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Neuropeptide-Y in the Trout Brain and Pituitary: Localization, Characterization, and Action on Gonadotropin Release*

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ABSTRACT. Using a specific antiserum raised against synthetic neuropeptide-Y (NPY), the distribution of NPY-like immunoreactivity in the brain and pituitary of the trout *Oncorhynchus mykiss* has been examined with the indirect immunofluorescence and peroxidase-antiperoxidase methods. The highest density of NPY-immunoreactive elements was found in the basal telencephalon and hypothalamus. In particular, NPY-immunoreactive neurons were located in the nucleus entopeduncularis and the preoptic nucleus. NPY-immunoreactive fibers were observed throughout the trout brain. The preoptic nucleus, the suprachiasmatic nucleus, and the nucleus entopeduncularis were densely innervated. In addition, NPY-positive fibers were detected in the nucleus lateralis tuberis and in the distal and intermediate lobes of the pituitary. The NPY-like peptide of the trout brain was characterized by combining HPLC analysis and radioimmunological detection. Serial dilutions of trout hypothalamus and pituitary extracts produced displacement curves that were parallel to the standard curve. HPLC analysis resolved a major peak which was slightly less hydrophobic than porcine NPY. The possible effect of NPY, either alone or in combination

with a GnRH antagonist, on gonadotropin (GtH) release from trout pituitaries was investigated using a perfusion system technique.

Graded concentrations of synthetic NPY induced a dose-dependent stimulation of GtH release. The stimulatory activities of NPY and various short chain analogs on GtH release were compared: the order of potency was NPY > NPY-(2-36) > NPY-(16-36) > NPY-(25-36). This result suggests that the biological determinant of NPY is located in the C-terminal part of the molecule. Administration of a short pulse of NPY or GnRH (10^{-7} M each) induced a marked stimulation of GtH release. Prolonged infusion of the GnRH antagonist D-Phe²⁻⁶,Pro³-GnRH induced a significant reduction of GnRH-evoked GtH secretion. In addition, the GnRH antagonist blocked NPY-induced GtH release. The widespread distribution of NPY in the trout brain suggests the involvement of this neuropeptide in a variety of physiological functions. The present data support the view that NPY, released by nerve terminals in the distal lobe of the pituitary, may act presynaptically on GnRH fibers to modulate GtH release. (*Endocrinology* 128: 2360-2368, 1991)

NEUROPEPTIDE-Y (NPY) is a 36-amino acid peptide which was first characterized from the porcine brain (1). NPY belongs to a family of related peptides that includes pancreatic polypeptide and peptide-YY (2). Immunohistochemical studies have shown that NPY is widely distributed in the central nervous system of mammals (3-5). On the basis of pharmacological studies,

several potential central functions have been proposed for NPY in mammals: stimulation of food consumption, inhibition of sexual behavior, regulation of autonomic nerves, and control of endocrine systems (6, 7). In particular, NPY has been shown to affect the secretion of LH in mammals (8-11). Although the site of action of NPY on the secretion of LH is still controversial, immunohistochemical (12) and pharmacological studies (9) suggest that NPY acts at the hypothalamic level to control LH release. In fact, NPY may play a physiological role on LH secretion via two distinct pathways: 1) a direct effect on the GnRH neuronal system and 2) a stimulatory effect on serotonergic fibers, which, in turn, inhibits GnRH release (12). However, a direct effect of NPY at the pituitary level cannot be ruled out in mammals, since high concentrations of NPY have been meas-

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ured in the portal blood (13) and since *in vitro* experiments have shown that NPY stimulates LH release from dispersed pituitary cells (8, 14). In addition, steroid hormones appear to play a crucial role in the neuroendocrine action of NPY; in rats and rabbits, NPY exerts opposite effects, depending on whether the animals are ovariectomized (15, 16).

