Growth-promoting and metabolic actions of recombinant ovine placental lactogen and bovine growth hormone in young lambs

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ABSTRACT

It has been shown previously that ovine placental lactogen (oPL) has a growth-promoting effect in the growth hormone (GH)-deficient dwarf rat. However, this effect has not been verified in an homologous system using animals with an intact somatotrophic axis. To examine the effects of oPL in the sheep, we injected lambs with recombinant oPL (0.1 mg/kg liveweight/day, n=16) or saline (n=16) for 21 days commencing on day 3 of life and compared their effects on body growth and energy intake with those in lambs treated with the same dose of recombinant bovine growth hormone (bGH, n=16). Circulating concentrations (ng/ml) of hormones in saline- vs bGH- vs oPL-treated lambs at day 20 of treatment were (mean±SE): oPL, <0.5 vs <0.5 vs 12.8±0.5; GH, 10.8±5.0 vs 48.4±5.0 vs 12.6±5.0, $P<0.001$; insulin-like growth factor-I (IGF-I), 267.6±14.4 vs 302.4±14.2 vs 291.7±14.4. Average daily gain (kg/day) during treatment was significantly ($P<0.05$) greater in oPL-treated lambs (0.28±0.01) than in saline-treated (0.25±0.01) or bGH-treated (0.24±0.01) lambs. Similarly, there were significant ($P<0.05$) increases in total energy intake (over the 21 day treatment period) during treatment with oPL (112.5±3.5 MJ ME), compared with bGH (102.0±3.5 MJ ME) or saline (102.0±3.5 MJ ME) treatment. It is concluded that oPL is somatogenic in young lambs. This effect may be mediated by stimulating voluntary feed intake rather than by elevating circulating concentrations of IGF-I.

Keywords: Ovine placental lactogen; bovine growth hormone; lambs; growth; voluntary intake.

INTRODUCTION

Ovine placental lactogen (oPL), a member of the growth hormone (GH)/prolactin (PRL) family, is a 198 amino acid protein (Colosi *et al.*, 1989) produced by the binucleate cells of the chorionic epithelium (Kappes *et al.*, 1992). Recent sequencing studies have shown that oPL has a higher degree of homology with ovine PRL (49 %) than with ovine or other species of GH (25-28 %) (Colosi *et al.*, 1989; Warren *et al.*, 1990).

Despite its low homology with oGH, oPL displays somatogenic activity. Administration of partially purified or recombinant oPL stimulates IGF-I production (Hurley *et al.*, 1977; Singh *et al.*, 1992) and weight gain in hypophysectomized and growth hormone-deficient dwarf rats with a similar or superior potency to bGH (Chan *et al.*, 1976; Singh *et al.*, 1992). Similar effects have been also reported with highly purified bovine PL (bPL) (Byatt *et al.*, 1991).

Although the above studies clearly demonstrate that oPL has somatogenic activities, these results have not been verified in an homologous system. In this paper, we report the first study to examine the effects on body growth and composition of recombinant oPL administered to milk-fed lambs.

MATERIALS AND METHODS

Animals and Treatment

Forty-eight twin-born Coopworth x (Border Leicester x Romney) lambs were used in the trial. All lambs were born to ewes at pasture over a 9 day period and remained with their dams until day 3 of life to ensure adequate intakes of colos-

trum. On day 3 of life the lambs were permanently separated from their dams, penned individually on slatted floors and assigned to one of three groups, each of 16 lambs, balanced for age and sex. Over the next three weeks, commencing on the evening of their arrival in the pens, one group of lambs was injected subcutaneously with recombinantly-derived oPL (0.10 mg/kg LW/day; Lot #M3RD86, Genentech, South San Francisco, CA, USA), one group with recombinantly-derived bovine growth hormone (bGH) (0.10 mg/kg LW/day; Lot 7368c-69Q, American Cyanamid, Princeton, NJ, USA) and the control group with sterile physiological saline (0.10 ml/kg LW/day). Subcutaneous injections were alternated between the right and left sides of the neck and administered twice daily, at 0800 and 1600h. oPL was dissolved at a concentration of 1 mg/ml in phosphate buffered saline (pH 7.6) while bGH was dissolved as a 1 mg/ml solution in carbonate buffered saline (pH 9.4). The solutions for injection were prepared fresh each 3-7 days and held at 4°C. Injection volumes were adjusted to a new liveweight every 5 days following reweighing of the animals.

