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# Neuropeptide Y affects secretion of luteinizing hormone and growth hormone in ovariectomized rats

(third cerebroventricular injections/hypothalamus/pituitary perifusion/follicle-stimulating hormone)

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**ABSTRACT** **Neuropeptide Y (NPY) has recently been localized in the rat hypothalamus. We have evaluated the effects of NPY on hypothalamic and pituitary function by injecting NPY into the third ventricle *in vivo* and by examining its action on perifused pituitary cells *in vitro*. Injection of NPY into the third ventricle of conscious ovariectomized rats led to a dramatic and highly significant reduction in plasma luteinizing hormone (LH) relative to pretreatment levels in these animals or to those of controls injected with physiological saline. Significant inhibition was obtained with doses ranging from 0.02 to 5.0 μg (4.7–1175 pmol) of NPY. These inhibitory effects on LH release were dose dependent and lasted for at least 120 min after injection of 5.0 μg of NPY. Intraventricular injection of NPY also significantly decreased plasma growth hormone; however, the threshold dose was 2.0 μg (470 pmol), a dose 100-fold greater than the lowest dose that inhibited LH release. Plasma follicle-stimulating hormone was unaffected by injection of NPY. NPY ($10^{-6}$ and $10^{-7}$ M) stimulated secretion of LH, growth hormone, and follicle-stimulating hormone from perifused anterior pituitary cells loaded in a Bio-Gel P-2 column. These results indicate that NPY acts on structures adjacent to the third ventricle to inhibit the secretion of LH and growth hormone but not follicle-stimulating hormone, whereas it can directly stimulate the secretion of all three hormones from the cells of the anterior pituitary *in vitro*. Since NPY has been found in the hypothalamus and median eminence, it is quite likely that it plays a physiologically significant role at both hypothalamic and pituitary sites: influencing secretion of pituitary hormones.**

Neuropeptide Y (NPY) was isolated from porcine brain by Tatemoto and co-workers (1, 2). It contains 36 amino acids and displays marked sequence homology to avian and bovine pancreatic polypeptide (APP and BPP). NPY-like immunoreactivity has been localized throughout the brain (3, 4, †, ‡), and especially high concentrations (5, 6) have been measured in the hypothalamus of the rat and human by RIA. NPY appears to be located in certain brainstem noradrenergic and adrenergic nuclei that project to the hypothalamus and also in intrinsic hypothalamic neurons (3, 4, †, ‡). Receptors for NPY have been measured in the rat brain, with high concentrations present in the hypothalamus.§ We have examined the effects of NPY on anterior pituitary function by evaluating its action on hypothalamic structures *in vivo* after third cerebroventricular (3V) injection and by testing its effects on hormone secretion by anterior pituitary cells perifused *in vitro*. These results have been compared to our previous observations on the effects of similar injections of APP and BPP.¶

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## MATERIALS AND METHODS

**Animals.** Adult female Sprague–Dawley rats (225–250 g) were bilaterally ovariectomized while they were under ether anesthesia, 3–5 weeks before implantation of a 3V cannula or before decapitation for collection of anterior pituitary glands. The animals were housed in group cages under controlled conditions of temperature (23–25°C) and illumination (lights on 0500–1900) and were provided food and water ad lib.

**Jugular Cannulation.** Twenty-four hours prior to 3V injection of NPY or saline (0.9% NaCl), a cannula was inserted into the right external jugular vein by using an established procedure (7). On the day of the experiment, the cannula was connected to a longer flexible tube (PE-50, Clay Adams) containing heparin and saline. This system allowed rapid collection of blood samples (0.6 ml) and replacement of blood volume with 0.9% NaCl without disturbing the animal. Rats were acclimated to these conditions for at least 1 hr prior to 3V injection of NPY.

