Human placental lactogen inhibits growth without changing serum levels of IGF-1 in rats: an apparent specific action of the hormone

Mimi H Chiang and Charles S Nicoll
Department of Integrative Biology, Cancer Research Laboratory and Graduate Group in Endocrinology, University of California, Berkeley, CA 94720, USA

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Previous work in our laboratory has shown that the internal environment of rats has reduced growth-promoting activity during the second half of gestation and this condition is associated with resistance to the anabolic effects of GH. The placenta appears to be responsible for this condition but injections of estradiol plus progesterone into virgin females did not mimic it. Accordingly, it seemed worthwhile to test the effects of a placental lactogen (PL) for possible growth inhibitory effects. In the present study the effects of human (h)PL on skeletal growth in young female rats and on the growth of embryonic tissue transplants under their kidney capsules were investigated. Human (h) and bovine (b) GH, and ovine prolactin (oPRL) were also tested to determine whether the results obtained with hPL were specific. Twice daily subcutaneous injections of a high dose of hPL (10 mg/day), but not oPRL (5 mg/day) for 7 days inhibited both host tail growth and tibial epiphyseal plate width, and growth of whole 10-day embryo transplants. Injections of hGH at 1 mg/day for 8 days significantly increased host skeletal growth and growth of 12-day embryonic head transplants: at the same dose, neither bGH nor oPRL affected growth of the embryonic heads or of the host tibial epiphyseal plate width, but the bGH increased host tail growth. By contrast, the 1 mg/day dose of hPL significantly reduced the host’s tibial epiphyseal plate width, tail growth, and transplant growth: lower doses of hPL (10 and 100 μg/day) were also inhibitory. Although all the hormone treatments increased total serum IGF-1 levels in the females, none of them had a significant effect when compared to saline injected control animals. Thus, the growth-inhibitory effects of hPL treatment appear to be specific to that hormone and they are not mediated by depression of serum IGF-1 levels. If these effects of hPL are mimicked by one or more of the rodent PLs, then the reduced growth-promoting activity and resistance to GH action that occurs in pregnant rats could be due to the rat PLs. These results indicate that in addition to having glucose-sparing effects in the mother, PLs could promote fetal growth by inhibiting growth of maternal tissues, which would thus spare other metabolites, such as amino acids and vitamins, for the conceptus.

Charles S Nicoll, Department of Integrative Biology, LSA 281, University of California, Berkeley, CA 94720, USA

Human placental lactogen (hPL) serves several important functions in pregnant women. Its diabetogenic action in the mother spares glucose for utilization by the conceptus (1), and it may interact with other hormones to promote the breast growth that occurs during pregnancy (2). Some evidence indicates that hPL may also function as a growth-promoting hormone, at least for the conceptus. Infusion of the hormone into pregnant rats between days 14 and 21 increased the weight of their fetuses on day 19 of gestation (3), and it increased DNA synthesis in human fetal cells in culture (4). In addition, hPL has weak GH-like effects in hypophysectomized rats (5, 6) and in hypotuitary and diabetic humans (7). These latter effects led to the proposal that hPL should be named human chorionic somatomammothropin (hCS) by Grumbach and Kaplan (5).

Although rodent PLs do not have significant GH-like activity (8), the idea that hPL functions as a gestational growth-promoting hormone received support from studies with ovine (o) and bovine (b) PL, which have appreciable GH-like effects (9–11). Other evidence indicates that the liver of fetal humans and sheep contains specific receptors for hPL and oPL, respectively (12, 13). Such receptors may mediate the GH-like effects of these hormones.

Bioassay and radioreceptor assay studies indicate that the internal milieu of pregnant mammals has increased GH-like activity (14, 15). These observations are consistent with the idea that PLs can mimic GH in the maternal compartment. However, when the internal milieu of late pregnant and lactating rats was evaluated by several indices, it was found to have reduced growth-promoting properties relative to those in non-pregnant females or in the first half of gestation (16, 17). The lowered growth-promoting properties of the second half of gestation in the rat were accompanied by a significant reduction in
serum levels of IGF-1, and a substantial decline in the responsiveness of maternal tissues to the somatotrophic action of GH (17). Other investigators have also reported that serum IGF-1 levels decline in pregnant rats (18–20). However, during lactation, serum IGF-1 returned to about prepregnancy levels (17, 18) despite the persistence of reduced growth-supporting activity of the internal milieu of the females (17).

