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PLACENTAL LACTOGENS, NEW DEVELOPMENTS

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SUMMARY

Recent radioreceptor assay data gathered on placental lactogens (PL) in several species has engendered many questions but relatively few answers concerning the roles played by these polypeptide hormones in the physiology of pregnancy and lactation. Certainly, there are many suggestions regarding possible functions assigned to this group of hormones, and some of these are considered in two excellent reviews [11, 77]. This paper focuses on the niche occupied by the lactogens and rat PL in particular, in the hormonal control of pregnancy, possible relationship to the metabolic adaptations of mother and fetus, and lactational development. Apparent biological and immunological similarities to pituitary growth hormone (GH) and prolactin (PRL) are reviewed, and the methodologies which underline the arguments for or against specific trophic influences of PL are analyzed. In addition, some interesting aspects of newly isolated PL (ovine placental lactogen, oPL and bovine placental lactogen, bPL) are discussed.

INTRODUCTION

As in most areas of endocrinology, classical extirpation experiments provided the primary impetus for the study of placental polypeptide hormones. Hypophysectomy or ovariectomy revealed a bimodal progestational control of pregnancy in the rat [1]; that is, hypophysectomy before day 11 of pregnancy or ovariectomy at any time during gestation resulted in abortion [1]. Following the finding that rat placental tissue maintained pseudopregnancy in the rat [2], Lyons[3] observed lobulo-alveolar mammary growth and early signs of lactation in hypophysectomized, ovariectomized pregnant rats treated with estrogen and progesterone. Furthermore, in 1950 Averill *et al.* [4] demonstrated that day 12 rat placental implants possessed luteotropic properties in the absence of the pituitary. Therefore, the crucial role played by the placenta in the latter half of gestation in the rat has been known for quite some time.

Although the foundation for placental lactogen (PL) research was laid with these early studies in the rat, a relatively quiescent period ensued until identification, purification and characterization of PL in primates (man [5–7], monkey [6–9] and baboon [10]) occurred. Most recently, similar studies using new techniques such as radioreceptor assays have led to the identification of PL in several domestic species (cow [11–15], goat [16] and sheep [17–20]). The identification of placental lactogens in earlier reports was based on cumbersome growth hormone (GH) or prolactin (PRL) bioassays (e.g. pigeon crop sac epithelium proliferation for PRL and tibial width or body weight gain in hypophysectomized rats for GH) and later on immunological similarities with human pituitary GH [5–9]. These assays served a useful purpose and the subsequent characterization of human PL (hPL) confirmed the homology between hPL and pituitary hGH [7, 21]. Even today, evidence for the occurrence of PL is based solely on qualitative methodologies [12, 15, 22–25]. However, with the advent of radioreceptor assays (RRA) using tissue receptors for PRL (mammary gland) or GH (liver) [26, 27] there was a rapid growth in the number of species in which placental lactogens were identified [18, 28]. On the other hand, the development of homologous radioimmunoassays (RIA) for PL has been limited to only a few species [13, 29–32]. Although reports have appeared of the purification and characterization of rat PL (rPL) [33, 34], a limiting factor in the development of a RIA for rPL is the limited availability of starting tissue. It seems to us that the rat is a promising model for the elucidation of the role placental lactogens play in pregnancy, parturition and lactation while the domestic ruminants may provide an equally useful tool in understanding the metabolic effects of placental lactogens.

RAT PLACENTAL LACTOGEN

The secretion of progesterone by the corpus luteum of the rat is controlled by both pituitary and placental hormones. However, at midpregnancy (approximately day 11) the major site of luteotropin production shifts from the pituitary to the placenta [1, 35]. Selye[35] first recognized the importance of the placenta for mammary growth during the second half of pregnancy in the rat, finding that neither hypophysectomy nor fetectomy affected mammary growth [36]. Following the observations of luteotropic activity in the day 12 rat placenta [2, 3] and implied mammotropic activity in the hypophysectomized, pregnant rat [31], numerous reports of luteotropic [37–42] and mammotropic [37–39, 42, 43] activity in both placenta and serum of the midpregnant rat appeared in the literature. In contrast, an absence of luteotropic or mammotropic activity in the rat placenta at stages of gestation beyond midterm was also noted [4, 37, 41, 44]. In Tables 1 and 2 we have summarized the amounts of rat sera or rat placentae or ovine PRL (oPRL)

Table 1. Luteotropic effects of rat serum and placenta from various stages of pregnancy and doses of ovine prolactin required to reach similar biological end points

(a) Rat placental lactogen

M.E.D.*, daily		RRA-oPRL Equivalents, reference: [33]					
Serum, ml	Placenta	Serum, ng	Placenta, μg	Days of treatment	Biological end point	Rat bioassay model	References
0.1 (12)†	1 p.e.‡ (12)	120	0.6	7	Deciduoma reaction	Hx̄††, immature female	[37, 38]
0.5 (12)	—	600	—	8	Deciduoma reaction, estrus inhibition	120 day old female	[39]
0.5 (12)	—	<600	—	8	Estrus inhibition		
0.5 (12)	1 p.e. (12)	600	0.6	3	Deciduoma reaction, progresterone secretion§	Hx̄, pseudopregnant	[41]
—	1–2 p.e. (12)	—	0.6–1.2	10	Estrus inhibition	Normale female	[44]
—	1 (12)	—	0.6	6	Histology of ovaries, uteri, vaginae	Hx̄, (day 6), pregnant	[14]
—	>5 (12)	—	>3.0	6	Pregnancy maintenance		
—	1 (12)	—	0.6	9 (days 12–20)	Pregnancy maintenance	Hx̄ (day 12), pregnant	[42]
—	1 p.e. (12) + 5 μg estradiol	—	0.6	4 (days 10–13)	Pregnancy maintenance	Hx̄ (day 10), pregnant	[41]
—	3 (15, fetal)	—	36	1	Pregnancy maintenance (prevention of ergocornine effect)	Day 7 pregnant treated with ergocornine	[63]
—	1 (15)	—	12	4 (days 12–15)	Progesterone secretion pattern	Hx̄ (day 12), pregnant	[69]
1.0 (12)	—	1200	—	4 (days 6–9)	Decidual tissue weight (day 12) (prevention of ergocornine effect)	Pseudopregnant, treated with ergocornine (day 7)	[54]