During the three week treatment period the lambs were individually fed *ad libitum* a mixture of ovine and bovine milk. Milk was bottle-fed four times daily (at 0700, 1030, 1400 and 1700h) and was warmed to 38°C prior to each feeding. A few days after the commencement of bottle feeding 5 lambs (distributed across groups) developed mild scouring. Therefore, Scourfix (Vetchem Laboratories Ltd, East Tamaki, Auckland, NZ) was added (1.5 ml/litre) to all milk and lambs were restricted to a maximum of 500 ml milk at the first feeding time (0700h). The ratio of ovine to bovine milk was 1:0 at days 3 to 5 of life, 7:3 at

days 6 to 7, 6:4 at days 8 to 12, 5:5 at days 13 to 17, and 3:7 at days 18 to 23 of life. The fat, protein and lactose content of milk was measured by using a Milkoscan 104 semiautomatic infrared analyzer (A/S N. Foss Electric, Denmark).

Blood Sampling and Hormone Analyses

Blood was taken from each lamb by jugular venipuncture at 0800, 1100 and 1500h on day 20 of treatment (day 23 of life). Samples (5 ml) were withdrawn into vacutainers (Nipro Medical Industries, Japan) containing EDTA as the anticoagulant and immediately placed on ice. Within 20 min the samples were centrifuged at 3000 g and 4°C for 20 min. Plasma was pipetted into duplicate vials and stored at -20°C for later analysis.

Placental lactogen concentrations in plasma were measured by homologous radioimmunoassay (Oliver *et al.*, 1992), using recombinant oPL (Lot # M3RD78) as standard. The minimal detectable dose was 0.05 ng/tube, half-maximal displacement was achieved at 0.6 ng/tube and intra- and inter-assay coefficients of variation were 6.1% and 8.7% respectively.

Growth hormone concentrations were measured using the double antibody radioimmunoassay described previously (Flux *et al.* 1984). The GH assay used bovine GH for iodination (USDA - bGH - I1, 3.2 IU/mg) and reference standards (USDA - bGH - B1, 1.9 IU/mg). Intra- and inter- assay coefficients of variation were 8.6 and 13.2% respectively.

Plasma IGF-I concentration was measured by RIA using a rabbit antiserum to recombinant human IGF-I (878/4) at a final titer of 1:250,000 (Breier *et al.*, 1991a). The antiserum has a cross-reaction with IGF-II of less than 0.05%, a minimum detectable dose of 0.06 ng/tube, and a half displacement dose of 0.30 ng/tube. Before RIA, plasma samples were subjected to acid-ethanol extraction with an additional cryo-precipitation step. The intra- and inter- assay coefficients of variation for IGF-I were 5.0 and 9.8%, respectively. IGF-I concentrations are expressed in terms of the international reference recombinant human IGF-I preparation 87/518 (National Institute for Biological Standards and Control, Potters Bar, Herts, U.K.).

Statistical Analyses

Data arising from repeated measurements on animals (liveweight and energy intake) were subjected to multivariate (repeated measures) analysis of variance to test the effects of treatment, time (repeated factor) and their interaction. Plasma hormone concentrations measured on three occasions on day 20 were averaged because there were no significant interactions between treatment and time (within a day). Prior to analysis, intakes of milk were converted to total metabolisable energy (ME) intakes based on assumed ME contents of fat(39.2 MJ/kg), protein (24.4 MJ/kg) and lactose (16.5 MJ/kg), respectively (Holmes and Wilson, 1987). All data are expressed as least squares means and standard errors. Statistical analyses were conducted using the computer package 'SAS' (1986).