Cooke et al. (16) showed that the reduced growth-promoting activity of the internal environment of female rats during the second half of gestation could be due to the placenta. They transplanted placentas or injected extracts of the organ into young females and showed clear growth-inhibitory effects (16). Injections of estradiol plus progesterone did not have such effects. Accordingly, another placental factor must be responsible for the growth inhibition. As PL is one of the major hormonal products of the placenta, it seemed worthwhile to test it for possible growth-inhibitory effects, despite the evidence that it may have growth-promoting activities, at least in the fetus (3, 21, 22).

In the present study, we determined the effects of injecting hPL on the growth of rapidly growing young virgin female rats, and of transplanted rat embryos grown on their kidneys. The embryo transplants were used as they grow rapidly and are sensitive to changes in the internal environment of the hosts (17, 23). Surprisingly to us, clear growth-inhibitory effects of hPL were observed, even at relatively low doses. In addition, this effect appears to be specific for that placental hormone.

Materials and methods

Virgin female rats of the Long-Evans strain 8 to 9 weeks of age, weighing 180–200 g (from our own colony) or 5 to 6 weeks of age, weighing 130–150 g (Simonsen Laboratories, Gilroy, CA), were used. Fisher 344 rats, 6 weeks of age and weighing 140–160 g (Simonsen Laboratories), were also used in one experiment because suitable Long-Evans rats were not available. The animals were maintained as described previously (17). All procedures used on the rats were described in detail in a protocol that was approved by our institutional Animal Care and Use Committee, and all experiments conformed to the regulations described in the NIH Guide to the Care and Use of Laboratory Animals.

Embryo transplants

In the first experiment, whole 10-day embryos were transplanted as described by Liu et al. (23). In two other studies, heads from 12-day embryos were transplanted because they were easier to handle than the 10-day embryos. Donor pregnant rats were anesthetized on day 12 of pregnancy and the embryos were removed and decapitated by a cut at the base of the head and the trunk. In one experiment, some trunk tissue was transplanted along with the head. On the day of surgery, the host rats were anesthetized, body weight (BW) and tail length (TL) were recorded, then two transplants were placed under the capsule of both of their kidneys.

Hormone treatments

The hormones were dissolved in a small volume of 0.01 N NaOH, then diluted in phosphate-buffered saline (PBS) to give the desired dose per day in two sc injections of 0.5 ml each. The hormone solutions were stored at 4°C and fresh solutions were made every three days. Control hosts received sc injections of PBS.
Hormone treatments of the hosts began on the day of surgery. In the first experiment, the effects of hPL (NIH C.8 B6) at 10 mg/day, or of oPRL (NIDDK-oPRL-18) at 5 mg/day, were tested on the 180–200 g BW Long-Evans rats. This high dose of hPL was selected for two reasons. First, we did not expect the hormone to have any effects as it generally has a low degree of activity compared to PRLs and GHs. The second was based on the estimated secretion rate of hPL of about 2 mg/100 g BW per day in late pregnant women (24). In the rats used in our first study, this secretion rate would equal about 4 mg/rat per day. As hPL may be less potent than rat (r)PRL in rats, the 10 mg/day dose of hPL was tested initially. The duration of treatment was for seven days.

Lower doses of hPL (radioiodination grade, NIDDK) ranging from 10 μg to 1 mg/day and of hGH (NIH AFP-9755A), bGH (USDA B1) or oPRL (NIH-19), all at 1 mg/day, were also tested, but in the younger females of either the Long-Evans or the Fisher 344 strains. Their treatment period was for eight days.

About 16 h after the last injection, the hosts were again anesthetized, BW and TL were remeasured, and blood samples were taken by cardiac puncture. Serum samples were stored at −20°C until they were assayed for IGF-1 levels by radioimmunoassay (RIA) using a modified acid-ethanol extraction procedure which gave results similar to those obtained with acid-ethanol gel filtration, as described (17). The transplanted whole embryos or heads were removed from the kidneys and weighed to the nearest 0.1 mg. Host tibiae were removed, the proximal ends were split longitudinally using a razor blade, and they were stored in acetone until they were stained with AgNO₃ (26). The width of each epiphysial cartilage plate (TEPW) was determined with an optical micrometer, with at least 40 measurements per bone.