**Ventricular Injections and Blood Sampling.** A stainless steel cannula (23 gauge) was implanted into the third ventricle 7–10 days before the experiment (8). Various doses of synthetic porcine NPY (lots 003778 and 004219, Peninsula Laboratories, Belmont, CA) or an equal volume (2 μl) of diluent (0.9% NaCl) was injected into the third ventricle of conscious, unrestrained animals after withdrawal of a preinjection blood sample (0.6 ml) through the jugular cannula. This blood sample was designated the 0-min sample and subsequent samples were obtained at various times after 3V injection. Samples were stored on ice and later spun at low speed at 4°C, the supernatant was removed, and plasma was divided into aliquots immediately or stored frozen for RIA to determine the concentrations of luteinizing hormone (LH) and growth hormone (GH) as well as follicle-stimulating hormone (FSH) in one experiment.

**Anterior Pituitary Cell Perifusion.** Ovariectomized rats

Abbreviations: NPY, neuropeptide Y; APP, avian pancreatic polypeptide; BPP, bovine pancreatic polypeptide; LH, luteinizing hormone; GH, growth hormone; FSH, follicle-stimulating hormone; 3V, third cerebroventricular; LHRH, luteinizing hormone-releasing hormone; MED, minimal effective dose.

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†Guy, J., Allen, Y. S., Polak, J. M. & Pelletier, G. (1983) Program of the 13th Annual Meeting of the Society for Neuroscience, Boston, p. 291 (abstr.).

‡O'Donahue, T. L., Chronwall, B. M. & Dimaggio, D. A. (1983) Program of the 13th Annual Meeting of the Society for Neuroscience, Boston, p. 290 (abstr.).

§Unden, A., Tatemoto, K. & Bartfai, T. (1983) Program of the 13th Annual Meeting of the Society for Neuroscience, Boston, p. 170 (abstr.).

¶McDonald, J. K. & Lumpkin, M. D. (1983) Program of the 65th Annual Meeting of the Endocrine Society, San Antonio, TX, p. 152 (abstr.).

were decapitated and the anterior pituitary glands were removed, minced, and dispersed in the presence of trypsin as described by Samson *et al.* (9) and cultured overnight. The next day, approximately $7 \times 10^6$ dispersed cells were loaded on a Bio-Gel P-2 column (0.4 × 1.5 cm; Bio-Rad) and perifused with medium 199 (GIBCO) containing 20 mM Hepes buffer, 0.1% bovine serum albumin, 20 μM bacitracin, and 1% penicillin–streptomycin, using the method of Gillies and Lowry (10). The cells were perifused at a flow rate of 0.5 ml/min and 5-min fractions were collected. NPY ($10^{-6}$ and $10^{-7}$ M) was included in the medium for 10-min exposure periods and synthetic luteinizing hormone-releasing hormone (LHRH) ($5 \times 10^{-8}$ M, Peninsula Laboratories) was added for 5 min at the end of the perifusion to determine responsiveness of the cells. The fractions were collected and divided into aliquots immediately or stored frozen for RIA of LH, GH, and FSH.

**Hormone RIA.** The concentrations of LH in plasma and medium were determined according to the method of Niswender *et al.* (11). LH antiserum was generously provided by G. Niswender (Colorado State University), and L. E. Reichert (University of Michigan) supplied LH for radioiodination. Results with the RP-1 reference standard were calculated in terms of the NIH-LH-S1 reference preparation. GH and FSH levels were determined by using RIA kits supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and the results are expressed in terms of the corresponding RP-1 reference standard.

**Statistics.** Data obtained from sequential measurements within a treatment group were examined by analysis of variance with repeated measures and a $\chi^2$ analysis, followed by a Student–Newman–Keuls test for multiple comparisons (12). Data from two different treatment groups at each time point were compared by using Student's *t* test and in some cases a Mann–Whitney *U* test. A one-way analysis of variance followed by a Student–Newman–Keuls test for multiple comparisons and a Kruskal–Wallis nonparametric analysis of variance were used to examine differences between three groups.

