Normal food intake and growth in hyperprolactinemic rats

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THE RECENT REPORT of Gerardo-Gettens et al. (24) that ovine prolactin (PRL) administration augmented food intake in rats followed another study (30) by the same group in which chronically hyperprolactinemic rats were found to eat more and grow heavier than control animals. The latter study utilized the nonhypophysectomized anterior pituitary-grafted rat model of chronic PRL excess. This model, as recently reviewed (1), is widely utilized partly because it has been reported to grow normally (10, 18, 19). The surprising findings of Gerardo-Gettens and colleagues (24, 30) prompted our analysis of food intake and weight gain in animals utilized for several different experiments. Other aspects of these studies have been published previously (4, 6, 7, 17).

METHODS

Pituitary-grafted rats. Litters of weanling Fischer 344 rats (Hilltop Lab Animals, Scottdale, PA) were used for these experiments. In each of several litters, four rats of the same sex and approximately equal weight were chosen to serve as hosts. Two of the four animals had three anterior pituitary glands from littermates placed under the kidney capsule. The two additional rats received littermate muscle tissue under the kidney capsule and served as controls. Surgery was performed under anesthesia with ketamine and pentobarbital sodium (1). Because it has been reported that pituitary-grafted animals have increased corticosterone secretion (2, 29), in some experiments animals were adrenalectomized at the time of pituitary or muscle implantation. These rats were then maintained with pellets of corticosterone and fludrocortisone (Innovative Research of America, Toledo, OH) at doses calculated to approximate physiological levels. The animals were killed 62 days later, at 102 days of age. Pituitary and muscle-grafted rats that were similarly prepared but not adrenalectomized were also studied. To determine the possible effect of PRL-induced hypogonadism on food ingestion, some animals were ovariec-tomized ~1 mo after pituitary or muscle implantation. Half of the gonadectomized animals received estrogen in the form of replacement subcutaneous Silastic capsules containing estradiol to be released at approximately physiological levels (6, 34). The other pituitary- and muscle-grafted rats received either a placebo pellet or oil-filled Silastic capsule subcutaneously.

A recent study (22) has suggested that the age of the pituitary graft donor may have an impact on host animal growth. To test this possibility, eight litters of Fischer rats were obtained. Half received pituitary or muscle grafts from littersmates just after weaning (age 28 days). The animals were killed 34 days later. The other half were implanted with subcapsular grafts of littermate pituitary or muscle tissue at the age of 61 days and were killed 35 days later.

Animals were housed two per cage except when undergoing metabolic experiments. They were maintained on a 12:12 h light-dark schedule with lights on at 0600 h each day. Except where noted in RESULTS, animals ate normal laboratory chow (Purina 5001) and drank water ad libitum. For studies of food intake, animals were housed in single metal metabolic cages and food was weighed with an electronic balance. This balance was also used for animal weights. Feces were weighed wet and after drying at 110°C for 2 days.

Injection of PRL. As part of studies on the acute effects of PRL, normal adult Sprague-Dawley rats (weight 180–220 g) and commercially hypophysectomized rats (weight 85–95 g) (Hilltop Lab Animals) were placed in metabolic cages for 2 baseline days without injections. For the next 2 days, all 12 animals were injected with 0.1 ml of polyvinylpyrrolidone (PVP-40, Sigma Chemical, St. Louis, MO). For the next 8 days, half of the normal animals continued to receive daily subcutaneous injec-
tions of PVP, and the other normals received 100 µg of rat prolactin [rPRL; biological grade rPRL-B6, National Hormone Program, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)] in 0.1 ml of PVP. The same dose of rPRL was similarly administered to all six hypophysectomized rats. This dosage regimen has been found to cause sustained elevation of PRL (25).

In another experiment, six normal (weight 188–220 g) and six hypophysectomized (weight 147–193 g) female Fischer rats were placed in individual metabolic cages for 4 consecutive days. On days 1 and 2, the animals received injections (0.1 ml ip) of 0.1 M NaHCO₃ at 0800, 1000, 1200, 1400, 1600, and 1800 h. On days 3 and 4, the animals received rPRL (rPRL-B6, NIDDK) injections (10 µg in 0.1 ml 0.1 M NaHCO₃) at similar times.

Serum PRL was measured by a homologous radioimmunoassay using materials provided by the NIDDK, as previously described (1). Data between groups were analyzed using Student’s t test, using programs of the Dartmouth Time Sharing System.

**RESULTS**

**Adrenalectomized rats with steroid replacement.** In these experiments, animals receiving pituitary or muscle grafts at weaning were weighed at least once per week for 72 days. As shown in Fig. 1 and Table 1, weight at implant, weight over time, weight at death, and weight change in grams per day were the same in muscle-implanted and in pituitary-implanted rats. All of the rats had been adrenalectomized at the time of implantation, and both groups received exactly the same corticosterone and fludrocortisone replacement pellets approximately every 3 wk.

