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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; 0.3nmol, 0.5nmol, 1.0nmol) 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.

Keywords

prepro-QRFP mRNA; Hypothalamus; high fat diet; Estrous cycle; 26RFa

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

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peptide exhibiting the Arg-Phe-NH₂ 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 [3,21,22,46]. 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 [1,2,6,11,23,24,36,40,44,45,47,53].

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), [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 2h 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 150-175g (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 12/12h 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, 80mg/ml; acepromazine, 1.6mg/ml; xylazine, 5mg/ml, i.p) and their heads were shaved, cleaned and injected with a local anesthetic (bupivacaine/lidocaine, 1mg/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-gauge stainless steel cannula, 7mm 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 (1.0mg/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 5.0μl of QRFP-26 (0.3nmol, 0.5nmol, 1.0nmol) 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 30min prior to beginning injection procedures. This was done to stimulate eating and promote satiety in these rats. Following this 30min period, female rats were injected with 5.0μl of QRFP-26 or vehicle using a 20-gauge injector (Plastics One), which extended 1mm beyond the guide cannula. Injections were made manually at a rate of 5.0μl/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 pre-weighed fresh HFD or LFD. All injections were made between 0900 and 1030. Food intake was measured at 1h, 2h, and 4h. Previous studies have indicated that the effects of QRFP-26 on food intake were undetectable at 24h following administration [36], therefore 24h 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 stage (diestrus n=12; proestrus n=10; estrus n=10) for analysis of prepro-QRFP, GPR103a, and GPR103b mRNA levels. Animals were sacrificed within 2h of determination of estrous cycle by decapitation and brains were removed, frozen on dry ice and stored at -80°C until further processing.

2.3.1 Real-time Polymerase Chain Reaction (PCR)—For gene expression analysis, bilateral 1mm diameter brain punches, approximately 2mm thick, were taken from the VMH/ARC and LH. Coordinates for punches were based on the Rat Brain Atlas by Paxinos and Watson [34]. RNA was isolated from bilateral punches of the specific hypothalamic regions using Tri-Reagent (Molecular Research Ctr, Cincinnati, OH USA) and RNeasy Minikit procedures (Qiagen, Valencia, CA USA) and based on previous experiments by Primeaux et al. [35-37]. Briefly, tissue was homogenized in Tri-Reagent, chloroform was added and the mixture was centrifuged ($12,000\times g$) in phase lock tubes to separate RNA. Ethanol (70%) was added to the upper aqueous phase and filtered by centrifugation ($8000\times g$). Following multiple washes, samples were subjected to an elution step using RNAase-free water. Reverse transcription (RT) was conducted using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). For RT, 1.0µg of RNA from each sample was added to random primers (10×), dNTP (25×), MultiScribe Reverse Transcriptase (50U/µl) and RT buffer (10×) and incubated in a thermal cycler (PTC-100, MJ Research, Inc, Watertown, MA, USA) for 10 min at 27°C , then for 120 min at 37°C . Taqman Gene Expression Assays (Applied Biosystems) were used to assess levels of prepro-QRFP (Rn01644297_s1), GPR103a (Rn01644293_mH), GPR103b (Rn01425102_m1) and the housekeeping gene, cyclophilin (Rn00574762_m1), therefore primer sequence information is unavailable. For Real-time PCR, Taqman Universal PCR Master Mix (Applied Biosystems), gene expression assay, and RT product (10ng) were added to a 384 well plate. The cycling parameters consisted of an initial 2 min incubation at 50°C , followed by 10 min at 95°C , then 15 sec at 95°C , and a 1 min annealing/extension step at 60°C (40 cycles). The quantity of prepro-QRFP, GPR103a, and GPR103b mRNA expression was based on a standard curve using pooled cDNA from the VMH/ARC of all experimental samples and normalized to cyclophilin levels (ABI Prism 7900 Sequence Detection System, Applied Biosystems).

