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Neuropeptide Y (NPY) modulates *in vitro* gonadotropin in release from rainbow trout pituitary glands

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Abstract

This work investigated the action of neuropeptide Y (NPY) on the *in vitro* pituitary release of the maturing gonadotropic hormone (GtH) of the rainbow trout using a perifusion system employing trout balanced salt solution (pH 7.5) at 15°C and a 12.5 ml/h flow rate. In vitellogenic females a 20 minutes NPY application (10^{-7} M) induced a 20–30% decrease in GtH secretion. Removal of NPY was followed by a rebound in GTH secretion. On the contrary, in ovulated females, NPY (15 minutes, 10^{-7} M) directly stimulated GTH secretion. The greatest stimulation was obtained the day of ovulation where the stimulatory effect of NPY was similar to those induced by s.GnRH in the same conditions, reaching 400% of the basal GTH level. In vitellogenic females treated with 1–4–6 androstadien 3–7 dione, an inhibitor of aromatase activity, the pituitary response to NPY was similar to that obtained in ovulated females. Thus the *in vitro* action of NPY might depend on the *in vivo* steroidogenic environment.

Introduction

Neuropeptide Y (NPY) is a 36 aminoacid peptide first isolated from porcine brain (Tatemoto *et al.* 1982). It belongs to a family of related peptides which include pancreatic polypeptide (PP) and peptide YY (Tatemoto 1982). Immunohistochemical studies have shown that NPY is widely distributed in the central nervous system of mammals (Allen *et al.* 1983; Pelletier *et al.* 1984; Chronwall *et al.* 1986) and amphibians (Danger *et al.* 1986). On the basis of pharmacological studies in mammals several potential central functions have been proposed for NPY including the stimulation of feeding behaviour, the inhibition of sexual behaviour, the regulation of autonomic nerves and regulation of neuroendocrine systems (Gray and Morley 1986).

NPY has been shown to inhibit the secretion of luteinizing hormone (LH) in mammals (McDonald *et al.* 1985; Kerkérian *et al.* 1985; Crowley *et al.* 1985), but until recently, the precise site of action at the pituitary or hypothalamic level remained controversial. There is now evidence that its action may result from the modulation of gonadotropin-releasing-hormone (GnRH) secretion from the medial basal hypothalamus (Crowley *et al.* 1987; Khorram *et al.* 1987). In fish, the existence of peptides related to the NPY-PP family have also been demonstrated. Recently, a peptide sharing 83% homology with porcine NPY has been isolated from the salmon pancreas (Kimmel *et al.* 1986). In fish, the presence of a peptide immunologically related to NPY was first demonstrated in the retina (Osborne *et al.* 1986). Using an antibody against porcine NPY, Pontet *et al.* (1988) determined the distribution of NPY in brain and pituitary of the gold-

fish. NPY has also been identified in the brain of trout by means of immunocytochemistry, radioimmunoassay and high performance liquid chromatography (Danger *et al.* 1988). In both goldfish and trout, NPY is widely distributed in the brain and high concentrations of the peptide have been detected in the pituitary. However, the physiological function of NPY in fish, in particular, the possible role of NPY in the control of gonadotropin secretion, remains unknown. The organization of the hypothalamo-pituitary complex of fishes differs from that of higher vertebrates by the absence of a portal vessel system. In contrast to mammals, the pars distalis of the fish pituitary is directly innervated by a number of aminergic and peptidergic fibers, including GnRH- and NPY-containing fibers, which originate from the hypothalamus and make direct contacts with hypophyseal endocrine cells (Kah *et al.* 1985). Thus, the fish pituitary represents an excellent model to study the respective roles of GnRH and NPY in the control of maturational gonadotropin II (GtH) secretion.

In the present study we have used an *in vitro* perifusion technique, to compare the actions of NPY and GnRH on GtH-release by the trout pituitary.

Material and methods

Animals

Female rainbow trout, 3 years old, 1–2 kg of body weight, of both winter and spring spawning strains, were obtained from a local experimental fish farm. The fishes were kept in recirculating water at 13 ± 1°C under a natural photoperiod. In these conditions fish developed a normal vitellogenesis, and ovulated naturally. Ovulated fish were still holding eggs.