Another group of similar muscle-implanted and pitui-

![FIG. 1. Body weight in hyperprolactinemic and normoprolactinemic rats. Body weight was measured at least weekly in 8 muscle-implanted control rats and 8 pituitary-implanted rats. Means are shown with SE bars. Day of muscle or pituitary grafting and adrenalectomy is shown by arrow marked “Surgery.” At that time and at other arrows, pellets of corticosterone and fludrocortisone were implanted subcutaneously.](http://ajpregu.physiology.org/)

**TABLE 1. Change in weight over time**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at Implantation, g</th>
<th>Weight at Death, g</th>
<th>Weight Change, g/day</th>
<th>Serum PRL at Death, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle-implanted (n = 8)</td>
<td>291.4±10.4</td>
<td>296.4±7.9</td>
<td>5.0±0.3</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>AP-implanted (n = 6)</td>
<td>297.5±10.3</td>
<td>297.5±10.3</td>
<td>5.0±0.3</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE from averages of 2 consecutive 24-h metabolic cage collections; n, no. of rats. There were no significant differences between groups.

**Effects of ovariectomy.** Because it is known that ovariectomized rats weigh more than controls (26), the possibility that PRL-induced functional hypogonadism leads to weight gain was explored in another set of rats placed in metabolic cages for three periods of 2–4 days. The results are shown in Table 2. At baseline (before ovariectomy) muscle-implanted and pituitary-implanted rats ate the same amount of food. Interestingly, ovariectomy led to a diminution of food intake, as measured by metabolic cage collections ~2 wk after ovariectomy. There was no difference between the muscle-implanted and pituitary-implanted groups. Finally, all animals were placed on a low (0.3%)-calcium diet for 2 wk. Food intake decreased to a comparable degree in both hyperprolactinemic and control rats.

**TABLE 2. Food ingestion and fecal weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Avg Food Intake, g/100 g body wt⁻¹·24 h⁻¹</th>
<th>Avg Wet Feces, g/100 g body wt⁻¹·24 h⁻¹</th>
<th>Avg Dry Feces, g/100 g body wt⁻¹·24 h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle-implanted (n = 8)</td>
<td>291.4±10.4</td>
<td>5.8±0.4</td>
<td>1.5±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>AP-implanted (n = 6)</td>
<td>297.5±10.3</td>
<td>5.0±0.3</td>
<td>1.9±0.1</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE from averages of 2 consecutive 24-h metabolic cage collections; n, no. of rats. There were no significant differences between groups.

**Effects of pituitary graft age.** Esquifino et al. (22) suggested that the pituitary grafts from adult rat donors might increase host weight, but grafts from weanling littermates did not. To test this possibility, eight litters of rats were obtained, but only four litters had implantation of three littermate pituitary glands or muscle tissue soon after weaning (28 days). The other litters received littermate pituitary or muscle tissue on day 61. Thus the latter group received adult pituitary grafts, whereas the former group received grafts from weanlings. Each set of rats was killed 34–35 days after implantation. The initial and final weights, as well as the weight gain over the 5-wk period after implantation, are listed in

**TABLE 3. Change in weight over time**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at Implantation, g</th>
<th>Weight at Death, g</th>
<th>Weight Change, g/day</th>
<th>Serum PRL at Death, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle-implanted (n = 8)</td>
<td>291.4±10.4</td>
<td>296.4±7.9</td>
<td>5.0±0.3</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>AP-implanted (n = 6)</td>
<td>297.5±10.3</td>
<td>297.5±10.3</td>
<td>5.0±0.3</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE from averages of 2 consecutive 24-h metabolic cage collections; n, no. of rats. There were no significant differences between groups.
of subcutaneous injection. However, daily injection for 8 days caused a variable weight gain response after 2 days known to cause a sustained serum PRL level (25) and homologous rat PRL. As shown in Table 5, the vehicle increased weight gain. We used an injection vehicle and neither the early implanted group nor the later implanted group did the pituitary-grafted rats gain more weight.

