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QRFP in female rats: Effects on high fat food intake and hypothalamic gene expression across the estrous cycle

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ABSTRACT

Pyroglutamylated arginine–phenylalanineamide peptide (QRFP) is a neuropeptide involved in feeding behavior. Central administration of QRFP selectively increases the intake of a high fat diet in male rats. QRFP administration also stimulates the hypothalamic-pituitary–gonadal axis via gonadotrophin-releasing hormone in male and female rats. Prepro-QRFP mRNA is expressed in localized regions of the mediobasal hypothalamus which are abundant in neurotransmitters, neuropeptides and receptor systems important for food intake regulation and reproductive behaviors. The current experiments were conducted to investigate the effects of centrally administered QRFP-26 on the intake of a high fat diet (HFD, 60% kcal from fat) in female rats and to investigate alterations in hypothalamic prepro-QRFP and its receptors, GPR130a and GPR103b, mRNA levels over the estrous cycle. In Experiment 1, female rats were administered QRFP-26 (intracerebroventricular; 0.3 nmol, 0.5 nmol, 1.0 nmol) in rats consuming either a HFD or a low fat diet. All doses of QRFP-26 selectively increased the intake of the HFD in female rats. These data suggest that QRFP-26 regulates the intake of energy dense foods in female rats, which is similar to previous findings in male rats. In Experiment 2, hypothalamic levels of prepro-QRFP mRNA and its receptors were assessed during diestrus, proestrus, or estrus. The level of prepro-QRFP mRNA in the ventromedial/arcuate nucleus (VMH/ARC) of the hypothalamus was increased during proestrus, which suggests that endogenous estrogen levels regulate QRFP expression in the VMH/ARC. These data suggest that QRFP may play a role in coordinating feeding behaviors with reproductive function when energy demand is increased.

1. Introduction

Members of the RFamide-related peptide family exert a large array of biological activities which include cardiovascular functioning, analgesia, food intake, blood pressure, locomotor activity and pituitary hormone regulation [10,18,19,33,36,54,55]. Recently, a 26-amino acid peptide exhibiting the Arg–Phe–NH_2 signature was isolated from frog brain, pyroglutamylated arginine–phenylalanineamide peptide (QRFP-26, also referred to as 26RFa) and the cDNA encoding QRFP-26 was characterized in rat, mouse, quail, chicken, goldfish and human [4,5,27,48]. The QRFP-26 precursor has been shown to generate an N-terminal extended form of 43-amino acids (QRFP-43, also referred to as 43RFa). Both QRFP-26 and QRFP-43 are potent ligands for GPR103a and GPR103b, G protein-coupled receptors, which are located throughout the brain [3,21,22,25,46]. Distribution of prepro-QRFP mRNA in the rat central nervous system is localized in the mediobasal hypothalamus, particularly the arcuate nucleus (ARC), lateral hypothalamus (LH), retrochiasmatic area, and ventromedial hypothalamus (VMH) [5,19,22]. These hypothalamic regions are important in the regulation of ingestive behaviors and reproductive behaviors and are abundant in neurotransmitters, neuropeptides, and receptor systems that influence feeding and reproduction [1,2,6,11,23,24,25,36,40,44,45,47,53].

QRFP-26 and QRFP-43 have been shown to increase food intake in mice and chickens and high fat food (HFD) intake in rats [5,9,30,36,46,48]. Administration of QRFP-26 led to a short-term increase in food intake in food restricted mice [9,46]. Chronic administration of QRFP-43 increased body weight over 14 days while only increasing food intake for the first few days [30]. In mice fed a moderately fat diet, chronic administration of QRFP-43 increased body weight, daily food intake, and body fat [30]. In male rats, central administration of QRFP-26 and QRFP-43 led to an increase in the consumption of HFD but not low fat diet (LFD),

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[36]. Kampe et al. [22] have reported that central administration of QRFP-26 moderately, though not significantly, increased standard chow (a diet low in fat) intake in rats at 2 h following administration [22]. Based on these studies, it is probable that the amount of fat in the diet is an important factor mediating the orexigenic actions of QRFP.

In addition to effects on feeding behavior, administration of QRFP-43 stimulates the hypothalamic-pituitary–gonadal axis via gonadotrophin-releasing hormone in rats [33]. In male rats, QRFP-43 administration increased plasma levels of luteinizing hormone and follicle stimulating hormone. In addition, QRFP-26 and QRFP-43 dose-dependently enhanced basal and stimulated luteinizing hormone secretion from the pituitary of male and cycling female rats [31]. Gonadotrophin-releasing hormone is important for reproduction and in female rats, luteinizing hormone triggers ovulation. Therefore, QRFP may play a role in reproduction and may have sex-specific effects on feeding behavior. Female rats show changes in feeding behavior across the estrous cycle and in response to ovariectomy (OVX) [12–14,16,28,29,41,43,51,52]. Following OVX, rats are hyperphagic and rapidly gain weight. The effects of OVX and estradiol treatment on the feeding effects of various peptides and drugs have been investigated and have led to numerous studies illustrating the complex role between feeding and reproduction [13,15,17,29,38,39,41,42].