(b) Ovine prolactin

M.E.D., daily (mg)	Days of treatment	Biological end point	Rat bioassay model	References
0.25¶	4 (days 6–10)	Maintenance of progesterone levels	Hx̄ (day 6), pseudopregnant	[50]
0.25 + 1 μg estrone	4 (days 6–10)	Decidual tissue weight		
0.25	4	Maintenance of progesterone	Hx̄, immature female	[80]
0.05	7	Deciduoma reaction	Hx̄, immature female	[49]
2.0**	4	Histology of ovaries, uteri, vaginae	Hx̄, pregnant	[4, 44]
>2.0		Pregnancy maintenance		
0.5–1.0	5–10	Prolongation of pregnancy	normal pregnant female	[114]

* Minimally effective dose. † Day of pregnancy in parentheses. ‡ Placental equivalent. § Day 11 placenta had more pronounced effect on blood progesterone concentrations. ¶ Even at 4 times this dose, oPRL ineffective in maintaining progesterone levels in Hx̄, day 12 pregnant rats [56]. ** Equivalent to 1 day 12 placenta. †† Hypophysectomized.

which yielded approximately equivalent biological results. The serum and placental contents of oPRL-like activity, as determined by RRA [33, 45] are included for comparison.

Upon examination of the data contained in Table 1, one finds that 0.1–0.5 ml of serum or 1 placental equivalent (p.e.) from day 12 of pregnancy is, in general, the minimally effective dose for the maintenance of corpus luteum function [37, 39, 40]. The dose of oPRL giving qualitatively similar results ranges from 50–250 μg. Based on this comparison, one ml of day 12 pregnant rat serum contains an equivalent of 100–2500 μg of oPRL, and estimates of the oPRL-equivalent content of 1 day 12 placenta vary from a low of 50–250 μg to as much as 2000 μg of oPRL (according to Ray *et al.*[44]). However, the RRA results of Kelly *et al.*[33, 45], in which oPRL was used as the standard, indicate much lower concentrations of rPL. These investigators utilized the binding of labelled oPRL to a particulate rabbit mammary gland receptor and found that day 12 serum from pregnant rats contained a maximum of 1.6 μg of oPRL-like activity per ml and day 12 placenta contained <1 μg/placenta. According to the bioassay data in Table 1, the RRA underestimates the oPRL-like activity in day 12 placenta and serum by greater than 50-fold. One might argue that the biological end points listed in Table 1 result from the cumulative effects of repeated injections. However, available information suggests that rPL is cleared rapidly from the circulation [33]. It is of interest to note that in experiments in which injections of placental extracts or pregnant rat serum were used to overcome the effects of ergocornine [54, 63] (a selective inhibitor of PRL secretion) larger amounts of these materials were required. In these experiments the RRA-oPRL estimates more closely approximate the actual doses of oPRL necessary to duplicate the luteotropic potency of pregnancy rat serum or placenta.

In order to explain the apparently greater luteotropic potency of day 12 pregnant rat serum and placental extracts by bioassay than radioreceptor assay, several possibilities must be considered: 1. The RRA detects only a portion of the luteotropic hormone present in day 12 serum or placenta; 2. additional factor(s) present in day 12 serum or placenta render the rPL highly active; 3. the biologically active half-life of rPL exceeds that of oPRL, especially in terms of luteotropic effects; 4. the dynamics of hormone–receptor interaction in the rat ovary are such that rPL has a greater luteotropic potency than oPRL.

It must be understood that the RRA-PRL does not purport to measure luteotropic activity. In comparing the earlier work of Averill *et al.*[4] to Kelly *et al.*'s receptor assay results [33], we find that the placenta at the stages of pregnancy at which high PRL-like activity was measured by RRA (day 15) was relatively inactive by bioassay for luteotropic activity.

Furthermore, it is unlikely that the RRA-PRL underestimates the lactogenic activity present in placental extracts or serum. We believe that the RRA-PRL is a quantitative technique in which substances with lactogenic activity compete with [^{125}I]-labelled oPRL for binding sites on the particulate receptor. However, the receptor assay is subject to the nonspecific interfering effects of pH, ionic strength and serum [26, 46] which can inhibit the binding of iodinated hormone to the receptor, resulting in artifactually high PRL-equivalent values. Precise estimates of rPL in placental preparations (especially homogenates) may be somewhat inaccurate since crude extracts do not always exhibit parallelism with the RRA standard curve, yet Kelly *et al.*[33] reported minimal lactogenic activity in day 12 rat placental extracts. Moreover, total PRL-like activity (rPL) in pregnant rat serum measured by RRA includes rPL and rat pituitary PRL (rPRL) in the circulation, but since the latter is consistently below 50 ng/ml until late in gestation [26, 47, 48], as measured by specific RIA, rPRL does not significantly contribute to RRA values except near term.