The distribution of NPY has been studied in the brain and pituitary of several lower vertebrates, including amphibians (17, 18) and fish (19, 20). In frog and toads, NPY acts as a MSH release-inhibiting factor (21–23). This effect appears to be specific to anurans, since NPY does not modify α -MSH secretion in the newt (18). In fish, NPY has recently been shown to regulate gonadotropin (GtH) secretion (24, 25). In the trout, the action of NPY is highly dependent on the steroidogenic environment of the animals (24), as previously reported in mammals (15, 16). For instance, NPY inhibits GtH secretion from the pituitary of vitellogenic trout, whereas it stimulates GtH secretion when the donor animals are taken within 1 week after ovulation (24). In addition, NPY has no effect on GtH release when trout are taken more than 3 weeks after ovulation (24).

The diencephalon-pituitary complex of teleostean fish is an interesting model, in that it does not possess a hypothalamo-hypophyseal portal system. In these animals, the pars distalis of the pituitary is directly innervated by aminergic and peptidergic fibers (26, 27). In the present study we have investigated the distribution of NPY-like neurons in the brain and pituitary of the trout by means of immunohistochemistry. The NPY-like peptide present in the fish brain has also been characterized by combining HPLC analysis and RIA. In addition, we have used an *in vitro* perfusion technique to investigate the site of action of NPY on GtH secretion in female trout.

Materials and Methods

Animals

Rainbow trout (*Oncorhynchus mykiss*), weighing 1–1.9 kg, were obtained from an experimental fish farm (INRA, Gournay, France). The animals were 3 yr old and were reproducing for the second time. In the laboratory, they were kept at least 3 weeks in a recirculating water system at a constant temperature (13 ± 1 °C) under a natural light-dark cycle. Experiments were conducted using three different groups of trout, reproducing in spring, summer, or winter. All fish were used at the time of ovulation.

Immunohistochemistry

Tissue preparation. Twelve trout were used to study the distribution of NPY-like immunoreactivity (NPY-LI) in the brain. The animals were anaesthetized by immersion in tricaine methane sulfonate (MS 222, Sandoz, Hanover, Germany; 100 mg/

liter) and perfused through the ventral aorta with 0.01 M cold saline buffer (PBS; pH 7.4). The perfusion was carried on with either Bouin's fixative or 4% paraformaldehyde in PBS. The brains with the attached pituitaries were removed and postfixed overnight in the respective fixatives. Bouin-fixed brains were dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Serial 5 μ m-thick sections were cut in frontal or sagittal planes.

Paraformaldehyde-fixed tissues were rinsed in 20% sucrose-PBS and then transferred to 30% sucrose-PBS. The brains were frozen on dry ice, and 20- μ m thick sections were cut in a cryostat. The slices were first incubated in PBS containing normal swine serum (0.7 mg/ml; Dakopats, Denmark). The sections were then processed by the indirect immunofluorescence (28) or the peroxidase-antiperoxidase (PAP) technique (29).

Immunohistochemical procedures. Tissue sections were incubated overnight at 4 °C in a humid atmosphere with the NPY antiserum diluted 1:400 or 1:800 in PBS containing 0.3% Triton X-100. The sections were rinsed and incubated with fluorescein isothiocyanate-conjugated swine antirabbit γ -globulins (Dakopats), diluted 1:500, for 1 h at room temperature. The sections were then rinsed, mounted in PBS-glycerol (1:1), and examined under a Zeiss epifluorescence microscope (Zeiss, New York, NY).

For the PAP technique, the sections were incubated for 1 h with swine antirabbit γ -globulins (Dakopats), diluted 1:500, and then with rabbit PAP-complex (Dakopats), diluted 1:300. The enzymatic activity was revealed using 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) containing 0.01% hydrogen peroxide in PBS. Specificity controls were performed by omitting one of the steps of the immunohistochemical procedure or preabsorbing the primary antiserum with synthetic NPY (10^{-6} M). Some sections were treated with preimmune rabbit serum (Dakopats) or PBS instead of the primary antiserum. Alternate sections were stained with cresyl violet to enable a better identification of the various brain areas. The nomenclature of the brain regions is based on the work of Peter and Gill (30).

Tissue extraction

Freshly ovulated trout from the summer spawning group were killed by decapitation. The brains were quickly dissected, placed in 1 ml boiling 2 N acetic acid, and maintained for 10 min in a boiling water bath to inactivate proteolytic enzymes (17). The samples were homogenized in a Potter-Elvehjem homogenizer and centrifuged at $10,000 \times g$ for 15 min. Supernatants were dried by vacuum centrifugation (Speed-Vac concentrator, Savant, Hicksville, NY).

