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Neuropeptide Y Inhibits Ca^{2+} Oscillations, Cyclic AMP, and Secretion in Melanotrope Cells of *Xenopus laevis* via a Y_1 Receptor

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SCHEENEN, W. J. J. M., H. G. YNTEMA, P. H. G. M. WILLEMS, E. W. ROUBOS, J. R. LIESTE AND B. G. JENKS. *Neuropeptide Y inhibits Ca^{2+} oscillations, cyclic AMP, and secretion in melanotrope cells of Xenopus laevis via a Y_1 receptor.* PEPTIDES **16**(5) 889–895, 1995.—The melanotrope cells in the pituitary gland of *Xenopus laevis* are innervated by neurons containing neuropeptide Y (NPY). In the present study, the mechanism of action of NPY on the melanotropes has been investigated. NPY inhibited in vitro secretion from melanotropes in intact neurointermediate lobes as well as from isolated, single melanotropes. Inhibition of secretion from neurointermediate lobes was mimicked by the NPY analogues PYY and [Leu^{31},Pro^{34}]NPY, whereas NPY(13–36) was inactive. Secretion from isolated melanotropes was inhibited by [Leu^{31},Pro^{34}]NPY and NPY(13–36), but NPY(13–36) was 10-fold less potent than [Leu^{31},Pro^{34}]NPY. Studies on isolated cells revealed that NPY and its analogues inhibited the occurrence of intracellular Ca^{2+} oscillations with the same potency as they inhibited secretion from isolated cells. In addition to inhibiting basal secretion and spontaneous Ca^{2+} oscillations, NPY inhibited the basal production of cyclic AMP. On the basis of these results it is proposed that NPY inhibits secretion from *Xenopus* melanotropes by inhibiting cyclic AMP-dependent spontaneous Ca^{2+} oscillations through a Y_1-like receptor.

Neuropeptide Y Melanotrope cells *Xenopus laevis* Ca^{2+} oscillations Cyclic AMP

MELANOTROPE cells in the pars intermedia of the pituitary gland of the amphibian *Xenopus laevis* convert neural input by classical neurotransmitters and neuropeptides into a hormonal output, viz. the secretion of α-melanophore-stimulating hormone (α-MSH). This hormone causes dispersion of pigment in dermal melanophores resulting in darkening of the skin (14,15). Recently, particular attention has been paid to the cellular signaling mechanisms that transduce the neural inputs into α-MSH secretion. Corticotropin-releasing hormone (CRH) stimulates α-MSH secretion by stimulating adenylyl cyclase (8), whereas thyrotropin-releasing hormone (TRH) stimulates this secretion by enhancing the production of inositol trisphosphate (11). On the other hand, melanotropes also receive various inhibitory inputs. It has been shown that γ-aminobutyric acid (GABA) inhibits α-MSH secretion via two receptor subtypes, a chloride channel containing a $GABA_A$ receptor, and a G-protein-coupled $GABA_B$ receptor that inhibits adenylyl cyclase (8,15). Dopamine inhibition of α-MSH secretion is achieved via a D_2-like receptor coupled to a G-protein and inhibiting adenylyl cyclase (8,15). The present study is concerned with the cellular signal transduction pathway by which neuropeptide Y (NPY) inhibits α-MSH secretion. The inhibitory effect of NPY is very characteristic as it lasts for many hours after removal of NPY, in contrast to the short-lasting inhibitions evoked by GABA and dopamine (17). Recent studies have revealed that NPY is also a very potent blocker of spontaneously occurring intracellular Ca^{2+} oscillations in isolated *Xenopus* melanotropes kept in vitro (25,27). Several studies in central and peripheral tissues indicate that NPY acts in both mammals and nonmammals (6,18,22), generally acting via inhibition of adenylyl cyclase activity (1,18,19,29,34). For *Xenopus* melanotropes it was found that spontaneous Ca^{2+} oscillations depend on Ca^{2+} influx through ω-conotoxin-sensitive Ca^{2+} channels (24,28). Furthermore, inhibition of the cAMP-dependent protein kinase led to an inhibition of Ca^{2+} oscillations (27). Therefore, in the present study we have investigated the possibility that NPY inhibits α-MSH secretion from *Xenopus* melanotropes by binding to a specific receptor that inhibits cAMP production and occurrence of the Ca^{2+} oscillations.

