Dissociation of Oxytocin Effects on Body Weight in Two Variants of Female Sprague-Dawley Rats

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Abstract—As a counterpoint to studies that make a case for the use of oxytocin in short-term inhibition of food intake, the aim of the present study was to determine whether the peptide facilitates weight gain in female rats in a more long-term perspective.

Two different variants of female Sprague-Dawley rats were used. The two variants differed in daily weight gain (0.38 g/day vs. 1.41 g/day during 28 days), and were designated slowly and rapidly growing rats (SGR and RGR) respectively.

Oxytocin 1 mg/kg given s.c. to SGR for a five-day period increased weight gain significantly in comparison to a previous five-day period with NaCl-treatment (18.0 g/5 days versus 5.0 g/5 days; p < 0.01).

In a separate study, oxytocin 1 mg/kg given for four days increased weight gain significantly in SGR versus saline-treated controls (7.5 g/4 days versus 1.6 g/4 days; p < 0.05). The weight-difference persisted six days later (p < 0.001). The weight increase of oxytocin occurred only during estrus (p < 0.05) and was not accompanied by any measurable increase in food intake.

In RGR, oxytocin-treatment decreased food intake significantly (p < 0.001) and tended to decrease weight gain, although not significantly.

The SGR and RGR also had different endocrine profiles with, for example, twice as high oxytocin (p < 0.01) and insulin levels (p < 0.01) in RGR compared to the SGR.

These data suggest that oxytocin influences weight gain and food intake differently in the two variants of Sprague-Dawley rats, perhaps depending on factors such as endocrine profile and oxytocin sensitivity.

Keywords—oxytocin, weight gain, food intake, estrous cycle, hormone levels

Introduction

OXYTOCIN HAS BEEN SHOWN to have an inhibitory influence on food intake in acute tests. Thus oxytocin given intracerebroventricularly (i.c.v.) or subcutaneously (s.c.) to male and female rats reduce their food intake during the following 1–3 hours. (For a review, see Argiolas & Gessa, 1990).

On the other hand, there is evidence pointing to the role of oxytocin as a hyperphagic agent. Oxytocin levels rise in the circulation and in the brain during pregnancy and lactation, both extremely energy-demanding periods (Kendrick et al., 1988; Silber et al., 1991). Experiments performed on lactating rats show that the hyperphagia associated with lactation is abolished if the neural pathways involved in the milk-ejection reflex are lesioned (Hansen & Ferreira, 1985). Furthermore, oxytocin in a dose of 10–20 μg given i.c.v. to
lactating estrogen-primed ewes increases food intake (Kendrick et al., 1987), supporting a possible role for oxytocin as a hyperphagic agent.

The enhanced calorie demand during lactation is, however, not met by an increased food intake alone. A more efficient use of calories for anabolic purposes applies to this period. Therefore neuroendocrine mechanisms that decrease catabolic and promote anabolic metabolism must be operating. Indeed, oxytocin has been shown to induce several energy-saving physiological adaptations such as induction of sedation, elevation of pain-threshold, reduction of tail-skin temperature (Uvnäs-Moberg, 1989, 1994) and endocrine changes, including increasing levels of insulin and cholecystokinin (CCK) (Lindén et al., 1990a), and a decreasing level of growth hormone (GH) (Hulting & Uvnäs-Moberg et al., in preparation), allowing anabolism to fat rather than to protein. These data taken together indicate that oxytocin might have a long-term effect on weight gain by influencing both food intake and the intensity of anabolic metabolism.

The present study was performed to analyze how oxytocin influences weight gain and food intake in female rats in a long-term perspective, i.e., over days and weeks rather than hours. For this purpose oxytocin 0.001—1 mg/kg was administered s.c. and weight gain and food intake were measured. The effects of oxytocin were related to the stage of the estrous cycle as determined by vaginal smears. Experiments were performed in two variants of Sprague-Dawley rats with different endocrine profile and growth rate.

**Materials and Methods**

**Animals**

Female Sprague-Dawley rats (280–300 g, ca. ten weeks old) were used (B&K Universal AB, Sollentuna, Sweden). During the study the original Sprague-Dawley strain was switched for a coronavirus-free strain because of a department-wide veterinary management decision. The new rats were found to differ from the old ones in several aspects. The average daily weight gain as calculated during a 28-day period, was 0.38 and 1.41 g respectively at an initial age of ten weeks. The two types of rats are referred to as slowly (SGR) and rapidly (RGR) growing rats (compared to the suppliers standard curve of weight increase). When the RGR were introduced, the SGR were no longer available to us. The animals of either strain arrived 5 days before experiments and were maintained under controlled conditions of light-dark cycle (12:12h, lights on 06.00h), temperature 20±2°C and relative humidity (55–60%). Food (R36, Ewos, Södertälje, Sweden) and tap water were available ad lib in the home cage. The animals were housed 5-6 per cage, except in one experiment, where the animals were housed in separate cages (Makrolone IV).