In vivo *treatments*

In order to study the influence of the *in vivo* steroidogenic environment on the *in vitro* response of the pituitary to NPY and GnRH, vitellogenic females were treated as follows (2 to 3 weeks before ovulation):

1. The firs group of trout received intraperitoneal injection of 17αhydroxy, 20βdihydroprogesterone (17α20β-OHP) dissolved in peanut oil; Sigma Chemicals, St Louis, MO) at a dose of 0.5 mg/kg body weight every two days for one week and the second group of trout received intramuscular injection of 1–4–6 androstadien 3–5 dione (ATD, Bacht 0371 Steraloid Wilton, N.H.) at a dose of 10 mg/kg body weight, twice a day at 12h intervals for 4 days, conditions which were demonstrated to inhibit the aromatase activity in the rat *in vivo* (Donaldson *et al.* 1981).

Perifusion technique

The animals (untreated or pretreated) were killed by decapitation at 08:00h. The pituitaries were quickly dissected and the pars distalis (PD) was minced with scissors. The pieces were pre-incubated for two hours in the perifusion medium consisting of Krebs-Ringer solution (NaCl, 140 mM; KCl, 2 mM; $CaCl_2$, 2 mM; HEPES, 15 mM) supplemented with glucose (2.5 mg/l) and bovine serum albumin (300 mg/l). The perifusion medium was gassed with O_2/CO_2 (95:5) prior to use and the pH adjusted to 7.5. The osmotic pressure was 300 mOsm.

The perifusion apparatus has been described previously in detail by Gonnet (1988).The perifusion chambers (1.5 ml) consisted of siliconized glass columns delimited by Teflon plungers. In each perifusion column, the equivalent of one pituitary was placed on a bed of Biogel P-2 matrix. The perifusion medium was pumped at a constant flow rate (12.5 ml/h) and the tissue was perifused for 2h before the experimental manipulation commenced. The effluent fractions were collected at 2.5 or 7.5 minute intervals. The concentration of GtH was determined in each fraction by means of a sensitive radioimmunoassay (Breton *et al.* 1978) immediately after collection.

Calculations

Each perifusion pattern was calculated as the mean profile of GtH-release (± SEM) established over at

least 4 columns from the same or different perifusions. The reference level of GtH-release (100% basal level) was calculated for each experiment as the mean secretion rate during the 22.5 minute period just preceding the infusion of the secretagogues. Mean GtH basal levels and GtH levels during secretagogue application were compared in paired Student's t-tests.

Results

Spontaneous release of GtH

A high rate of GtH secretion occurred immediately after the pituitary fragments were packed on the column, and rapidly fell to a steady rate of secretion within 2h. Thereafter, the spontaneous secretion rate remained stable for up to 7h. The mean basal rate of GtH secretion calculated from independent perifusion columns was 12.64 ± 4.76 ng/ml (n = 17) per pituitary in vitellogenic females, 30.62 ± 10.53 (n = 23) in ovulated females and 20.03 ± 5.05 ng/ml (n = 13) in vitellogenic ATD treated females.

Effect of NPY or GnRH upon GtH secretion from PD of vitellogenic females

A 15 minute pulse (Fig. 1A) of NPY (10^{-7} M) caused a significant inhibition (20%) of GtH release, followed by a rebound of GtH secretion and then a steadily decreasing GtH-release.

A 15 minute pulse of GnRH (10^{-7} M) induced a rapid and sustained increase of GtH release (Fig. 1B). The removal of GnRH resulted in a progressive decline of GtH secretion which, was still higher than the basal level 1h after GnRH withdrawal (Fig. 1B).

Effect of NPY and GnRH upon GtH secretion from PD of ovulated females

NPY (10^{-7} M) had no effect on GtH secretion when the PD's were taken from females which had