## RESULTS

**Effects of 3V Injections of NPY on Plasma LH.** The highest intraventricular dose of NPY (5 μg; 1.175 pmol) induced a rapid decline in plasma LH that began at the initial bleeding (5 min) and reached statistical significance by 30 min. Levels remained low until the final sampling at 120 min after injection (Fig. 1 *Upper*). The smaller dose of 0.5 μg (118 pmol) produced a similar result, with a significant lowering at 30 min and recovery toward initial levels by 120 min. Plasma LH was not altered after intraventricular injection of saline.

Similar results were obtained in a second experiment; 2 μg of NPY reduced LH significantly by 30 min (Fig. 1 *Lower*). There was a slight recovery by 120 min, by which time LH was no longer significantly lowered. At the smallest dose, 0.02 μg (4.7 pmol), the lowering of plasma LH was also significant at 30 and 60 min, and there was a slight recovery, such that there was no significant difference at 120 min.

**Effects of 3V Injections of NPY on Plasma GH.** After 3V injection of saline there was a decline in plasma GH concentration at 5 min, which was followed by a return to initial values 15 to 30 min after injection (data not shown). Intraventricular injection of the 0.5-μg dose of NPY did not alter the values from those of saline-injected controls; however, the injection of the higher dose of 5.0 μg of NPY resulted in a sustained reduction in plasma GH concentration that persisted until the end of the experiment. These values were less than those in control animals at 30, 60, and 120 min and were significantly reduced below control values at 120 min ($P < 0.05$, data not shown). In a second experiment (Fig. 2), neither saline nor the minimal dose of 0.02 μg of NPY altered plasma GH levels. In contrast, intraventricular injection of 2.0 μg (470 pmol) of the peptide lowered plasma GH in all six rats at 5 min, and these values remained low for the duration of the experiment. They were significantly lower than the values in saline-injected controls at 60 min ($P < 0.025$). Thus, the minimal effective dose (MED) of NPY to lower GH was 470 pmol, whereas it was ≤4.7 pmol to lower plasma LH.

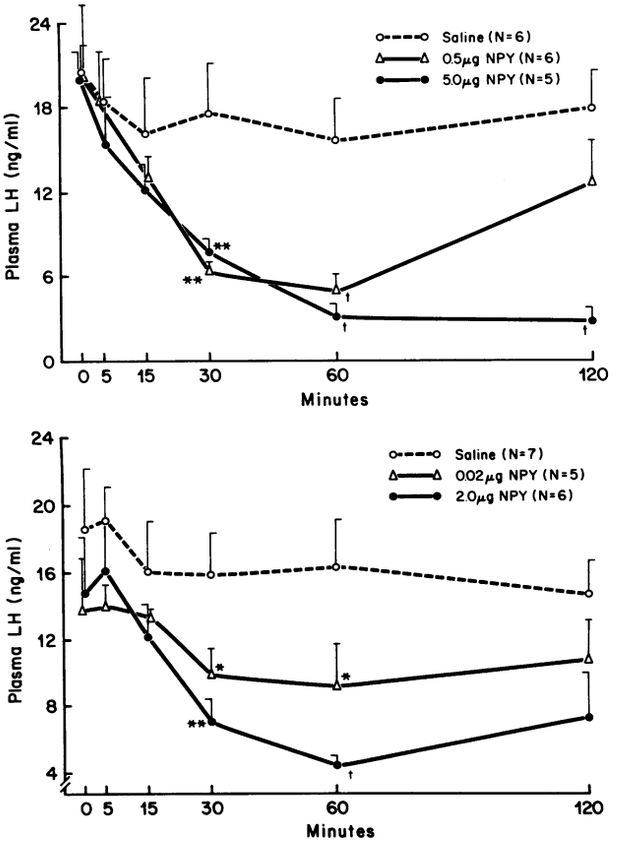


FIG. 1. Effect of the 3V injection of 0.9% saline and NPY on plasma levels of LH in conscious, unrestrained, ovariectomized rats. In this and the subsequent figure, points and vertical bars represent mean values ± SEM. Symbols adjacent to points represent the level of significance when compared to saline-injected controls: *, $P < 0.05$; **, $P < 0.025$; †, $P < 0.005$. (*Upper*) NPY doses were 0.5 and 5.0 μg. (*Lower*) NPY doses were 2.0 and 0.02 μg.