2.4 Histology

Rats in Experiment 1 were sacrificed by CO_2 asphyxiation; brains were removed and placed in 4% paraformaldehyde/0.1M sodium phosphate solution for 24 hours at 4°C . Brains were subsequently placed in a 15% sucrose/0.1M sodium phosphate buffer solution at 4°C until slicing (50µm sections) using a freezing microtome (Microm HM400, Waldorf, Germany). Sections were thaw mounted on slides, stained with cresyl violet histological stain, and examined under a low power microscope. Cannula placement was plotted using the rat atlas

of Paxinos and Watson [34]. One animal was removed from analyses due to improper cannula placement.

2.5 Statistical Analysis

In Experiment 1, cumulative food intake in kilocalories (kcal) was assessed for each dietary condition and at each time point (1h, 2h, 4h) following QRFP-26 or vehicle injection using a repeated measures ANOVA (QRFP is the between subjects factor and time is within subjects factor). In Experiment 2, a one way ANOVA was used to analyze differences in prepro-QRFP, GPR103a and GPR103b mRNA levels over the estrous cycle, for each brain region. Bonferroni post-hoc tests were used to compare groups when a significant main effect or interaction was detected. A significance level of $p < .05$ was used for all tests.

3. Results

3.1 Experiment 1: Effects of central QRFP-26 administration on food intake in female rats

As expected, in female rats consuming a HFD, significant main effects were detected for QRFP-26 ($F(4,35) = 2.78, p < .05$) and time ($F(2,35) = 55.08, p < .0001$). Administration of 0.3nmol, 0.5nmol, and 1.0nmol QRFP-26 increased HFD intake in female rats at 1h, 2h, and 4h, compared to vehicle administration ($p < .05$; Figure 1A). In rats consuming a LFD, a main effect of time was detected ($F(2,30) = 35.05, p < .0001$; Figure 2B), suggesting that rats consumed more LFD over the course of the experiment. However, as seen previously in male rats [36], QRFP-26 administration did not significantly increase LFD intake at any time point in the current experiment.

3.2 Experiment 2: Influence of estrous cycle on hypothalamic prepro-QRFP, GPR103a and GPR103b mRNA levels

Prepro-QRFP and its receptors, GPR103a and GPR103b, mRNA levels were assessed in the VMH/ARC and LH of cycling female rats. These regions in the mediobasal hypothalamus regions were selected due to their role in feeding and reproductive behaviors and because previous studies have demonstrated the presence of prepro-QRFP, GPR103a and GPR103b mRNA in these regions. In the VMH/ARC, prepro-QRFP mRNA level was significantly affected by estrous cycle ($F(2,23) = 3.77; p < .05$). Prepro-QRFP mRNA expression in the VMH/ARC of proestrus rats was significantly higher than prepro-QRFP mRNA levels in the VMH/ARC of rats in diestrus or estrus (Figure 2A; $p < .05$). Prepro-QRFP mRNA levels in the LH did not differ across the estrous cycle. Gene expression levels of the receptors, GPR103a and GPR103b, were not altered by estrous cycle in either the VMH/ARC or the LH (Figures 2B, 2C, $p < .05$).

4. Discussion

The neuropeptide, QRFP, is expressed in the mediobasal hypothalamus, which is important for feeding and reproductive behaviors [1,2,5,6,19,22,44]. Previous studies have reported increased food intake following QRFP-26 and QRFP-43 administration in rodents [9,30,36,46]. In male rats given a single diet choice, QRFP-26 and QRFP-43 (26-amino acid peptide and 43-amino acid peptide, respectively) administration into the lateral ventricles selectively increased HFD intake, but not LFD intake [36]. In addition to its effects on feeding behavior, QRFP has been linked to the hypothalamic-pituitary-gonadal axis. In male rats, QRFP-43 administration increased circulating luteinizing hormone and follicle stimulating hormone levels. In male and cycling female rats, QRFP-26 and QRFP-43 administration enhanced basal and stimulated luteinizing hormone from the pituitary [31]. These hormones are important for a successful reproductive cycle. These results suggest that QRFP may be involved in mediating the reproductive cycle. The purpose of the current

series of experiments was investigate the effects of QRFP-26 on feeding behavior in female rats and to determine if prepro-QRFP and the receptors, GPR103a and GPR103b, mRNA levels in the VMH/ARC and LH were influenced by estrous cycle in female rats. To our knowledge, this is the first investigation of QRFP on feeding behavior in female rats and the first assessment of QRFP, GPR103a, and GPR103b gene expression in the mediobasal hypothalamus of female rats.