HPLC

Dry hypothalamic extracts were resuspended in distilled water and centrifuged at $10,000 \times g$ for 15 min. The extracts were then analyzed by HPLC on a Beckman liquid chromatograph (model 114 M, Fullerton, CA) equipped with a gradient controller (model 421 A) and an Interchim C-18 column (250 $\times$ 4.1 mm). The samples were eluted at 22 °C, using a gradient of acetonitrile-0.1% trifluoroacetic acid with a flow rate of 1

ml/min. The gradient used is shown in Fig. 6. One-milliliter fractions were collected, dried by vacuum centrifugation, and kept dry until RIA. HPLC standards consisted of 1 μ g synthetic porcine NPY.

RIA

RIA for NPY was carried out in duplicate, as described previously (17). Serial dilutions of tissue homogenates were assayed in order to compare the slopes of the inhibition curves to that of the standard curve. All HPLC fractions were resuspended in 500 μ l veronal buffer (20 mM veronal), 0.4% BSA, and 2 ml/liter mercaptoethanol (pH 8.6), and the concentration of NPY was measured on 100- μ l aliquots of each fraction in duplicate.

Perfusion technique

For perfusion experiments, female rainbow trout were killed within 1 week after ovulation. The animals were killed by decapitation at 0800 h. The pituitaries were quickly dissected, and the pars distalis were minced with scissors. Tissue slices were preincubated in the perfusion medium consisting of Krebs-Ringer solution (140 mM NaCl, 2 mM KCl, 2 mM CaCl_2 , and 15 mM HEPES) supplemented with glucose (2.5 mg/liter) and BSA. Before use, the perfusion medium was gassed with 95% O_2 -5% CO_2 , and the pH was adjusted to 7.4. The osmotic pressure was 300 mosmol.

The perfusion apparatus has previously been described in detail (31, 32). The perfusion chambers consist of siliconized glass columns delimited by Teflon plungers. Pituitary fragments, equivalent to one whole pars distalis, were transferred into each perfusion column between two beds of Bio-Gel P2. The perfusion medium was pumped at a constant flow rate (12.5 ml/h), and the tissues were perfused for 2 h before the experiment commenced. Then, the effluent fractions were collected at 2.5- or 7.5-min intervals. The concentration of GtH was determined in each fraction by means of a sensitive RIA (33) on the same day as the perfusion experiment.

Each perfusion pattern was calculated as the mean profile of GtH release ($\pm$ SEM) established over at least four independent experiments. The reference level of GtH release (100% basal level) was calculated as the mean secretion rate during the 22.5-min period just preceding the infusion of the secretagogues.

Peptides and chemicals

NPY and NPY short chain analogs were synthesized on a benzhydrylamine resin developed by Bio-Mega (Montreal, Quebec, Canada). Simultaneous cleavage and removal of the protective groups were performed in liquid hydrogen fluoride. Purification of the compounds was performed by ion exchange chromatography and preparative reverse phase HPLC. All chemicals were purchased from Merck (Darmstadt, Germany), except for BSA (Boehringer, Paris, France) and trifluoroacetic acid (Prolabo, Paris, France).

Results

Immunohistochemistry

The antiserum against synthetic porcine NPY has been applied successfully to localize NPY-containing

neurons in human, frog, and fish brains (5, 17, 19, 20). This antiserum appears to be highly specific for NPY. In particular, it exhibits no cross-reaction with avian pancreatic polypeptide and peptide-YY. Preabsorption of the primary antiserum with synthetic NPY (10^{-6} M) resulted in the complete loss of immunoreaction. When the NPY antiserum was substituted with either preimmune rabbit serum or PBS, no labeling was observed.

The distribution of NPY-immunoreactive neurons is illustrated on Figs. 1 and 2. Cell bodies are shown as *large filled circles*; the number of circles approximates the number of cells in a given section. Immunoreactive fibers are represented as *small dots*. NPY-immunoreactive cell bodies were exclusively located in the basal telencephalon and the anterior part of the hypothalamus.

