Developmental reversal in neuropeptide Y action on feeding in an amphibian

Erica J. Crespi1,2,⁎, Robert J. Denvera,b,*
1Department of Molecular, Cellular, and Developmental Biology, The University of Michigan, Ann Arbor, MI 48109, USA
2Department of Ecology and Evolutionary Biology, The University of Michigan, Ann Arbor, MI 48109, USA

A R T I C L E   I N F O

Article history:
Available online 26 April 2012

Keywords:
Neuropeptide Y
Appetite
Feeding
Amphibian
Tadpole
Development
Hypothalamus

A B S T R A C T

Neuropeptide Y (NPY) is expressed in the hypothalamus where it exerts orexigenic actions within the feeding control circuit. While NPY stimulates feeding in juvenile and adult animals, it is not known whether NPY influences food intake at earlier life stages. We investigated a role for NPY in regulating feeding at two stages of the life cycle of an amphibian, the Western spadefoot toad Spea hammondii. We administered NPY by intracerebroventricular (i.c.v.) injection to juvenile toads or prometamorphic tadoles, and monitored locomotion, feeding behavior and/or food intake. Injection of NPY (0.02–20 ng/g BW) into juvenile toads decreased the latency to, and increased the number of strikes at prey, and the number of crickets eaten compared to uninjected or vehicle-injected controls. By contrast, injection of NPY (0.02–20 ng/g BW) into prometamorphic tadpoles caused a dose-dependent decrease in time spent foraging compared to controls. Blocking NPY signaling in the prometamorphic tadpole brain by i.c.v. injection of a general NPY receptor antagonist increased foraging, and partly blocked the action of exogenous NPY on foraging. Taken together, our findings show a developmental reversal in NPY actions on feeding in an amphibian, with the peptide having a characteristic orexigenic action in the juvenile toad, but an inhibitory action on foraging in the prometamorphic tadpole. The anorexigenic action of NPY in the tadpole correlates with a decrease in feeding that occurs during metamorphic climax when the tadpole’s gut and cranium remodels for the transition to a carnivorous diet.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Neuropeptide Y (NPY) is a neurotransmitter/neuromodulator that acts within hypothalamic feeding control centers to increase appetite and food intake [28]. The peptide has orexigenic actions in juveniles and adults of diverse vertebrate species, suggesting that its role in the regulation of appetite and feeding is ancient, and has been evolutionarily conserved (mammals: [35]; birds: [23,41]; amphibians: [9]; fishes: [30,34,38]). In mammals, expression, and maturation of the feeding control circuitry prior to weaning correlate with the change in dietary needs between a milk-based diet to the independent ingestion of solid foods, particularly carbohydrates, and discrete meal times [19]. However, data on the development of the hypothalamic NPY system and its role in feeding are largely correlative and restricted to mammals, and to our knowledge it is not known whether and how NPY might influence feeding during early development in any vertebrate.

In the current study we investigated a role for NPY in the control of feeding at two amphibian life history stages, the prometamorphic tadpole and the juvenile stage of the Western spadefoot toad Spea hammondii. Earlier, we found that NPY stimulated food intake in juvenile Xenopus laevis [9], but it is not known whether NPY has similar or different actions on feeding in tadpoles. In addition to the morphological changes and habitat shift that occur during anuran metamorphosis, the feeding strategies employed at these two life history stages differ dramatically. The spadefoot toad tadpole is primarily a detritivore/herbivore that grazes often and grows rapidly to reach a minimum body size to initiate metamorphosis [2]; although some species have evolved the ability to exploit carnivorous diets [25]. By contrast, the post-metamorphic spadefoot toad is a sit-and-wait predator that often experiences long periods of food deprivation until rainfall signals foraging.
behavior [3]. We predicted that, given the dramatic change in feeding ecology that occurs during the metamorphic transition in the spadefoot toad, that the neuroendocrine controls of food intake, specifically the role of NPY, also differ between the two life history stages.