Other luteotropic substances in the rat, such as PRL [49–52], luteinizing hormone (LH) [53–55], estrogen [56] and follicle-stimulating hormone (FSH) [50], coexist with rPL in day 12 serum [47, 57, 58]. However the respective concentrations of these hormones in the blood of the day 12 pregnant rat (PRL, LH, FSH: <30 ng/ml) [47, 57, 59]; estrogen: 0.3 ng/ml in ovarian venous plasma [58]) would be insufficient to elicit the luteotropic effects reported by the investigators listed in Table 1. Of course, one cannot rule out possible synergistic effects of the gonadotropins and estrogens on luteal function. Combinations of PRL and estrone (or LH or FSH) are effective in increasing progesterone levels and decidual weights in hypophysectomized, pseudopregnant rats [50]. Yet these hormones demonstrate negligible synergism in luteotropic action in hypophysectomized, hysterectomized rats [56].

It is of course possible that another factor apart from rPL is present in placental tissue which also exerts an effect on the ovary. This factor which has LH-like activity can be detected by radioreceptor assay and would be analogous to human chorionic gonadotropin (hCG) in "rescuing" the corpus luteum. The presence of "LH-like" activity in rat placental extracts has been reported by two groups [60, 61]. Thus when crude placental extracts are administered at least 2 luteotropic factors may be present: rat placental lactogen and rat chorionic gonadotropin.

The half-time disappearance rate of endogenous rPL from day 12 pregnancy rat serum is approximately 20 min as compared to 1.2 min during late pregnancy [33]. Upon Sephadex G-100 fractionation of day 17 pregnancy rat serum, all the lactogenic activity (RRA) is found in fractions with a molecular weight slightly less than that of oPRL; whereas in similar experiments using day 12 serum, the lactogenic substance elutes with fractions of much greater molecular weight than 22,000 [33]. It is the day 12 serum, containing the large molecular weight rPL, which exhibits great luteotropic activity [37, 39, 41]. The small

Table 2. Mammotropic effects of rat serum and placenta from various stages of pregnancy and doses of ovine prolactin required to reach similar biological end points

(a) Rat placental lactogen

M.E.D.*, Daily		RRA-oPRL Equivalents: reference: [33]					
Serum, ml	Placenta	Serum, ng	Placenta, μg	Days of treatment	Biological end point	Rat bioassay model	References
0.05 (12)†	0.5 p.e.‡ (12)	60	0.3	7	Mammary parenchymal proliferation	Hx̄**, immature female	[37]
—	0.5 p.e. (12)	—	0.3	7	Mammary parenchymal proliferation	Hx̄, immature female	[38]
—	1.0 p.e. (11)	—	0.5				
0.1 (12)	—	120	—	3	Lobulo-alveolar development	30 day old male	[39]
—	4.0 p.e. (18)	—	60.0	5	Histology of mammary gland	Virgin, estrogen-primed subcutaneous injections	[43]
—	3–5 (12–20)	—	1.8–80.0	12–20	Mammary proliferation on day 20	Hx̄, pregnant	[42]
—	1.0 p.e.§ (12)	—	0.6	10	Lobulo-alveolar development	Hx̄, ovx̄††, virgin	[44]

(b) Rat placental lactogen or ovine prolactin

Placenta	Equivalents oPRL (μg)	Days of treatment	Biological end point	Bioassay model	References
4.0 p.e. (18)	18	5	Epithelial proliferation	Pigeon crop sac	[43]
1.0 (12)	0.01¶	10	Local growth response	Pigeon crop sac	[3, 4, 44]

* Minimally effective dose. † Day of pregnancy in parentheses. ‡ Placental equivalent. § Injected with 1 μg estrone and 4 mg progesterone daily. ¶ "Mammotropic hormone".
** Hypophysectomized. †† Ovariectomized.

molecular weight rPL predominates in placental extracts from days 11–13 as well as days 17–21 [33]. Paradoxically, luteotropic activity determined by bioassay is not demonstrable in placental extracts obtained after day 13 when rPL (mostly small molecular weight species) content by RRA is high. Moreover, placental luteotropic activity is highest [37, 39, 41, 42, 62, 63] when placental rPL, measured by RRA, is relatively low (days 10–13, see Fig. 1). The longer half-life of the large molecular weight rPL present in day 12 pregnancy serum (20 min) as compared to oPRL (6–8 min) may explain the more potent luteotropic properties of rPL at this stage but the physiological milieu of the pregnant rat on day 12 may also be a factor [33]. Thus in summary, the ability of the midpregnancy rat placenta to support luteal function in the face of an apparently low content of lactogenic hormone remains an enigma. The availability of purified rPL for the development of a homologous radioimmunoassay and for binding studies of luteal tissue may be helpful in explaining the apparent differences in potency estimates obtained by classical bioassays and RRA.