**Drugs**

Oxytocin (Ferring, Malmö, Sweden) and the oxytocin antagonist (1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin) (Ferring, Malmö, Sweden) were dissolved in physiological saline and injected s.c. in a volume of 1 ml/kg. The animals were treated during four- or five-day periods to include a whole estrous cycle. Injections were given at 18.00, i.e., before onset of the dark period when the animals feed.
Body Weight and Intake Measurement

The animals were weighed each morning, except in the first experiment where they were weighed after each five-day period. Food and water consumption was measured by weighing what was left in the cage of the food and water given on the previous day. Weight measurements (Mettler PE200) were accurate to a tenth of a gram.

Determination of Stage of the Estrous Cycle

The stage of the estrous cycle was determined daily by microscopical examination of vaginal smears. The cycles were found to be 4–5 days long. Only rats showing regular cycles were included in the study.

Methods for Hormone Measurement

SGR (n=15) and RGR (n=14) were decapitated between 11.00 and 14.00, the day after the last injection. The blood was collected in ice-chilled tubes containing heparin (10 IU/ml) and Trasylo1 (500 IU/ml). Plasma levels of gastrin and insulin (Nilsson & Uvnäs-Wallensten, 1974) were measured with radioimmunoassay directly in plasma, and the concentration of oxytocin (Stock & Uvnäs-Moberg, 1988), somatostatin (Efendic et al., 1980) and CCK (Himeno et al., 1983) were measured after SEP-PAK extraction. The concentration of unextracted plasma prolactin was measured by a radio-immunoassay kit (RPA 553, Amersham Sweden AB, Solna, Sweden). Glucose was measured with GOD-PAP spectrophotometric method (cat. no. 14365; Diagnostica Merck, FRG).

Statistics

- The results are presented as means ± SD.
- The statistical methods used are given in table and figure legends.
- P-values of 0.05 or less were regarded as statistically significant.

Results

Effect of Oxytocin on Weight Gain and Food Intake

Oxytocin (1 mg/kg) caused an increased weight gain over a five-day period in the SGR, which was highly significant compared to the previous five-day period when NaCl-injections were delivered (18 g/5 days versus 5.0 g/5 days; p < 0.01) (Fig. 1).

In a second study, 1 mg of oxytocin increased weight gain in SGR versus NaCl-treated controls (7.5 g/4 days versus 1.6 g/4 days; p < 0.05) (Fig. 2a). The weight increase was not paralleled by an increased food intake (Fig. 2b). The weight difference persisted six days after the end of the treatment period. At that point in time, rats that had been treated with oxytocin weighed on average 4.2 g more than did NaCl-treated rats (p < 0.001) (data not shown). No significant effect was seen by a four-day treatment with 0.1 mg/kg of oxytocin or by a one-day treatment with 1 mg/kg of oxytocin. The oxytocin antagonist (1.5 mg/kg) did not influence weight gain by itself but counteracted the effect on weight gain caused by treatment with 1 mg/kg of oxytocin (Fig. 2a).

In contrast, in the RGR, oxytocin 1 mg/kg caused a slight but not significant decrease in
weight gain compared to saline-treated controls (Fig. 3a). However, there was a significant
decrease in food intake (p < 0.001) after oxytocin treatment in these rats (Fig. 3b).

Effect of oxytocin on weight gain, food and water intake in relation to stage of estrous cycle

Both NaCl-treated SGR and RGR there was increased their weight during metestrus and
diestrus, whereas a decrease in weight occurred during estrus. The difference in weight
gain occurring during proestrus (P) and estrus (E) compared to metestrus (M) and diestrus
(D) was highly significant. In SGR, a significant difference between P vs. D (p < 0.05), E
vs. M (p < 0.05) and E vs. D (p < 0.001) was established (Fig. 4a), and in RGR there was a
significant difference between P vs. M (p < 0.001), P vs. D (p < 0.001), E vs. M (p <
0.001) and E vs. D (p < 0.001) (Fig. 4b).

In the SGR administration of oxytocin (1 mg/kg), there was increased weight gain in
comparison to the weight gain during a pretreatment period and to controls during estrus (p
< 0.05). No further increase of weight gain occurred during metestrus and diestrus (Fig.
4a). Thus, the significant difference in weight gain between the different stages of the
estrous cycle disappeared.

For the RGR, by contrast, the difference in weight gain between the different stages of
the estrous cycle was enhanced during oxytocin treatment. In fact a significantly (p < 0.05)
larger decrease of weight occurred during estrus in the oxytocin-treated animals. However,
when weight gain during the entire cycle was compared to controls, no significant effect
could be demonstrated (Fig. 4b).