First, the NPY receptor subtype involved in inhibition of α-MSH secretion was identified. At least three receptor subtypes have been described for NPY, as reviewed by Michell (18). The subtypes can be distinguished on the basis of their differential

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responses to agonistic NPY analogues; the Y_1 receptor subtype is activated by [Leu^{31},Pro^{34}]NPY but not by NPY(13–36), whereas, conversely, the Y_2 receptor subtype is activated by NPY(13–36) but not by [Leu^{31},Pro^{34}]NPY (3,18). Peptide YY (PYY) activates both the Y_1 and the Y_2 receptors but has no effect on the Y_3 receptor (18). To determine the receptor subtype that is responsible for the inhibitory action of NPY on *Xenopus* melanotropes, the effects of NPY and NPY analogues on α-MSH secretion from superfused neurointermediate lobes were studied. Furthermore, to substantiate the hypothesized relation between spontaneous Ca^{2+} oscillations and hormone secretion (25,27), the effects of NPY and the analogues on oscillations and secretion were investigated with in vitro superfusion and dynamic video imaging techniques, using isolated, single melanotropes. Finally, the possible involvement of cAMP in NPY-induced inhibition of α-MSH secretion was tested by determining the effects of a membrane permeable cAMP analogue on spontaneous Ca^{2+} oscillations, and by measuring cAMP release from neurointermediate lobes.

METHOD

Animals

Young adult *Xenopus laevis* were taken from laboratory stock and adapted to a black background for 3 weeks under continuous illumination, at 22°C. They were fed weekly with beef heart.

In Vitro Secretion Studies With Neurointermediate Lobes

Neurointermediate lobes were rapidly dissected and placed in 10-μl superfusion chambers. Four chambers were superfused simultaneously with incubation medium (IM) consisting of 112 m*M* NaCl, 2 m*M* KCl, 2 m*M* $CaCl_2$, 15 m*M* Ultral-HEPES (pH 7.4; Calbiochem, La Jolla CA), 2 mg/ml glucose, 0.3 mg/ml bovine serum albumin (Sigma, St. Louis MO), and 1 μg/ml ascorbic acid, at a rate of 1.5 ml/h, at 22°C. To establish stable release, lobes were superfused for at least 75 min (10 fractions of 7.5 min) with IM before NPY and its analogues were added. The concentration of α-MSH in each fraction was determined by radioimmunoassay (31). Basal (spontaneous) α-MSH release was defined as the average secretion in the three fractions preceding the first application. Details on concentrations of the NPY analogues used and their administration protocols are given in the results section. In experiments in which cAMP secretion was measured, superfusion was performed in the presence of 0.1 m*M* 3-isobutyl-1-methylxanthine (IBMX, Sigma) and fractions were collected every 15 min (8).

Preparation of Single Melanotropes

Isolation of melanotrope cells was performed as described previously (24). In short, after anesthetization in 0.1% MS222 (Sigma) animals were perfused with *Xenopus* Ringer's solution, containing 112 m*M* NaCl, 2 m*M* KCl, 2 m*M* $CaCl_2$, and 15 m*M* Ultral-HEPES, to remove blood cells. Then neurointermediate lobes were dissected and incubated for 45 min in Ringer's solution without $CaCl_2$ to which 0.25% (w/v) trypsin (Gibco, Renfrewshore, UK) had been added. Cells were subsequently dispersed in Leibovitz's L15 medium, which had been adjusted to *Xenopus* blood osmolality (L15:ultrapure water, 2:1) and contained 10% fetal calf serum (Gibco). Single cells were plated on glass cover slips coated with poly-L-lysine (Sigma; mol.wt. > 300 kDa) at a density of 10,000 cells/slip. At least 95% of the cells prepared in this way are melanotropes (9).

Measurement of Peptide Release From Single Melanotropes

To measure secretion from single cells, freshly isolated cells were cultured in 75 μl IM containing 22.5 μCi [^{3}H]lysine (86 Ci/mmol; Amersham, Buckinghamshire, UK) for 18 h. After washing, coverslips were placed in a superfusion chamber with a volume of 200 μl. The superfusion flow rate was 1 ml/min. Cells were superfused for 1.5 h before NPY analogues were added and 1-min fractions were collected. To each fraction 1 ml scintillation fluid (Optiphase 'HiSafe' 3, LKB-Wallac, Turku, Finland) was added and radioactivity was determined using a β-counter (1216 Rackbeta, LKB-Wallac).