In the telencephalon, a first group of NPY-immunoreactive perikarya was detected in the nucleus entopeduncularis. These cell bodies were located in the dorsal extension of the nucleus entopeduncularis and were found coursing in a band parallel to the telencephalic ventricle. They were round or oval in shape with a fine fluorescent cytoplasmic rim (Fig. 3, a and b). In the hypothalamus, a few immunoreactive perikarya were stained in the preoptic nucleus, where they were mainly located in front of the preoptic recess. These perikarya formed small clusters in the dorsal side of the preoptic nucleus. Scattered positive perikarya were also seen ventrally to the preoptic recess. Some of the latter cell bodies clearly corresponded to magnocellular neurons, which were characterized by an abundant and intensely stained cytoplasm surrounding a large nucleus (Fig. 3, c and d). The other brain regions did not contain any NPY-immunoreactive perikarya.

NPY-immunoreactive fibers were widely distributed in the trout brain. In the telencephalon, the whole basal region contained a dense network of fine beaded nerve processes, which were found coursing in the antero-posterior direction (Fig. 4a). The medioventral part of the entopeduncular nucleus was also densely innervated. Scarce immunoreactive fibers were occasionally visualized in other telencephalic regions. In the diencephalon, a high density of NPY-containing nerve processes was detected in the hypothalamus. These fibers were abundant in the preoptic area and ran toward the suprachiasmatic region, where they formed a dense network (Fig. 4b). These NPY-immunoreactive fibers were found to penetrate into the retrochiasmatic area and invaded the whole posterior part of the hypothalamus (Fig. 4c). Another tract of NPY-containing fibers was also visualized in the lateral part of the nucleus lateralis tuberis. In the pituitary, numerous nerve processes and terminals were localized in the neurointermediate lobe and posterior pars distalis (Fig. 4, d and e). Some NPY-positive nerve processes were also detected around the posterior recess.

FIG. 1. Schematic frontal sections through the brain of *Oncorhynchus mykiss* depicting the distribution of immunoreactive NPY. The levels of the sections are indicated on the sagittal scheme on the top of the figure. The distribution of NPY-containing perikarya (filled circles) and fibers (small dots) is shown on the right side. The relative density of NPY-containing structures is meant to be proportional to the density of the symbols. Ac, Anterior commissure; Dcd, area dorsalis telencephali, pars centralis dorsalis; Dd, area dorsalis telencephali, pars dorsalis; Dm, area dorsalis telencephali, pars medialis; Dld, area dorsalis telencephali, pars lateralis; Dlv, area dorsalis telencephali, pars lateralis ventralis; Hy, pituitary; Nah, nucleus anterioris hypothalami; Nap, nucleus anterioris periventricularis; Nat, nucleus anterioris tuberis; Ndm, nucleus dorsomedialis thalami; Ndtl, nucleus diffusor tori lateralis; Nlta, nucleus lateralis tuberis, pars anterioris; Nltl, nucleus lateralis tuberis, pars lateralis; Nltp, nucleus lateralis tuberis, pars posterioris; Npo, nucleus preopticus; Ne, nucleus entopeduncularis; Nrlt, nucleus recessus lateralis; Nrp, nucleus recessus posterioris (the Nrp is part of the posterior lobe noted in Fig. 2); Nrpg, nucleus glomerulosus, pars rostralis; Nvm, nucleus ventromedialis thalami; Oc, optic chiasma; Sv, saccus vasculosus; Tec, tectum mesencephali; Vd, area ventralis telencephali, pars dorsalis; Vl, area ventralis telencephali, pars lateralis; Vp, area ventralis telencephali, pars postcommissuralis; Vv, area ventralis telencephali, pars ventralis.

Finally, scattered immunoreactive fibers were visualized in the thalamus and the mesencephalon, where they were restricted to the tegmental gray.