**Data presentation and statistical analysis**

The data are presented as the means±SEM and the significance of differences was determined by the Student’s t-test, or by analysis of variance using Duncan’s Multiple Range test (27), as appropriate.

**Results**

Fig. 1 shows that after seven days of treatment, the oPRL injections (at 5 mg/day) had no effect on the growth of the whole embryo transplants or on the two growth indices in the hosts. However, the final wet weight of transplants in hPL-treated hosts (at 10 mg/day) was reduced by 35% compared to the control transplants. Furthermore, the hPL-treated hosts had TEPWs and tail growth that were reduced by 20% (p<0.01) and 36% (p<0.05), respectively.

The effects of hPL and the other hormones (hGH, bGH and oPRL) at a dose of 1 mg/day are compared in Fig. 2. Because the amount of embryonic tissue that was transplanted differed among the experimental groups, the data on transplant growth for each treatment are expressed as a percentage of their own controls, which were size matched. Human GH significantly increased the growth of the transplants and the host TEPW and tail length (p<0.01); the latter effect was particularly striking. Neither bGH nor oPRL affected growth of the embryos or of the host TEPW, but the bGH increased tail growth significantly (p<0.01). By contrast, although the 1.0 mg/day dose of hPL had only slight but significant inhibitory effects on the hosts’ tail growth and TEPW (p<0.05), it had a strikingly suppressive effect (−40%) on growth of the embryo transplants (p<0.01).
Discussion

The results presented in this report show that systemic administration of hPL inhibits skeletal growth in rapidly growing virgin female rats, but it had no effect on their body weight gain. Growth of embryonic transplants in these hosts was also depressed and by an amount similar to that observed with fetal paws or whole 10-day embryo transplants in late pregnant and lactating hosts (16, 17). Injections of hGH increased embryo transplant, host tail and host epiphyseal plate growth. The rats given bGH injections showed only an increase in tail growth and oPRL had no effect on any of the growth parameters. Thus, among the members of the PRL-GH-PL family of hormones tested, the growth inhibition observed appears to be a specific action of hPL.

These results with hPL, in conjunction with previous data obtained in our laboratory, suggest that a PL from the rat placenta could be responsible for the growth inhibition in late pregnant females (16, 17). Using the same transplantation system, Cooke et al. (16) showed that growth of fetal tissue transplants was inhibited in hosts bearing placental co-transplants, or in hosts injected with placental extract. Of the two known rat PLs, hPL is structurally more similar to rat placental lactogen II (rPL-II), and hPL has biological activities in rats and mice that are qualitatively similar to those of the rodent PLs (8). Therefore, hPL and rPLII may serve similar physiological functions during late pregnancy.

Since maternal serum IGF-1 levels decline dramatically during late gestation in the rat (17–20), we expected that the growth-inhibitory effects of PL would be mediated by depression of this growth-promoting factor. However, administration of hPL did not alter total IGF-1 levels in the treated rats (Table 1), even though growth of the host and the embryo transplants was reduced. Thus, the depressed serum level of IGF-1 that occurs in late pregnancy (17–20) may be due to a placental factor other than PL.

Fig. 3. The effects of twice daily subcutaneous injections of hPL at 10 and 100 µg/day on the growth of host skeletal structures and transplanted 12-day embryo heads in female Fisher 344 host rats over an eight-day period. Otherwise, as in Fig. 1. **p < 0.01.

Table 1. Total serum IGF-1 levels in female Long-Evans rats treated with 10 mg/day hPL or 1 mg/day hPL, hGH, bGH or oPRL, and in female Fisher 344 rats treated with 10 and 100 µg/day of hPL.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Hormone dose (mg/day)</th>
<th>N</th>
<th>Serum IGF-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Control</td>
<td>—</td>
<td>10</td>
<td>937 ± 49</td>
</tr>
<tr>
<td>*hPL</td>
<td>10</td>
<td>5</td>
<td>1213 ± 91</td>
</tr>
<tr>
<td>*hPL</td>
<td>1</td>
<td>9</td>
<td>1066 ± 64</td>
</tr>
<tr>
<td>*hGH</td>
<td>1</td>
<td>10</td>
<td>1081 ± 57</td>
</tr>
<tr>
<td>*bGH</td>
<td>1</td>
<td>5</td>
<td>1026 ± 74</td>
</tr>
<tr>
<td>*oPRL</td>
<td>1</td>
<td>7</td>
<td>1103 ± 92</td>
</tr>
</tbody>
</table>