Table 4. Effect of implant age on weight gain

<table>
<thead>
<tr>
<th>Implantation at day 28 with weaning tissue</th>
<th>Weight at Implantation, g</th>
<th>Weight at Death, g</th>
<th>Weight Gain, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle-grafted rats (n = 8)</td>
<td>76.0±3.1</td>
<td>226.3±4.8</td>
<td>149.3±2.3</td>
</tr>
<tr>
<td>AP-grafted rats (n = 8)</td>
<td>76.0±3.0</td>
<td>216.9±5.0</td>
<td>140.8±3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Implantation at day 61 with day 61 tissue</th>
<th>Weight at Implantation, g</th>
<th>Weight at Death, g</th>
<th>Weight Gain, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle-grafted rats (n = 8)</td>
<td>199.1±3.1</td>
<td>291.1±3.6</td>
<td>92.0±2.7</td>
</tr>
<tr>
<td>AP-grafted rats (n = 8)</td>
<td>193.6±3.0</td>
<td>279.1±3.9</td>
<td>85.5±3.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of grafted rats. Rats implanted at 28 days were killed 34 days later. Rats implanted on day 61 were killed 35 days later. There were no significant differences between the 2 groups of animals gaining weight in early muscle-grafted group tended to be greater than in pituitary-grafted group (P = 0.0626).

Table 5. Weight gain after PRL or PVP injection

<table>
<thead>
<tr>
<th>Rats</th>
<th>Weight Gain, g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Injection</td>
<td>PVP Injection</td>
</tr>
<tr>
<td>PVP-injected normal rats (n = 6)</td>
<td>6.3±1.3</td>
</tr>
<tr>
<td>PRL-injected normal rats (n = 6)</td>
<td>8.8±1.1</td>
</tr>
<tr>
<td>PRL-injected hypophysectomized rats (n = 6)</td>
<td>0.2±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of rats. All rats were weighed each morning after no injections for 2 days, polyvinylpyrrolidone (PVP) vehicle only for 2 days, and either PVP or PRL in PVP daily for 8 days. There were no significant differences between the 2 groups of normal rats during any of the 3 phases of experiment.

Table 4. All rats gained more weight in the 5 wk after weaning. However, in neither the early implanted group nor the later implanted group did the pituitary-grafted animals gain more weight.

Effects of PRL administration. Only some studies (24, 28) of PRL administration to normal rats have shown increased weight gain. We used an injection vehicle known to cause a sustained serum PRL level (25) and homologous rat PRL. As shown in Table 5, the vehicle PVP caused a variable weight gain response after 2 days of subcutaneous injection. However, daily injection for 8 additional days resulted in a weight gain in all animals. A similar pattern was found in animals receiving 2 days of PVP followed by 8 days of PRL in PVP. In hypophysectomized rats (Table 5), PRL did not cause a substantial weight gain.

Because pulsatile PRL may have effects different from those of sustained hyperprolactinemia, normal and hypophysectomized rats were injected at 2-h intervals from 0800 to 1800 h for 4 days. On the first 2 days, 0.1 mL of 0.1 M NaHCO₃ was injected intraperitoneally, and on the following 2 days 10 µg rPRL in 0.1 mL of 0.1 M NaHCO₃ were similarly administered. Four of six normal rats, but none of the hypophysectomized rats, ate more on the days PRL was injected. The average food intake tended to be greater after the PRL treatment (10.9 vs. 6.7 g/day), but this was not statistically significant (P = 0.074 by paired t test). In contrast, a similar trend in the opposite direction was found in hypophysectomized rats (3.6 ± 1.3 g/day after PRL vs. 4.6 ± 1.3 g/day before, P = 0.065 by paired t test).

Discussion

The pituitary-grafted rat model of chronic hyperprolactinemia has been utilized in our laboratory for almost 15 yr. Studies in rats in our laboratories, substantiated by reports from other laboratories (reviewed in Ref. 1) and in pituitary-grafted hypophysectomized orchidectomized hamsters (35) provide evidence that pituitary-grafted animals grow normally despite the evidence that another hyperprolactinemic state (lactation) is associated with hyperphagia (13, 23). We therefore analyzed data from several studies using pituitary-grafted rats. We attempted to avoid the possible shortcomings (1) of this rat model by surgical modifications. For example, pituitary-grafted rats may have excess corticosterone secretion (2, 29). The mechanism of this finding is still unclear, although there is some evidence that PRL has an adrenocorticotropic hormone (ACTH)-like or ACTH-potentiating effect (20) and may alter corticosterone catabolism (16). To avoid this problem, both muscle-grafted and pituitary-grafted animals were adrenalectomized and given replacement pellets of corticosterone and fludrocortisone. This eliminated any hyperphagia (33) or catabolic (11) effects that might be attributed to...
changes in corticosterone levels. The hyperprolactinemic rats gained weight in a manner similar to the control, normoprolactinemic rats. In a previous study of nonadrenalectomized rats (27), 20 anterior pituitary-implanted male rats grew at a rate similar to that of 20 nonadrenalectomized muscle-implanted rats (4.05 ± 0.11 vs. 4.32 ± 0.11 g/day). The antigonal action of PRL is well known and leads to a relatively constant diestrous vaginal smear in pituitary-grafted female rats (32). Because hypogonadism also results in a relative weight gain in female rats (26), muscle-implanted and pituitary-implanted rats were ovariectomized. Both weight gain and food intake were similar in hyperprolactinemic and control ovariectomized rats. Food intake decreased when these rats were placed on a low-calcium diet, but there was no difference between muscle-implanted and pituitary-implanted ovariectomized rats. In male rats, castration leads to decreased weight (6). We have found that muscle-grafted and pituitary-implanted male rats have similar and greater body weight than castrated muscle-grafted and pituitary-grafted rats (6).