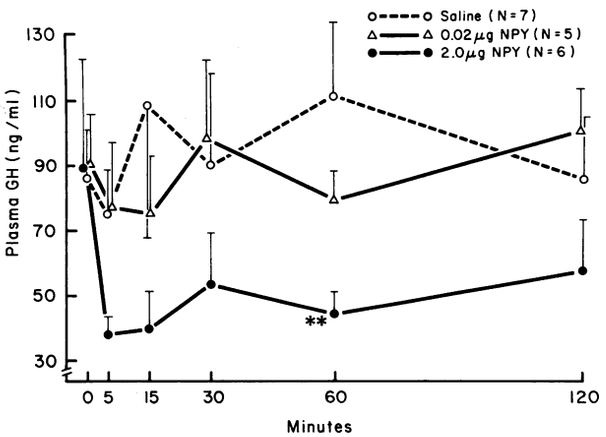


FIG. 2. Effect of the 3V injection of 0.9% saline and NPY (2.0 and 0.02 μg) on plasma levels of GH in conscious, unrestrained, ovariectomized rats.

**Effect of 3V Injection of NPY on Plasma FSH.** Injection of 2.0 or 0.02 μg of NPY did not alter plasma FSH relative to pretreatment levels or values in saline-injected controls (data not shown).

**Effect of NPY on Perifused Anterior Pituitary Cells.** Dispersed anterior pituitary cells perifused in a Bio-Gel P-2 column released LH, FSH, and GH in response to NPY in a dose-related fashion (Fig. 3). The lower dose of NPY ($10^{-7}$ M) stimulated rapid increases in the amount of LH (1.5-fold), FSH (1.6-fold), and GH (1.9-fold) released during the 15 min prior to NPY exposure relative to the amount released during the 10-min exposure and the next 5-min period. Greater increases were seen after exposure to $10^{-6}$ M NPY (LH, 5.0-fold; FSH, 2.4-fold; GH, 6.5-fold). Hormone levels rapidly returned to preexposure control values over the next 15 min of perifusion. In the same preparation, synthetic LHRH ($5 \times 10^{-8}$ M) induced increases in LH and FSH similar to those seen with the lower dose of NPY. Similar results with NPY exposure were observed in a second experiment (data not included).

## DISCUSSION

This study clearly indicates that NPY has a powerful and long-lasting (2 hr) action to inhibit LH secretion in the ovariectomized rat after its 3V injection. Since the MED was only 4.7 pmol, the sensitivity to this effect is great. Also notable was the failure of NPY to inhibit FSH release in spite of the dramatic inhibition of LH release. This would fit with the concept that FSH release is at least partially under the control of a FSH-releasing factor other than LHRH (13). Otherwise one would expect that FSH would decline along with LH after inhibition of LHRH release.

The site of action of NPY to inhibit LH release after its 3V injection is presumably on LHRH neurons located near the third ventricle (14). NPY immunoreactivity has been reported in cell bodies located in the arcuate (3, 5) and periventricular nuclei as well as in the septum (5, ‡) and bed nucleus of the stria terminalis (3), and labeled fibers have been observed in these regions and in the paraventricular, suprachiasmatic, and medial preoptic nuclei (3, 5, †, ‡). The median eminence, particularly the internal layer, reportedly contains NPY immunoreactive fibers (3). Presumably the injected NPY is taken up from the ventricle and acts on NPY receptors, which are present in the rat brain, with particularly high concentrations in the hypothalamus.§