NPY concentrations in tissue extracts

The displacement curves obtained with serial dilutions of trout hypothalamic and pituitary extracts were parallel to the standard curve prepared with synthetic por-

FIG. 2. Schematic parasagittal section through the brain of *Oncorhynchus mykiss* depicting the distribution of NPY-immunoreactive perikarya (filled circles) and neuronal processes (small dots). Hy, Hypophysis; Ne, nucleus entopeduncularis; Npo, nucleus preopticus; Oc, optic chiasma; Pl, posterior lobe; Pr, preoptic recess; Sv, saccus vasculosus.

cine NPY (Fig. 5). The concentrations of NPY-LI material in various brain regions are reported in Table 1. High concentrations of NPY were found in both hypothalamic and pituitary extracts.

Reverse phase HPLC

The elution profiles of trout NPY-immunoreactive material and synthetic porcine NPY are compared in Fig. 6. The synthetic standard had an elution time of 52 min, whereas the major peak of immunoreactive NPY in trout hypothalamic extracts had a retention time of 50 min. In addition, HPLC analysis resolved three minor peaks in tissue extracts (retention times, 5, 10, and 15 min), which may correspond to degradation forms generated during the extraction procedure.

Effect of NPY on GtH release

Administration of a 15-min pulse of NPY (10^{-7} M) induced a rapid stimulation of GtH release from trout pituitary fragments, and the maximum response was reached within 10 min (Fig. 7). Thereafter, despite continued NPY infusion, GtH secretion declined gradually. The rate of GtH release returned to the basal level within 15 min after the end of NPY administration. NPY concentrations ranging from 10^{-9} – 10^{-6} M induced a dose-dependent stimulation of GtH release. Half-maximum stimulation was observed at a concentration of 5×10^{-8} M (Fig. 8). The potency of various short chain analogs in stimulating GtH release was compared to that of NPY [NPY-(1-36)]. The order of potency was NPY-(1-36) > NPY-(2-36) > NPY-(16-36) > NPY-(25-36); the short chain analog NPY-(2-36) was almost as potent as NPY (Fig. 8). As expected, a short pulse of GnRH (10^{-7} M) also induced a robust stimulation of GtH release (Fig. 9). Administration of the GnRH antagonist D-Phe²⁻⁶,Pro³-GnRH (10^{-6} M) for 30 min resulted in a significant reduction of GnRH-evoked GtH release (Fig. 9). In addition, the GnRH antagonist significantly reduced the stimulatory effect induced by a 15-min pulse

FIG. 3. Photomicrographs of NPY-immunoreactive perikarya revealed by means of indirect immunofluorescence (a and b) or PAP techniques (c and d). a, Transverse section through the nucleus entopeduncularis showing immunofluorescent perikarya and fibers ($\times 420$). b, Sagittal section through the anterior part of the nucleus entopeduncularis showing two positive perikarya and numerous immunoreactive fibers ($\times 420$). c, Group of stained perikarya located in the anterior part of the nucleus preopticus ($\times 125$). Pr, Preoptic recess. d, Higher magnification of c, showing some immunoreactive magnocellular elements in the preoptic nucleus ($\times 680$).

of NPY ($P < 0.005$, by two-way analysis of variance; Fig. 10).

Discussion

The results of the present investigation demonstrate the occurrence of NPY-LI in the brain and pituitary of the rainbow trout *Oncorhynchus mykiss*. NPY-immunoreactive structures were detected mainly in the telencephalon and the hypothalamo-hypophyseal complex. In particular, NPY-containing neurons were observed in the entopeduncularis and preoptic nuclei. While dense tracts of fibers were seen in the caudal region of the basal telencephalon and hypothalamus, only scattered fibers were visualized in the thalamus and the mesencephalon. The distribution of NPY-immunoreactive neurons is in good agreement with that recently reported in the brain of the elasmobranch fish *Scyliorhinus canicula* (20), but appears to be rather limited compared with that found in tetrapod vertebrates (4, 17, 34, 35). The distribution of NPY-immunoreactive perikarya in the trout hypothalamus is consistent with the location of NPY-positive cell bodies in the mediobasal and paraventricular regions of human (35), cat (36, 37), rat (3, 4), and frog brains (17, 38). These studies have shown that numerous im-