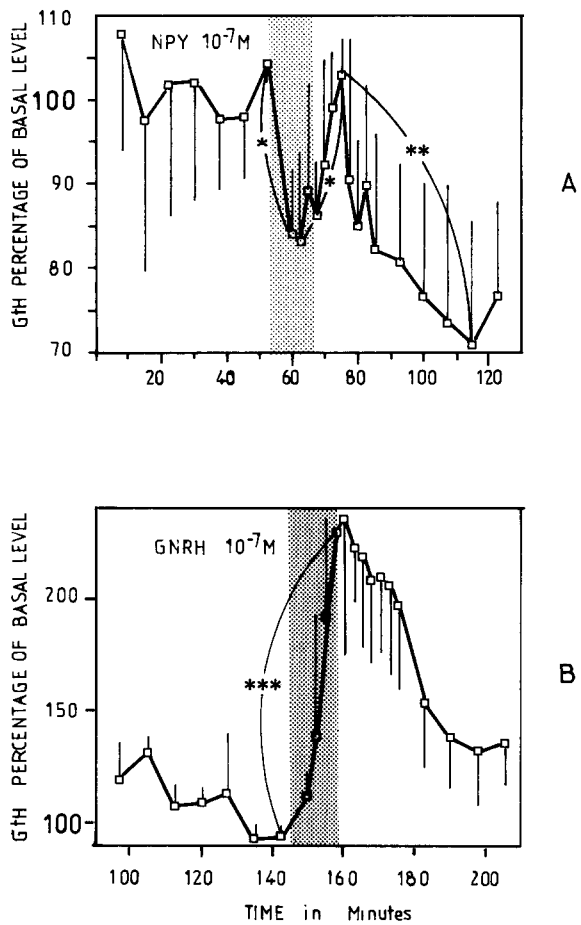

Fig. 1. Effects of NPY (A) and GnRH (B) on GtH release from PD's of vitellogenic rainbow trout. After a 52.5 minute equilibration period, NPY (10^{-7} M) or GnRH (10^{-7} M) was infused for 15 minutes (shaded area). The profile represents the mean ± SEM of 7 different columns. * $p < 0.01$ ** $p < 0.005$ *** $p < 0.001$

ovulated more than 3 weeks before the experiment (data not shown). In contrast, when the animals were sampled within one week after ovulation NPY induced a significant stimulation (163 ± 16%; $p < 0{,}01$) of GtH secretion (Fig. 2). The rate of GtH-release returned to basal level within 15 minutes of ending NPY stimulation.

A 15 minute pulse of GnRH (10^{-7} M) induced a massive stimulation of GtH (318 ± 28%; $p < 0{,}001$). The kinetics of the response to GnRH by pituitaries from ovulated females were similar to those obtained with females sacrificed during vitel-

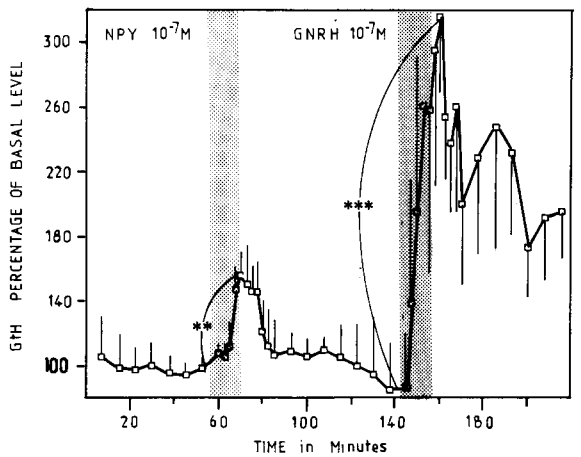

Fig. 2. Effects of NPY (10^{-7} M) and GnRH (10^{-7} M) on GtH release from PD's of females taken more than 3 weeks after ovulation. The profile represents the mean ± SEM of 5 independent experiments. See Fig. 1 for details.

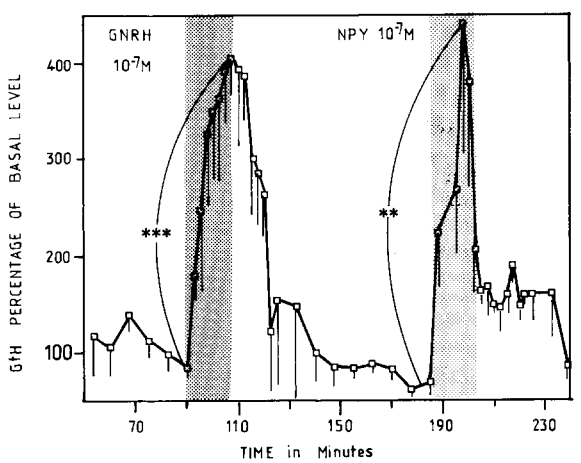

Fig. 3. Effects of GnRH (10^{-7} M) and NPY (10^{-7} M) on GtH-release from PD's of animals taken within 24h following ovulation. NPY and GnRH were administered for 20 minutes. The profile represents the mean ± SEM of 4 independent experiments. See Fig. 1 for details.

logenesis. The secretion rate peaked 20 minutes after the onset of GnRH administration and GtH-levels were higher than the basal level 1h after GnRH withdrawal (Fig. 2).

