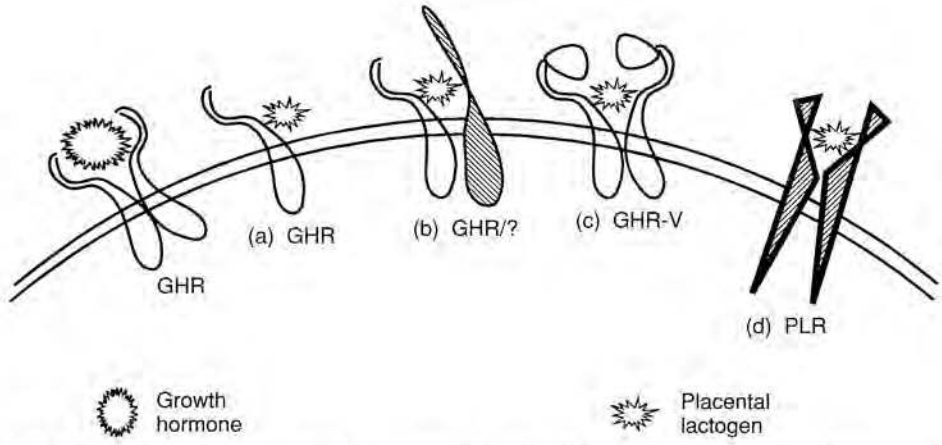

Fig. 5. Schematic representation of the possible identity of the ruminant placental lactogen (PL) receptor. (a) Ruminant PLs may act by binding to one monomer of the growth hormone receptor (GHR). (b) Ruminant PLs may act by binding to one monomer of the GHR and a yet undescribed monomer. (c) Ruminant PLs may act by binding to two monomers of a variant form of the GHR (GHR-V), such as a GHR with an amino-terminal extension. (d) Ruminant PLs may act through a receptor (PLR) that is structurally distinct from the GHR.

value 0.30 nmol l^{-1}) for the oGH receptor than does oPL (K_d value 0.76 nmol l^{-1}), yet both ligands bind approximately the same number of oGH receptor molecules per cell (Fiddes *et al.*, 1992). If the common receptor for oGH and oPL present in fetal liver (Breier *et al.*, 1994b) is the oGH receptor, why does oGH not bind with equal or greater affinity than oPL. Furthermore, the sequence of the oGH receptor mRNA present during mid-gestation (day 60 to day 120 of gestation) in fetal liver differs within the 5′-untranslated region from the oGH receptor mRNA present at 135 days of gestation and in adult liver (Pratt and Anthony, 1993). The major oGH receptor mRNA transcript present during mid-gestation is about 300 base pairs larger than oGH receptor mRNA expressed in maternal liver (Pratt and Anthony, 1993). These results suggest that fetal liver oGH receptor mRNA is not derived from the recently described (O'Mahoney *et al.*, 1994) exon 1A of the oGH receptor gene. This implies a developmental switch in oGH receptor gene transcription, from usage of a variant-exon 1 to the adult liver-specific exon 1A, during late gestation. The increase in oGH binding (22.0 ± 1.9%; Breier *et al.*, 1994a) that occurs in postnatal liver (18 days of age) does not occur in post-mature fetal liver (166–175 days of gestation; 1.7 ± 0.29%), further suggesting a periparturient switch in transcription and possibly the structure of the oGH receptor.

From the data available, firm conclusions cannot be drawn about the structure of the receptor for ruminant PLs. However, there are several possibilities about the identity of the PL receptor (Fig. 5). One possibility is that PL binds a single monomer of the GH receptor; another possibility is that it binds a single monomer of the GH receptor and a yet undescribed receptor monomer. It is possible that PL acts by binding a modified GH receptor, such as one that has an extended amino terminus, that through conformational changes loses its affinity for GH but retains its affinity for PL (Fig. 5). Finally, there may be a structurally distinct receptor for ruminant PLs. The structural characterization of the PL receptor may finally be accomplished using expression-cloning methods.

