NPY-leptin: opposing effects on appetitive and consummatory ingestive behavior and sexual behavior

A. A. AMMAR, 1*, F. SEDERHOLM, 1*, T. R. SAITO, 2*
A. J. W. SCHEURINK, 3 A. E. J. JOHNSON, 3 AND P. SÖDERSTEN 1
1 Section of Applied Neuroendocrinology, Karolinska Institute, Novum, S-141 57 Huddinge, Sweden; 2 Division of Veterinary Ethology, Department of Laboratory Animal Sciences, Nippon Veterinary and Animal Science University, Musashino-shi, Tokyo 180, Japan; and 3 Department of Animal Physiology, University of Groningen, 9750 AA Haren, The Netherlands

Ingestive behavior is controlled by a variety of hormonal messengers and neurotransmitters. Of these, neuropeptide Y (NPY) is thought to have a particularly important role as a stimulator of food intake (12). The evidence on which this hypothesis is based, recently reviewed in depth (12, 14), includes the observations that intracerebral injection of NPY potently stimulates food intake and that antagonists of NPY receptors prevent this effect. Furthermore, endogenous levels of NPY are maximal at the time of day when feeding is initiated, and deprivation of food increases the synthesis and release of NPY by the hypothalamus, an effect that is reversed by the intake of food (14).

Thus the evidence for the hypothesis that NPY is a naturally occurring orexigen is compelling. However, in a widely cited study mice that do not synthesize NPY, due to replacement of the signal peptide of NPY in embryonic stem cells, showed normal food intake and body weight (8). Thus, at least in this animal model, NPY is not an absolute requirement for normal ingestive behavior. Also, and not surprisingly, NPY has effects in addition to affecting food intake. For example, NPY can both suppress and stimulate reproductive neuroendocrine function (26) and behavior (5, 20) and, in high doses, cause taste aversion (35). NPY is therefore not merely an orexigen (35).

Most experiments report that NPY is one of the stimulators of food intake, whereas other modulators are inhibitors (12). However, the stimulator-inhibitor categorization may be overly simplistic because ingestive behavior can be partitioned into distinct activities. Appetitive activities include searching for and approaching food. Consummatory activities include chewing and swallowing, i.e., consumption of the food (29, 35). It has been reported that whereas rats consume more sucrose from a bottle following intracerebroventricular injection of NPY, they do not ingest more if the sucrose is administered directly into their oral cavities by introrastral infusion (29). Hence, NPY appears to stimulate appetitive but not consummatory ingestive behavior.

We have tested this possibility further in the present series of experiments and also examined the role of leptin in these two aspects of ingestive behavior. Leptin exerts an effect that is opposite to that of NPY by reducing the synthesis of NPY in the hypothalamic arcuate nucleus (3). In addition, we have examined the effect of NPY and leptin on the sexual behavior of male rats to test the specificity of the effects of NPY and leptin on ingestive responses.

Methods

Animals and Operations

Female and male Wistar rats (200–220 and 320–350 g, respectively, Møllegård Breeding Laboratories; Ejby, Denmark) were maintained individually in an air-conditioned, temperature-controlled (22°C) colony room in which the lights were off between 1200 and 2400. The rats had free access to food and water except on days of behavioral testing when food was removed at 0700.
All surgery was performed under pentobarbital sodium anesthesia (60 mg/kg ip, Mebuneral, NordVacc; Stockholm, Sweden). A 21-gauge stainless steel guide cannula (Plastics One; Roanoke, VA) was placed in the right lateral ventricle of the brain, 1.0 mm posterior and 1.4 mm lateral to the bregma and 3.5 mm ventrally from the surface of the skull, and anchored to the skull using dental cement. Female rats were provided with intracranial cannulas as described by Grill et al. (11). The animals were allowed 2 wk to recover.