Daily food and water intake were measured only in the RGR. When measured during
three consecutive cycles, food intake was significantly less in E vs. M (p < 0.05) and E vs.
Fig. 2a. Weight gain per 4 days in SGR during 4-day s.c. treatment periods. A. NaCl (n=5); B. oxytocin 0.1 mg/kg (n=5); C. oxytocin 1.0 mg/kg (n=10); D. the oxytocin antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin 1.5 mg/kg (n=5); E. the oxytocin antagonist 1.5 mg/kg together with oxytocin 1.0 mg/kg (n=5); and F. oxytocin 1.0 mg/kg for 1 day and NaCl for 3 days (n=5). Statistical analysis was performed with the Mann-Whitney U-test. * p < 0.05

Fig. 2b. The effect on food intake measured as group totals in the same experiment as described above. Statistical analysis was performed with the Mann-Whitney U-test.
Fig. 3a. The effect on weight gain per four days in RGR (n=18) in response to oxytocin 1.0 mg/ kg s.c. (OX). NaCl-treated animals (C) served as controls (n=18). Statistical analysis was performed with the Mann-Whitney U-test.

Fig. 3b. The effect on food intake in the same experiment as described above. Statistical analysis was performed with the Mann-Whitney U-test. *** p < 0.001.
Figs. 4a and 4b. Differences in daily weight gain between proestrus (P), estrus (E), metestrus (M) and diestrus (D) in a: SGR (n=10) and b: RGR (n=18) in response to oxytocin. Pretreatment values (NaCl) are shown in white and treatment values (oxytocin 1.0 mg/kg s.c.) in black. Statistical analysis was performed with the Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test and the Wilcoxon signed rank test. * p < 0.05.
OXYTOCIN AND WEIGHT GAIN

Fig. 5. Daily food intake during proestrus (P), estrus (E), metestrus (M) and diestrus (D) in RGR (n=18) in response to oxytocin. Pretreatment values (NaCl) are shown in white and treatment values (oxytocin 1.0 mg/kg s.c.) in black. Statistical analysis was performed with the Kruskal Wallis one-way ANOVA followed by the Mann-Whitney U-test and the Wilcoxon signed rank test. * p < 0.05, ** p < 0.01, *** p < 0.001.

D (p < 0.05) (data not shown). After treatment with oxytocin 1 mg/kg s.c, a significant decrease in food intake occurred during proestrus (p < 0.001), estrus (p < 0.01) and metestrus (p < 0.05) compared to pretreatment values (Fig. 5).

The water intake during the estrous cycle varied more than food intake between individual rats, and tended to decrease during estrus although not significantly (E 20.2 g/day vs. P 24.3 g/day, M 23.8 g/day and D 24.5 g/day).

No significant difference in water intake was observed after oxytocin treatment.

Endocrine Profile

The two groups of female Sprague-Dawley rats had a very different endocrine profile. In the RGR, the levels of oxytocin (p < 0.01), CCK (p < 0.01) and insulin (p < 0.01) were twice as high, the level of glucose significantly higher (p < 0.05), and the level of somatostatin significantly lower (p < 0.05) compared to the SGR (Table 1).

Discussion

In the present study oxytocin in a dose of 1 mg/kg given s.c. caused a sustained weight gain in slowly growing female rats (SGR) without any increase in food intake. In contrast, in the rapidly growing rats (RGR), oxytocin caused a decreased food intake and also tended to decrease weight gain.

In the present study oxytocin was administered s.c. However, the effect of oxytocin on weight gain is likely to be exerted centrally, because two 0/00 of a dose given s.c. reaches
TABLE 1
Basal Hormone Levels in Slowly Growing Rats (SGR) (n=15) and in Rapidly Growing Rats (RGR) (n=14)

|                | Slowly growing rats | Rapidly growing rats |
|----------------|---------------------|----------------------|
| CCK (pmol/l)   | 14.7 ± 5.2          | 25.0 ± 4.8           |
| Gastrin (pmol/l) | 114.1 ± 47.1       | 120.0 ± 35.2         |
| Oxytocin (pmol/l) | 31.2 ± 13.6        | 64.3 ± 11.8          |
| Somatostatin (pmol/l) | 24.3 ± 10.9    | 14.6 ± 2.9           |
| Insulin (ng/ml) | 3.2 ± 0.8          | 5.7 ± 2.4            |
| Glucose (nmol/l) | 6.7 ± 0.8          | 7.4 ± 0.5            |
| Prolactin (ng/ml) | 5.0 ± 2.6         | 3.7 ± 1.2            |

Statistical analysis was performed with the Mann-Whitney U-test. ** p > 0.05, * p < 0.05, ** p < 0.01.

