Effects of recombinant ovine placental lactogen and recombinant ovine growth hormone on growth of lambs and milk production of ewes

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Abstract

The somatogenic and galactopoietic effects of recombinant ovine placental lactogen (oPL) were compared with the effects of recombinant ovine growth hormone (oGH) in post-weaned growing lambs and in lactating ewes. In two experiments that each lasted 35 days, 2-month-old lambs were given daily subcutaneous injections (0.1 mg/kg live weight) of oPL or oGH, and their daily growth rates were compared with those of non-treated control lambs. Ovine GH and oPL had similar profound (P < 0.01) growth-stimulating effects, enhancing lamb growth by 10 to 25%. In two other experiments, lactating ewes were injected with oGH or oPL (0.1 mg/kg live weight/day) for 14 days in mid-lactation. Treatment with oGH increased (P < 0.001) daily milk production by up to 55% over control ewes. Ovine PL increased (P < 0.01) milk production by up to 25%. In all experiments, treatment of lambs or lactating ewes with oGH, but not with oPL increased (P < 0.05) serum insulin-like growth factor-I concentrations. It is concluded that oPL and oGH have similar somatogenic effects in lambs. Both hormones exhibited galactopoietic effects, but oGH was considerably more potent than oPL. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Placental lactogen; Growth hormone; Growth; Milk production; Sheep; IGF-I

1. Introduction

Similar to other mammalian species, placentae of ruminants synthesize and secrete unique proteins that belong to the growth hormone/prolactin (GH/PRL) family and are called placental lactogens (PLs) (Byatt et al., 1992b). Ovine (o) (Martal and Djiane, 1975; Warren et al., 1990) and bovine (b) (Murthy et al., 1982) PLs are 22 to 23 kDa proteins which are, based on structural analysis and gene mapping, more closely related to their respective PRL than GH (Byatt et al., 1992b; Gootwine and Yossefi, 1998).
One unique property of ruminant PL which was observed early on is their ability to bind to both PRL and GH receptors, including receptors for human (h)GH (Forsyth, 1986; Anthony et al., 1995a,b). This observation suggested that GH and PL may have similar biological activities. However, attempts to prove this hypothesis using in vitro experimental systems yielded contradictory results depending on whether a homologous or a heterologous model system was chosen. Both bPL and oPL were potent agonists in FDC-11P cells stably transfected with human or rabbit GH receptors, and their activities were comparable with those of hGH and oGH, respectively (Helman et al., 1997, 1998; Sakal et al., 1997). In contrast, in “293” cells transiently transfected with oGH or hGH receptors, ruminant PL were active only in cells transfected with hGH receptors (Herman et al., 1999). In cells transfected with oGH receptors, only oGH acted as an agonist, whereas ruminant PL, despite their ability to bind to those receptors, exhibited antagonistic activity. Therefore, it was concluded that ruminant PL can bind to, but not homodimerize, oGH receptors (Herman et al., 1999).

Results from in vivo comparisons of PL and GH bioactivities are also inconsistent. Whereas administration of both bPL or bGH increased growth and milk production in rats, only bGH affected milk yield in dairy cows (Byatt et al., 1992a). Furthermore, oPL failed to show GH-like galactopoietic properties when injected into ewes (Min et al., 1995). Bovine GH, but not bPL, had stimulatory effects on lamb growth rate in one study (McLaughlin et al., 1993) while, in another study, administration of oPL to growing lambs significantly increased their growth rate, whereas bGH did not (Min et al., 1996). This latter result contradicts several other studies where bGH had a growth-promoting effect in lambs (Wagner and Veenhuizen, 1978; Rosemberg et al., 1989).

Recombinant oPL (Colosi et al., 1989; Sakal et al., 1997) and bPL (Krivit et al., 1989) have been prepared, and were produced in our laboratory (Gertler et al., 1992; Sakal et al., 1997) in amounts sufficient for in vivo studies with a large number of animals. Therefore, the present study compared the effects of oPL and oGH in a homologous in vivo system, using two experimental models: growing lambs and lactating ewes.

2. Materials and methods

The Volcani Center Animal Care Committee approved the experimental protocols used in this research.

2.1. Materials

Recombinant oPL and oGH were prepared as described previously (Sakal et al., 1997). Human insulin-like growth factor-I (IGF-I) and antiserum against IGF-I were obtained from Fugisawa Pharmaceutical (Osaka, Japan). Antiserum against oGH was obtained from the National Hormone Pituitary Program (University of Maryland School of Medicine, MD, USA). Goat anti-rabbit IgG and normal rabbit serum were purchased from Bio-Makor (Rehovot, Israel). Antiserum against oPL was prepared in rabbits in our laboratory. Carrier-free Na[125]I was purchased from New England Nuclear Corp. (Boston, MA, USA).