Peptide Administration

Porcine NPY (Bachem; Bubendorf, Switzerland) was dissolved in sterile artificial cerebrospinal fluid (aCSF; 8.98 g NaCl, 0.25 g KCl, 0.14 g CaCl$_2$, 0.11 g MgCl$_2$, 0.07 g Na$_2$PO$_4$, 0.13 g urea, and 0.61 g glucose, dissolved in triple-distilled water to 1,000 ml) to a concentration of 2 µg/µl (pH 7.4). Starting 8 min before testing, 10 µg NPY were injected over a 2- to 3-min period through a 28-gauge cannula (Plastics One) with a 25-µl Hamilton syringe. Recombinant human leptin (PeproTech EC; London, UK) was dissolved in aCSF to a concentration of 1 µg/µl (pH 7.29). Rather than injecting 10 µl into the ventricular space at one time, two injections of 5 µg leptin were given 30 min apart. Leptin was administered 4-6 h before testing because the effect of leptin on food intake has a 4- to 6-h latency (S. P. Kalra, personal communication, and Ref. 13). The forms and doses of NPY (29) and leptin (13) were chosen on the basis of previous work.

Behavioral Tests

Behavioral observations were made by direct observation and manually recorded starting 1 h after lights off.

Intraoral intake. Female, rather than male, rats were used for these experiments because our work aims at understanding eating disorders that mainly afflict women.

A 1 M (343 g/l) solution of sucrose (140 kcal/100 ml) was prepared before each daily test. Sucrose was used because NPY has been reported to stimulate carbohydrate intake (34). The animals were tested in circular (30-cm diameter) Plexiglas arenas 1 h after the onset of darkness using the intraoral infusion method developed by Grill and collaborators (11), which is ideally suited to measure consummatory ingestive behavior. The sucrose solution was delivered via an intraoral cannula connected to a peristaltic pump (Alitea XV, Ventur Alitea; Stockholm, Sweden). Intraoral infusion of sucrose activates ingestive responses, which continue until the rat becomes satiated and lets the solution passively drip from the side of the mouth. Active rejection of the solution is less common. The infusion was then terminated and restarted 30 s later and continued until the rat had rejected the solution within 1 min of the restart of the infusion.

Intake from a bottle and visits to the bottle. The rats were also trained to ingest the sucrose solution from the spout of a bottle protruding into the test cage. The number of visits to the bottle was also recorded. A visit to the bottle was defined as a complete circular movement by the animal inside the arena away from and back to the bottle.

Before the actual experiment rats were given a series of five to seven preliminary daily tests until the amount of intraoral intake and intake from the bottle had stabilized. The rate of intraoral infusion in these tests was 1.0 ml/min (experiment 1) or 0.5 ml/min (experiments 2 and 3), and the tests with the bottle were 15 min long.

Sexual behavior. Male rats were tested individually for sexual behavior with regularly cycling sexually receptive female rats in the cages used for testing ingestive behavior. The number of mounts with or without intromission, the latency to the first intromission, and the latency between the first intromission and ejaculation were recorded over a 15-min period (17). The rats were used after showing a stable sexual behavior in two daily tests.

All rats served as their own controls, and treatments were given in random order. Because the rats were tested repeatedly, 3-4 days elapsed between each experimental manipulation to minimize the effect of the previous treatment. The rats were tested for intraoral and bottle intake in the intervening days to ensure a stable intake.

Procedure

Experiment 1: Effect of NPY and different rates of intraoral infusion. A group of rats was injected with NPY or aCSF and tested for intraoral intake (infusion rate, 1 ml/min), intake from the bottle, and visits to the bottle.

Another group of rats was tested for intraoral intake and intake from the bottle at the following rates of intraoral infusion: 0, 0.125, 0.25, 0.5, and 1.0 ml/min. The tests were 15 min long and performed to find an infusion rate slow enough to allow simultaneous intake from the bottle.

A third group of rats was injected with NPY or aCSF and tested for intraoral intake (infusion rate, 0.5 ml/min), intake from the bottle, and visits to the bottle.

Experiment 2: Effect of NPY and leptin on intraoral intake, intake from the bottle, and visits to the bottle. A group of rats was injected with NPY or aCSF and tested under three conditions: 1) intake from the bottle without intraoral infusion, 2) intraoral intake (infusion rate, 0.5 ml/min) in the absence of the bottle, and 3) simultaneous intraoral intake and intake from the bottle.

Experiment 3: Effect of an empty bottle and NPY on intraoral intake. A group of rats was tested under three conditions: 1) intraoral intake (infusion rate, 0.5 ml/min) in the absence of the bottle, 2) intraoral intake and intake from the bottle and visits to the bottle, and 3) intraoral intake and visits to an empty bottle. The rats were also injected with NPY or aCSF and tested for intraoral intake and visits to an empty bottle.