In Experiment 1, randomly cycling females were habituated to a HFD or a LFD prior to testing. Since this was the first study conducted investigating the effects of QRFP-26 on food intake in female rats, and since the consumption of a HFD may alter estrous cycle, this study was designed to examine the effects of QRFP-26 administration in randomly cycling female rats. During testing, QRFP-26 was administered into the lateral ventricles of satiated female rats and food intake was measured at varying time points (1h, 2h, 4h). As predicted, central QRFP-26 (0.3nmol, 0.5nmol, 1.0nmol) administration in female rats significantly increased the intake of HFD, but not LFD (Figure 1A and 1B). Though there are several significant differences between the current experiment and the experiment reported by Primeaux et al. [36], using male rats, it is important to note that the dose response curve differed substantially. In male rats, a higher dose of QRFP-26 (3.0nmol) was needed to increase HFD intake, compared to the females used in the current experiment (0.3nmol). Though no direct comparisons between male and female rats were made, these data suggest that female rats are more sensitive to the feeding effects of QRFP-26.

In Experiment 2, the influence of estrous cycle on prepro-QRFP and its receptors, GPR103a and GPR103b, mRNA levels in the VMH/ARC and LH regions of the mediobasal hypothalamus was assessed. Prepro-QRFP mRNA levels in VMH/ARC fluctuated across the estrous cycle, and were highest during proestrus (Figure 2A). Prepro-QRFP mRNA levels in the LH did not differ across the estrous cycle. The receptors for QRFP, GPR103a and GPR103b, are expressed throughout the brain [3,22,21,46]. In the current study, GPR103a and GPR103b mRNA levels in the VMH/ARC and LH were not altered by estrous cycle (Figure 2B, 2C), suggesting that GPR103a and GPR103b receptor expression is not altered by estrogen/progesterone levels in the hypothalamic regions which have been shown to express their endogenous ligand, prepro-QRFP. However, further studies are needed to examine alterations in GPR103a and GPR103b gene expression in other brain regions across the estrous cycle.

Previous studies have reported significant effects of estrous cycle on the expression of hypothalamic neuropeptides involved in feeding behavior [26,32,49,50]. Galanin and neuropeptide Y protein levels peak during proestrus, when circulating estrogen and progesterone also rise. The peak in peptide level is accompanied by an increase in caloric intake in rats eating a chow diet [26]. Hypothalamic neuromedin S mRNA levels, a neuropeptide which decreases food intake, are significantly increased during proestrus [50]. In female rats, food intake varies across the estrous cycle [12,14,20]. In most strains, female rats consume the most food during diestrus, followed by proestrus, and the smallest amount of food during estrus. Interestingly, the efficacy of peptides associated with food intake varies across the estrous cycle (e.g. CCK, MCH) [13,20,42]. Further studies are needed to examine the role of QRFP across the estrous cycle, and the effects of endogenous estrogen and progesterone levels on the expression and behavioral effects of QRFP.

Though not investigated in these experiments, it is likely that the consumption of the HFD significantly altered aspects of the neural chemistry, which in turn may have altered estrogen/progesterone levels, the estrous cycle and QRFP and GPR103 expression. Previous studies have investigated the effects of various peptides on feeding behavior following ovariectomy and estradiol-replacement following ovariectomy [7,8,41,42]. These studies

lend valuable insight into the relationship between gonadal hormones and feeding behavior and need to be applied to QRFP. Additional studies are also needed to examine the circuitry of QRFP and its relationship to neurotransmitter, neuropeptide, hormone and receptor systems involved in feeding and reproduction.