2.2. Animals, housing and feeding

The experiments were conducted with the Katzenelbugen dairy flock, which includes 600 Assaf breeding ewes, at Moshav Talmai Elazer. This flock is kept indoors all year round and the ewes are fed according to Israeli Ministry of Agriculture recommendations (Landau and Leibovich, 1992). All the lambs of this flock are routinely separated from their dams on the day of lambing, and are moved to an artificial rearing unit where they receive a commercial milk replacer ad libitum until weaning at approximately 25 days of age. Following weaning, lambs have free access to concentrates, hay and water. Ewes are milked at 08:00 and 16:00 daily from the day of lambing until milk yield decreases to approximately 0.5 l/day.

2.3. Growth-promoting effects following administration of oPL and oGH

Sixty male lambs born in February 1995 and 36 male lambs born in March 1997 were used in two experiments. In each experiment, 2-month-old lambs were allocated to experimental groups according to their weight, post-weaning growth rate, litter size and age. The lambs were fed concentrates (16% crude
protein) ad libitum and hay at 0.1 kg/head/day. Lambs were treated daily at 10:00 for 35 days with saline solution containing 0.1 mg/kg body weight of oPL (oPL group) or oGH (oGH group), or received saline only (control group). Lambs were weighed at the beginning of the injection period and once a week thereafter until the end of the injection period. Group feed consumption was monitored daily. Blood samples for hormone assays were collected from the jugular vein on the morning time of day 34 of the injections, prior to the daily hormone administration, to obtain serum, which was stored at \(-20^\circ C\) until assayed.

2.4. Galactopoietic effects following administration of oPL and oGH

Forty-five multiparous ewes that lambed during 2 weeks in October 1997 and 143 multiparous ewes that lambed during 2 weeks in October 1998 were used in two experiments to determine daily milk production, recorded weekly from lambing. In each experiment, at about 8 weeks after lambing and when all ewes had passed their peak of lactation, they were assigned to three groups. Distribution of ewes into the different groups was according to their weight and their daily milk production averaged across the last three daily milk records. The ewes were treated daily at 17:00 for 14 days with saline solution containing 0.1 mg/kg body weight of either oPL (oPL group) or oGH (oGH group), or received saline only (control group). Milk production was monitored the day before the injections were begun (day 0) and then every 3 to 5 days, for up to 28 days. Body weight was recorded for all ewes before and at the end of the hormonal injection periods. Group feed consumption was monitored daily in the first experiment only. Blood samples were collected 10 to 11 days after initiation of hormone treatments at 10:00 to obtain serum for assay for concentrations of IGF-I, oGH and oPL.

2.5. Milk composition analysis

Milk composition was evaluated on day 13 of the injection period in the second experiment only. Concentrations of fat, protein, lactose and total solids in milk were measured using a semi-automated infrared analyzer (Milkoscan 134; N. Foss Electric, Hillerod, Denmark).

2.6. Radioimmunoassays

Serum concentrations of oGH (Peri et al., 1993), IGF-I (Breier et al., 1991) and oPL (Kann, 1971) were determined as described previously. Iodination of oGH and oPL was performed as described previously (Gertler et al., 1984). The inter- and intra-assay coefficients of variation were 12.4% and 8.7% for oGH, 10.4% and 6.2% for oPL, and 8.8% and 6.4% for oPL, respectively.

2.7. Data analyses

Lamb growth rates were calculated by fitting linear regressions to the body weight measurements and then subjecting them to analysis of variance. The model included the main fixed effects of experiment (1, 2) and experimental group (oPL, oGH and control). Lamb weight at the beginning of the experiment was used as a covariate. Results from each of the milk production experiments were analyzed separately. Data on milk production on each day of recording was analyzed by analysis of variance. The model included experimental group as the main effect, and milk production on day 0, days from lambing and ewe body weight at the beginning of the injection period were taken as covariates. Serum hormone concentrations and milk composition were subjected to analysis of variance to test for effects of treatment within day of sampling. Statistical analyses were conducted using the Statistical Analysis Institute (SAS) computer package (1985). All data are expressed as least squares means and standard errors. Differences of \( P < 0.05 \) were considered significant.