Another series of experiments was conducted with PD's taken from animals less than 24h after ovulation (Fig. 3). A 20 minute pulse of GnRH (10^{-7} M) induced a massive stimulation of GtH release (444 ± 130%; $p < 0.01$). The rate of GtH

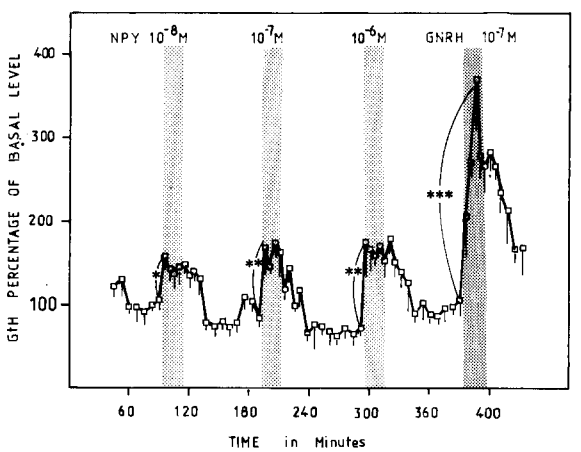

Fig. 4. Effect of administration of NPY (10^{-8} to 10^{-6} M) and of a single pulse of GnRH (10^{-7} M) on GtH release from PD's of animals taken within a week following ovulation. The profile represents the mean ± SEM of 5 independent experiments. Secretagogues were administered as 20 minute pulses. (** $p < 0.01$). See Fig. 1 for details.

secretion returned to basal values within 35 minutes of ending GnRH stimulation. As shown in Fig. 3, perifusion with NPY (10^{-7} M) for 20 minutes stimulated GtH-release to a similar magnitude as that induced by GnRH. GtH-secretion also returned to basal values within 35 minutes following NPY administration.

The effects of repetitive administration of NPY at doses ranging from 10^{-8} to 10^{-6} M was studied in PD's of fish sampled within a week after ovulation (Fig. 4). NPY was administered as 20 minute pulses at 75 minute intervals. Regardless of the dose infused, NPY induced a stimulation of GtH release which reached 150% of basal level (peak values: 156%, 163% and 166% for doses of 10^{-8}, 10^{-7} and 10^{-6} M respectively). The amounts of GtH secreted during the stimulations (areas under the peaks) where not significantly different.

Effect of steroid treatment

Treatment of vitellogenic females with 17α20β-OHP did not alter the ability of the pituitary to respond to NPY. In animals treated with ATD (5 among 9 vitellogenic females treated), NPY (10^{-7}

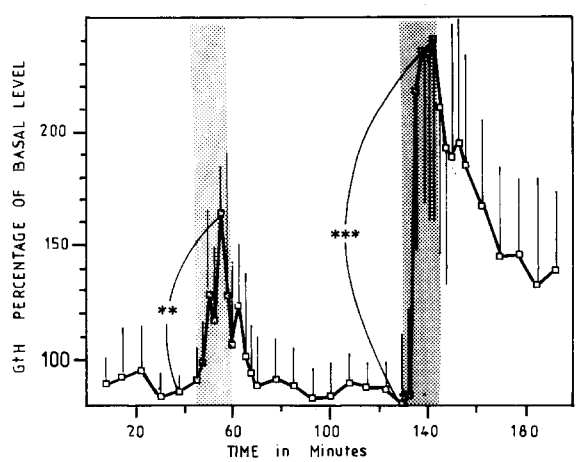

Fig. 5. Effects of synthetic NPY (10^{-7} M) and GnRH (10^{-7} M) on GtH secretion from PD's of a ATD-treated vitellogenic female. The figure represents the mean ± SEM of 5 independent experiments. NPY and GnRH were administered as 15 minute pulses. (** $p < 0.01$; *** $p < 0.001$).