Function of Ruminant Placental Lactogens

Although the existence of ruminant PLs has been known for some time, their specific biological function(s) is not known. This is due to two factors, the first and more important is that classical ablation–replacement experiments are not feasible with placental hormones. This situation may be rectified in the future if ruminant embryonic stem cell lines become available to allow gene ablation

experiments to be conducted. The second factor is the lack of highly purified biologically active ruminant PLs for functional studies. However, recombinantly derived bPL and oPL are now being used to determine their biological functions. Consequently, a more thorough understanding of the biological functions of these placental hormones should be forthcoming.

Mammotrophic actions

As their name implies, ruminant PLs may act on the mammary gland. Induction of mammogenesis in nonpregnant ewes is inhibited if prolactin secretion is prevented by administration of bromocryptine (Schams *et al.*, 1984), but in pregnant sheep, mammary development occurs in the absence of prolactin secretion, suggesting the presence of a conceptus-derived mammotrophic agent (Schams *et al.*, 1984). However, oPL is not as efficient as prolactin in stimulating β-casein mRNA transcription in ewe mammary gland explants (Serverly *et al.*, 1983), and does not compete for prolactin binding sites in mammary tissue (Emane *et al.*, 1986). The latter data were interpreted to suggest that oPL does not play an important role in mammogenesis and lactation. It is possible that the conceptus-derived mammotrophic agent (Schams *et al.*, 1984) is not PL but a prolactin-related protein, although there is no evidence to support this hypothesis.

In cattle, as in sheep, inhibition of prolactin secretion during pregnancy does not impede mammary development (Schams *et al.*, 1984). However, in contrast to sheep, bPL can bind the bPRL receptor with high affinity (Scott *et al.*, 1992). Furthermore, bPL stimulates DNA synthesis in bovine mammary gland explants maintained in athymic nude mice (Vega *et al.*, 1989). Recent evidence (Byatt *et al.*, 1994) demonstrated the ability of bPLs to stimulate mammary growth and DNA synthesis. Bovine prolactin also stimulated mammary growth but did not significantly increase DNA synthesis. Both hormones induced milk synthesis, although prolactin was more potent than bPL, and bPL stimulated greater mammary secretion of the insulin-like growth factor binding proteins (IGFBP-2 and -3) than did prolactin (Byatt *et al.*, 1994). These data suggest that bPL may stimulate mammogenesis and lactation.

Luteotrophic actions

Several lines of evidence support the hypothesis that rodent PLs or prolactin-related proteins play a luteotrophic role during pregnancy. However, evidence for a similar role for ruminant PLs is lacking. Ovarian infusion with oPL does not increase progesterone secretion (Schramm *et al.*, 1984) and short-term immunoneutralization of oPL does not alter progesterone concentrations in pregnant ewes (Waters *et al.*, 1985). Furthermore, oPL does not appear capable of inhibiting the luteolytic actions of $PGF_{2\alpha}$ (Schramm *et al.*, 1984). These data shed doubt on any proposed action of oPL as a luteotrophin. However, recent evidence in cattle (Lucy *et al.*, 1994) indicates that bPL injections will increase luteal size and plasma concentrations of progesterone, and reduce the size of the largest follicle. Some, but not all, of these effects were obtained with bGH injections (Lucy *et al.*, 1994). It is not yet clear whether the bGH receptors present in bovine luteal tissue (Lucy *et al.*, 1993) are functional receptors capable of mediating these actions, or whether the larger bGH receptor transcript is encoding a receptor responsible for these actions. Until more is known about the structure and function of bPL-binding sites in corpora lutea, firm conclusions cannot be drawn about the role of bPL as a luteotrophic hormone.

Maternal and fetal metabolism

Efforts to define the effects of PLs on maternal and fetal metabolism have focused primarily on the effects of human PL and oPL, but much of the available information was obtained using interspecific assays. Figure 6 shows the possible role of oPL in repartitioning maternal nutrients to the fetus and how oPL stimulates the fetus to use these substrates. However, as will be discussed, there is insufficient data to support or refute many of the hypotheses about the role of PL during pregnancy.