Radioimmunoassays

Radioimmunoassay for α-MSH was performed as described previously, using an antiserum raised in our laboratory (31), which has equal affinity for the acetylated and nonacetylated forms of α-MSH. Cross-reactivities with ACTH(1–24) and ACTH(1–39) were below 0.01%. Bound and free antibodies were separated by polyethylene glycol/ovalbumin precipitation. Detection limit was 2 pg α-MSH per sample. Superfusion fractions were assayed in duplicate. Radioimmunoassay for cAMP was performed as described previously (8), using a cAMP-RIA kit from Amersham. Detection limit was 1 fmol cAMP per sample.

Ca^{2+} Measurements

Ca^{2+} measurements in isolated melanotrope cells were performed as described previously (24,25). In short, single cells were cultured for three days at 22°C. After culturing, cells were loaded with 2 μ*M* Fura-2/AM (Molecular Probes, Eugene, OR) in IM containing 1 μ*M* pluronic F127 (23) (Molecular Probes) for 20 min at 22°C. After loading, cells were washed with IM in a Leiden perifusion chamber (13) (volume 800 μl) at a rate of 1 ml/min, for 25 min. During this wash unattached cells were sucked off and attached cells were allowed to equilibrate. The chamber was placed on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and the light from a 100-W xenon lamp was directed through a quartz neutral density filter (ND 2, Ealing Electro-Optics, Holliston, MA) to reduce bleaching of the intracellularly trapped fluorochrome. The excitation bandpass filters, mounted in a motor-driven rotating wheel, had transmission maxima at 340 and 380 nm (± 12 nm) (Ealing Electro-Optics). The fluorescence emission ratio at 492 nm was used as a measure of $[Ca^{2+}]_i$ after excitation at 340 and 380 nm (21). An epifluorescent 40× magnification oil immersion objective was used. Dynamic video imaging was carried out using the MagiCal hardware and TARDIS software of Joyce Loebl (Dukesway, Tyne & Wear, UK) as described by Neylon et al. (20). The interframe interval between the ratio frames was 6.4 s with a maximal sampling time of 32 min. Test substances were added to the bath using a perifusion pump.

Compounds

Substances tested were NPY, NPY(13–36), PYY (Bachem Feinchemikalien AG, Bubendorf, Switzerland), and [Leu^{31},Pro^{34}]NPY (Bachem California, Torrance, CA). 8-Br-cAMP was obtained from Sigma. In some experiments a test-pulse of dopamine (Sigma) was included.

Calculations and Statistics

Results of superfusion experiments with neurointermediate lobes are shown as the average ± SEM of four lobes superfused