** Control — 10 | 875 ± 58
** hPL — 0.1 | 6 | 903 ± 56
** hPL — 0.01 | 6 | 808 ± 49

* All treatments were for 8 days except for the 10 mg/day dose of hPL, which was for 7 days.
* Long-Evans rats; ** Fisher 344 rats.
Metabolic studies with hPL have shown that it reduces maternal glucose utilization and increases the catabolism of triglycerides while promoting maternal utilization of free fatty acids (1, 28). This glucose-sparing effect provides increased energy sources for the conceptus, and thus promotes fetal growth. Collins et al. (3) suggest that hPL could also function as a fetal growth hormone by acting either directly or indirectly, through stimulation of IGF secretion. The anabolic effects of hPL on fetal tissue (10, 29) contribute to the idea that the PLs may act as fetal growth-promoting hormones. Furthermore, hPL promotes DNA synthesis in cultured human fetal cells (4) and it has some growth-promoting activity in rats (6, 30) and humans (7). However, these positive effects with hPL were obtained in systems in which growth was retarded. The culture experiments measured proliferation of cells grown in low serum conditions (4) and the in vivo studies involved hypophysectomized rats (6, 30) or either hypopituitary or diabetic humans (8). In addition, the growth-stimulatory effects of hPL in vivo were meagre in comparison to responses obtained with hGH or other GHs (7). Furthermore, there are in vitro reports of no anabolic effects of hPL on fetal rat diaphragm (10) and hepatocytes (31).

In our previous studies on the growth of fetal and embryo transplants in pregnant rats, clear inhibition of growth was observed during the second half of gestation, while the fetuses in the uterus of these hosts grew rapidly (16, 17). These results indicate that the placenta protects the conceptus from the anti-anabolic actions of PL (or other placental factors) in the maternal compartment. It is also possible that hPL has growth-promoting effects on the fetus in utero, even though we found it to be growth-inhibiting on fetal transplants in the extrauterine compartment.

In an earlier study, Josimovich (32) reported that injections of high doses of hPL could augment the growth-promoting and diabetogenic actions of low doses of hGH in hypophysectomized rats. These results would appear to contradict our findings of a growth-inhibitory effect of hPL. However, the preparation of hPL used by Josimovich (32) was probably less pure than those currently available. The earlier preparations may have been contaminated by the placental hGH variant (33), which would account for some of the apparent synergism. In addition, as Josimovich (32) himself suggested, the high doses of hPL may have reduced the clearance rate of the injected hGH and thus, augmented its effects.

There are several other proteins in the PRL-GH-PL family of hormones that are produced by the placenta, the functions of which are currently being investigated. Amongst these are proliferin (34) variant forms of GH (33, 35, 36) and PL (37–39). Of particular interest here is the hGH-variant (hGH-V) that differs in amino acid sequence from pituitary hGH at 13 residues (40). Serum levels of hGH-V increase from mid-gestation to term (41), following a pattern similar to that of hPL (42). As placental hGH-V, like pituitary hGH, has significant somatotropic and lactogenic activities (43), it was suggested that it may be responsible for the elevation of serum IGF-1 levels in pituitary GH-deficient women (35). It may also be responsible for the rise in serum IGF-1 levels that occurs in late gestation in normal women (35, 44, 45). Because of these various placental hormones, it is difficult to directly compare the results obtained in our experiments with hPL in virgin female rats, with events occurring in pregnant women or in other mammals.

The well characterized ungulate PLs have biological properties more similar to those of hGH-V (i.e. lactogenic and somatotrophic), than to those of hPL, which is essentially devoid of growth-promoting activity, even though it is diabetogenic (1, 8, 28). It will be of interest to learn whether any of the biologically uncharacterized variant forms of ungulate PL prove to be functionally more like hPL than hGH-V in having growth-inhibitory activity. Although we did not expect to observe growth inhibition when we began these experiments with hPL, in retrospect this finding should not be surprising. The hormone has been shown to antagonize the actions of insulin in humans and animals (1, 8, 28), and it is well established that insulin is necessary for normal growth both pre- and post-natally (46). This effect would be important in the mother as it would reduce maternal use of substrates in addition to glucose that are needed for fetal anabolism, such as amino acids and vitamins.

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