Infusion of partially purified oPL for 8 h to fasting pregnant and nonpregnant ewes decreased plasma concentrations of non-esterified fatty acids, glucose and amino nitrogen, but an increase in plasma insulin

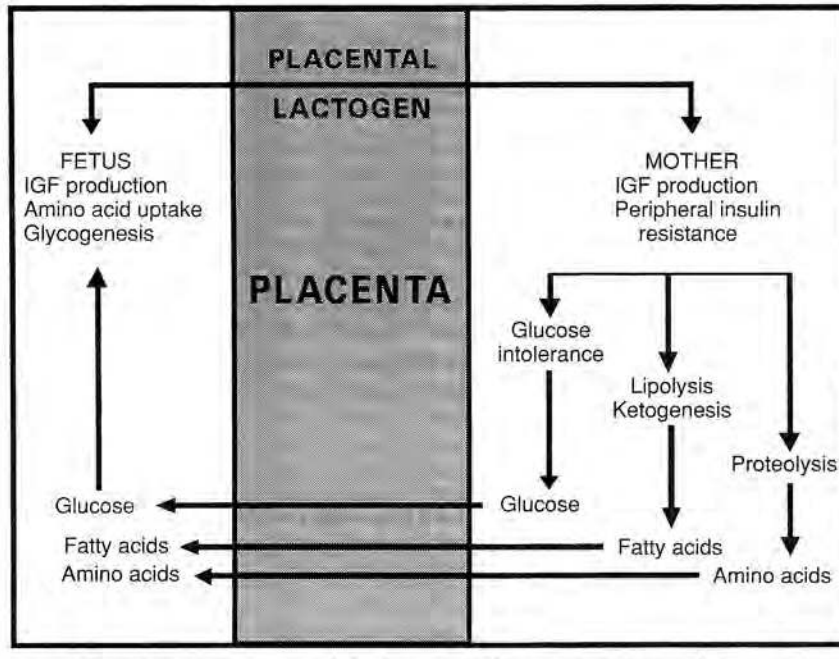

Fig. 6. Schematic representation of the hypothesis that ruminant placental lactogens (PLs) may act to repartition nutrients from the maternal system to the fetus. Redrawn from Handwerger (1991).

was observed (Handwerger *et al.*, 1976). In contrast, infusion of a placental extract, enriched for oPL, for 36 h into nonpregnant ewes resulted in increased plasma non-esterified fatty acids, glucose and urea nitrogen, with no effect on plasma insulin (Thordarson *et al.*, 1987). In addition, short-term (12 h) immunoneutralization of maternal oPL did not significantly affect whole body glucose metabolism, but there was a trend for lower plasma non-esterified fatty acids and a significant increase in plasma insulin (Waters *et al.*, 1985). It is difficult to reach any firm conclusions from the results of these three reports as the experimental approach differed, especially in regard to dose and purity of oPL, pregnancy status and gestational age. Further research is required to determine the effects of oPL on maternal metabolism. The nutritional status of the ewes must be normalized across experiments, as fasting will increase oPL concentrations in both maternal and fetal circulation (Brinsmead *et al.*, 1981), and reduce the concentration of oPL binding sites in maternal and fetal liver (Freemark *et al.*, 1992). The latter effect of fasting can be reversed by glucose infusion (Freemark *et al.*, 1992), suggesting not only nutritional regulation of oPL secretion but also of its function.

In cattle, administration of bPL to pregnant or nonpregnant, non-lactating cows did not increase concentrations of non-esterified fatty acids, glucose, tri-iodothyronine and insulin in plasma, whereas administration of bGH increased all of these parameters (Byatt *et al.*, 1992). However, bGH and bPL increased plasma concentrations of IGF-I and IGF-II, and decreased the concentrations of blood urea nitrogen and IGFBP-2. Lucy *et al.* (1994) also noted an increase in circulating IGF-I when nonpregnant heifers were injected with bPL. These data from cattle are in contrast to at least some of the data available for sheep (see above), and the reasons for these differences are not known. However, Byatt *et al.* (1992) concluded that bPL was not necessarily acting as a GH agonist, but rather bPL elicited distinct effects on intermediary metabolism that may be mediated through another specific receptor.