Currently, there are no reports of the effects of QRFP on the consumption of HFD or LFD in female rats. Furthermore, there are no reports on the effects of estrous cycle on hypothalamic prepro-QRFP, GPR103a or GPR103b mRNA levels. Therefore the purpose of the current series of experiments was to investigate the effects of centrally administered QRFP-26 on HFD and LFD consumption in female rats, and to investigate fluctuations in hypothalamic prepro-QRFP and its receptors, GPR103a and GPR103b mRNA expression across the estrous cycle. We hypothesized that central administration of QRFP-26 would selectively increase HFD in female rats; supporting the idea that QRFP is involved in the intake of energy dense foods. This hypothesis is based on our previous experiment in male rats, which demonstrated that central administration of QRFP-26 and QRFP-43 (26 amino acid sequence and 43 amino acid sequence, respectively) increased HFD intake, but not LFD intake [36]. In Experiment 2, prepro-QRFP, GPR103a and GPR103b gene expression was measured in the ventromedial hypothalamus/arcuate nucleus and lateral hypothalamus. We hypothesized that QRFP gene expression would be affected by estrogen and/or progesterone and therefore fluctuate across the estrous cycle.

2. Materials and methods

2.1. Animals

Adult female Long Evans rats (Harlan, Inc., Indianapolis, IN) weighing between 150 and 175 g (8 weeks of age) upon arrival were used for Experiments 1 and 2. Rats were housed in standard shoebox cages in an AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care) approved animal facility on a 12/12 h light/dark cycle (lights on at 0700) with food and water available *ad libitum*. All procedures were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

2.2. Experiment 1: effects of central QRFP administration on food intake in female rats

Individually housed female rats were adapted to a pelleted HFD (60% kcal from fat/20% kcal from carbohydrates; kcal = 5.24/g; D12492; Research Diets, New Brunswick, NJ) or a LFD (10% kcal from fat/70% kcal from carbohydrates; kcal = 3.85/g; D124508; Research Diets) for two weeks prior to surgery for the implantation of an indwelling cannula aimed at the lateral ventricle. Estrous cycle was not monitored during this experiment. Therefore, the effects of QRFP-26 on food intake were determined in randomly cycling females.

2.2.1. Indwelling cannula surgery

Rats were anesthetized with a ketamine cocktail (ketamine, 80 mg/ml; acepromazine, 1.6 mg/ml; xylazine, 5 mg/ml, i.p.) and their heads were shaved, cleaned and injected with a local anesthetic (bupivicane/lidocaine, 1 mg/kg, s.c) [36]. Rats were placed in a stereotaxic apparatus (David Kopf, Tujunga, CA) and a midline incision was made. Bregma was measured and a single hole was drilled into the skull. A 22-gage stainless steel cannula, 7 mm in length (Plastics One, Roanoke, VA) was implanted into the lateral ventricle, using the coordinates AP −0.9, LM −1.5, DV −3.0 [34] and anchored with orthodontic resin (Dentsply Caulk, Milford, DE). Following surgery, carprofen (1.0 mg/kg, s.c.) was given for postoperative analgesia. Rats were allowed to recover for 7–10 days and were habituated to handling procedures prior to testing.

2.2.2. Drug treatment

QRFP-26 (purity ≥ 95%; Phoenix Pharmaceuticals, Inc., Burlingame, CA) was dissolved in vehicle (30% propylene glycol in 0.9% sterile saline). On test day, female rats were injected with 5.0 μl of QRFP-26 (0.3 nmol, 0.5 nmol, 1.0 nmol) or vehicle.

2.2.3. Measurement of food intake

Testing began 10 days following cannula implantation surgery. On test day, all rats were given access to fresh HFD or LFD for 30 min prior to beginning injection procedures. This was done to stimulate eating and promote satiety in these rats. Following this 30 min period, female rats were injected with 5.0 μl of QRFP-26 or vehicle using a 20-gage injector (Plastics One), which extended 1 mm beyond the guide cannula. Injections were made manually at a rate of 5.0 μl/min. The injectors remained in the cannula for an additional minute to allow for diffusion. QRFP-26 and vehicle were administered using a Latin square design (n = 6–7/dose for LFD; n = 8–9/dose for HFD). Each rat received each dose of QRFP-26 and vehicle (4 injections), 3–4 days apart. Following injections, rats were immediately returned to their home cage and given preweighed fresh HFD or LFD. All injections were made between 0900 and 1030. Food intake was measured at 1 h, 2 h, and 4 h. Previous studies have indicated that the effects of QRFP-26 on food intake were undetectable at 24 h following administration [36], therefore 24 h intake was not measured in the current study. In this experiment, rats had minimal spillage of diet from the food hopper during testing. When spillage was detected, care was taken to collect and measure any spillage from the bottom of the shoebox cage.

2.3. Experiment 2: influence of estrous cycle on hypothalamic prepro-QRFP and GPR103a mRNA levels

Two weeks after arrival to the animal facilities, determinations of estrous cycle began in a separate group of female rats fed a standard chow diet. In this experiment, rats were pair housed (2/cage) for the duration of this experiment. Daily vaginal smears were taken via lavage from cycling female rats between 0900 and 1000. Estrous phase determination was based on vaginal smear cytology of cells viewed under a low-power microscope. Diestrus was identified by the presence of leukocytes and nucleated epithelial cells. Proestrus was identified by large clusters of round nucleated cells and the absence of leukocytes. Estrus was identified by the presence of cornified cells. Estrous cycle was monitored for 2 weeks prior to sacrifice. Female rats were euthanized based on their estrous