The role of neuropeptide Y in the regulation of the stress response and food intake in the brown anole (*Anolis sagrei*).

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Abstract

Neuropeptide Y (NPY) is a conserved hypothalamic regulator of food intake in vertebrates, where it promotes the hunger response to encourage foraging. In addition, NPY has a complex interaction with components of the stress response both within the brain and on peripheral tissues. During a stress response, a hormonal cascade initiating in the hypothalamus, and acting through the anterior pituitary gland, eventually results in the release of glucocorticoid steroid hormones, such as corticosterone (CORT) into general circulation. Recent studies suggest that NPY can interact directly with the adrenal gland to facilitate CORT secretion. This interaction has been explored in mammals, where NPY receptors have been found on adrenal tissue, yet the general role of NPY in reptiles remains understudied. This raises the question of whether NPY can stimulate a stress response and whether a functioning stress response is required for NPY to exert its effects on food intake. Using the invasive brown anole (Anolis sagrei) we tested the hypothesis that NPY promotes both food intake and also CORT release. First, to test whether NPY can activate the stress response, we injected male anole lizards with either: 1) saline; 2) NPY; 3) dexamethasone, a glucocorticoid agonist which suppresses CORT release; or 4) both NPY and DEX. One hour after injection a blood sample was collected to measure plasma CORT concentrations. Second, we tested how the above treatments influenced food intake in captive anoles, by measuring the number of mealworms consumed post-injection. Injection with DEX did not increase food intake above control animals, suggesting CORT release does not itself alter feeding. Current studies are continuing to elucidate the dynamic and complex relationship between NPY, food intake, and stress.

Introduction

Neuropeptide Y (NPY) is an orexigenic (i.e., appetite-stimulating) hypothalamic peptide that is ubiquitous in the vertebrates. Although widely distributed in the central nervous system it is also present in circulation, however its role here has not been fully elucidated. Experimental studies have suggested that NPY signaling interacts with components of the stress response (Krysiak et al., 1999) and research in lab rodents has demonstrated both direct and indirect effects on the adrenal release of glucocorticoids, such as corticosterone (CORT: Carlson et al. 2009). Dexamethasone (DEX) is an agonist that initiates the endogenous negative feedback inhibition of CORT secretion. Thus DEX serves as a useful tool to separate the direct actions of NPY on the adrenal gland, from indirect actions of NPY within the brain. *We tested the hypothesis that exogenous NPY could interact with the stress response to promote CORT secretion, as well as food intake* in the brown anole (*Anolis sagrei*), an abundant invasive lizard species in Florida. We further *tested whether CORT secretion is necessary for the appetite inducing effect of NPY*.

Methods

Study 1: NPY and DEX effects on CORT secretion in the field

- Adult male anoles were captured by noosing at Rollins College from July-Sept 2017.
- Anoles received one of six intraperitoneal injections (100µl) of one of six treatments (*N* =7-11 per treatment): 1) 0.9% saline vehicle as a ***control***; 2) a ***low NPY*** dose of 4 µM; 3) a ***high NPY*** dose of 8 µM; 4) a ***low DEX*** dose of 1.6 µg; 5) a ***high DEX*** dose of 16 µg.
- Snout-vent length (SVL); head width (HW) and head depth (HD) measurements were obtained (all to ±1 mm).
- After one hour post-injection, a blood sample was collected from the post-orbital sinus using a heparinized capillary tube.
- CORT was assayed using enzyme-linked immunoassay (Arbor Assays).
- Hormone data were log-transformed and analyzed using 2-way ANOVA with treatment and SVL as main factors.

Study 2: NPY and DEX effects on Food Intake

- Adult male lizards were capture by noosing at Rollins College from June-July 2017 and live transported to our lizard colony.
- Anoles were permitted to acclimate for 2 weeks prior to any testing and fed two crickets every other day and weighed weekly (±0.1 g).
- Lizards were fasted for either 24 or 48 hours and then received one of four intraperitoneal injections (100µl) (*N* =8-22 per treatment): : 1) saline as ***control***; 2) 16 µg ***DEX;*** 3) 8 µM ***NPY;*** and 4) pretreatment with 16 µg ***DEX*** followed by 8 µM ***NPY*** after 1 hour.
- Lizards returned to their respective cage with 10 mealworms and left for 2 hours, after which the mealworms eaten was recorded.
- Food intake was analyzed using 2-way ANOVA with treatment and time fasted as main factors .

Results

Study 1. Treatment with NPY and DEX, promoted and inhibited CORT secretion, respectively.

Measurements of body size were highly correlated with each other ($r > 0.485$), and thus SVL was used for further analyses. Treatment significantly affected plasma CORT concentrations ($F = 13.11$, $p < 0.001$; Figure 1), but SVL did not ($F = 0.787$, $p = 0.671$). Injection with NPY increased CORT levels relative to both controls (all $p < 0.019$) and DEX-treated (all $p < 0.001$). No difference between NPY doses was observed ($p = 0.636$). As expected, DEX prevented the stress related increase in CORT concentrations with levels equivalent to controls ($p > 0.770$).