If the role of PL is to repartition nutrients for fetal use (Fig. 6), catabolic actions within the maternal system should be offset by anabolic actions within the fetus. Ovine PL stimulates ornithine decarboxylase activity, amino acid uptake, IGF-II secretion and glycogen synthesis by fetal rat tissues (reviewed by Handwerger, 1991). Analogous treatment with GH is without effect. However,

Table 2. Concentrations of ovine placental lactogen (oPL), insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein 2 (IGFBP-2) as a function of oPL infusion into fetal sheep vasculature

	Pre-infusion (ng ml^{-1})	Saline infusion (ng ml^{-1})	oPL infusion (ng ml^{-1})
oPL	48.5 ± 6.8	na	186.3 ± 28.1
IGF-I	34.8 ± 3.2	31.9 ± 4.1	na
IGF-I	30.3 ± 5.7	na	43.1 ± 1.7
IGFBP-2	1271 ± 86	1323 ± 56	na
IGFBP-2	1401 ± 109	na	1266 ± 63

Data derived from Schoknecht *et al.* (1992b).
na: not available.

interpretation of these results must be tempered by the realization that oPL is not acting through oPL-specific receptors in these tissues. At least one of these actions occurs in fetal sheep tissues. Treatment of ovine fetal hepatocytes with oPL stimulated dose-dependent increases of glucose incorporation into glycogen and total cellular glycogen content (Freemark and Handwerger, 1986). Both oGH and oPRL stimulated glycogen synthesis by fetal hepatocytes, but their potencies were considerably less than that of oPL (12% and 4% of oPLs potency, respectively). In addition, infusion of oPL at a rate of 1.2 mg day^{-1} for 14 days into fetal vasculature (days 122–135 of pregnancy) increased fetal serum IGF-I concentrations, but did not affect serum IGFBP-2 (Table 2; Schoknecht *et al.*, 1992b). Although these last results are not conclusive, they support the hypothesis that oPL may directly or indirectly stimulate IGF production by the fetus, thereby providing anabolic stimulation to the fetus.

The hypothesis shown in Fig. 6 is neither conclusively supported or refuted by the available data. It is clear that ruminant PLs can bind the GH receptor, and it is plausible that the role of PL in the maternal circulation is to augment or modulate the actions of GH within the mother. This may be accomplished by mediating its action through the GH receptor rather than by a structurally distinct receptor. However, some of the actions of GH (for example diabetogenic actions) may not be advantageous to the fetus, and actions of PL through a structurally distinct receptor may provide a more generalized anabolic fetal environment. Future research on the biological function of ruminant PLs must take into account that during pregnancy there are two separate metabolic units (mother versus fetus).

Conclusions

Placental members of the GH/prolactin gene family are true placental hormones that are not synthesized or secreted by other endocrine glands. In ruminants, these include the PLs and prolactin-related proteins. Considerable progress has been made in defining the primary structure and gene organization of these placental hormones. However, we do not know what specifically regulates production of these hormones, their mechanism of action or their specific biological functions. This is particularly true for the family of ruminant prolactin-related proteins. Ruminant PLs may act through receptors other than GH or prolactin receptors, but this remains controversial and will be resolved only when specific PL binding sites are structurally characterized. Methods are now available to incorporate a functional binding assay into the search for PL receptors, and these methods should be used for both maternal and fetal tissues. Finally, there is a need to define the function(s) of this placental gene family. These research efforts must take into consideration the pregnancy status, gestational age and nutritional status of the experimental animals. With the availability of recombinantly derived hormones, and availability of gene ablation on the horizon, the function of this placental gene family may finally be resolved.

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