5. Conclusions

The intake and regulation of food and the initiation and maintenance of reproduction are complex behaviors which are intimately related to the successful survival of the species. Though there are several neuronal systems involved in both behaviors, the complexity of the behaviors has hindered understanding of the coordination of food intake regulation and reproduction. QRFP is a neuropeptide that has been conserved across species, suggesting that this neuropeptide has important implications for behavior. The role of QRFP in female rats is unclear. Based on the current experiments, it is possible that QRFP serves as a 'trigger' for reproduction in female rats and promotes a successful reproductive cycle by increasing the intake of calorically dense foods during an increase in energy demand. This data is supported by previous reports linking QRFP administration to a release of luteinizing hormone, which stimulates ovulation [31]. The alterations in prepro-QRFP mRNA levels across the estrous cycle suggest that QRFP's orexigenic effects fluctuate across the estrous cycle, which is congruent with data demonstrating estrous cycle-induced fluctuations in food intake in female rats. These relationships are not completely clear at this time, and require future studies investigating the coordination of gonadal hormones, hypothalamic QRFP and other established neuropeptides known to influence feeding behavior and reproductive parameters.

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References

- [1]. Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. *Nat Rev Genet.* 2002; 3(8):589–600. [PubMed: 12154382]
- [2]. Bray GA. Peptides affect the intake of specific nutrients and the sympathetic nervous system. *Am J Clin Nutr.* 1992; 55(1 Suppl):265S–271S. [PubMed: 1728838]
- [3]. Bruzzone F, Lectez B, Alexandre D, Jegou S, Mounien L, Tollemer H, Chatenet D, Leprince J, Vallarino M, Vaudry H, Chartrel N. Distribution of 26RFa binding sites and GPR103 mRNA in the central nervous system of the rat. *J Comp Neurol.* 2007; 503(4):573–591. [PubMed: 17534937]
- [4]. Chartrel N, Bruzzone F, Dujardin C, Leprince J, Tollemer H, Anouar Y, Vallarino M, Costentin J, Vaudry H. Identification of 26RFa from frog brain: a novel hypothalamic neuropeptide with orexigenic activity in mammals. *Ann N Y Acad Sci.* 2005; 1040:80–83. [PubMed: 15891009]
- [5]. Chartrel N, Dujardin C, Anouar Y, Leprince J, Decker A, Clerens S, do-Rego JC, Vandesande F, Llorens-Cortes C, Costentin J, Beauvillain JC, Vaudry H. Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. *Proc Natl Acad Sci U S A.* 2003; 100(25):15247–15252. [PubMed: 14657341]
- [6]. Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology.* 1984; 115:427–429. [PubMed: 6547387]
- [7]. Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes.* 2006; 55(4):978–987. [PubMed: 16567519]