Moore et al. (30) found increased weight gain in rats implanted with pituitary glands from female retired breeders. Because most of our studies use rats implanted with weanling tissue, this could possibly explain why our results differ. In support of this concept, Esquifino et al. (22) reported that adult anterior pituitary glands implanted into weanling rats may increase body weight in comparison with weanling pituitary grafts. In a modification of their protocol, rats were implanted with muscle or anterior pituitary glands either at 28 or at 61 days of age. In neither group did the pituitary-grafted rats grow heavier than the muscle-grafted rats. Esquifino et al. used littermate pituitary glands for weanling donor tissue but unrelated adult pituitary glands for the older grafts. In our studies, littermate tissue was used for all grafts. In addition, Esquifino et al. used Wistar rats (22), whereas our experiments utilized Fischer 344 rats.

In a set of experiments utilizing Sprague-Dawley rats, PRL was injected into normal rats for 8 days. PVP was used as a vehicle to cause sustained high PRL levels (14). There was no difference in the weight gain between PRL-injected and PVP-injected rats. PRL administration to hypophysectomized rats caused no significant weight gain. To determine whether repeated PRL pulses might augment food intake and weight gain, normal and hypophysectomized rats were injected with rat PRL in an aqueous buffer at 2-h intervals from 0800 to 1800 h for 2 days. No significant changes in eating behavior or weight gain were found in either set of rats compared with multiple injections of vehicle alone.

The reports that led to these analyses found that pituitary-grafted and PRL-injected rats ate more and weighed more than their respective controls. Moore et al. (30) claimed that pituitary-grafted rats weighed more and ingested more than controls. Our results are consistent with the preponderance of data on normal growth in chronically hyperprolactinemic rats. The more recent report of a dose dependent food intake response to PRL is also difficult to reconcile with our negative findings. The dose in our experiments utilizing PVP vehicle, 100 µg/day, was approximately the same as the low dose in the study by Gerardo-Gettens et al. (24). Rats receiving the low dose did not show increased body weight until after 12 days of injection, whereas our rats were injected for only 8 days. In the study of Gerardo-Gettens et al. (24), the weight increase was mostly due to increased total body water, which may have been caused by the vasopressin contamination of PRL preparations (5, 9). In addition, the authors of this study reported surprisingly elevated serum rPRL levels after administration of ovine PRL (oPRL). In other studies, PRL injection has led to suppressed serum rPRL levels (15). PRL does not cross-react substantially in rPRL immunoassays (31). In one study (21), in which oPRL levels were measured in rats receiving oPRL, the serum oPRL levels declined after several days, suggesting an immunological response to the heterologous hormone. Thus it might be speculated that the immune response to the foreign hormone or perhaps a growth-hormone (GH)-like response may have caused the weight gain noted by Gerardo-Gettens et al. (24). Supporting a GH-like effect of oPRL, Buntin and Figge (14) found that ovine PRL and GH, human GH, and turkey GH but not turkey PRL caused weight gain in ring doves. Pulses of homologous PRL did not increase weight, although the rats were injected for only 2 days.

If increased growth in pituitary-grafted rats can somehow be substantiated, one possible mechanism might be increased growth hormone secretion by the pituitary grafts. Somatotropes can be demonstrated to be present in the ectopic hypophyses (3, 27). Although there is evidence that the ectopic glands retain the ability to secrete GH (3), basal GH levels are similar in pituitary-grafted and muscle-grafted rats (3, 8). Moreover, the intrasellar pituitary GH content is not affected by the presence of ectopic pituitary grafts (8).

In summary, chronically hyperprolactinemic animals did not increase food intake or excessive weight gain compared with controls. In addition, 8 days of continuous or 2 days of pulsatile homologous PRL administration caused no increased weight gain. These findings are compatible with the work of Fleming (23), who found that mechanisms other than hyperprolactinemia appear to be responsible for the hyperphagia of lactation in rats. Consistent also are reports in normal lactation demonstrating that as milk production rises (13), serum PRL does not increase (12).

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