RESULTS

Plasma Hormone Concentrations

Concentrations of oPL in plasma on day 20 of treatment were below the assay detection limit in the bGH- and

saline-treated groups, whereas oPL treatment increased circulating concentrations of oPL to 12.8±0.5 ng/ml (Table 1). Plasma concentrations of GH were increased four-fold (P<0.05) by bGH treatment compared with the saline or oPL groups. Treatment of lambs with bGH or oPL produced a small but non-significant elevation of circulating IGF-I concentrations on day 20 of treatment.

TABLE 1: Plasma hormone concentrations (ng/ml) on day 20 of treatment in lambs treated with saline, recombinant bGH (0.1 mg/kg/day) or recombinant oPL (0.1 mg/kg/day) from day 3 to 24 of life (mean±SE).

Hormone	Saline	bGH	oPL
n	16	16	16
PL	<0.5	<0.5	12.8±0.5
GH	10.8±5.0[a]	48.4±5.0[b]	12.6±0.5[a]
IGF-I	267.6±14.4	302.4±14.2	291.7±14.4

[a, b] Means with different superscripts are significantly different (P<0.05)

Liveweight gain, Feed Intake and Feed Conversion Ratio

Liveweights of oPL-treated lambs progressively diverged from those of the other treatment groups (Figure 1), as indicated by a significant (P<0.01) treatment x time interaction. Average daily gain during the three weeks of treatment was significantly (P<0.05) greater in oPL-treated lambs than in saline-treated or bST-treated lambs (Table 2).

TABLE 2: Average daily gain, energy intake and feed energy/gain ratios in lambs treated with saline, recombinant bGH (0.1 mg/kg/day) or recombinant oPL (0.1 mg/kg/day) from day 3 to 24 of life (mean±SE).

| Hormone	Saline	bGH	oPL
n	16	16	16
Daily gain (kg/day)	0.25±0.01[a]	0.24±0.01[a]	0.28±0.01[b]
Energy intake (MJ ME/day)	4.9±0.2[a]	4.9±0.2[a]	5.4±0.2[b]
Ratio (MJ ME/kg)	19.8±0.5	20.0±0.5	19.5±0.5

[a, b] Means with different superscripts are significantly different (P<0.05)

The increased rate of gain observed in the oPL group was accompanied by an increased energy intake (Figure 1). Daily energy intakes during treatment of oPL-treated lambs were significantly (P<0.05) greater than those of saline-treated or bST-treated lambs (Table 2). This increased energy intake with oPL treatment led to feed energy/gain ratios similar to those with saline or bGH treatments.

DISCUSSION

It has been reported previously that recombinant oPL has a potent growth-promoting effect in GH-deficient dwarf rats (Singh *et al.*, 1992). However, there is some evidence that the biological activities of oPL could vary depending on the animal species studied. For example, oPL is equipotent to PRL and GH in stimulating lactogenic activity in the mammary gland of the rabbit (Servely *et al.*, 1983), but possesses low lactogenic activity in sheep mammary gland (Servely *et al.*, 1983; Emane *et al.*, 1986). It is well documented that