- [8]. Clegg DJ, Brown LM, Zigman JM, Kemp CJ, Strader AD, Benoit SC, Woods SC, Mangiaracina M, Geary N. Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. *Diabetes*. 2007; 56:1051–1058. [PubMed: 17251274]
- [9]. do Rego JC, Leprince J, Chartrel N, Vaudry H, Costentin J. Behavioral effects of 26RFamide and related peptides. *Peptides*. 2006; 27(11):2715–2721. [PubMed: 16730856]
- [10]. Dockray GJ. The expanding family of -RFamide peptides and their effects on feeding behaviour. *Exp Physiol*. 2004; 89(3):229–235. [PubMed: 15123557]
- [11]. Dourmashkin JT, Chang GQ, Gayles EC, Hill JO, Fried SK, Julien C, Leibowitz SF. Different forms of obesity as a function of diet composition. *Int.J Obes*. 2005; 29(11):1368–1378.
- [12]. Eckel LA. Ingetive behaviour in female rats: influence of the ovarian cycle. *Appetite*. 1999; 32:274. [PubMed: 10097032]
- [13]. Eckel LA, Geary N. Endogenous cholecystokinin's satiating action increases during estrus in female rats. *Peptides*. 1999; 20(4):451–456. [PubMed: 10458514]
- [14]. Eckel LA, Houpt TA, Geary N. Spontaneous meal patterns in female rats with and without access to running wheels. *Physiol Behav*. 2000; 70(3-4):397–405. [PubMed: 11006440]
- [15]. Eckel LA, Houpt TA, Geary N. Estradiol treatment increases CCK-induced c-Fos expression in the brains of ovariectomized rats. *Am J Physiol Regul Integrative Comp Physiol*. 2002; 283(6):R1378–R1385.
- [16]. Eckel LA, Moore SR. Diet-induced hyperphagia in the rat is influenced by sex and exercise. *Am J Physiol Regul Integrative Comp Physiol*. 2004; 287(5):R1080–R1085.
- [17]. Eckel LA, Rivera HM, Atchley DP. The anorectic effect of fenfluramine is influenced by sex and stage of the estrous cycle in rats. *Am J Physiol Regul Integrative Comp Physiol*. 2005; 288(6):R1486–R1493.
- [18]. Fang Q, Liu Q, Li N, Jiang TN, Li YL, Yan X, Wang R. Cardiovascular effects of intravenous administered 26RFa, a novel RFamide peptide ligand for GPR103, in anaesthetised rats. *Eur J Pharmacol*. 2009; 621(1-3):61–66. [PubMed: 19747477]
- [19]. Fukusumi S, Fujii R, Hinuma S. Recent advances in mammalian RFamide peptides: the discovery and functional analyses of PrRP, RFRPs and QRFP. *Peptides*. 2006; 27(5):1073–1086. [PubMed: 16500002]
- [20]. Huang YS, Doi R, Chowdhury P, Pasley JN, Nishikawa M, Huang TJ, Rayford PL. Effect of cholecystokinin on food intake at different stages of the estrous cycle in female rats. *J Assoc Acad Minor Phys*. 1993; 4(2):56–58. [PubMed: 8490287]
- [21]. Jiang Y, Luo L, Gustafson EL, Yadav D, Lavery M, Murgolo N, Vassileva G, Zeng M, Laz TM, Behan J, Qiu P, Wang L, Wang S, Bayne M, Greene J, Monsma F, Zhang FL. Identification and characterization of a novel RF-amide peptide ligand for orphan G-protein-coupled receptor SP9155. *J Biol Chem*. 2003; 278(30):27652–27657. [PubMed: 12714592]
- [22]. Kampe J, Wiedmer P, Pfluger PT, Castaneda TR, Burget L, Mondala H, Kerr J, Liaw C, Oldfield BJ, Tschop MH, Bagnol D. Effect of central administration of QRFP(26) peptide on energy balance and characterization of a second QRFP receptor in rat. *Brain Res*. 2006; 1119(1):133–149. [PubMed: 16996040]
- [23]. Konturek PC, Konturek JW, Czesnikiewicz-Guzik M, Brzozowski T, Sisto E, Konturek SJ. Neuro-hormonal control of food intake: basic mechanisms and clinical implications. *J Physiol Pharmacol*. 2005; 56(Suppl 6):5–25.
- [24]. Kyrkouli SE, Stanley BG, Seirafi RD, Leibowitz SF. Stimulation of feeding by galanin: anatomical localization and behavioral specificity of this peptide's effects in the brain. *Peptides*. 1990; 11(5):995–1001. [PubMed: 1704616]
- [25]. Lectez B, Jeandel L, El-Yamani FZ, Arthaud S, Alexandre D, Mardargent A, Jegou S, Mounien L, Bizet P, Magoul R, Anouar Y, Chartrel N. The orexigenic activity of the hypothalamic neuropeptide 26RFa is mediated by the neuropeptide Y and proopiomelanocortin neurons of the arcuate nucleus. *Endocrinology*. 2009; 150(5):2342–2350. [PubMed: 19164468]
- [26]. Leibowitz SF, Akabayashi A, Alexander JT, Wang J. Gonadal steroids and hypothalamic galanin and neuropeptide Y: role in eating behavior and body weight control in female rats. *Endocrinology*. 1998; 139(4):1771–1780. [PubMed: 9528961]