The final notion which may serve to resolve the apparent difference in estimated circulating luteotropic activity measured by bioassay and RRA may involve an alteration in luteal tissue receptor sensitivity to PRL and rPL during pregnancy in the rat. We know that prolactin is essential for maintenance of luteal progesterone secretion during the first 7–8 days of pregnancy in the rat [51, 54, 63, 64] and the last of 2 daily prolactin surges vanishes by day 10 [65]. LH is necessary for the support of the corpus luteum between days 8 and 11 [66], and there is evidence suggesting that the conceptus and hence rPL is required for LH to exert its luteotropic effect [67, 68]. Contrariwise, other investigators (most notably Madhwa Raj *et al.*[53]) indicate that LH is the most important stimulus to the rat ovary up to day 12 of pregnancy since neither day 12 placental extracts nor oPRL (up to 2 mg) is capable of preventing fetal resorption resulting from the administration of anti-LH serum on day 8 [53]. Yet Rothchild's group [69, 70] demonstrated that rPL (in the absence of LH) raises the secretion rate of progesterone from the pregnant rat ovary to a major degree. Hypophysectomy on day 12 of pregnancy results in a prolonged gestation, and although progesterone levels remain uncompromised for 3 days (with no further corpus luteum growth) following hypophysectomy and hysterectomy on day 12 [70], the progesterone secretion rate is well below that of the intact or hypophysectomized day 12 pregnant rat [69]. Significantly, the retention of just 1 placenta in hypophysectomized, pregnant rats supports ovarian progesterone output on day 15. In addition, peripheral 20α-hydroxypregn-4-ene-3-one (20α-OHP) levels are only slightly reduced from day 12 values 7 days following removal of the pituitary and ovaries [70].* Furthermore, Takayama *et al.*[56] have shown that the rapid increase in luteal weight which occurs between days 12 and 16 is unaffected by hypophysectomy or fetectomy but is blocked by hysterectomy. Therefore, since the rat placenta exhibits luteotropic properties in the absence of the pituitary, LH is probably not essential for support of the corpus luteum during the second half of pregnancy which is likely a function of rPL alone. In fact, both LH and PRL blood levels are low during most of rat gestation (see Fig. 1) increasing (only slightly for LH) shortly before parturition at which time they may have a luteolytic function [70, 76]. RPL is also a potential luteolytic agent, depending on the temporal pattern of ovarian exposure to rPL [37]. The rPL peak during late pregnancy [26, 33] could (in combination with PRL) cause lysis of refractive luteal tissue [77].

The action of polypeptide hormones is generally thought to be mediated through plasma membrane receptors [78] and the content and sensitivity of these receptors is considered crucial to hormone–effector interaction. For our purposes, an attempt to delineate the differences in rat ovarian receptor dynamics towards rPL and oPRL would be most fruitful in explaining the unaccounted for luteotropic potency

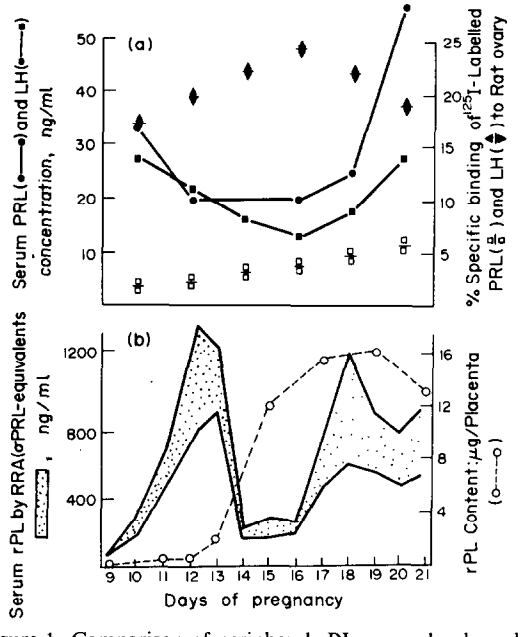

Figure 1. Comparison of peripheral rPL serum levels and placental content with peripheral serum levels of PRL or LH and specific binding of labelled PRL or LH to rat ovaries from days 9–21 of gestation in the rat. Panel A adapted from Cheng, 1976 [47]; panel B from Kelly *et al.*, 1975 [33].

* PRL [71] and, indirectly, rPL [72] have been implicated as depressors of ovarian 20α-hydroxysteroid dehydrogenase (20α-HSD) activity. The effect is considered a direct one since progesterone and 20α-OHP levels are inversely related during pregnancy [73]. However, the importance of the direct action of these luteotropic agents on 20α-HSD activity has been questioned recently. Instead, Veomett and Daniel feel that PRL acts directly on progesterone secretion which in turn alters 20α-HSD activity [74]. In the rat placenta, an end product inhibition of 20α-HSD by 20α-OHP may be a more significant reaction in the control of progesterone synthesis [75].

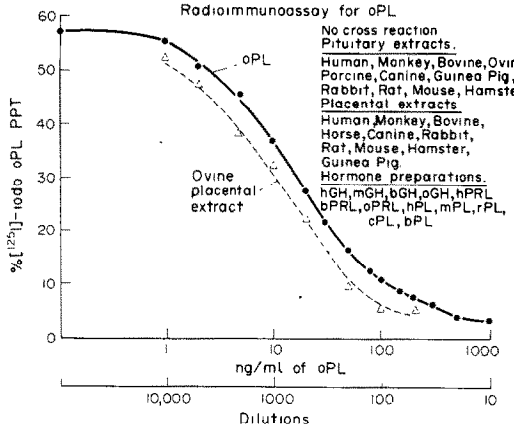

Figure 2. Double antibody radioimmunoassay of oPL. Preparations listed were tested at 10 μg/ml without significant inhibition of [^{125}I]-iodo-oPL binding to dilutions of rabbit anti-oPL serum. From Chan *et al.*, 1976c [32].

of day 12 serum and placenta. Unfortunately, little evidence exists regarding hormone receptors in the rat corpus luteum, but a few points are worth mentioning.