2. Methods

2.1. Animals and animal husbandry

Feeding experiments were conducted on *S. hammondii* tadpoles and juvenile toads (approximately 3 months post-metamorphosis). Egg masses were collected from Riverside County, CA, under a California scientific collecting permit (#802003-01) issued to R.J.D., and transported to the laboratory where tadpoles were reared through metamorphosis. Tadpoles were reared in a 20-gallon aquarium at 22–23 °C with a light:dark cycle of 12L:12D, and fed a composite of rabbit chow, agar, and gelatin. Individuals of the appropriate development stage were chosen haphazardly from this group for each experiment. Juveniles were housed in plastic shoeboxes with wet toweling, and were fed first instar crickets. Animal husbandry and use followed a protocol approved by the University of Michigan Animal Care and Use Committee.

2.2. Effect of intracerebroventricular (i.c.v.) injections of NPY on feeding in juvenile toads

The juvenile spadefoot toad feeding assay was conducted as described by Crespi and Denver [7]. Juvenile toads (3–4 g BW) were fed 4 h before the experiment to standardize the time since the last meal. Toads were randomly assigned to unhandled, vehicle (0.6% saline)-injected, or NPY-injected (20 or 200 ng/g body weight – BW) groups (n = 5–6/treatment). The synthetic frog NPY (origin: *Rana ridibunda*) was a generous gift of Dr. Hubert Vaudry; the doses of NPY used were within the range of doses previously shown to stimulate food intake in *X. laevis* [9]. Animals were anesthetized by immersion in 0.01% benzocaine (Sigma, St. Louis, MO) and given i.c.v. injections (150 nl) into the region of the third ventricle using a Drummond Nanoject injector. They were then placed in aquaria containing 3 L of purified water until they recovered from the anesthesia (approximately 5–10 min). Thirty minutes after recovery toads were placed individually into the center of a plastic container (25 × 19 × 12.5 cm), into which approximately 50 crickets were scattered along the edges. Time until first movement and time until the first strike at crickets were recorded for each treatment and time until the first strike at crickets were reduced by NPY injection (first movement: P = 0.008; Fig. 1A; first strike: P = 0.013; data not shown). The NPY-injected toads made more strikes at crickets compared with the saline-injected group (P = 0.012; ANOVA; Fig. 1B) and ate about twice as many crickets as vehicle-injected animals at both 3 and 30 min observation times (3 min: P = 0.003; Fig. 1C; 30 min: P = 0.0059; Fig. 1D).

2.3. Effect of i.c.v. injections of NPY or NPY receptor antagonist on tadpole foraging behavior

We examined the effects of NPY or a general NPY receptor antagonist on spadefoot tadpole foraging using a behavioral assay described previously [6]. The general NPY receptor antagonist (D-Tyr27,36, D-Thr32)-NPY (27–36) was from BACHEM (Torrance, CA). Prometamorphic *S. hammondii* tadpoles (Gosner stages 35–37; 5–6 g BW) were anesthetized by immersion in 0.005% benzocaine, then given i.c.v. injections (150 nl) of NPY (0.02 ng, 0.2 ng, 2 ng, or 20 ng/g BW) or NPY receptor antagonist (1 µg/animal) into the region of the third ventricle. After injection tadpoles were placed into plastic cages (25 × 19 × 12.5 cm) containing 2 L purified water until behavioral observations commenced (approximately 30 min. after injection); all tadpoles were observed within 2 h after injection [6]. Three tadpoles were observed in each container; 2–4 replicates containers per treatment were used in each experiment, resulting in a sample size of 6–11 tadpoles per treatment. Each tadpole was observed for 2 min, and the amount of time spent foraging was recorded [6,18]. Foraging behavior was scored as the scraping of mouthparts on food or along the container wall. Earlier we showed, using food impregnated with lead beads, that the measured foraging behavior in this assay is directly proportional to the actual food intake [6]. The number of seconds foraging, swimming and resting during the 2 min. trial was recorded.

2.4. Data analysis and statistics

We statistically analyzed the dependent variables in each experiment using one-way analysis of variance (ANOVA); the data were log10-transformed if the variances were found to be heterogeneous. If the ANOVA revealed a significant treatment effect (P × 0.05) we used Duncan’s multiple comparisons test (P × 0.05) to determine significant differences among treatments. All statistical analyses were conducted using the statistical software package SAS v8.0.

