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Effects of rat- prolactin on ingestive behavior and leptin levels in adult male rats

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Summary
Previous studies have indicated that ovine- or bovine-prolactin stimulates ingestive behavior in female, but not in male rats; i.e., that prolactin has sex-specific effects on ingestive behavior. The question addressed here was whether ingestive behavior in male rats would be induced by rat-prolactin. In a preliminary test male rats were allowed to ingest a 1 M solution of sucrose from a drinking spout. After daily intake of sucrose became stabilized, the males received rat-prolactin by pituitary grafting. The results showed that pituitary grafting both stimulates ingestive behavior and increases serum leptin levels in male rats. These findings coupled with previous findings suggest that prolactin has species-specific effects on ingestive behavior.

Introduction
Prolactin (PRL) is a polypeptide hormone which plays a critical role in initiation and maintenance of lactation. In rats, lactation is characterized by a marked hyperphagia and significant increases in plasma levels of PRL. A number of studies suggest that peripheral administration of PRL facilitates the onset of maternal behavior *(Moltz et al. 1970, Bridges et al. 1985)* and causes a dose-dependent increases in food intake by virgin female rats *(Leon 1974, Gerardo-Gettens et al. 1989, Byattet al. 1993, Noel & Woodside 1993)*. While the effects of PRL on food intake are fairly consistent in female rats, the effects in male rats are relatively unexplored. Recently Heil (1999) reported that there is a sex-specific feeding response to PRL by rats. Female rats significantly increased their food intake in response to exogenous ovine-PRL administration, while male rats maintained baseline levels of food intake despite increased treatment during or doses of ovine-PRL.
In related work, previous observations from our laboratory suggest that mouse-PRL itself, but not ovine-PRL nor bovine-PRL promotes maternal behavior in the mouse *(Saito et al. 1983)*.
The aim of the present experiment was simply to establish whether rat-PRL induced by grafting pituitary glands, which release rat-PRL into the circulation, could increase ingestive behavior by male rats.

Materials and Methods

Animals
Specific-pathogen-free Iar: Wistar-Imamichi male rats were obtained from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan) at 7 weeks of age. These animals were seronegative for *Mycoplasma pulmonis, Bacillus piliformis, Boldetella bronchiseotica, Streptococcus pneumoniae,* and Sendai virus. Rats were individually housed in suspended wire-mesh cages (width x depth x height, 205 x 250 x 180 mm), kept in an animal room with controlled temperature (24 ± 2°C) and humidity (55 ± 10%). A 12:12 -h light:dark cycle, with lights on at 24:00, prevailed. Food (Oriental MF, Oriental

Yeast Co., Ltd., Tokyo, Japan) and tap water were always available. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Nippon Veterinary and Animal Science University.

Ingestive behavior
On reaching the age of 8 to 9 weeks, 12 males were subjected to an ingestive behavior test. The pellets were removed at 07:00, but water was available at all times. Tests were conducted 6 hr later, i.e., 1 hr after the lights were switched off. The animals were placed in circular (30 cm diameter) Plexiglas arenas and trained to ingest the 1 M solution of sucrose from a bottle protruding into the test cage. Before being used in the experiments the animals were given a series of 7 preliminary daily tests until intake from the bottle had stabilized.

Pituitary grafting
Twelve male rats that had a stable intake of sucrose solution in the preliminary test were randomly divided into two groups. One group of 6 males received one pituitary gland, obtained from one rat of the same sex and age, under the kidney capsule, whereas the other group underwent sham surgery. The surgical procedure we used followed the description provided by Aoki et al. (1992). The animals were allowed a post-operative recovery period of 4 days. Post-operative body weight had regained the pre-operative weight by the time of the experiment.

Blood sampling
After the end of the testing period, the males were decapitated and blood was collected. Blood samples were centrifuged at 3,500 rpm for 15 min at 4°C, and serum was stored at –50°C until analyzed for determination of serum PRL and leptin.

Serum PRL determination
Serum concentrations of PRL were measured by RIA using the method of Furudate et al. (1989) with reagents provided from NIADDK. The standard reference used was rPRL-3 for PRL. The intra- and inter-assay coefficients of variation were 3.2% and 13.9 %, respectively.

Serum leptin determination
Serum concentrations of leptin were measured using an ELISA kit (Morinaga rat leptin kit, Yokohama, Japan).

Experimental procedure
After the post-operated recovery, the grafted and non-grafted animals were introduced into the circular Plexglas arenas and tested for sucrose intake for 7 consecutive days. Tests lasted for 30 min after the introduction of the animals.

Statistics
The results are expressed as means ± s.e.m. and analyzed by one-way ANOVA for repeated measures with the aid of the GSB-Stat statistical program for Macintosh computers (Dynamic Microsystems, Inc., Silver Spring, MD, USA).

Results

Sucrose intake before and after grafting
When the male rats had access to a bottle filled with sucrose, the daily intake of sucrose during 7 days before grafting was found to stabilize in those subsequently grafted or sham-operated at 3.6 and 3.7 ml, respectively (Fig. 1).
Figure 2 presents the result of the daily amount of sucrose ingested during the 30-min test in male rats after being grafted or sham-operated. The pituitary grafting resulted in a significant increase in the daily amount of sucrose intake during 7 days after grafting, compared with the sham-operated ($p<0.05$).