3. Results

3.1. Comparison of oGH and oPL effects in growing lambs

Least-squares means and levels of significance for growth rates, and oGH, oPL and IGF-I serum concentrations are presented in Table 1. Experimental group and initial weight of lambs had significant effects on growth rate. Both oGH and oPL had
Table 1
Growth performance, and oGH, oPL and IGF-I serum concentrations (LS mean±S.E.M.) of control lambs and lambs treated daily for 35 days with 0.1 mg/kg body weight of oPL or oGH

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Control</th>
<th>oPL</th>
<th>oGH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of lambs</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Initial body weight (kg)</td>
<td>23.5±0.6</td>
<td>23.2±0.6</td>
<td>24.1±0.6</td>
</tr>
<tr>
<td>Growth rate (kg/d)</td>
<td>0.48±0.01</td>
<td>0.53±0.01</td>
<td>0.53±0.01</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>7.5±1.0</td>
<td>8.1±1.0</td>
<td>12.2±1.2</td>
</tr>
<tr>
<td>PL (ng/ml)</td>
<td>N.D.</td>
<td>56.1±12.3</td>
<td>N.D.</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>304±23</td>
<td>321±25</td>
<td>466±35</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of lambs</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Initial average body weight (kg)</td>
<td>25.0±0.9</td>
<td>24.4±0.9</td>
<td>24.6±0.9</td>
</tr>
<tr>
<td>Growth rate (kg/d)</td>
<td>0.36±0.02</td>
<td>0.45±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>2.4±0.3</td>
<td>2.8±0.6</td>
<td>7.2±1.2</td>
</tr>
<tr>
<td>PL (ng/ml)</td>
<td>N.D.</td>
<td>58.5±15.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>310±15</td>
<td>308±20</td>
<td>365±16</td>
</tr>
</tbody>
</table>

1, a,b Within rows, means with different superscripts are different (P < 0.05).

2 Measured 34 days after the beginning of the injection periods.

N.D., Not detectable.

3.2. Comparison of oGH and oPL effects in lactating ewes

At 6 weeks after lambing, ewes reached their peak lactation at an average of 2.4 and 2.1 kg/d milk yield in experiments 1 and 2, respectively. The experiment was started 8 weeks after lambing when the body weight of ewes averaged 80±2.6 kg. Daily milk production prior to and during the experimental period is presented in Fig. 1. There were no pre-treatment differences in milk yield among the treatment groups for ewes in either experiment. For control ewes, milk production gradually decreased during the injection periods in both experiments. In contrast, ewes injected with oGH experienced a dramatic increase (P < 0.001) in daily milk production within 5 to 6 days after the first day of injection. The maximum percentage increase in milk production due to oGH was observed 10 days after the first injection, being 45 to 55% above controls. At that point, the absolute daily yield was higher than that at peak lactation prior to the oGH injections. Injection with oPL also increased milk yields in both experiments, but the effect was moderate. The maximum percentage increase above control ewes was observed 11 days after the first
Table 2
Milk yield and composition (LS mean ± S.E.M.) on the 13th day of the injection period of ewes belonging to the control, oPL and oGH groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Control</th>
<th>oPL</th>
<th>oGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ewes</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Milk yield (l)</td>
<td>1.91 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.97 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>17.1 ± 0.3</td>
<td>17.0 ± 0.2</td>
<td>16.4 ± 0.2</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>5.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>5.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.1 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>5.6 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>1</sup><sup>a</sup><sup>b</sup>Within rows, means with different superscripts are different (P < 0.05).

Fig. 1. Milk production in ewes treated daily for 14 days with 0.1 mg/kg body weight of oPL (triangles), oGH (open circles) or saline (squares). The values are given as LS means ± S.E.M. The numbers of ewes in each treatment were 15 in experiment 1 and 47–48 in experiment 2.

Injection (P < 0.01), being 25 and 20% in experiments 1 and 2, respectively. Upon cessation of the hormonal treatments, there was a decrease in milk production within 10 to 14 days and daily milk yields for ewes in the treatment groups were not different from those of the control ewes. IGF-I concentrations in control ewes on days 10 to 11 of the injection periods were 88 ± 11 and 126 ± 10 ng/ml in experiments 1 and 2, respectively. While oPL administration did not significantly change serum concentrations of IGF-I (101 ± 11 and 121 ± 9 ng/ml in experiments 1 and 2, respectively), concentrations of IGF-I in oGH-treated groups increased (P < 0.05) to 265 ± 11 and 294 ± 14 ng/ml in experiments 1 and 2, respectively.

Ovine GH and oPL serum concentrations were determined in ewes only in experiment 1, 10 to 11 days after the initiation of the injection periods. In the control and oPL groups, concentrations of oGH in serum were similar (2.4 ± 0.18 and 2.4 ± 0.28 ng/ml, respectively) and less (P < 0.01) than in ewes injected with oGH (3.4 ± 0.2 ng/ml). Ovine PL was not detectable in control ewes or ewes treated with oGH, but averaged 73 ± 11 ng/ml in ewes treated with oPL. Feed intake for the oGH and oPL-treated ewes averaged 3.6 and 3.8 kg DM/d, respectively, numerically higher than the feed intake in control ewes (3.1 kg DM/d).

Results on milk composition are summarized in Table 2. The increase (P < 0.05) in milk yield in the oPL and the oGH groups did not affect total solids or fat concentration. However, percent protein was lower (P < 0.05) in the oGH group and percent lactose was lower (P < 0.05) in both the oPL and oGH groups.