3. Results

3.1. Intracerebroventricular injection of NPY stimulated food intake in juvenile toads

Injection of NPY (20 ng/g or 200 ng/g BW i.c.v.) in juvenile toads increased feeding behavior (strikes at crickets) and food intake (number of crickets eaten); there was no difference in response between the two NPY doses tested (Fig. 1). Time until the first movement and time until the first strike at crickets were reduced by NPY injection (first movement: P = 0.008; Fig. 1A; first strike: P = 0.013; data not shown). The NPY-injected toads made more strikes at crickets compared with the saline-injected group (P = 0.012; ANOVA; Fig. 1B) and ate about twice as many crickets as vehicle-injected animals at both 3 and 30 min observation times (3 min: P = 0.003; Fig. 1C; 30 min: P = 0.0059; Fig. 1D).

3.2. Intracerebroventricular injection of NPY reduced foraging time in prometamorphic tadpoles

Injection of NPY i.c.v. in prometamorphic tadpoles caused a dose-dependent decrease in time spent foraging (P = 0.0016; Fig. 2A). This action was confirmed in a second experiment (using a 20 ng/g BW dose of NPY; Fig. 2B). By contrast, injection of the general NPY receptor antagonist (D-Tyr27,36, D-Thr32)-NPY (27–36) increased foraging and decreased swimming relative to controls (foraging: P < 0.0001; swimming P < 0.0001; Fig. 2B). Co-injection of the NPY receptor antagonist (1 µg/animal) with NPY (20 ng/g BW) partly blocked the reduction in foraging caused by NPY.

4. Discussion

Neuropeptide Y has well known orexigenic actions in juvenile and adult vertebrates [9,23,30,35], but less is known about NPY actions during postembryonic development. Our findings support that there is a developmental reversal that occurs during anuran metamorphosis in the nature of NPY actions within the hypothalamic feeding control circuit. We confirmed that NPY stimulates feeding in juvenile spadefoot toads, but we found that NPY decreased foraging in prometamorphic tadpoles. A physiological role for endogenous NPY in reducing food intake in the tadpole is supported by the increase in foraging that we observed upon injection of the general NPY receptor antagonist.

The stimulatory effect of central NPY injection on food intake in the juvenile spadefoot toad agrees with our previous findings in juvenile *X. laevis* where we showed that i.c.v. injection of NPY...
increased the amount of food eaten (quantity of beef liver eaten in a 10 min period 1 h after i.c.v. injection; [9]). In the spadefoot toad, NPY injection decreased the latency to strike and increased the number of strikes at prey, leading to an increase in total number of prey eaten. This behavior, and that of *X. laevis* juveniles [9] is consistent with NPY stimulating appetite in juvenile toads, likely through actions within the hypothalamic feeding control circuit. However, NPY has also been shown to influence prey catching ability in anuran amphibians via actions within the retino-tectal circuit. For example, Carr and colleagues [5], working with adult Texas toads (*Bufo speciosus*) showed that NPY neurons extending from the pretectum to the tectum were involved with sharpening motor output to enhance prey catching ability, and in enhancing visual stimulation caused by the moving prey [5]. In mammals, NPY has been shown to increase exploratory and motivational behaviors that enhance feeding [11,15,36]. Thus, NPY may act within both the hypothalamic feeding circuit and the visuomotor system to promote feeding in vertebrates.

By contrast to its orexigenic action in juvenile and adult vertebrates, NPY caused a dose-dependent reduction in foraging behavior in prometamorphic spadefoot tadpoles. Importantly, the general NPY receptor antagonist increased foraging, supporting a role for endogenous NPY in the tonic suppression of food intake in the prometamorphic tadpole. Tuinhof and colleagues [37] reported an increase in NPY immunoreactivity in the *Xenopus* tadpole hypothalamus during metamorphosis (we found a similar increase in NPY mRNA in *X. laevis*; M. Cui and R. Denver, unpublished data). The increase in NPY expression corresponds with increased expression of other genes involved with feeding controls within the POA/hypothalamus such as CRF, POMC and leptin receptor [13] (M. Cui, C. Pelletier, C. Hu and R. Denver, unpublished data). Developmental expression of NPY has been reported in mammals; for example, the number of hypothalamic NPY neurons and fibers increased during late gestation in the fetal sheep [29,39], and a dramatic increase in hypothalamic NPY mRNA expression was seen just before birth in sheep [39] and at weaning in rodents [26].