Prolactin and leptin concentrations
The concentration of prolactin in the serum of grafted males increased two-fold, compared with the sham-operated animals ($p<0.05$) (Fig. 3). In addition a significant increase ($p<0.05$) in serum leptin concentration was noted in grafted males (4.1 ± 0.2 ng/ml) in comparison to sham-operated (3.3 ± 0.2 ng / ml) (Fig. 4).

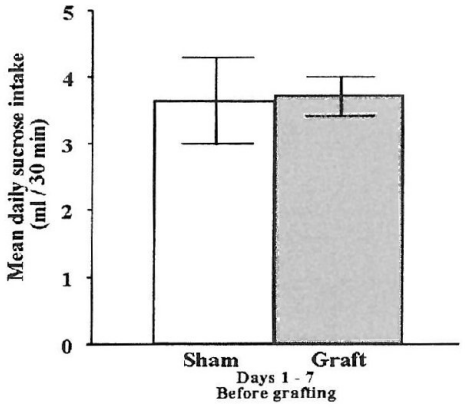

Fig. 1 Daily intake of the 1.0 M sucrose solution from a drinking spout before grafting. Data are means ± s.e.m.

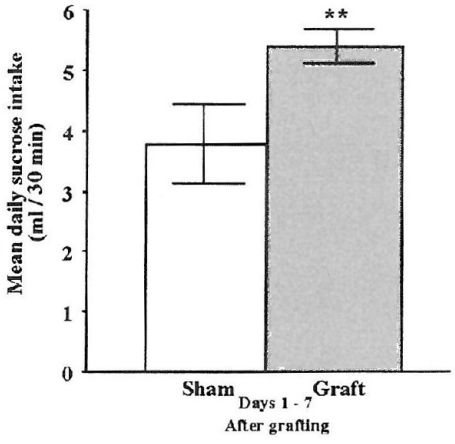

Fig. 2 Effect of pituitary grafting on intake of sucrose solution. Data are means ± s.e.m. **, $p<0.01$

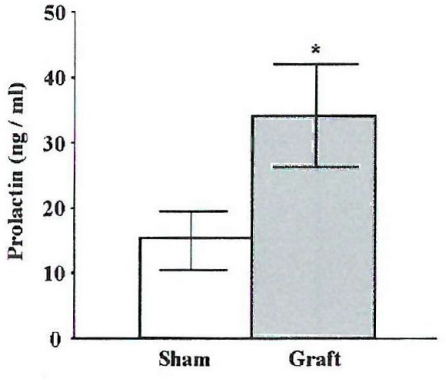

Fig.3 Effect of pituitary grafting on serum prolactin
Data are means ± s.e.m. *, p<0.05

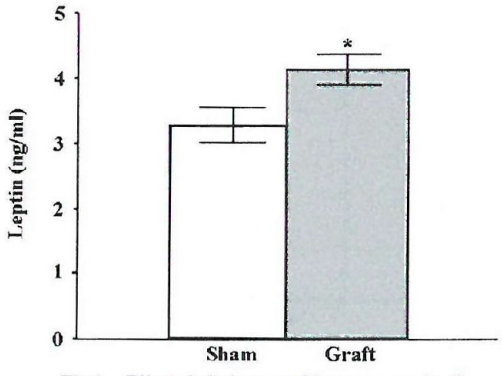

Fig.4 Effect of pituitary grafting on serum leptin.
Data are means ± s.e.m. *, p<0.05

Discussion

A preliminary (i.e., baseline) experiment showed that male rats provided with access to a bottle of sucrose solution stabilized their intake after seven days. Half these were then pituitary-grafted and the remainder sham-operated. After post-operative recovery, the effect of the pituitary grafting on the ingestive behavior of the two groups was measured using a bottle intake test *(Gill et al., 1987)*. This measures appetitive ingestive behavior (i.e., the responses used to obtain food).

The results showed that the daily amount of sucrose ingested by the pituitary-grafted male rats was significantly higher than the sham-operated males.

In the former the serum rat-PRL increased, compared with sham-operated. Furthermore, ELISA confirmed that serum rat leptin secretion was markedly increased in pituitary-grafted males (as also observed by *Gualillo et al. 1999*). These findings suggest that rat-PRL itself increases ingestive behavior and acts on adipose tissue increasing leptin synthesis and secretion in adult male rats.

As to other effects, Heil et al. (1999) demonstrated that ingestive behavior in female, but not in male rats was induced by PRL. Our data in present experiment showed that PRL administration increased ingestive behavior in both female (unpublished data) and male rats. However, while Heil et al. used ovine- or bovine-PRL, we increased rat-PRL through pituitary-grafting. It is well known that PRL has species-specific effects on hormonal responses in mice and rats, which may explain the difference in results. That this is a likely explanation is supported by our findings that mouse nest- building was induced by mouse-PRL, but not by ovine-PRL *(Saito et al. 1983)*. The present study also suggests that PRL has species-specific effects on ingestive behavior of male rats. Detailed experiments will be necessary in order to clarify this point.

In conclusion, the results of the present experiments are the first to show that rat-PRL itself accelerates appetitive ingestive behavior by adult male rats.

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