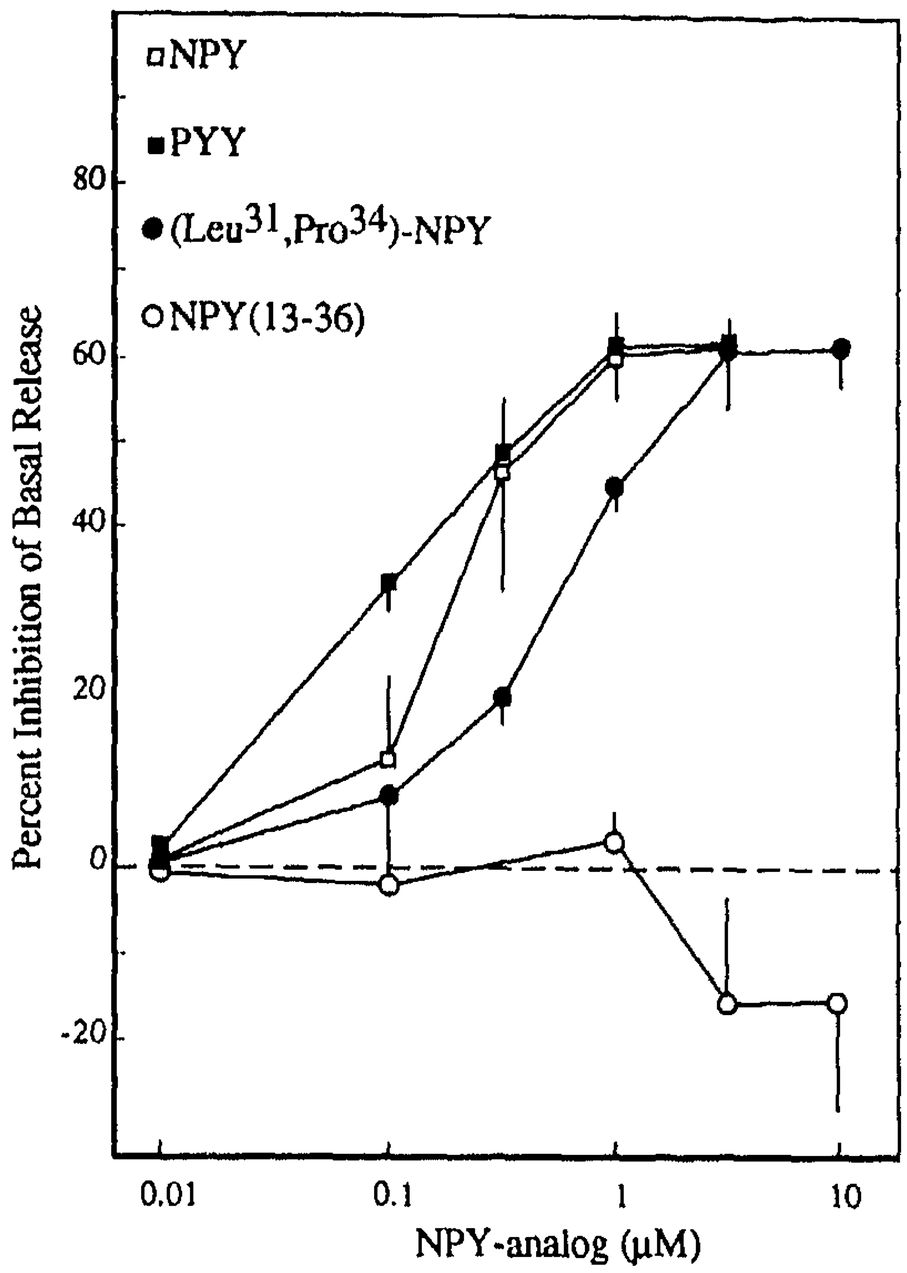

FIG. 1. Dose–response relationship of NPY analogues on α-MSH secretion from neurointermediate lobes. The efficacies of PYY and NPY were similar; both analogues produced a maximal inhibition of 60% at 1 μM. [Leu^{31},Pro^{34}]NPY was less potent that NPY and PYY at 0.1 and 1 μM, but eventually reached a similar efficacy at 3.3 μM. NPY(13–36) did not inhibit α-MSH secretion at any concentration tested. At 3.3 and 10 μM NPY(13–36) caused a stimulation of 16%.

in one experiment, unless stated otherwise. Each experiment was performed at least two times independently. Percentages of inhibition and stimulation of α-MSH or cAMP release were calculated on the basis of integration of the respective peak areas in the graphs, using three fractions preceding and three fractions following the onset of experimental treatment. In superfusion experiments with isolated melanotropes, pulses of 5 min were given (five fractions), and the inhibition was calculated using the five fractions before and the five fractions after the onset of treatment. The graph of a representative experiment is shown. Each experiment was performed at least two times independently. The integrated areas were analyzed with the paired Student's t-test. A value of $p < 0.05$ was considered to indicate statistical significance.

For Ca^{2+} imaging representative profiles of single cells are given. Each experiment was conducted a minimum of three times. The total number of cells examined for each treatment is given in the Results section, as is the total number of cells that gave a response.

RESULTS

Effects of NPY and NPY Analogues on α-MSH Secretion From Neurointermediate Lobes

NPY dose-dependently inhibited basal α-MSH secretion with a minimum effective concentration of 0.1 μM, leading to an inhibition of 12 ± 8%. Maximum inhibition (60 ± 7%) was achieved at a concentration of 1 μM (Fig. 1). PYY was slightly more potent than NPY in inhibiting α-MSH secretion, giving 33 ± 3% inhibition at a concentration of 0.1 μM (Fig. 1). Although the efficacy, achieved over a period of 22.5 min, was similar for NPY and PYY, the recovery from inhibition clearly differed; NPY had a long-lasting effect (more than 1 h), whereas PYY-induced inhibition fully disappeared within 37.5 min following PYY removal (five superfusion fractions) (Fig. 2). [Leu^{31},Pro^{34}]NPY inhibited α-MSH secretion at a minimal effective concentration of 0.1 μM, giving 10 ± 6% inhibition, and maximum inhibition (60 ± 7%) was achieved at a concentration of 3.3 μM (Fig. 1). NPY(13–36) was ineffective in inhibiting α-MSH secretion in concentrations up to 10 μM (Fig. 1).

