Development of the sheep as an animal model to study placental lactogen physiology

Ovine placental lactogen, purified from term sheep cotyledons, has been found to have chemical and biologic properties similar to those of human placental lactogen, ovine growth hormone, and ovine prolactin. OPL stimulates lactation in vivo and in vitro and binds to prolactin and growth hormone membrane receptors. Its binding to growth hormone receptors is approximately 20 times greater than that of hPL, suggesting that its somatotrophic potency is greater than that of hPL. Preliminary in vivo studies in the sheep indicate that oPL affects maternal carbohydrate, lipid, and protein metabolism and that its effects are, in part, similar to those of hPL and growth hormone.


Numerous studies indicate that human placental lactogen (human chorionic somatomammotropin), a polypeptide hormone secreted by the syncytiotrophoblast, plays an important role in the regulation of maternal carbohydrate, lipid, and protein metabolism during pregnancy.1-5 When administered to nonpregnant and pregnant subjects, hPL, like human growth hormone, causes enhancement in insulin secretion, impairment of glucose tolerance, release of free fatty acids, and retention of urea nitrogen.1-5 Because of the similarity of the physiologic effects of hPL and hGH, Grumbach and associates1 have suggested a major role for hPL as a “growth hormone” of the second half of pregnancy. They suggest that the concerted action of hPL and insulin during pregnancy promotes conservation and synthesis of protein and helps to meet the large amino acid requirements of the fetus. In addition, they postulate that, by stimulating the release of free fatty acids from maternal adipose tissue and by diminishing maternal glucose utilization, hPL permits delivery of an adequate glucose supply to the fetus. Thus, by its actions on maternal metabolism, hPL may play an important role in the regulation of substrates available to the fetus. This interesting hypothesis, however, has not been tested and the physiologic role of hPL during pregnancy remains unclear.

Abbreviations used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>oPL</td>
<td>ovine placental lactogen</td>
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<tr>
<td>hPL</td>
<td>human placental lactogen</td>
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<tr>
<td>oGH</td>
<td>ovine growth hormone</td>
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<tr>
<td>hGH</td>
<td>human growth hormone</td>
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<tr>
<td>oPR</td>
<td>ovine prolactin</td>
</tr>
<tr>
<td>hPR</td>
<td>human prolactin</td>
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Although the biologic activities of hPL and hGH are similar, the acute stimuli of hGH secretion, such as arginine and insulin-induced hypoglycemia, have no effect on the maternal concentration of hPL.1-5 In addition, most investigators have failed to demonstrate suppression of hPL levels following administration of glucose.7 Concentrations of hPL, however, have been noted to increase 30-40% in women fasted 84-90 hours during weeks 16-20 of pregnancy (prior to therapeutic abortion), suggesting that prolonged hypoglycemia in the
mother or hypoglycemia in association with alterations in other metabolic fuels may be an important stimulus to secretion of hPL. The specific fuels or other factors regulating secretion of hPL have not been elucidated.

Aberrations in maternal hPL concentrations have been noted in several pathologic conditions of pregnancy, including diabetes mellitus, pre-eclampsia, hypertensive vascular disease, erythroblastosis fetalis, sickle cell anemia, intrauterine growth retardation, and some instances of spontaneous abortion. The incidence of abnormal hPL concentrations in these conditions, however, has varied considerably among different clinical studies. A declining maternal hPL concentration during the course of pregnancy is associated with a high risk of fetal morbidity or mortality. In a recent study, a single observation of an abnormally low plasma hPL (less than 4 µg/ml) in the last five weeks of pregnancy was associated with a 30% risk of fetal distress and/or neonatal asphyxia. When low concentrations were noted on two occasions, the risk increased to 50%; with three or more such measurements, the risk increased to 71%.

Because of the potential risk of physiologic studies to the human fetus, fundamental questions about the biologic role of placental lactogen during pregnancy will have to be answered in an animal model. Several studies have been performed in the monkey, but expense and technical factors make this model unavailable to most investigators. Consequently, the development of a subprimate animal model which is readily accessible, relatively inexpensive, and which permits sampling of both the maternal and fetal circulations would contribute significantly to the understanding of the physiology of placental lactogen. With this in mind, a major goal of our laboratories has been to isolate ovine placental lactogen (oPL) from sheep cotyledons, to characterize its chemical, biologic and immunologic properties, and to initiate physiologic studies of oPL in nonpregnant and pregnant sheep.