M) administered as a 15 minute pulse induced a stimulation of GtH release which was similar to that obtained with animals taken within a week after ovulation (Fig. 5).

Discussion

The present study demonstrates that NPY modulates the *in vitro* secretion of GtH from the trout pituitary. In mammals, the action of NPY on gonadotropin secretion is still controversial; some studies indicate that NPY does not stimulate LH-secretion from anterior pituitary cells from castrated male rats (Kerkerian *et al.* 1985) or from hemipituitary glands from normal rats incubated *in vitro* (Rodriguez-Sierra 1987), while others suggest that NPY stimulates both LH and FSH release from dispersed pituitary cells obtained from ovariectomized female rats (McDonald *et al.* 1985). However, in all cases, NPY administered *in vivo* inhibits LH-seretion.

In intact rats and rabbits, NPY stimulates GnRH release at the hypothalamic level (Crowley *et al.* 1987; Khorran *et al.* 1987). The anatomical basis for the regulatory action of NPY on hypothalamic GnRH neurons is now clearly established since Li *et al.* (1988) have recently shown that NPY endings make direct contact with GnRH neurons in the preoptic area of the rat.

The present results suggest that in vitellogenic rainbow trout, the primary action of NPY might be to inhibit GtH-release. In contrast NPY appears to stimulate GtH-release in ovulated animals. From the present results, it cannot be established whether NPY acts directly on pituitary gonadotrophs or presynaptically on GnRH-ergic fibers.

The anatomical organization of the hypothalamo-pituitary complex supports the possibility that, in the trout, NPY can control GtH-release by modulating GnRH-release, since GnRH fibers directly innervate parenchymal cells of the pituitary (Kah *et al.* 1986). In the trout, (Breton *et al.* 1986), goldfish (Yu *et al.* 1987) and roach (Breton *et al.* 1988), the pituitary concentration of GnRH is higher than in any other brain area. The pituitary of fish might thus be considered as an extension of the hypothalamus-median eminence complex. A presynaptic action of NPY is also supported by the differences between vitellogenic and ovulated females in the response of the pituitary to NPY. The GnRH content in the pituitary is low during vitellogenesis and increases to a maximum during ovulation (Breton *et al.* 1986, 1988). Thus, a sufficient amount of GnRH could be released under NPY action to enable, *in vitro* stimulation of GtH release. More recent results (Breton *et al.*, unpublished data) have shown that NPY has no effect upon GtH secretion from dispersed pituitary cells from ovulated trout and that the stimulatory action of NPY on ovulated animals is abolished in the presence of a GnRH antagonist. These data suggest that, in fish, NPY exerts a presynaptic control on the release of GnRH by nerve terminals located at the level of the rainbow trout pituitary.

In fish, as in mammals (Crowley *et al.* 1987; Kalra *et al.* 1987), the action of NPY may depend on the steroidogenic environment. *In vivo* treatment of vitellogenic females by 17α20β-OHP did not induce a stimulatory effect of NPY on GtH secretion. Thus, this steroid which is, in the trout, the maturation inducing steroid, does not appear to be involved in the pituitary response to NPY stimulation. In contrast, NPY stimulated GtH secretion

both in ovulated and vitellogenic ATD treated female trout. ATD has been shown to inhibit *in vivo* the aromatase action in the rat (Donaldson *et al.* 1981) and to partially inhibit the enzymatic activity in microsomal preparations of trout ovary (De Mones 1987). The inhibition of estrogen synthesis from testosterone during maturation could be one of the major events responsible for the change in pituitary responsiveness to NPY. Testosterone has been shown to stimulate GtH synthesis in the pituitary and to drastically increase the content of GnRH in the telencephalon and pituitary of a strain of triploid rainbow trout (Breton, unpublished data). Since, in fish treated with ATD, the magnitude of the response to NPY remains lower than that obtained with fish just after ovulation, other factors might be involved in the control exerted by GnRH on the release of GtH during ovulation. These factors would either inhibit the effect of NPY or act on GnRH-containing fibers. Alternatively, inhibitory signals such as dopamine may inhibit the action of GnRH at the pituitary level.

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