Binding sites for hPRL are present in rat ovarian homogenates and their numbers are highest at proestrus [79]. At midpregnancy in the rat, the progestational 'need' for pituitary PRL is minimal and peripheral PRL levels are low. Indeed, PRL receptor content in the pregnant rat ovary is on the decline after day 8 and stays relatively low until term, whereas LH receptors peak between days 11 and 16 and remain high until parturition [47, 80] (see Fig. 1). Since PRL receptor activity is quite low at day 12, we might reasonably expect that large doses of oPRL (> 1 mg/day) are needed to support luteal progesterone secretion at this time, and this is the case [56]. However, smaller doses of oPRL (50–250 μg/day) increase progesterone production and LH receptor activity in FSH/estradiol-primed, hypophysectomized immature rats [80], block the loss of LH receptor associated with luteolysis [81], and, in combination with estrone or LH, elevate peripheral progesterone levels in pseudopregnant rats [50]. The induction of LH (and rPL?) receptors by PRL in the pregnant rat ovary might explain, in part, the increased luteotropic activity of day 12 serum and the ineffectiveness of even large doses of oPRL to facilitate progesterone secretion in the hypophysectomized day 12 pregnant rat [56]. However, crucial studies on the binding of rPL to the pregnant rat ovary and the effectiveness of equivalent amounts of oPRL, rPL and day 12 serum on progesterone secretion have not been performed. In addition, it must be kept in mind that luteal tissue presents a heterogenous population of cells and therefore the binding properties of various hormones to the ovary must be accepted with some reservations.

Comparing the mammotropic potency estimates of rat placentae using RRA or bioassay (although the data is limited; see Table 2), we see less divergence than in the case of luteotropic activity (Table 1). For example, Shani *et al.*'s[43] estimate of PRL-like activity in the day 18 rat placenta (4.5 μg/placenta) using the pigeon crop sac is approximately 4-fold less than the RRA activity reported by Kelly *et al.*[33]. However, the preparation of rPL tested by Shani *et al.*[43] was previously lyophilized and the pH lowered to 4.5. In our laboratory, we have observed a loss of activity with time in lyophilized crude rat placental extracts stored at −20° or in fresh extracts in which the pH is decreased below 5.0. Another point of interest is that Shani *et al.*[43] utilized the pigeon crop sac bioassay in which some PL preparations are less reactive [2, 5, 77].

On the other hand, Shani *et al.*[43] reported that four day 18 p.e. were required for a mammotropic effect whereas a minimally effective dose in Matthies' study [37] was 0.5 day 12 p.e. The difference in types of assays could explain some of the differences between the two experiments, but it is more difficult to explain the requirement for larger amounts of day 18 placentae [43] which have elevated RRA contents of rPL than for day 12 placentae [37, 38] which contain small amounts of rPL by RRA-PRL. A day 12 placenta, according to Kelly *et al.*[33], contains < 1 μg oPRL equivalents compared to 16 μg in a day 18 placenta (Fig. 1). In addition, Anderson [42] reported that while the retention of only one placental unit is adequate for pregnancy maintenance after day 12, 3–5 placentae are needed to prevent a fall-off in mammary gland proliferation on day 20. The foregoing data implies that rPL at day 12 has greater luteotropic as opposed to mammotropic potency. It may be that lactogenic stimulation of the rat mammary gland by rPL only becomes important late in pregnancy. In this regard, significant luteotropic activity has only been demonstrated in day 11–13 serum or placenta [4, 37–42] while Shiu *et al.*'s[26] finding of lactogenic (RRA) activity in day 19 pregnancy rat serum was later confirmed by both Matthies[77] and Talamantes[24] using lactogenic bioassays. Furthermore, taking into account the known metabolic effects of PL [82], the finding of increased insulin and insulin-resistance in the day 19 fed pregnant rat may suggest that rPL has a metabolic function at this time [83].

Only 0.05 ml of day 12 pregnant rat serum injected for 7 days into immature female rats leads to partial maintenance of mammary parenchymal proliferation [37]. Cohen *et al.*[39] substantiated this finding in a male rat bioassay but failed to observe secretory activity in the mammary gland as previously noted at the 0.1 ml dose by Matthies[37]. The above comparisons underscore the importance of the assay model on the interpretation of experimental results. The finding that less serum, placenta or PRL is necessary to engender a mammotropic than a luteotropic effect should also be interpreted with caution, since several of the mammotropic assay models employ local injections over the mammary gland. In studying luteotropic effects of placental extracts, we, of course, must administer the compounds parenter-

ally and sustain reasonable systemic levels for an adequate period of time. Hence it is likely that minimal effective doses are much lower in the case of mammogenic assays. Such critical analysis of these methodologies can help explain wide disparities in mammotropic (0.01 μg) and luteotropic (2 mg) contents of day 12 placenta as reported by Lyons and colleagues [3, 4, 44]. Suffice it to say that unexplained differences between radioreceptor assay data and bioassay results for rPL, in terms of both its luteotropic and mammotropic potencies, raise interesting and productive questions. Properly designed experiments will no doubt clarify the issue, but a more complete understanding of the complex interaction between rPL and its target tissues awaits receptor binding studies and the development of a homologous RIA for this placental polypeptide.

Future studies should also be directed toward the clarification of Contopoulos and Simpson's[84] report of elevated somatotropic activity in pregnant rat serum. Although preliminary results from our laboratory support such a claim, the finding in rat serum obtained during pregnancy of a growth-binding protein clouds the original finding (see review by Friesen *et al.*, 1975[85]).