Experiment 4: Effect of NPY and leptin on sexual behavior and intake from a bottle. A group of male rats with a stable intake from a bottle and a stable sexual behavior was injected with NPY, leptin, or aCSF and tested under three conditions: 1) with a sexually receptive female rat in the absence or 2) presence of a bottle and 3) with a bottle in the absence of a female.

This experiment was performed to investigate whether NPY- or leptin-treated male rats choose to copulate or ingest sucrose from a bottle. We used male rats for this experiment, because in a previous experiment using the same experimental conditions, female rats attempting to drink sucrose from a bottle were constantly interrupted by the mounting attempts of the male (15). Untreated male rats spend most of their time copulating and consume little sucrose solution if tested in this manner (15).

Analysis of Data

The results are expressed as means ± SE and analyzed using one-way ANOVA for repeated measures with the aid of the GB-Stat statistical program for Macintosh computers (Dynamic Microsystems; Silver Spring, MD). Subsequent within- and between-group comparisons were made using Scheffe comparisons. There were 6-10 rats/group.

RESULTS

Experiment 1

Intracerebroventricular injection of NPY had no effect on the intake of a solution of sucrose infused at a
rate of 1.0 ml/min. However, when the rats were treated with NPY they visited the bottle from which they had been trained to ingest the sucrose solution more often than when they were treated with aCSF (Fig. 1A). Yet, they did not ingest the sucrose solution from the bottle. However, when the rate of intraoral infusion was systematically reduced, rats compensated by gradually ingesting more from the bottle (Fig. 1B). Compared with no intraoral infusion, infusion at low rates significantly reduced the amount of intake from the bottle (Fig. 1B) and, consequently, the total amount ingested was reduced (0.25 ml/min, 5.2 ± 0.3 ml; 0.125 ml/min, 5.0 ± 0.6 ml vs. 0 ml/min, 8.5 ± 0.6 ml/ml; P < 0.01 for both comparisons). A similar behavioral shift, i.e., an increased number of visits to the bottle, increased intake from the bottle, and reduced intake from the intraoral infusion was found after injection of NPY when the rate of the intraoral infusion was 0.5 ml/min (Fig. 1C). As a consequence, the total amount ingested decreased (NPY 17.0 ± 2.2 ml vs. aCSF 20.7 ± 1.1 ml; P < 0.01).

**Experiment 2**

NPY stimulated bottle intake in the absence of intraoral infusion (Fig. 2A) but reduced intake when

![Fig. 1. Intake of a 1 M solution of sucrose infused intraorally or available from a bottle. Effect of neuropeptide Y (NPY, 10 µg icv) on intraoral (IO) intake (infusion rate, 1 ml/min) and visits to the bottle (A). Intraoral intake and intake from the bottle at various infusion rates (B). Effect of NPY (10 µg icv) on intraoral intake (infusion rate, 0.5 ml/min), visits to the bottle, and intake from the bottle (C). Values are means ± SE; n = 6 rats/group. **P < 0.01 compared with vehicle [artificial cerebrospinal fluid (aCSF)] or 0 ml/min.

![Fig. 2. Intake of a 1 M solution of sucrose available from a bottle or infused intraorally in 8 rats. Effect of NPY (10 µg icv) and leptin (10 µg icv) on intake from the bottle (A), intraoral (IO) intake (infusion rate, 0.5 ml/min; B), and simultaneous intraoral and bottle intake (C). Values are means ± SE. **P < 0.01 and *P < 0.05 compared with vehicle (aCSF).](http://ajpregu.physiology.org/).
rats were infused intraorally at a rate of 0.5 ml/min and not given access to a bottle (Fig. 2B). Under the same experimental conditions, leptin reduced intake from the bottle (Fig. 1B) but markedly stimulated intraoral intake (a 50% increase, Fig. 2B). When the rats were infused intraorally and simultaneously allowed access to a bottle filled with sucrose, NPY increased intake from the bottle and decreased intraoral intake. Leptin caused a behavioral shift in the opposite direction although the decrease in intake from the bottle did not reach statistical significance (Fig. 2C). As a consequence, NPY reduced intake from the bottle (Fig. 1B) but markedly stimulated intraoral intake (a 50% increase, Fig. 2B). When the rats were infused intraorally and simultaneously allowed access to a bottle filled with sucrose, NPY increased intake from the bottle and decreased intraoral intake. Leptin caused a behavioral shift in the opposite direction although the decrease in intake from the bottle did not reach statistical significance (Fig. 2C). As a consequence, NPY reduced intake from the bottle (Fig. 1B) but markedly stimulated intraoral intake (a 50% increase, Fig. 2B).