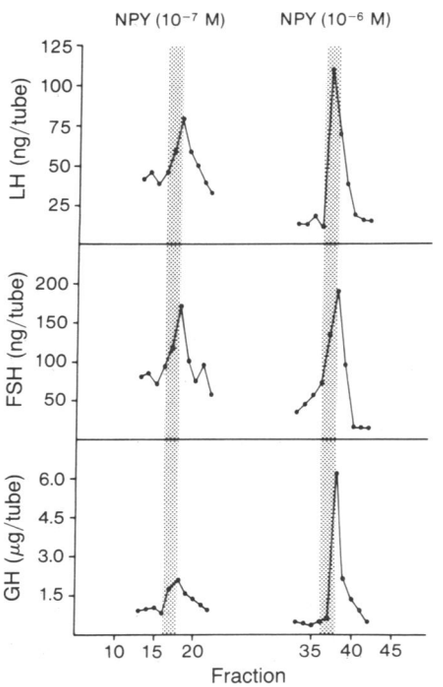


FIG. 3. LH, FSH, and GH released from a perifused cell column (Bio-Gel P-2, 0.4 × 1.5 cm) loaded with $7 \times 10^6$ dispersed anterior pituitary cells (ovariectomized female rat donors). Five-minute fractions were collected and NPY ($10^{-6}$ and $10^{-7}$ M) was included in the medium for the 10-min exposure periods indicated by the stippled bars.

The inhibitory action of NPY on LH release after 3V administration contrasts sharply with its dose-related stimulatory action on both FSH and LH release from perifused anterior pituitary cells obtained from ovariectomized rats. This action was unexpected, because there is little or no homology between NPY and LHRH. The potency is quite high, since the release of both gonadotropins obtained by a dose of $10^{-7}$ M NPY matched that obtained by $5 \times 10^{-8}$ M LHRH. In view of the localization of NPY in the median eminence, it may be found in portal blood and could have a physiological action to stimulate gonadotropin release from pituitaries under certain conditions. Note that the stimulatory action on LH release by the cells *in vitro* was opposite to the inhibitory action after 3V injection. This is reminiscent of the effects of several other peptides, which frequently have opposite actions at the hypothalamic and pituitary levels (15). This may be indicative of an ultrashort-loop negative feedback action by which the peptide released in the brain inhibits further release, perhaps via recurrent collaterals.

Intraventricular administration of only the higher doses of 2.0 or 5.0 μg of NPY decreased plasma GH. Thus the sensitivity for the inhibitory effect of NPY on GH release was less than that for LH by at least a factor of 100; however, this threshold is not different from that of a number of other peptides that may have physiological effects on the release of hypothalamic factors controlling GH, such as corticotropin-releasing factor (16). In view of the localization of NPY cell bodies and terminals in hypothalamic areas that control GH release (17, 18), this action may be exerted via a stimulation of somatostatin release, by an inhibition of GH-releasing factor release, or by a combination of these two actions. The action of NPY administered in the third ventricle was opposite to its effects on the pituitary, a dose–related stimulation of GH release. The sensitivity appeared to be similar for direct stimulation of the release of all three pituitary hormones. Since NPY is present in the median eminence (3), it is quite possible that stimulation of GH release also may be a physiologically significant effect of the peptide. As in the case of LH release, there may be a negative ultrashort-loop feedback of the peptide at the hypothalamic level, which could explain its opposite action on GH release when injected into the ventricle.

NPY is contained in endogenous hypothalamic neurons and also in afferents from several brainstem catecholaminergic nuclei (3, 4). Numerous reports have described the influence of norepinephrine in the control of somatostatin and LHRH secretion (19, 23). NPY might act as a neuromodulator of noradrenergic input to these releasing and inhibiting factor-containing neurons. Precedent for a modulatory role for NPY on noradrenergic transmission has been obtained from studies of the isolated rat vas deferens (24) and the vascular supply to the cat submandibular gland (25).

These effects of NPY on plasma LH and GH are remarkably similar to those previously observed for 3V injections of APP and BPP.¶ In view of the structural similarity of these peptides, it is possible that APP and BPP might bind to the NPY receptor and exert similar activities. Other reports indicate that the rat brain has little, if any, binding capacity for APP and BPP (26, 27). Evidence obtained from several sources suggests that NPY is the endogenous peptide that accounts for previous descriptions of APP and BPP-like im-

munoreactivity in brain (28, 32). Thus, the actions of the pancreatic polypeptides may mimic the action of the endogenous peptide, NPY.

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