ISOLATION AND PURIFICATION OF OVINE PLACENTAL LACTOGEN

Highly purified oPL has been isolated from term sheep cotyledons by a protocol which includes neutral pH extraction, ammonium sulfate precipitation, and chromatography on Sephadex G-150, DEAE cellulose, and CM cellulose. The biologically active material elutes from Sephadex G-150 in a major peak with an elution volume similar to that of ovine prolactin and ovine growth hormone (Fig. 1). After purification by ion exchange chromatography, the oPL migrates as a single major band by gel electrophoresis at pH 9.0 with a mobility similar to that of oGH. Its molecular size, estimated by chromatography and electrophoretic determination of Stokes radius, is consistent with a molecular weight of approximately 24,000 daltons; its amino acid composition is similar to that of hPL and oGH. Immunodiffusion studies with rabbit oPL antiserum demonstrate partial identity between oPL and oGH. Rabbit oPL antiserum, however, does not react with hPL, hGH, or oPR.

BIOLOGIC PROPERTIES OF OVINE PLACENTAL LACTOGEN

Like prolactin and hPL, oPL was noted to stimulate lactation in vivo in the rabbit intraductal assay and in vitro in the mouse mammary gland assay. In both assays, oPL stimulated ductal proliferation and the lumina contained moderate amounts of colloidal secretions. In addition, mammary explants cultured with oPL in the incubation media synthesized 2,172 ± 200 cpm 14C-casein/mg tissue protein, whereas explants cultured without oPL in the media synthesized only 1,042 ± 114 cpm 14C-casein/mg tissue protein. In the prolactin radioreceptor assay, oPL competed with 125I-human prolactin for prolactin-binding sites on mammary gland membranes (Fig. 2). The displacement of 125I-hPR from the prolactin receptor by increasing quantities of highly purified oPL parallels the displacement of 125I-hPR by increasing quantities of hPL and oPR. Ovine growth hormone, however, did not displace 125I-hPR from the prolactin receptor. Shiu and...
Use of sheep in studying placental lactogen

Utilization of the pregnant sheep as an animal model for the study of the physiology of placental lactogen

The maternal concentration of oPL has been measured with the prolactin radioreceptor assay at frequent intervals between 30 and 140 days of gestation in six pregnant ewes with single gestations and one pregnant ewe with twins (Fig. 4). The length of gestation in the sheep is 140 to 145 days. In pregnant ewes with single gestations, the maternal oPL concentration at 30 to 50 days of gestation ranged from 0.4-0.9 µg/ml. Thereafter, the maternal concentration of oPL increased progressively until term, when it reached concentrations of 3.2-5.0 µg/ml (mean, 4.3 µg/ml). In two of the five pregnant ewes with single
Table I. The effect of oPL on plasma free fatty acid, glucose, α-amino nitrogen, and insulin levels in two nonpregnant sheep

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Free fatty acid (μEq/ml)</th>
<th>Glucose (mg/dl)</th>
<th>α-Amino nitrogen (mg/dl)</th>
<th>Insulin (μU/ml)</th>
<th>Free fatty acid (μEq/ml)</th>
<th>Glucose (mg/dl)</th>
<th>α-Amino nitrogen (mg/dl)</th>
<th>Insulin (μU/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>994 ± 121</td>
<td>57.2 ± 3.2</td>
<td>4.06 ± 0.20</td>
<td>7.0 ± 6.6</td>
<td>833 ± 85</td>
<td>62.3 ± 5.6</td>
<td>4.37 ± 0.20</td>
<td>22.1 ± 3.0</td>
</tr>
<tr>
<td>1</td>
<td>229 ± 40†</td>
<td>53 ± 6.1</td>
<td>3.70 ± 0.28</td>
<td>687 ± 122</td>
<td>60.3 ± 11.0</td>
<td>4.14 ± 0.06</td>
<td>4.4 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>251 ± 8†</td>
<td>54.2 ± 6.3</td>
<td>3.68 ± 0.25</td>
<td>767 ± 9</td>
<td>64.5 ± 8.9</td>
<td>3.76 ± 0.08</td>
<td>6.4 ± 4.7</td>
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</tr>
<tr>
<td>3</td>
<td>229 ± 40†</td>
<td>48.4 ± 8.2†</td>
<td>3.48 ± 0.03†</td>
<td>739 ± 67</td>
<td>62.7 ± 9.5</td>
<td>3.88 ± 0.07</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>436 ± 51†</td>
<td>39.8 ± 6.8†</td>
<td>3.21 ± 0.08†</td>
<td>799 ± 151</td>
<td>60.3 ± 6.8</td>
<td>3.97 ± 0.13</td>
<td>4.0 ± 1.1</td>
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<tr>
<td>6</td>
<td>1,078 ± 159</td>
<td>32.6 ± 5.9‡</td>
<td>2.69 ± 0.10‡</td>
<td>880 ± 37</td>
<td>56.0 ± 6.8</td>
<td>4.16 ± 0.10</td>
<td>3.2 ± 2.0</td>
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<tr>
<td>8</td>
<td>1,233 ± 255</td>
<td>33.8 ± 8.8‡</td>
<td>2.19 ± 0.15‡</td>
<td>1,028 ± 16</td>
<td>54.0 ± 8.0</td>
<td>4.07 ± 0.47</td>
<td>6.5 ± 0.8</td>
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At time 0, 50 mg oPL purified by ammonium sulfate precipitation and gel filtration or vehicle alone was administered through an indwelling catheter. Each animal received oPL and vehicle on two or more occasions. Values are expressed as mean ± SD; significant difference from control: *p < 0.05, †p < 0.01, ‡p < 0.005.