Experiment 3

Rats visited the sucrose-filled bottle somewhat more often than the empty bottle. However, the presence of either bottle reduced intraoral intake (Fig. 3A). Treatment with NPY caused a further increase in the number of visits to the empty bottle and a further decrease in intraoral intake (Fig. 3B). Note that in the absence of a bottle, rats ingested about 25 ml via intraoral infusion (Fig. 3A) and that they ingested markedly less (about 40%) after treatment with NPY when presented with an empty bottle (Fig. 3B).

Experiment 4

NPY had no effect on the capacity of male rats to ejaculate in the absence of a bottle (Fig. 4A). However, when given the choice between a female and a bottle, only two of ten NPY-treated rats ejaculated (Fig. 4A). The presence of the bottle did not affect the number of ejaculations when the rats were treated with aCSF. Treatment with leptin caused the rats to ejaculate twice as often as when they were given aCSF whether or not the bottle was present (Fig. 4A).

Intake from the bottle was increased by NPY and reduced by leptin, and whereas the presence of a female reduced intake when the rats were treated with aCSF or leptin it had a less marked, statistically insignificant effect when they were treated with NPY (Fig. 4B). Although treatment with NPY did not interfere with the capacity to ejaculate it increased the latency to intromission and ejaculation and reduced the number...
of mounts and intromissions before ejaculation (Table 1). Leptin reduced the number of mounts and intromissions and the latency to ejaculation (Table 1).

**DISCUSSION**

The first experiment in this study confirms the results of an experiment by Seeley et al. (29), which showed that intracerebroventricular injection of NPY does not affect intake of a sucrose solution that is infused intraorally at a high rate. Elaborating on that observation, this study demonstrates that during intraoral infusion NPY-treated rats visited a bottle, from which they had been trained to ingest the sucrose solution more often than when they were treated with aCSF. But as the rate of intraoral infusion was decreased, rats also ingested from the bottle. However, intake from the bottle was less than 50% of that ingested when the rats were not infused intraorally even when the rate of the intraoral infusion was as low as 0.125 ml/min. This suggests that slowly filling the mouth of the rat with a test solution can inhibit appetitive behaviors used to obtain that solution. When infused at 0.5 ml/min the rats visited the bottle and ingested a small amount of the solution from the bottle. Treatment with NPY doubled the number of visits to the bottle and the amount ingested from the bottle and decreased ongoing intraoral intake. Thus appetitive ingestive behavior affects consummatory ingestive behavior and vice versa.

Seeley et al. (29) hypothesized that NPY stimulates food intake only in tests in which rats need to search for the food. We confirmed that whereas NPY markedly increases intake from a bottle, it actually inhibits intraoral intake at an infusion rate of 0.5 ml/min. When rats were infused intraorally at this rate and simultaneously tested with a bottle, NPY caused a behavioral shift so that the frequency of appetitive behavior increased while that of consummatory ingestive behavior decreased. As a consequence, the total amount ingested decreased.

Similarly, we found that the effect of leptin, like that of NPY, is dependent on the testing paradigm. As expected, leptin decreased intake of the sucrose solution if the rats had to obtain the solution from a bottle. However, leptin markedly increased intraoral intake of the same solution. When rats were infused intraorally and simultaneously had access to a bottle, leptin-treated rats ingested a minimal amount from the bottle and a large amount from the intraoral infusion so that the total amount ingested increased. These results suggest that NPY and leptin exert opposing effects on appetitive and consummatory ingestive behavior: NPY stimulates appetitive and inhibits consummatory ingestive responses, whereas leptin has the opposite effect. This hypothesis departs considerably from the prevailing view that NPY merely stimulates and leptin merely inhibits food intake (12, 14). However, as the rate of intake from a drinking bottle differs from intake during intraoral infusion at a constant rate, a more detailed comparison between the two methods might be necessary to further test this hypothesis.