- [27]. Liu Y, Zhang Y, Li S, Huang W, Liu X, Lu D, Meng Z, Lin H. Molecular cloning and functional characterization of the first non-mammalian 26RFa/QRFP orthologue in Goldfish, *carassius auratus*. *Mol Cell Endocrinology*. 2009; 303(1-2):82–90.
- [28]. McElroy JF, Wade GN. Short- and long-term effects of ovariectomy on food intake, body weight, carcass composition, and brown adipose tissue in rats. *Physiol Behav*. 1987; 39(3):361–365. [PubMed: 3575476]
- [29]. Messina MM, Boersma G, Overton JM, Eckel LA. Estradiol decreases the orexigenic effect of melanin-concentrating hormone in ovariectomized rats. *Physiol Behav*. 2006; 88(4-5):523–528. [PubMed: 16793070]
- [30]. Moriya R, Sano H, Umeda T, Ito M, Takahashi Y, Matsuda M, Ishihara A, Kanatani A, Iwaasa H. RFamide peptide QRFP43 causes obesity with hyperphagia and reduced thermogenesis in mice. *Endocrinology*. 2006; 147(6):2916–2922. [PubMed: 16543370]
- [31]. Navarro VM, Fernandez-Fernandez R, Nogueiras R, Vigo E, Tovar S, Chartrel N, Le MO, Leprince J, Aguilar E, Pinilla L, Dieguez C, Vaudry H, Tena-Sempere M. Novel role of 26RFa, a hypothalamic RFamide orexigenic peptide, as putative regulator of the gonadotropic axis. *J Physiol*. 2006; 573(Pt 1):237–249. [PubMed: 16543265]
- [32]. Olofsson LE, Pierce AA, Xu AW. Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. *Proc Natl Acad Sci U S A*. 2009; 106(37):15932–15937. [PubMed: 19805233]
- [33]. Patel SR, Murphy KG, Thompson EL, Patterson M, Curtis AE, Ghatei MA, Bloom SR. Pyroglutamylated RFamide peptide 43 stimulates the hypothalamic-pituitary-gonadal axis via gonadotrophin-releasing hormone in rats. *Endocrinology*. 2008; 149(9):4747–4754. [PubMed: 18535111]
- [34]. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press; San Diego: 1997.
- [35]. Primeaux SD, Barnes MJ, Bray GA. Olfactory bulbectomy increases food intake and hypothalamic neuropeptide Y in obesity-prone, but not obesity-resistant rats. *Behav Brain Res*. 2007; 180(20):190–196. [PubMed: 17420059]
- [36]. Primeaux SD, Blackmon C, Barnes MJ, Braymer HD, Bray GA. Central administration of the RFamide peptides, QRFP-26 and QRFP-43, increases high fat food intake in rats. *Peptides*. 2008; 29(11):1994–2000. [PubMed: 18765262]
- [37]. Primeaux SD, Wilson SP, Bray GA, York DA, Wilson MA. Overexpression of neuropeptide Y in the central nucleus of the amygdala decreases ethanol self-administration in “anxious” rats. *Alcohol Clin Exp Res*. May; 2006 30(5):791–801. [PubMed: 16634847]
- [38]. Rivera HM, Eckel LA. The anorectic effect of fenfluramine is increased by estradiol treatment in ovariectomized rats. *Physiol Behav*. 2005; 86(3):331–337. [PubMed: 16139852]
- [39]. Rivera HM, Oberbeck DR, Kwon B, Houtt TA, Eckel LA. Estradiol increases Pet-1 and serotonin transporter mRNA in midbrain raphe nuclei of ovariectomized rats. *Brain Res*. 2009; 1259:51–58. [PubMed: 19168037]
- [40]. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998; 92(4):573–585. [PubMed: 9491897]
- [41]. Santollo J, Eckel LA. Estradiol decreases the orexigenic effect of neuropeptide Y, but not agouti-related protein, in ovariectomized rats. *Behav Brain Res*. 2008; 191(2):173–177. [PubMed: 18453005]
- [42]. Santollo J, Eckel LA. The orexigenic effect of melanin-concentrating hormone (MCH) is influenced by sex and stage of the estrous cycle. *Physiol Behav*. 2008; 93(4-5):842–850. [PubMed: 18191424]
- [43]. Schneider JE, Palmer LA, Wade GN. Effects of estrous cycles and ovarian steroids on body weight and energy expenditure in Syrian hamsters. *Physiol Behav*. 1986; 38(1):119–126. [PubMed: 3024192]