The possible role of rPL in maternal and fetal metabolism is unclear. The luteotropic and mammotropic potency of the midterm rat placenta appears unaltered by dietary protein restrictions [38], but newer data suggests that the major effect of reduced protein intake on fetal resorption occurs after midpregnancy [86]. Pregnancy in the rat results in significant increases in glucose uptake by adipose tissue [87] and an increased amino acid uptake into liver proteins [88]. Finally, the earlier finding by Jost[89] that the rat placenta in combination with adrenocortical steroids supports glycogen deposition in the fetal liver deserves further investigation.

Other indirect evidence provides support for the view that rPL is closely related immunologically to hPL. Rabbit antiserum to hPL cross reacts with rat placental extracts [87, 90] and these antibodies bind to the rat placenta *in vivo* [91]. However, the inhibition of hPL antibody binding to placenta [91] or hPL binding to the lactating rat mammary gland [92] by purified rPL has not been shown. Further support for the immunological relationship between rPL and hPL is the deleterious effects on pregnancy and lactation which are observed after passive or active immunization of pregnant rats with hPL [93, 94]. Yet care must be taken in interpreting these latter results, for neutralization of endogenous PRL by anti-hPL might account for some of the observed effects.

OVINE PLACENTAL LACTOGEN

In studies conducted in our laboratory over the past few years, both a GH and PRL receptor assay were utilized to quantify and monitor the purification of ovine placental lactogen (oPL) from sheep cotyledons for the development of an oPL-RIA [20, 31, 32, 95, 96]. The two receptor assays reveal that purified oPL has at least a 150-fold greater GH:PRL ratio than hPL. In addition, the somatotropic potency is approximately 1.5 times that of bGH in either the hypophysectomized rat body weight gain [20] or tibial width assays (unpublished observations).

Chan's preparation of oPL is nearly equipotent with hGH in the liver receptor assay [96], whereas Handwerger's oPL preparation has one-fifth the activity of oGH in the GH-RRA [19]. The studies of Martal and Djiane[17] agree more closely with our own findings in that their oPL preparation is almost equipotent in receptor assays for PRL and GH. Although Handwerger *et al.*[97] reported a partial immunological identity between oPL and oGH, Chan *et al.*[32] failed to show cross reaction between these ovine polypeptide hormones in a radioimmunoassay for oPL (see Fig. 2). On the other hand, oGH and hGH compete with [^{125}I]-iodo-oPL for binding sites on ovine liver or adipose tissue membrane receptors [31]. Observations made in both Friesen's and Handwerger's laboratories support the notion that oPL and hPL are immunologically distinct entities [19, 31], but Gusdon *et al.*[90] claim some similarities based on indirect evidence.

Radioreceptor assay data of PRL-like activity (rabbit mammary gland) present in the plasma of pregnant sheep show that oPL is first detectable on days 50–60 of gestation. Peak peripheral serum concentrations of oPL (1–2 μg/ml) are found between days 95–114 of gestation followed by a gradual decline towards term with a more rapid disappearance 12 h before parturition [95]. Others report a continuous increase in RRA levels of oPL to term (peak concentrations: 3.2–5.0 μg/ml) [98]. It should be noted that oPRL and hPL were used as standards in Kelly *et al.'s*[95] and Handwerger *et al.'s*[98] assays, respectively. In addition, a major component of the prolactin-like activity detected by RRA shortly (6 h) before parturition in the ewe results from pituitary PRL (300–600 ng/ml) as noted by Kelly *et al.*[95]. Furthermore, no clear-cut relationship was demonstrated between progesterone levels and changes in oPL concentrations in the Kelly study [95].

Using a homologous RIA (sensitivity = 2 ng/ml), Chan *et al.*[31, 32] report that oPL is detectable in sheep chorionic membranes and allantoic fluid as early as 20 days of gestation. Whereas monkey PL [30] and hPL [99, 100] concentrations in the blood rise progressively to term with hPL levels reportedly falling off during labor [101], the pattern of oPL secretion during pregnancy, as determined by both RRA [95] and RIA [32], differs from that of primate PL. OPL is detectable by RIA in peripheral serum by day 46 and uterine venous serum by day 35 rising to maximum peripheral concentrations between days 110 and 135. Generally, a decline in peripheral serum oPL concentrations is observed a few days before parturition in the ewe, and during the postpartum period oPL levels fall off rapidly [32].

In addition, fetal peripheral serum concentrations are greater than maternal concentrations between 50 and 80 days of pregnancy [31, 32]; quite opposite to the maternal and fetal hPL concentrations during human pregnancy [29]. The early detection of oPL in maternal peripheral and uterine venous blood [32] and the specific binding of [^{125}I]-iodo-oPL to corpus luteum membrane fractions [31] suggest that oPL might affect ovarian function during sheep pregnancy. However, a direct effect of oPL on luteal progesterone secretion in the ewe has not been shown.

Recently, Handwerger *et al.*[98, 102] demonstrated that when 50 mg of oPL (60% pure, based on hPL equivalents in a rabbit mammary gland receptor assay) were injected into the femoral vein of fasted pregnant and nonpregnant ewes, dramatic effects on the blood levels of several metabolic substrates and insulin were noted. Significant decreases in the plasma concentrations of free fatty acids (FFA) and insulin were noted 1 h following the injection, and several hours after the oPL injection, plasma glucose (3 h) and amino nitrogen (6 h) concentrations declined significantly. Two hours after the injection, plasma insulin levels were significantly elevated while plasma FFA did not return to basal values for 6 h. Although the fall in amino nitrogen began after plasma insulin concentrations were elevated (and might be attributable to insulin [103]), a lower dose of oPL (5 mg) had a similar effect on amino nitrogen concentrations without altering plasma insulin. Similarly, the plasma FFA decline preceded the rise in plasma insulin and is therefore probably not causally related. In contrast, the fall in plasma glucose might be attributable to the potentiated insulin output.