A possible mechanism for the developmental switch in the action of NPY on feeding in the spadefoot toad could be differential expression of NPY receptors in the hypothalamus, specifically Y2 and Y1/Y5. Frogs have six NPY receptors orthologous to receptors identified in other vertebrates (Y1, Y2, Y4, Y5, Y7 and Y8) [1,17,21] (G. Sundström, B. Xu, T. Larsson, J. Heldin, C. Bergqvist, R. Fredriksson, J. Conlon, I. Lundell, R. Denver, and D. Larhammar, unpublished data). Activation of the Y1 and Y5 receptors promotes food intake [4]. However, anorexigenic effects of NPY signaling have been reported in rodents that involves pre-synaptic auto-inhibition of the expression and release of NPY mediated by the Y2 receptor expressed on hypothalamic NPY neurons [16]. NPY signaling can also inhibit food intake through activation of post-synaptic Y2 receptors expressed on pro-opiomelanocortin (POMC) neurons, and subsequent release of α-melanocyte-stimulating hormone (α-MSH) [16], a potent inhibitor of food intake in mammals and amphibians [5]. These data suggest the hypothesis that the Y2 receptor is dominant in the prometamorphic tadpole, while the Y1 and Y5 receptors may predominate in the juvenile (either due to differences in expression levels, or spatial expression within the feeding control circuitry).

Another mechanism for the anorexigenic actions of NPY in the tadpole could be the activation by NPY of CRF neurons located in the anterior preoptic area (POA)/hypothalamus. In rodents, central administration of NPY increased CRF mRNA expression in the PVN and activated the hypothalamic–pituitary–adrenal axis, specifically...
through the Y5 receptor [20,22]. We showed that CRF has potent anorexigenic actions in amphibian tadpoles and juvenile toads [6,9]. Although it is not known whether NPY receptors are expressed on CRF neurons in amphibians, NPY fibers from perikarya in the neighboring ventral hypothalamic area extend fibers into the POA in climax-stage *Rana esculenta* tadpoles [10] and in adult frogs [24]. With regard to a possible role for CRF in mediating the anorexigenic actions of NPY in the tadpole, it would be interesting to determine if the Y5 receptor (or other NPY receptors) is expressed on CRF neurons in the tadpole POA (homologous to the rostral PVN) [40], and whether this receptor can mediate NPY actions on CRF biosynthesis and release.

The behavior of the premetamorphic tadpole is directed towards maximizing growth to achieve a minimum body size to initiate metamorphosis. However, as a tadpole proceeds through metamorphosis its food intake declines and ceases as it reaches metamorphic climax (M. Cui and R. Denver, unpublished data). This decline in feeding is correlated with dramatic remodeling of the gut and the cerebral cortex as the animal transitions from an herbivorous/omnivorous diet to a carnivorous diet. Prometamorphosis is a period of tadpole development marked by maturation of the hypothalamic hypophysiotropic [14] and feeding control circuits [7]. Negative feeding controls appear to be absent in the premetamorphic tadpole, but develop during metamorphosis [6–8]. Indeed, our findings with NPY suggest that negative control of feeding predominates in the prometamorphic/metamorphic climax tadpole. This may reflect an adaptation to suppress food intake while the jaw and the gut remodel to a carnivorous morphology.

Acknowledgments

We are grateful to Dr. Hubert Vaudry for providing synthetic frog NPY. We thank Holland Quick for assisting with behavioral observations. We thank Jeff Arendt for providing *S. hammondii* egg masses used in this study. This research was supported by NSF Grants IBN 0235401 to RJD and IOS 0818212 to EJC.

References


