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Neuropeptide Y influences acute food intake and energy status affects NPY immunoreactivity in the female musk shrew (*Suncus murinus*)

Karolina Bojkowska [1,2], Magdalena M. Hamczyk [1,3], Houng-Wei Tsai, Anna Riggan, Emilie F. Rissman *

Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, PO Box 800733, VA 22908, USA

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Abstract

Neuropeptide Y (NPY) stimulates feeding, depresses sexual behavior, and its expression in the brain is modulated by energetic status. We examined the role of NPY in female musk shrews, a species with high energetic and reproductive demands; they store little fat, and small changes in energy can rapidly diminish or enhance sexual receptivity. Intracerebroventricular infusion of NPY enhanced acute food intake in shrews; however, NPY had little affect on sexual receptivity. The distribution of NPY immunoreactivity in the female musk shrew brain was unremarkable, but energy status differentially affected NPY immunoreactivity in several regions. Similar to what has been noted in other species, NPY immunoreactivity was less dense in brains of *ad libitum* shrews and greater in shrews subjected to food restriction. In two midbrain regions, both of which contain high levels of gonadotropin releasing hormone II (GnRH II), which has anorexigenic actions in shrews, NPY immunoreactivity was more sensitive to changes in food intake. In these regions, acute re-feeding (90–180 min) after food restriction reduced NPY immunoreactivity to levels noted in *ad libitum* shrews. We hypothesize that interactions between NPY and GnRH II maintain energy homeostasis and reproduction in the musk shrew.

Keywords: Obesity; Anorexia; Orexins; Energy; Feeding disorder; Mating behavior; Reproduction; Gonadotropin releasing hormone; GnRH II

Introduction

The two behaviors that are the most highly conserved in all animals are feeding and mating (Bronson, 1985; Schneider, 2004). One neuropeptide that is abundant in brains and influences both behaviors is neuropeptide Y (NPY), a 36 amino acid peptide member of the pancreatic hormone family (Allen et al., 1983; de Quidt and Emson, 1986; Tatemoto, 1982). NPY is a potent stimulant of feeding behavior and lipogenesis (Clark et al., 1984; Stanley and Leibowitz, 1985; Zarjevski et al., 1993). It also plays an important role in maintaining energy intake and body weight under conditions of food restriction and decreased energy expenditure (Herzog, 2003; Kalra and Kalra, 2003; Thorsell and Heilig, 2002). In contrast in rats and hamsters, NPY infusions inhibit sexual behaviors (Clark et al., 1985; Corp et al., 2001; Jones et al., 2004; Kalra et al., 1988). These data taken together suggest that NPY is one of the neuropeptides that acts as an interface between reproduction and energy intake.

NPY is widely distributed within vertebrate species as its presence in the brain and role in stimulating food intake has been confirmed in various vertebrates from fish to humans (Allen et al., 1983; Crespi et al., 2004; Goldstone, 2006; Grove et al., 2003; Inui et al., 1991; Kuenzel et al., 1987; Morley et al., 1987; Volkoff et al., 2005). While the function of NPY has been extensively studied in a few temperate zone mammals, to our knowledge no data have been collected in tropical or semi-tropical mammals that have less predictable food supplies and as such may have differences in neuropeptide regulation of appetite and feeding. Here we addressed the role of this well-

* Corresponding author. Fax: +1 434 243 8433.
E-mail address: efr2f@virginia.edu (E.F. Rissman).
[1] Equally contributing authors.
[2] Present address: School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland.
[3] Present address: Polish Academy of Sciences, Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warszawa, Poland.

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characterized peptide in the musk shrew (*Suncus murinus*). Shrews are primitive mammals belonging to order Insectivora. Musk shrews reside in the tropics and semi-tropics where they breed year round. Their reproduction is exquisitely tied to food availability, having low body fat stores and high metabolic rates (Temple, 2004). The hypothalamic–pituitary–gonadal (HPG) axis can be shut down by 2 days of mild food restriction (60% of *ad libitum*) and re-activation begins after 90 min of *ad libitum* feeding (Temple and Rissman, 2000a). To date, the only neuropeptide that has been examined for its role in feeding in this species is gonadotropin releasing hormone II (GnRH II), which depresses food intake and enhances female sexual behavior (Kauffman and Rissman, 2004; Temple et al., 2003; Temple and Rissman, 2000a). NPY stimulates feeding and inhibits sexual behavior (Clark et al., 1985) and thus appears to act in an opposite direction to GnRH II.

In the first two experiments, we evaluated several doses of NPY, infused into the lateral ventricles, on acute food intake and sexual receptivity. Next the distribution of NPY immunoreactivity in the musk shrew brain and the effect of nutritional status on staining densities were quantified.

Materials and methods

Animals

All experiments were conducted with adult (2–3 months of age), sexually naive, female musk shrews (*S. murinus*) weighing between 20 and 28 g. All animals were born in the breeding colony at the University of Virginia (Charlottesville, VA). After weaning at 21 days of age, the animals were housed individually in cages (28×17×12 cm) with food (Purina Cat Chow, Nestle Purina PetCare, St. Louis, MO) and water available *ad libitum*(except in Experiment 3 in which food restriction was required). The room, containing only females, was maintained on a 14:10 light:dark photoperiod at temperature of 23± 2 °C. All experiments were performed in compliance with regulations of the Animal Care and Use Committee of the University of Virginia.