Effects of NPY and NPY Analogues on Secretion From Single Cells

During a 5-min pulse NPY inhibited release of radioactive peptides from single cells cultured on coated coverslips by 50% when applied at a concentration of 1 nM [Fig. 3(A)]. At 10 nM the percentage of inhibition was 60%. Compared to the dopamine-induced inhibition, recovery from inhibition with NPY was long lasting after removal of the inhibitor. Both NPY analogues inhibited secretion; after removal of the inhibitor the inhibition by [Leu^{31},Pro^{34}]NPY was readily reversible whereas the effect of NPY(13–36) was long-lasting. At 1 nM [Leu^{31},Pro^{34}]NPY inhibited secretion by 45% [Fig. 3(B)]; when a concentration of 10 nM was given maximum inhibition was reached (55%) [Fig. 3(B)]. NPY(13–36) at 1 nM had no effect on secretion; 10 nM inhibited secretion by 35%; and 100 nM NPY(13–36) induced an inhibition of 50% [Fig. 3(C)].

Effects of NPY and NPY Analogues on Spontaneous Ca^{2+} Oscillations in Single Melanotropes

Both NPY and PYY appeared to be very potent in blocking spontaneous Ca^{2+} oscillations in single melanotropes. Complete inhibition in all cells by NPY was reached at a concentration of 1 nM [Fig. 4(A), $N = 29$]. PYY was similarly effective in inhibiting the spontaneous Ca^{2+} oscillations, causing a complete inhibition at 1 nM [Fig. 4(B), $N = 11$]. [Leu^{31},Pro^{34}]NPY completely inhibited spontaneous Ca^{2+} oscillations at a concentration of 10 nM [Fig. 4(C), $N = 18$], whereas in 4 of the 18 cells oscillations were already blocked at 1 nM. NPY(13–36) inhibited oscillations in 5 out of 13 cells when tested at a concentration of

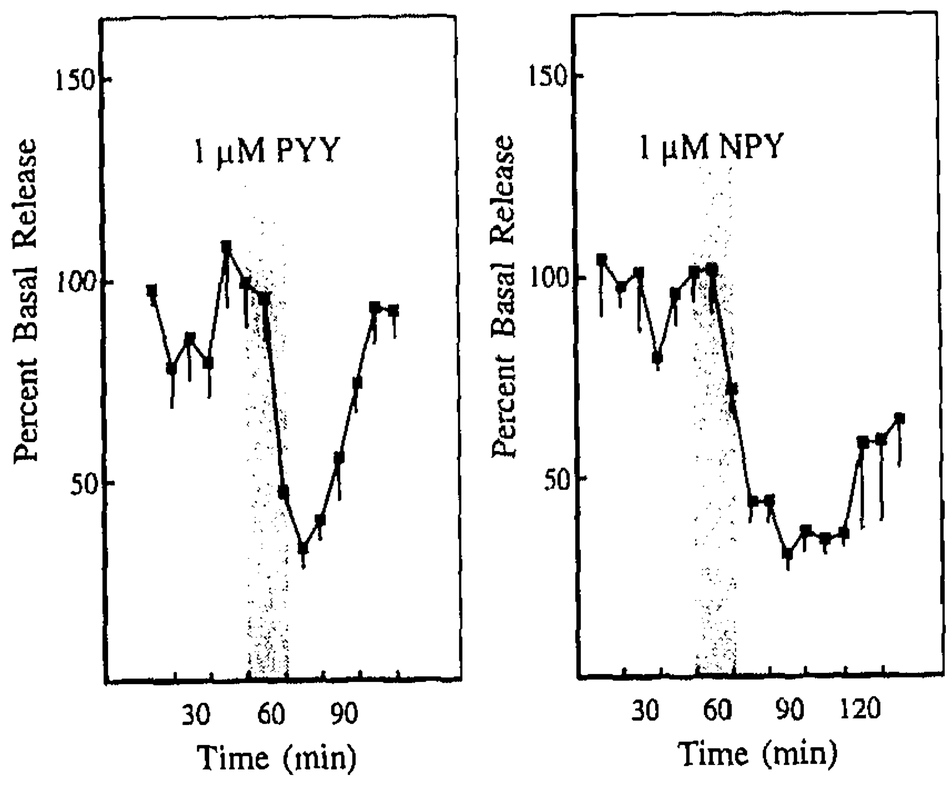

FIG. 2. Reversibility of PYY- and NPY-induced inhibition of α-MSH secretion in freshly dissected neurointermediate lobes. The PYY-induced inhibition was completely reversed within five superfusion fractions (37.5 min) after removal of the test substance. NPY-induced inhibition was only reversed by 45% after nine superfusion fractions (67.5 min).