While oPRL or oGH administration does not affect serum insulin in fasted ewes, oGH results in *increased* plasma concentrations of plasma FFA after 8 h and decreased amino nitrogen after 10 h [104]. The effect of oPL on FFA concentrations, as reported by Handwerger *et al.*[98, 102], is quite opposite to the diabetogenic, anti-insulin effects of hPL or hGH administration in humans [82, 105], although maternal amino acids and glucose are reported slightly decreased during the second half of human gestation [82]. Furthermore, oral glucose administration does not alter serum concentrations of hPL [106], but prolonged fasting may increase hPL levels by 30–40% during midterm human pregnancy [107]. On the other hand, hPL *does* enhance amino acid incorporation into rat liver slices [88] and the insulin response to glucose in rat pancreatic slices [108], providing support for the supposition that oPL is hPL-like in terms of its anabolic effects [98, 102].

Studies on the carbohydrate metabolism of pregnant and lactating sheep on a constant food intake reveal increased plasma FFA concentrations during late pregnancy which correlate with total fetal weight and the level of milk production during the first month of lactation [109]. On a restricted diet, pregnant animals have significantly elevated plasma FFA levels yet these levels are also elevated (2–2.5 mequiv./liter) in well-fed, late pregnant and lactating ewes [110]. It is somewhat puzzling then, considering the increased metabolic requirements of the pregnant state that Handwerger's group [102] observed no differences in response to oPL between advanced pregnant and nonpregnant ewes. Perhaps oPL is important to metabolic adjustments only during early pregnancy in sheep. On the other hand, the decrease in plasma amino nitrogen occurring 8 h after oPL injection in fasted ewes [98, 102] corresponds to the effects of a restricted diet on protein metabolism in pregnant ewes [109], but the same acute effects of oPL on lipid metabolism requires more study since the nutritional and emotional state of ruminants is an essential determinant of blood FFA levels [110, 111]. Likewise, the absorption of fatty acids (acetate, propionate and butyrate) from the gut of the ruminant [112] for energy utilization must be considered since absorbed acetate (the predominant fatty acid in the rumen) has a fat-sparing effect [110].

BOVINE PLACENTAL LACTOGEN

The occurrence of a placental lactogen in the cow (bPL) is detectable in cotyledons co-cultured with mouse mammary gland explants [11, 12] or when placental extracts are injected directly into the rat mammary gland [15], but lactogenic activity in peripheral blood samples is low (bioassay) or undetectable (RRA-PRL) [12, 18]. Gusdon *et al.*[90] report that extracts of term bovine placentae cross react with anti-hPL serum (hemagglutination inhibition) but are far less potent than hPL. It seems clear that bPL is immunologically distinct from either bPRL or bGH [12, 14], but the recent report [14] of a purified bPL preparation raises interesting questions. Bolander and Fellows'[13, 14] bPL preparation was reported to be homogeneous by several criteria which is somewhat surprising in view of the fact that a mere 40-fold purification from the original extract had occurred. In the case of oPL and other lactogens a purification factor of 1,000 or more is required to obtain homogeneity [34, 96]. Although Bolander and Fellows [14] point out that inactivation of the hormone during purification appears unlikely as they obtained a highly active oPL preparation utilizing a similar procedure [113], the resolution of the problem of low activity awaits the purification and characterization of bPL by other groups.

CONCLUSION

Placental lactogens have significant effects on pregnancy maintenance, lactation and fetal/maternal nutrition. At present these activities are under investigation, but the elucidation of the precise roles played by PL during gestation and lactation awaits the further purification of PL in a variety of species with the concomitant characterization of their target tissue binding and development of homologous immunoassays.

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DISCUSSIONS

Posner. In a number of species pregnancy seems to be associated with an increase in either lactogen specific or growth hormone specific binding sites. Is there any data in the sheep as to whether these OPL binding sites change as a function of pregnancy?

Friesen. This study is just under way at the moment. I don't have all of the facts at my finger tips. Rather than making erroneous statements about specific changes I might say that the changes in some respects are similar to those found in the rat. Binding of OPL in fetal liver is one-third that of the maternal liver at various stages of pregnancy.

Grumbach. I think the last point that you made as far as I am concerned is exceedingly important, namely, testing hormone in receptor preparations from the same species. I know that you have had an opportunity to look at this in regard to monkey placental lactogen and also to the human preparation in contrast to the rabbit liver and rabbit mammary gland. In comparison to estimates of potency obtained with rabbit liver, what estimates were obtained with human and the monkey liver receptor?

Friesen. I am not quite sure that I understood the question. If you are asking what is the estimated potency of HPL in the rabbit liver compared to monkey and human

liver, I can tell you that using either tissue HPL has perhaps 1% or somewhat less the activity of human growth hormone. We had had great difficulty in identifying prolactin sites in any human tissue thus far so I can't give a comparable figure in terms of prolactin-like activity. In the case of the monkey we have had great difficulty in demonstrating either GH or prolactin binding sites so I can't offer any comparative estimate of potency.

Grumbach. Have you looked at the pregnant monkey liver or the fetal monkey liver?