munoreactive neurons are located in hypophysiotropic areas, especially within the preoptic nucleus, and that NPY-immunoreactive fibers are present in the infundibulum and median eminence. In trout, NPY is also located in all regions of the preoptic-hypophyseal complex, suggesting that NPY may act as a neurohormone. In fact, the existence of a large number of immunoreactive fibers running through the hypothalamic floor and the density of NPY-containing nerve terminals within the proximal pars distalis and pars intermedia of the pituitary support the view that in trout, NPY may regulate the secretion of pituitary hormones at both the hypothalamic and pituitary levels. In the pituitary, we found a good correlation between the distribution of NPY-immunoreactive fibers and terminals and the localization of gonadotropic, melanotropic, and GH cells, suggesting that in fish, NPY may exert regulatory effects on these three categories of cells. It is of interest to note that in the trout hypothalamus, the distribution of NPY immunoreactivity coincides with that of GnRH, in particular in the ventral telencephalon, preoptic region, and basal hypothalamus, as previously described in the trout *Salmo trutta* (39). In the rat, double staining techniques have shown that NPY-immunoreactive fibers terminate in the close vicin-

FIG. 4. Photomicrographs of NPY-immunoreactive fibers in the brain (a, b, and c) and pituitary (d). a, Network of beaded immunofluorescent fibers innervating the pars ventralis of the basal telencephalon. Sagittal section ($\times 460$). b, Transverse section through the supra-chiasmatic region showing numerous PAP-stained fibers ($\times 460$). c, Sagittal section showing the presence of a long beaded immunofluorescent fiber within the hypothalamus ($\times 460$). d, Sagittal section ($\times 420$) of the caudal region of the pituitary labeled with the PAP technique; numerous NPY-immunoreactive fibers (arrows) are observed among melanotropic cells of the pars intermedia (PI; right side) and within the posterior pars distalis (PPD; left side). NHE, Neurohypophyseal extension (caudal branch).

FIG. 5. Semilogarithmic plots comparing competitive inhibition of antibody-bound ^{125}I -labeled NPY by synthetic NPY and acetic acid extracts of various regions of trout pituitary and hypothalamus.

ity of GnRH-containing neurons in the preoptic area of the hypothalamus (12).

These morphological studies support the view that in trout, as in mammals, NPY may regulate GtH secretion

TABLE 1. NPY content in hypothalamic and pituitary extracts

Region	NPY content (ng/extract)
Hypothalamus	4.5 ± 0.9
Pituitary, pars intermedia	4.4 ± 0.3
Pituitary, rostral pars distalis	2.7 ± 0.4
Pituitary, posterior pars distalis	3.1 ± 0.4

Values are given as mean $\pm$ SEM.

via presynaptic control of GnRH neurons. The presence of numerous NPY-positive fibers and terminals in the pars intermedia of the pituitary observed in the present study is consistent with previous results obtained in elasmobranchs and tetrapods (20–22, 40). These data indicate that in trout, NPY may be involved in the control of melanotropic cells, as previously shown in anuran amphibians (21–23, 41, 42). On the other hand, the occurrence of NPY-containing neurons in the telencephalon and the distribution of extrahypothalamic immunoreactive fibers in the trout brain suggest that NPY may also act as a neuromodulator and/or a neurotransmitter in fish.

The results of our RIA investigation extend the immunocytochemical data and indicate that high amounts of NPY are found in the hypothalamus and pituitary.

FIG. 6. Reverse phase HPLC analysis RIA quantification of NPY-like peptides in a trout hypothalamic extract. The broken line represents the gradient of acetonitrile in 0.1% trifluoroacetic acid- H_2O used over 80 min. The arrow indicates the retention time of synthetic porcine NPY.

FIG. 7. Effect of NPY on GtH release from slices of trout pars distalis. NPY (10^{-7} M) was infused for 15 min. The data represent the mean $\pm$ SEM of four independent perfusion experiments. The reference level of GtH release (100% basal level) was calculated for each experiment as the mean secretion rate during 30 min (four consecutive fractions) just preceding the infusion of NPY.