4. Discussion

In this study, we have compared for the first time the in vivo somatogenic and lactogenic activities of oGH and oPL in a homologous system. Our results indicate that the growth-promoting activity of oPL in lambs is similar to that of oGH. However, the galactopoietic effects of oPL in ewes were significantly less than for oGH. The mechanism(s) responsible for the stimulatory effects of oPL on growth and milk production may differ from those of oGH, as only oGH treatment resulted in an increase in serum IGF-I concentrations.

In the present study, oPL and oGH were found to have a similar growth-promoting effect. To our knowledge, a similar homologous comparison of the somatogenic activity of bGH and bPL has not been reported. However, in a non-homologous system,
bGH, but not bPL, stimulated growth of lambs (McLaughlin et al., 1993). The growth-promoting effects of oPL in our study (10–25%), obtained by treating 2-month-old lambs for 35 days, are similar to the 12% increase in growth rate of 3-day-old lambs following treatment with oPL for 21 days (Min et al., 1996). In contrast, Ogawa et al. (1995) saw no somatogenic and anticatabolic effects of oPL following daily injection of 2-month-old lambs for 5 days. Thus, collectively, these results indicate that the somatogenic effect of PL (as compared with that of GH) may depend on the age of the lambs, the duration of the injection period and the homology of the experimental system.

In two experiments conducted with lactating ewes injected for 14 days, both oGH and oPL had galactopoietic activity (Fig. 1), with oGH being consistently much more potent. Similar results showing positive but different galactopoietic effects of bGH and bPL were also reported by Byatt et al. (1992a) in dairy cows. In addition, we recently compared in vivo effects of oGH and oPL and determined that both hormones exhibit mammogenic activity in pseudo-pregnant ewes (Kann et al., 1999).

Our results concerning the galactopoietic activity of oPL contradicts the results of Min et al. (1995), who reported no galactopoietic effect following five consecutive daily injections of lactating ewes with oPL. The differences between the two studies may be related to the short duration of the oPL treatment in the Min et al. (1995) study. As shown in Fig. 1, the development of the oPL galactopoetic effect in our study was relatively slow and, although evident in both experiments after 5 days, it was only significant in experiment 1. The effect of oPL became significant only after 10 days in experiment 2.

The strong galactopoietic effect of oGH in the present study (45–55% above control) resembles the effect of bGH in sheep (Fernandez et al., 1995; Chiofalo et al., 1999) and was much higher than the typical effects of bGH in cows (15 to 25%; Bauman and Vernon, 1993). While different biological potencies of the bGH and oGH preparations may account for some differences in biological effects, an alternative explanation may be related to the fact that dairy sheep have not undergone the same extensive genetic selection for high milk production as dairy cows. Thus, the high response to the oGH in ewes may reflect the difference between the actual levels of production and the physiological potential for production. Interestingly, injection of bGH into lactating goats has also increased milk production by 50–60% (Jammes et al., 1996).

It should be emphasized that the in vivo somatogenic and lactogenic effects of oPL in the present study occurred during physiological states of growth and lactation when oPL is not naturally present. Thus, our results advance our understanding of the biological potency of oPL, but its physiological role during pregnancy needs to be investigated further.

In both the growth and milk production experiments, administration of oGH but not oPL increased IGF-I serum concentrations. The fact that oPL administration does not increase circulating blood concentrations of IGF-I was also reported in other studies (Ogawa et al., 1995; Min et al., 1996). Collectively, these results raise the question as to the way oPL effect is transduced, as it appears to be different from that of oGH. One unique property of ruminant PL, which was observed early on, is their ability to bind to both prolactin (PRL) and GH receptors (Forsyth, 1986; Anthony et al., 1995a,b). Thus, several alternatives concerning the mechanism of oPL action may be considered: (a) the PL signal is transduced through homologous PRL receptors; (b) PL is capable of heterodimerization with the homologous GH receptor through site I and the PRL receptor through site II; (c) a unique unidentified PL receptor exists but has not yet been identified; and (d) an unknown variant(s) of GH receptor, mutated at the extracellular domain allows dimerization of GH receptor by PL and this variant GH receptor is expressed in specific tissues or under unique physiological conditions. Recently, we (Herman et al., 2000) have shown that oPL heterodimerizes the extracellular domains (ECDs) of ruminant GH receptor (GHR) and PRL receptor (PRLR). We also showed that PL or PL analogues that exhibit little or no activity in cells transfected with PRLR and no activity in cells transfected with oGHR, do exhibit largely enhanced activity in cells co-transfected with both PRLR and GHR. Those results may provide
new ideas on the oPL specific mechanism of action, which could explain the similar but different oGH and oPL activity in the present study.

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