gestations who subsequently delivered normal lambs, a slight decline in oPL concentrations was noted prior to delivery. In one ewe, the maternal concentration of oPL decreased from 2.9 μg/ml on Day 100 to 1.4 μg/ml on Day 127. The ewe aborted on Day 128. In the pregnant ewe with a twin gestation, the maternal oPL concentrations during pregnancy were approximately twice those noted during single gestations, with a maximal oPL concentration of 9.7 μg/ml.

In preliminary studies, 50 mg of oPL purified by ammonium sulfate fractionation and Sephadex chromatography were administered systemically to two nonpregnant female sheep through indwelling venous catheters. Concentrations of plasma glucose, insulin, free fatty acid, and alpha amino nitrogen were measured prior to and at frequent intervals following the oPL injection, and the results were compared to experiments in which the vehicle alone was administered (Table I). Ovine placental lactogen caused a significant decrease in free fatty acids during the first two hours with return to normal by eight hours; plasma glucose and alpha amino nitrogen values decreased only after the second hour. In contrast, insulin levels rose significantly from two to eight hours after the administration of oPL.

**DISCUSSION**

Ovine placental lactogen has been found to have chemical properties resembling those of human placental lactogen and ovine growth hormone; OPL, hPL, and oGH have similar estimated molecular weights, and amino acid compositions, and oPL and oGH have similar electrophoretic mobilities. The immunodiffusion studies with rabbit oPL antisera suggest that the oPL and oGH molecules have homologous antigenic sites. This observation is of particular interest since hPL and hGH have been demonstrated to be identical in approximately 86% of their primary structure.17

These studies indicate that oPL, like hPL, has biologic properties similar to both prolactin and growth hormone. Like hPL and prolactin, oPL stimulates lactation and competes with prolactin for binding sites to prolactin receptor sites on mammary membranes. In pregnant sheep serum and in ovine placental extracts OPL has also been reported by Kelly and associates18 to bind to prolactin receptors. Like hPL and growth hormone, oPL competes with growth hormone for growth hormone-binding sites in liver membranes. The somatotropic potency of hPL, however, is only about 1% as great as that of hGH. Since Tsushima and associates19 have demonstrated an excellent correlation between binding to growth hormone receptors and somatotrophic potency by bioassay, our results in the binding assay suggest that oPL may prove to have as much as 20 times greater somatotrophic potency than hPL. Although hPL does not appear to stimulate growth in human subjects,20 our findings raise the possibility that oPL might be useful as a somatotrophic agent in man.

In the sheep, the pattern of oPL secretion during pregnancy is similar to that of hPL secretion during pregnancy.1 In the human, hPL is first detected in the maternal circulation in approximately five to six weeks of gestation.1 The maternal concentration of the hormone then increases progressively until about the thirty-second week of pregnancy and plateaus until near term, when a slight decrease in the concentration of the hormone is noted. The maximal concentration of hPL in single gestations is approximately 5-6 μg/ml; the maximal concentration of oPL in single gestations in the sheep
(expressed in hPL equivalents) is 3.2-5.0 μg/ml. The maternal concentration of hPL during twin gestations is approximately twice that noted in single gestations; this also appears to be the case in the sheep.

The concentrations of oPL in maternal plasma during pregnancy have also been measured in three sheep by Kelly and associates using the mammary gland radioreceptor assay and oPR rather than hPL as a standard. Peak oPL concentrations of 1-2 μg/ml were observed on days 95-114 of gestation. In contrast to our findings, however, they noted a decline in oPL concentration after the initial peak followed by one or more additional peaks prior to parturition.

The preliminary experiments in the nonpregnant ewe indicate that oPL affects carbohydrate, lipid, and protein metabolism. The early decline in free fatty acid concentration after administration of oPL is similar to the early insulin-like effect observed with growth hormone. The subsequent rise in free fatty acids suggests a predominant anti-insulin effect in the later time period similar to the late effects of growth hormone and hPL.

The striking chemical and biologic homologies between oPL and hPL clearly establish the sheep as an appropriate animal model for investigation of the physiology of placental lactogen during pregnancy. Thus, ongoing studies in the pregnant sheep should provide insight into the physiologic role of hPL in the regulation of carbohydrate, lipid and protein metabolism in the mother and fetus during human pregnancy.

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REFERENCES