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Developmental reversal in neuropeptide Y action on feeding in an amphibian

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ABSTRACT

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 tadpoles, and monitored locomotion, feeding behavior and/or food intake. Injection of NPY (20 or 200 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 at metamorphic climax when the tadpole's gut and cranium remodels for the transition to a carnivorous diet.

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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 of hypothalamic NPY mRNA varies positively with appetite within daily feeding patterns [35] and with long-term changes in feeding associated with seasonal weight gain [12,27,32], and in birds with weight gain prior to migration [33] and reproduction [31].

In the neonatal rat, hypothalamic NPY mRNA expression increases, and projections develop from NPY neurons in the arcuate and paraventricular (PVN) nuclei, during the transition from suckling to weaning [19,26]. The increase in hypothalamic NPY 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

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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, time until first cricket eaten, and the number of crickets eaten within 3- and 30-min time intervals were recorded.

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 toad tadpole foraging using a behavioral assay described previously [6]. The general NPY receptor antagonist (D-Tyr^{27,36}, D-Thr^{32})-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 replicate 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 $\log_{10}$-transformed if the variances were found to be heterogeneous. If the ANOVA revealed a significant treatment effect ($\alpha \leqslant 0.05$) we used Duncan's multiple comparisons test ($\alpha = 0.05$) 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-Tyr^{27,36}, D-Thr^{32})-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

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