There is now a considerable amount of evidence that intake in the intraoral infusion test has many similarities with normal ingestion, results with this method can also be obtained with other methods (11, 29). One advantage of the method is that it measures consummatory ingestive behavior selectively. By contrast, the bottle test confounds the distinction between appetitive and consummatory ingestive behavior because once the rat has reached the bottle it consumes the solution. To control for this effect we tested rats either with a filled or an empty bottle while simultaneously infusing the sucrose solution intraorally. The presence of a filled as well as an empty bottle significantly stimulated visits to the bottle and reduced intraoral intake. Clearly, the display of appetitive ingestive responses, in the absence of consummatory responses, inhibits consummatory ingestive behavior. Treatment with NPY caused a further increase in the number of visits to an empty bottle (appetitive responses) and a further decrease in intraoral intake (consummatory responses).

The hypothesis that NPY selectively stimulates appetitive and inhibits consummatory ingestive behavior is paradoxical in view of the extensive evidence that NPY stimulates food intake in a variety of tests (12, 14). Testing a wider spectrum of appetitive ingestive behaviors might resolve this paradox. However, it is noteworthy that rats respond to restricted feeding by hoarding, not ingesting, food (4). Because deprivation of food increases NPY levels in the brain (14) it seems likely that this occurs also during restricted feeding. Hoarding is an example of appetitive ingestive behavior, which can be expressed in many different ways (7). Seeley et al. (29) found that deprivation of food, and therefore supposedly an increase in brain levels of NPY, increased consummatory ingestive behavior, i.e., intraoral intake. Yet, exogenous administration of NPY did not. However, we failed to observe an effect of food deprivation of the same duration as used by Seeley et al. (29) on intraoral intake (18, 19), possibly because of the difference in the molarity of the sucrose solutions used in the different experiments. It must be added, however, that deprivation of food causes endocrine adaptations in addition to altering endogenous levels of NPY, and exogenous administration of NPY therefore does not precisely mimic the physiological effects of fasting (35). Also, it should be added that the effects of NPY and leptin reported here may very well be medi-

---

**Table 1. Sexual behavior in ten male rats treated with aCSF, 10 μg NPY, or 10 μg leptin**

<table>
<thead>
<tr>
<th>Behavior Pattern</th>
<th>aCSF</th>
<th>NPY</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mounts</td>
<td>16.7 ± 1.1</td>
<td>8.1 ± 0.6*</td>
<td>3.8 ± 0.8*</td>
</tr>
<tr>
<td>Intromissions</td>
<td>13.1 ± 0.8</td>
<td>7.0 ± 0.5*</td>
<td>9.8 ± 0.6*</td>
</tr>
<tr>
<td>Intromission latency, min</td>
<td>0.4 ± 0.1</td>
<td>2.1 ± 0.2*</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Ejaculation latency, min</td>
<td>8.0 ± 0.5</td>
<td>12.4 ± 0.7*</td>
<td>2.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. aCSF, artificial cerebrospinal fluid; NPY, neuropeptide Y. Measurements were taken in 15-min-long tests with sexually receptive female rats. Data were first analyzed with ANOVA (P < 0.0001 for all comparisons) and thereafter with the Scheffé test. *P < 0.01 compared with aCSF. All leptin values are significantly different from NPY values.
ated indirectly by one or more of the neuroendocrine effects that follow intracerebral administration of these peptides (12, 14).

To test for the specificity of the behavioral effects of NPY and leptin we examined the behavior of male rats that were trained to ingest from a bottle and to copulate with a sexually receptive female. In the absence of a bottle, all rats ejaculated once in the 15-min test period when treated either with NPY or aCSF. The latencies to intromission and ejaculation were prolonged in NPY-treated rats and they showed fewer mounts and intromissions before ejaculation, confirming a previous report (22). Interestingly, in the presence of the bottle, only two of ten NPY-treated males ejaculated. Irrespective of the presence or absence of a sexually receptive female, treatment with NPY markedly stimulated intake from the bottle. Thus, although NPY has minor effects on the reproductive behavior in males, it shifts interest from sexual stimuli to a food stimulus if the male is offered the choice. A similar conclusion was reached by Clark et al. (5), who suggested that NPY inhibits sexual motivation but has no effect on sexual responses.