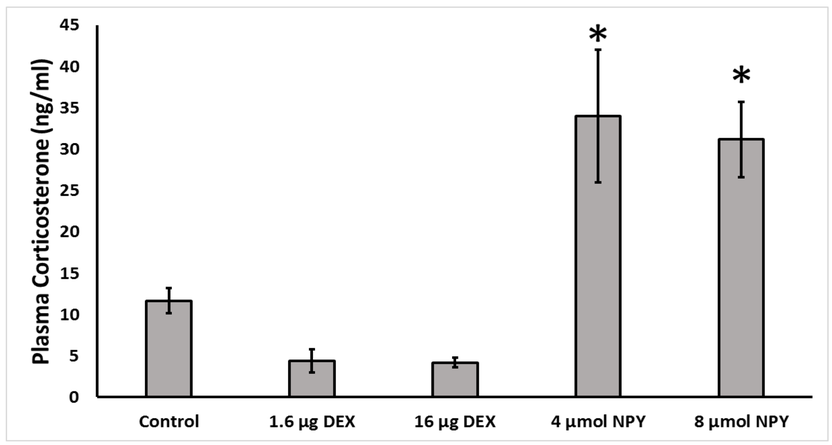

Figure 1. Plasma CORT increases with NPY injection in field-sampled brown anoles. Both doses of NPY increased plasma CORT above saline injected controls and those injected with two doses of dexamethasone (DEX) lizards. * indicates significance at $p < 0.05$.

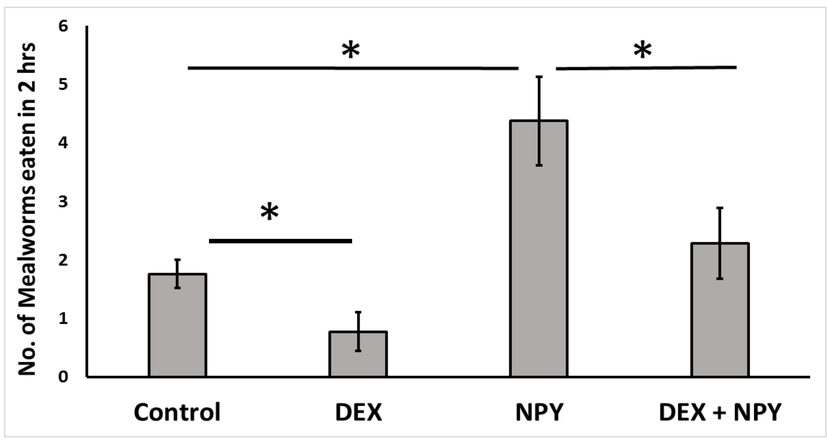

Figure 2. Food intake decreases and increases with DEX and NPY treatment, respectively in captive brown anoles. Injection with DEX suppresses food-intake, as measured by mealworm consumption where as NPY promotes food-intake. Treatment with both produces results comparable to controls. * indicates significance at $p < 0.05$.

Study 2. NPY and DEX had opposing effects on food intake.

Body mass did not differ between treatment groups of captive anoles ($t = 0.207$, $p = 0.973$; Figure 2). Injection with DEX decreased food intake relative to controls ($p = 0.0104$). In contrast, NPY treatment caused higher food intake, but only when anoles were fasted for two days prior, as opposed to one day, to the experiment ($F = 8.315$; $p = 0.007$). Treatment with DEX, followed by NPY resulted in food intake comparable to that of controls ($p = 0.692$), however still considerably lower than that of NPY-treated anoles ($p = 0.028$).

Discussion

We tested the hypothesis that NPY both promotes food intake and activates the CORT stress response, and the interactions between adrenal CORT release and circulating NPY are codependent. Injection with NPY increased CORT levels in field sample anoles and in captivity stimulated food intake. Treatment with DEX, that was shown in the field to decrease CORT, suppressed food intake. When administered together, with DEX given first to suppress CORT secretion, NPY did not elicit a robust increase in food intake. This suggests adrenal activation may be necessary to permit NPY's famed orexigenic effects. The doses of NPY administered here have been previously shown to increase food intake in another lizard species, the Italian wall lizard, *Podarcis sicula* (Yajurvedi and Chandramohah, 1994), but this is the first time changes in CORT have been demonstrated. Although CORT data was not available for the captive study, the vertebrate adrenal cortex is known to possess NPY receptors (see review by Renshaw and Hinson, 2001), thus a direct effect on function is likely. Although studies have focused on the role of NPY on adrenal catecholamines, its effects on CORT also warrant study.

Acknowledgments

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