Experiment 1: food intake and NPY

In Experiment 1, the impact of NPY on acute feeding was examined. Adult females were subjected to stereotaxic surgery, and a cannula was placed in the lateral ventricle. After 7–10 days of recovery from the surgery, basal food intake was measured for 4 days. Animals were briefly anesthetized and received an intracerebroventricular (icv) infusion with one of four doses of NPY (20, 2, 0.2 and 0.02 nmol; $n=8$–10 per dose group). Control animals received an infusion of vehicle, artificial cerebral spinal fluid (aCSF; $n=8$). Next, food intake was measured 1, 3 and 24 h after the infusion. All shrews were infused at the same time of the day about 30 min before lights in the animal room go off. This time was selected because the majority of feeding occurs in the dark portion of the day (Kauffman and Rissman, 2004). All feeding groups were initially matched for average body weight and baseline food intake.

Experiment 2: sexual behavior and NPY

In Experiment 2, the effect of NPY on female receptivity was examined. Adult females were subjected to stereotaxic surgery, and a cannula was placed in the lateral ventricle. After 7–10 days of recovery from the surgery, animals were briefly anesthetized and received an icv infusion with one of two doses of NPY (2 nmol, $n=12$; and 0.2 nmol, $n=10$). Control animals received an infusion of vehicle, aCSF ($n=12$). Fifteen minutes after infusion, females were paired with a stud male that was habituated to a neutral test box. All shrews were infused and tested at the same time of the day, during the last 4 h prior to when the room lights went off; musk shrews can display mating behavior at any time in the day (Rissman, unpublished observation). All groups were initially matched for average body weight.

Experiment 3: immunocytochemistry for NPY

Adult female musk shrews were assigned to one of four feeding groups ($n=6$ per group): AL, receiving unlimited access to food at all times; FR, food restricted to 60% of baseline intake for 48 h prior to sacrifice; and RF-90 or RF-180, receiving *ad libitum* access to food for 90 or 180 min, respectively, after 48 h of food restriction. These time points were selected based on our past work in which we found that this schedule of food restriction suppressed mating behavior which was reversed in the majority of females by 90 min of *ad libitum* access to food (Temple and Rissman, 2000b). Upon sacrifice, brains were removed and fixed, and the tissue was processed for immunocytochemistry with a primary antibody against NPY peptide. Pattern of NPY immunoreactivity and differences in the staining between groups were determined. All musk shrews were sacrificed at the same time of the day.

Experiment 4: immunocytochemistry for NPY and GnRH II

To assess the colocalization of NPY and GnRH II, we conducted a study with brains from 4 females that were food restricted for 12 h prior to perfusion. The tissue was processed for dual-labeled immunocytochemistry in sequence with first a primary monoclonal antibody against NPY peptide and then a primary rabbit polyclonal antibody made against GnRH II. Presence of NPY-immunoreactive (ir) fibers near GnRH II-ir perikarya was assessed qualitatively. All musk shrews were sacrificed at the same time of the day.

Stereotaxic surgery

In Experiments 1 and 2, the animals underwent stereotaxic implantation of cannula into the lateral ventricle as previously described (Temple et al., 2003). Briefly, animals were anesthetized with sodium pentobarbital (4.5 mg/ml/kg body weight) and/or isoflurane inhalant. Shrews received a midline incision along the top of the head, and bupivacaine (0.25% in 0.1 ml) was injected into the muscles above the skull. Shrews were fitted into a modified mouse stereotaxic apparatus and a guide cannula (26 gauge from Plastics One) containing an internal dummy cannula was centered on bregma and positioned −4.5 mm rostral–caudal, −1.0 mm medial–lateral. A hole was drilled in the skull, and the cannula was lowered to a depth of 2.2 mm aimed at the lateral ventricle. The cannula was fixed to the skull with glue and dental acrylic, and the tip of cannula was secured with dummy cannula (33 gauge). After the surgery, the animal received an injection of saline (sc) and analgesic (Ketoprofen, 2 mg/kg body weight).

Peptide infusions

In Experiments 1 and 2, on the day of infusion, females were briefly anesthetized with isoflurane inhalant (Burns Veterinary Supplies, Inc., Paul, MN, USA) and infused with 2 μl of either artificial cerebral spinal fluid (aCSF, control group) or different doses of NPY, using an internal cannula (33 gauge) with a 0.5-mm projection attached to a syringe and delivered slowly with an infusion pump over the course of 1 min in Experiment 1 and 2 min in Experiment 2. The internal cannula was left in place for an additional 30–45 s after the infusion in order to prevent backflow. Then the dummy cannula was replaced and the shrew returned to its cage. At the end of the study, to confirm the placement of cannula, shrews were anesthetized with an overdose of sodium pentobarbital, and 10 μl of India ink was injected into the cannulas. The brains were removed and sectioned on a cryostat. Cannulas were considered placed correctly if ink was present in the ventricular linings.

Food intake measurement and food restriction

In Experiments 1 and 3, animals received pre-weighed food in excess of their normal 24-h intake. The uneaten food was weighed at 24 h intervals for 4 days, and the difference was used to calculate the daily average baseline food intake for each individual. In Experiment 1, after the icv infusion, individual food