Although it is known that leptin can accelerate sexual development (1), there is only one previous report on the effect of leptin with regard to sexual behavior. That study showed that the effect of leptin was dependent on nutrient availability in female hamsters (33). In the present study we found that leptin-treated males ejaculated twice independently of the presence or absence of the bottle and consumed very little sucrose from the bottle irrespective of the presence or absence of the female. The main effect of leptin was a reduction in the latency to ejaculation. A reduction in ejaculation latency is often associated with a reduction in the number of intromissions (17). This situation can interfere with reproduction because intromissions are required for activation of the corpora lutea and therefore pregnancy (9). However, the number of intromissions before ejaculation in leptin-treated rats was relatively high. Leptin therefore might enhance reproduction in rats by increasing the number of ejaculations while maintaining a high rate of intromissions.

The mechanisms by which leptin stimulates consummatory ingestive behavior in female rats and consummatory sexual behavior in male rats are unknown. It appears unlikely that these effects are mediated by peripheral leptin receptors. Most work on leptin receptors in the brain has concentrated on the arcuate and paraventricular hypothalamic nuclei (12, 14). It is possible that the reduced synthesis and release of NPY within this hypothalamic circuit, which follows exogenous administration of leptin (14), may in part be related to the increase in consummatory ingestive behavior in female rats. It seems less likely that this effect of leptin is involved in sexual behavior of male rats. Interestingly, both the long and the short form of the leptin receptor are present in the corticotropin-releasing hormone producing neurons in the amygdala of the rat brain (24), and the amygdala is activated by intracerebroventricular injection of NPY as evidenced by an increase in Fos-like immunoreactivity (23). Lesioning the amygdala has long been known to cause alterations in consummatory behavior related to both reproduction (reviewed in Ref. 25) and ingestion (reviewed in Ref. 16). More recent experiments have confirmed the involvement of the amygdala in both appetitive and consummatory aspects of both kinds of behaviors (6, 10, 16, 28, 30). Whether the effects reported here can be related to the amygdala is, however, an open question.

Rats can show consummatory ingestive and sexual behavior simultaneously (15). However, display of sexual behavior normally inhibits appetitive ingestive behavior (15). Conversely, in this study NPY stimulated appetitive ingestive behavior, thereby interfering with sexual behavior, without exerting a major inhibitory effect on consummatory sexual behavior. These results offer a preliminary insight into the mechanisms that allow rats to choose between behavioral actions. We suggest that these mechanisms most likely operate via appetitive behaviors. Thus NPY and leptin might activate these mechanisms, thereby directing attention to food-related and sex-related stimuli, respectively. The presence of NPY in the neural systems related to attention such as the locus ceruleus in the brain stem (2, 27), which receives afferent input from the corticotropin-releasing hormone containing cells in the amygdala (32) is consistent with this hypothesis. However, in the absence of further data on the networks involved in the regulation of appetitive and consummatory ingestive and sexual behaviors, this hypothesis remains speculative. It is also important to determine whether the behavioral effects reported here, although highly specific, can be obtained with methods that more precisely mimic the neuroendocrine events that occur under physiological conditions such as energy depletion and repletion.

Perspectives

The current intense interest in the hypothalamic circuity of food intake (12, 14) is reminiscent of the interest in hypothalamic centers of motivation in the 1950s (31). The main difference is that excitatory and inhibitory centers have been replaced by excitatory and inhibitory peptides. A broader concept of the role of peptides in the behavioral adaptations needed during energy depletion and repletion might be useful. As a start, it is tempting to speculate that the leptin-NPY neuroendocrine system serves the purpose of directing attention to food acquisition when energy stores are depleted, i.e., when leptin levels are low and NPY levels are high, and to other activities when energy levels are high, i.e., when leptin levels are high and NPY levels are low.

We thank Drs. G. van Dijk, J. H. Strubbe, and S. C. Woods for valuable comments.

A. A. Ammar is supported by the Ninni von Heijne research student stipend. The work was supported by the Swedish Medical Research Council (K2000-04X-13410-01A) and the Karolinska Institute.
REFERENCES