the brain (Jones & Robinsson, 1982). Also supporting the central role of oxytocin in weight gain and food intake is the fact that i.c.v. injections of 5–10 μg of oxytocin is accompanied by a higher weight gain and food intake than that of NaCl-treated control rats (Björkstrand & Uvnäs-Moberg, 1995). Other central effects can also be induced by both s.c. and i.c.v. administration of oxytocin. In a previous experiment we have shown that oxytocin may induce an anxiolytic-like effect and a sedative effect by both s.c. and i.c.v. injections. Again, 100 to 1,000-fold higher doses were required for s.c. administration (Uvnäs-Moberg et al., 1994). Here s.c. administration of oxytocin was used, since this route of administration is less stressful to the animals than i.c.v. administration, and allows long-term studies on development of weight over several estrous cycles.

It is not known by which mechanisms oxytocin stimulates weight gain in the SGR. The differential effect of oxytocin during various stages of the estrous cycle deserves some attention. As shown by others, and in the present study, female rats lose weight in proestrus-estrus and gain weight in metestrus-diestrus (Tarttelin & Gorski, 1971). No
further effects from the use of oxytocin were seen in metestrus and diestrus, when maximal weight gain occurred spontaneously. Possibly endogenous levels of progesterone and estrogen may have optimized endogenous oxytocinergic effects on weight gain during this phase of the cycle. In contrast, oxytocin could increase weight gain during periods of the cycle when weight gain is low.

It has been suggested that the inhibited food intake during estrus, and the concomitant fall in weight during this period, is due to a potentiation of estradiol on CCK-mediated satiety (Butera et al., 1993; Lindén et al., 1990c). The present results indicate that this effect can be overcome by high levels of oxytocin. Indeed, CCK levels in CSF of lactating rats that are hyperphagic are lower than those in normal cycling rats (Lindén et al., 1990b), suggesting that oxytocin may influence the CCK-level under physiological conditions.

No parallel increase in food intake measured over a four-day period was seen in s.c. oxytocin-treated rats, in spite of an increase in daily weight gain. Therefore, the weight-promoting effect of s.c. administered oxytocin in SGR is likely to be due to a transfer of energy normally used for catabolic activity to anabolic metabolism. In line with this, we have shown that oxytocin 1 mg/kg s.c. causes physiological and metabolic changes aimed at energy conservation (Uvnäs-Moberg, 1994). Oxytocin also has an insulin-like activity in isolated rat adipocytes—an effect that may have contributed to weight gain in the present experiments (Hanif et al., 1982).

During the experimental period a change of type of Sprague-Dawley rats used in our department occurred. The reason for this was that the rats were infected with coronavirus, and the SGR were no longer available. It was not possible to induce any weight promoting effects by oxytocin in the new animals. In contrast oxytocin inhibited food intake and tended to decrease weight gain.

When subjected to a closer examination, the new rats were found to differ in several aspects from the SGR previously used. The new rats of a comparable age (RGR) had a basal growth rate that was 4-fold higher and, moreover, oxytocin, CCK and insulin levels that were twice as high, and somatostatin levels that were significantly lower compared to the SGR. It is possible that no further growth-promoting effect could be induced against this neuroendocrine background, perhaps mediating maximal growth by itself. Thus, different types of oxytocin-mediated effects may be expressed depending on the neuroendocrine profile of the rats receiving oxytocin. In the SGR with low levels of CCK, insulin and oxytocin, the weight-promoting effects based on an enhanced anabolic metabolism may become apparent, whereas in the RGR the satiety effect dominates.

Prolactin has been suggested to cause hyperphagia and weight gain in female rats (Noel & Woodside, 1993). Oxytocin stimulates prolactin secretion, and prolactin is also shown to stimulate the release of oxytocin in lactating rats (Parker et al., 1991). The present data suggest that oxytocin, perhaps together with prolactin—both of them released by suckling during lactation—may contribute to female reproductive adaptations of weight and food intake.

Whether or not oxytocin also influences weight gain in humans remains to be established. It has been shown that women reduce energy expenditure during pregnancy and lactation, periods characterized by high oxytocin levels (Uvnäs-Moberg, 1989), suggesting that oxytocin may also have an energy-saving function in women during reproductive periods. Indeed, women with high circulatory oxytocin levels give birth to babies with a higher birth weight than do those who have lower levels (Uvnäs-Moberg et al., 1990).

It is interesting that grossly obese women and men have higher average oxytocin levels than do normal weight controls (Stock et al., 1989). Obese people also have low soma-
tostatin, high insulin and high glucose, as do the RGR. Oxytocin may therefore promote storing of energy and weight gain in humans during non-reproductive situations, perhaps both by stimulating food intake and by anabolic metabolism.

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