The fact that the dilution curves obtained with trout brain extracts were parallel that obtained with synthetic NPY indicated that the trout peptide is closely related to porcine NPY. However, concentration-dependent displacement of labeled NPY by tissue extracts does not provide information concerning the chemical structure of the immunoreactive peptide detected. In fact, a peptide homologous to porcine NPY has recently been characterized from the salmon (*Oncorhynchus kisutch*) pancreas (43). HPLC analysis revealed that the trout peptide is slightly less hydrophobic than porcine NPY. This result is consistent with those obtained in sharks (20)

FIG. 8. Semilogarithmic plot comparing the effects of NPY and various NPY short chain analogs on GtH release. All experimental values were calculated from data similar to those represented in Fig. 7. Each point corresponds to the area under the peak, i.e. the net increase in GtH release during and after infusion of NPY or NPY analogs (mean $\pm$ SEM of four independent experiments).

FIG. 9. Effect of synthetic GnRH alone or during infusion of the GnRH antagonist D-Phe²⁻⁶,Pro³-GnRH. GnRH (10^{-7} M) was infused for 15 min; the GnRH antagonist (10^{-6} M) was infused for 30 min. See Fig. 7 for other details.

and goldfish (19), which indicated that fish NPY has a retention time shorter than that of porcine NPY. Both human (44) and frog NPY (17, 45) also appear to be less hydrophobic than porcine NPY.

Recent studies have shown that in fish, NPY modulates GtH secretion *in vitro* (24, 25, 46). However, the mechanism of action of NPY on pituitary gonadotrophs has not so far been investigated. The data presented herein confirm that NPY exerts a stimulatory effect on

FIG. 10. Effect of the GnRH antagonist D-Phe²⁻⁶,Pro³-GnRH on NPY-induced stimulation of GtH release. The GnRH antagonist (10^{-6} M) was infused for 25 min. NPY (10^{-7} M) was administered for 15 min. See Fig. 7 for other details.

pituitary gonadotrophs from female trout that had ovulated 1 week previously. The kinetics of the response were very similar to those observed with GnRH, *i.e.* both peptides induced a rapid stimulation of GtH release, which peaked within 10 min, and then the secretion of GtH decreased in spite of continued exposure to neuropeptides. This desensitization phenomenon can be accounted for either by internalization of the receptors or conversion of the receptors from high to low affinity conformational states. The order of potency of the various NPY short chain analogs as stimulator of GtH release [NPY > NPY-(2-36) > NPY-(16-36) > NPY-(25-36)] is in total agreement with those reported in the frog pars intermedia (47). The fact that NPY-(16-36) and NPY-(25-36) were also potent stimulators of GtH release suggests that the biological activity of NPY is contained within the C-terminal region of the molecule. The results of our perfusion experiments indicate that NPY-induced GtH secretion can, at least partly if not totally, be accounted for by presynaptic stimulation of GnRH fibers, since the stimulatory effect of NPY was significantly blocked by the GnRH antagonist D-Phe²⁻⁶,Pro³-GnRH. Consistent with this hypothesis was the observation that NPY had no effect on acutely dispersed pituitary cells, a preparation in which afferent fibers are disconnected (data not shown). However, a direct effect of NPY on pituitary gonadotrophs cannot be totally ruled out, since recent studies indicate that NPY induces a more rapid desensitization than GnRH on GtH secretion from perfused pituitary gland. In addition, the presence of NPY-containing nerve terminals in the preoptic region, where GnRH cell bodies are located, supports the concept that NPY may also regulate the activity of GnRH neurons at the hypothalamic level, as previously

demonstrated in mammals (12, 16, 48). Therefore, immunocytochemical studies will be undertaken in order to determine whether synaptic contacts occur between NPY terminals and GnRH elements in both the preoptic nucleus and pituitary gland of fish.

In conclusion, the present results show that a peptide immunologically and biochemically related to porcine NPY is present in discrete neuronal populations of the trout brain. Our data also indicate that in fish, NPY-evoked GtH secretion is mediated through presynaptic stimulation of GnRH neurons. Taken together, these data suggest that NPY may play an important role in the regulation of the hypothalamo-pituitary-gonadal axis in teleostean fish.

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