FIGURE 1: Effects of saline (●), bovine growth hormone (▲) and ovine placental lactogen (◆) on live weight and metabolisable energy intake of milk-fed lambs. Each point represents the mean of 16 observations. Vertical bars represent the pooled standard error of the mean.
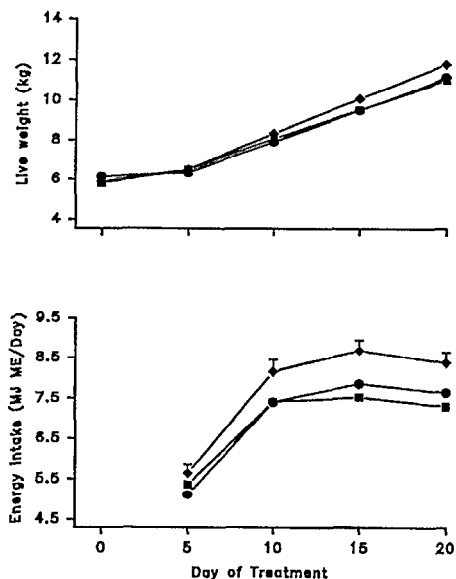
bGH has somatogenic activities in ruminants, including sheep (Enright, 1989). Thus, the objective of this study was to investigate whether the somatogenic activity of oPL observed in dwarf rats also occurs in intact animals of the homologous species (ovine) and to compare its effects on body growth with those of bGH.

Pell *et al.* (1990) have shown that treatment of lambs with bGH from 9 to 19 weeks of age significantly increased growth rate and lean tissue content, but such effects are not always evident (Pell *et al.*, 1987; Sun *et al.*, 1992). Our results are consistent with the latter studies since they showed no effects of exogenous bGH on growth rate of milk-fed lambs. In contrast, oPL treatment significantly increased the live weights of lambs from day 10 after commencement of treatment. This finding confirms a previous report on somatogenic effects of recombinant oPL in a heterologous (dwarf rat) system (Singh *et al.*, 1992) and provides the first evidence that oPL also has growth-promoting effects in the ovine.

The mechanism by which oPL stimulates body growth is unclear. In this study, bGH and oPL treatment both elevated circulating concentrations of IGF-I in young lambs although such effects were small and non-significant. Although previous reports indicate that serum and hepatic tissue concentrations of IGF-I are regulated in part by GH and that GH binding to the hepatic GH receptor increases markedly at birth (Breier *et al.*, 1991b), treatment of young lambs with 0.1 mg/kg/d bGH or oPL in this study invoked a much smaller increase in circulating IGF-I concentrations (24-34 ng/ml or 9-13 % relative to saline-treated lambs) than did treatment of pregnant ewes with 0.15 mg/kg/d of the same bGH preparation (ca. 200 ng/ml or 130%) in our previous study (Min *et al.*, 1994). Thus changes in circulating IGF-I concentrations cannot account for the growth-promoting effects of oPL in these lambs.

It is well documented that bGH treatment increases voluntary feed intake in sheep and cattle, but only after

several weeks of continuous treatment (Bauman *et al.*, 1985; Sandles *et al.*, 1988). In contrast, administration of recombinant bPL stimulates feed intake in dairy cattle within 9 days after commencement of treatment (Byatt *et al.*, 1992). Such acute increases in feed intake have also been observed in rats treated with highly purified bPL (Byatt *et al.*, 1991), indicating that there are marked differences between bGH and bPL in their effects on voluntary feed intake. The present results showed that treatment with oPL, but not bGH, significantly increased feed intake in milk-fed lambs from 10 days after commencement of treatment. Thus, our data are consistent with previous reports and suggest that oPL, like bPL, stimulates energy intake. It has been suggested previously that the higher feed intakes of bPL-treated rats are partly mediated through the PRL receptor (Byatt *et al.*, 1991) since administration of oPRL stimulates feed intake in mature female rats (Gerardo-Gettens *et al.*, 1989). However, it seems unlikely that the effect of oPL is similarly mediated. oPL binds to the GH receptor with a high affinity, but not to the PRL receptor, in hepatic tissue from lactating ewes (Emane *et al.*, 1986). Furthermore, administration of exogenous PRL to lactating goats and dairy cows failed to alter feed intake (Plaut *et al.*, 1987; Jacquemet and Prigge, 1990). It thus seems likely that oPL exerts direct effects on voluntary intake. This effect on voluntary intake, rather than changes in circulating IGF-I concentrations, may be responsible for the growth-promoting actions of oPL.

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