- [44]. Schwartz MW, Woods SC, Porte JD, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000; 404:661–671. [PubMed: 10766253]
- [45]. Smith BK, Berthoud HR, York DA, Bray GA. Differential effects of baseline macronutrient preferences on macronutrient selection after galanin, NPY, and an overnight fast. *Peptides*. 1997; 18(2):207–211. [PubMed: 9149292]
- [46]. Takayasu S, Sakurai T, Iwasaki S, Teranishi H, Yamanaka A, Williams SC, Iguchi H, Kawasaki YI, Ikeda Y, Sakakibara I, Ohno K, Ioka RX, Murakami S, Dohmae N, Xie J, Suda T, Motoike T, Ohuchi T, Yanagisawa M, Sakai J. A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. *Proc Natl Acad Sci U S A*. 2006; 103(19):7438–7443. [PubMed: 16648250]
- [47]. Tiesjema B, la Fleur SE, Luijendijk MC, Brans MA, Lin EJ, During MJ, Adan RA. Viral mediated neuropeptide Y expression in the rat paraventricular nucleus results in obesity. *Obesity*. 2007; 15(10):2424–2435. [PubMed: 17925468]
- [48]. Ukena K, Tachibana T, Iwakoshi-Ukena E, Saito Y, Minakata H, Kawaguchi R, Osugi T, Tobari Y, Leprince J, Vaudry H, Tsutsui K. Identification, localization, and function of a novel avian hypothalamic neuropeptide, 26RFa, and its cognate receptor, G protein-coupled receptor 103. *Endocrinology*. 2010; 151(5):2255–2264. [PubMed: 20308530]
- [49]. Vigo E, Roa J, Castellano JM, Navarro VM, Aguilar E, Pinilla L, Tena-Sempere M. Novel role of the anorexigenic peptide neuromedin U in the control of LH secretion and its regulation by gonadal hormones and photoperiod. *Am J Physiol Endocrinol. Metab*. 2007; 293(5):E1265–E1273. [PubMed: 17726140]
- [50]. Vigo E, Roa J, Lopez M, Castellano JM, Fernandez-Fernandez R, Navarro VM, Pineda R, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Neuromedin s as a novel putative regulator of luteinizing hormone secretion. *Endocrinology*. 2007; 148(2):813–823. [PubMed: 17110433]
- [51]. Wade GN. Some effects of ovarian hormones on food intake and body weight in female rats. *J Comp Physiol Psych*. 1975; 88(1):183–193.
- [52]. Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav. Rev*. 1992; 16(2):235–272. [PubMed: 1630733]
- [53]. Woods SC, Figlewicz DP, Madden LJ, Porte D, Sipols AJ, Seeley RJ. NPY and food intake: Discrepancies in the model. *Regul Pept*. 1998; 75-76:403–408. [PubMed: 9802436]
- [54]. Yamamoto T, Miyazaki R, Yamada T. Intracerebroventricular administration of 26RFa produces an analgesic effect in the rat formalin test. *Peptides*. 2009; 30(9):1683–1688. [PubMed: 19520126]
- [55]. Yamamoto T, Wada T, Miyazaki R. Analgesic effects of intrathecally administered 26RFa, an intrinsic agonist for GPR103, on formalin test and carrageenan test in rats. *Neuroscience*. 2008; 157(1):214–222. [PubMed: 18824219]

Figure 1.

Randomly cycling female rats were a single choice diet of either HFD (60% kcal from fat) or LFD (10% kcal from fat). **A.** Central administration of QRFP-26 at varying doses (0.3nmol, 0.5nmol, 1.0nmol) significantly increased the intake of HFD at 1h, 2h, and 4h. **B.** QRFP-26 administration did not increase LFD intake in female rats at any dose or time point measured. Data is expressed as kilocalories due to differences in kilocalories/gram. * $p < .05$, data shown as mean $\pm$ SEM.

Figure 2.

Estrous cycle was monitored daily in female rats. Levels of prepro-QRFP, GPR103a and GPR103b mRNA expression were determined during diestrus, proestrus and estrus using Real-Time PCR. **A.** Prepro-QRFP mRNA levels were significantly elevated during proestrus in the ventromedial hypothalamus/arcuate nucleus (VMH/ARC). **B.** GPR103a mRNA levels were not altered by estrous cycle in the VMH/ARC or LH. **C.** GPR103b mRNA levels were not altered by estrous cycle in the VMH/ARC or LH. Data are normalized to cyclophilin levels and shown as arbitrary units. * $p < .05$, data shown as mean $\pm$ SEM.