Friesen. Only very inadequately. We have studied, I believe, one or two pregnant monkeys and as I recall there was very little binding either of growth hormone or prolactin. For those not in the field I believe it is worth stating that if you find no binding one has to be very careful about drawing any conclusions because the negative result may simply be a methodological problem. There may be proteases which destroy either the tracer or the receptor and thus negative data is not as helpful as positive data. To conclude confidently that in fact no receptors are present in any given tissue would require very extensive studies.

Kann. Dr Friesen, I would like to ask you two questions. First, the first slide you have shown were the results on mouse or rat expressed in terms of rat prolactin or ovine prolactin?

Friesen. No, our standards for all studies have been NIH ovine prolactin (28 IU/mg) or NIH bovine growth hormone (bGH) (2.6 IU/mg).

Kann. The second question is related to the question of Dr Grumbach; have you looked at the liver of the sheep and the ovaries of the sheep?

Friesen. The data I showed was for binding of OPL to sheep liver, which was displaced by growth hormone (OGH) but not by OPRL. There is very little binding of sheep prolactin tracer to sheep liver just as there is very little displacement by sheep prolactin of labeled OPL. The binding to ovine liver is much greater by ovine growth hormone, and this binding can be reduced by OPL but OPL binds considerably better than ovine growth hormone. It seems to be predominately a GH binding site. You also asked about the ovine corpus luteum. There is some binding, again displaceable by GH to a greater extent but also by prolactin.

Thorburn. Dr. Freisen, you said that in your assay, ovine placental lactogen did not cross-react with ovine growth hormone. So when you measured fetal levels of ovine placental lactogen there was no chance of any cross-reaction with the large amounts of ovine growth hormone that are present in fetal plasma.

Friesen. No, there was no inhibition in the OPL radioimmunoassay, not even by concentrations of OGH as great as 10 μg/ml.

Thorburn. Now as far as the effects of hypophysectomy on fetal growth, there is some growth retardation of the fetal lamb following fetal hypophysectomy. Do you think that if there was not a placental lactogen that the dwarfing could be even greater. Do you know anything about the action of placental lactogen on thymidine uptake in cartilage. Does it only act on the liver or on any other sites where growth hormone acts.

Friesen. I can only provide a vague answer to the first part of your question because I don't know whether OPL is acting as a somatogenic agent in the fetus. Although I didn't show any data on bioassays of OPL it is certainly a very potent growth promoting agent as judged by its effect on body weight gain or tibia assay in hypophysectomized rats. Potency estimates range from 1–2 units/mg.

Thorburn. It makes it difficult therefore to decide that the fetal pituitary has no effect on fetal growth if placental lactogen is present in the fetal circulation.

Freisen. Yes, and I think it is worthwhile underscoring what I said in my introductory remarks. It is difficult, if not impossible, to selectively ablate a placental hormone particularly when the latter is circulating in relatively high concentrations. You really can not neutralize these high concentrations of placental hormones with antibodies like one might be able to with pituitary hormones that circulate at concentrations of 1–10 ng/ml. One of the approaches we were hopeful might work was in fact to use antibodies to prolactin receptor, thinking at that time that the effects of placental lactogens might well be mediated through prolactin receptors. Now recognising that at least some effects of OPL might be mediated to somatogenic effects the use of antibodies to a growth hormone receptor might be more appropriate. Using this approach it may be possible to selectively neutralize the effect of OPL. I might just add that if one uses a growth hormone and prolactin receptor assay placental lactogen from each species has different relative ratios of activity. Thus in the case of human placental lactogen the predominant activity is prolactin-like whereas in the goat if anything there is more growth hormone-like than prolactin-like activity. The ratio might be 2:1 in favour of somatogenic activity.

Naftolin. Can you tell us about possible sexual differences in the effects of the hormones.

Friesen. Well, I can simply speculate, extrapolating I suppose from data on human placental lactogen, where there are no sex differences in levels. We have not looked at the effect of sex differences of the offspring in various species on the concentration of placental lactogens. However, tissue responsiveness may vary because receptor number may vary greatly among the two sexes in fetal life. In the case of prolactin the binding is rather low in fetal life but I am not aware of any good data on differences in specific receptors for hormones during fetal life. Perhaps Dr. Posner is. Certainly I am not aware of any sex differences in expression of receptors in the case of polypeptide hormones at least during fetal life. There may well be, but I don't think it has been really looked at very extensively.

Grumbach. The observation that serum OPL levels are higher in the sheep fetus at one time is really unique as far as I know, certainly in contrast to the human; I don't know if we have had an opportunity to measure it in the serum of any other species that have chorionic hormones. Do you think, have you excluded the possibility, that there may be a binding protein?

Friesen. These measurements were made using a radioimmunoassay.

Grumbach. That is right, that was not a receptor assay.

Friesen. The same caution applies to measurements by radioimmunoassay. It is really a question of affinity of the antibody. For example, in the mouse you can very nicely measure human growth hormone when it is added to mouse serum. The antibody to human growth hormone has such a high affinity compared to the affinity of the serum binding protein that for growth hormone interference is negligible. In the mouse during pregnancy a growth hormone binding protein appears. This can be demonstrated by adding [^{125}I]-labelled growth hormone to serum prior to separation of serum proteins on Sephadex. Under these circumstances growth hormone, but not prolactin, elutes with proteins in the void volume suggesting that the growth hormone is bound. Incidentally, I might mention that Dr. Posner independently has noted the induction of growth hormone receptors in the mouse liver beginning around day twelve of gestation which then increases and declines rapidly one or two days post partum, almost the same temporal sequence as we observed for the circulating GH binding protein. It is still not clear whether what he has observed and what we found in the circulation are related GH binding factors.