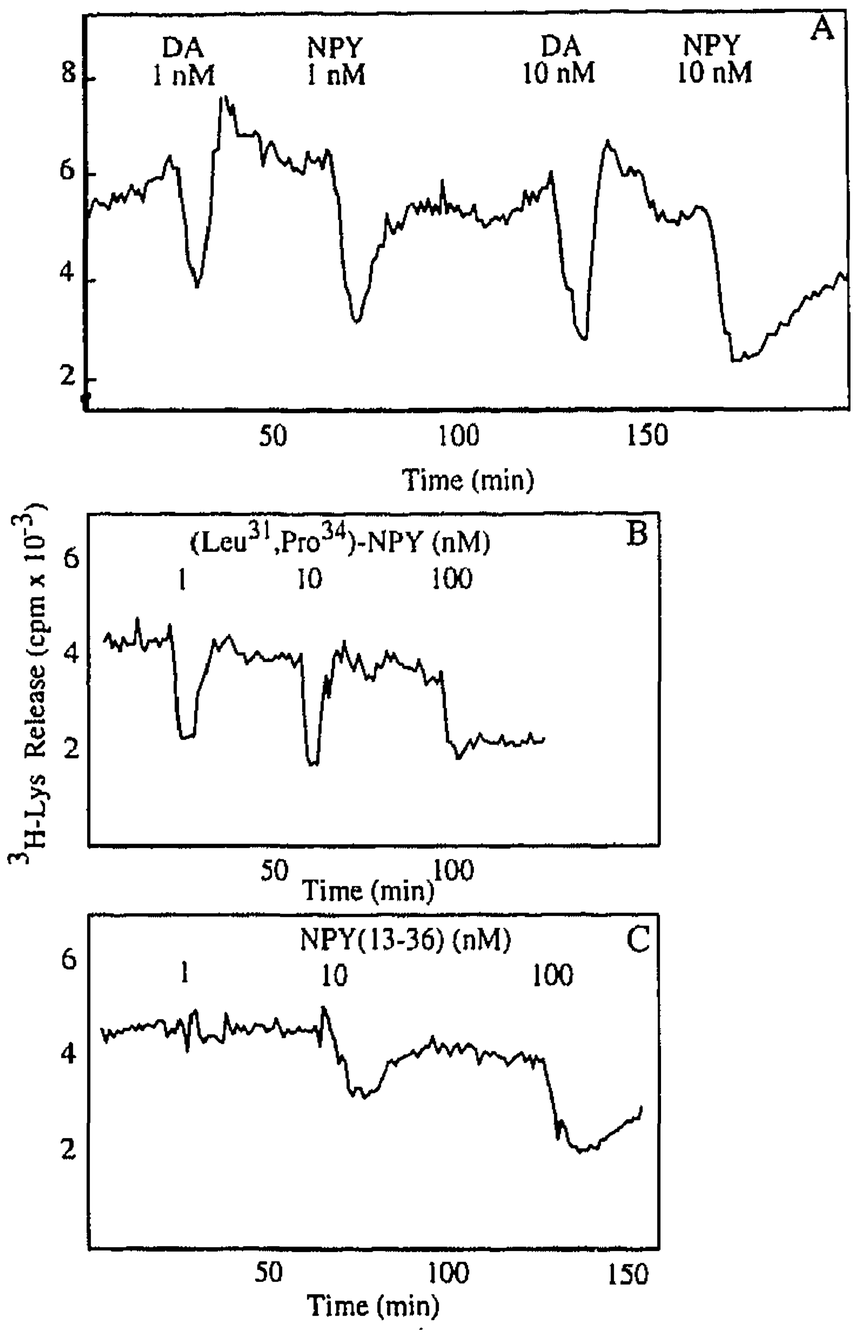

FIG. 3. Effect of dopamine and NPY, [Leu^{31},Pro^{34}]NPY and NPY(13–36) on radioactive peptide release from single melanotropes. (A) Dopamine and NPY inhibit secretion from isolated melanotropes. Dopamine (10 n*M*) inhibition was reversed 6 min after dopamine removal; removal of 10 n*M* NPY caused a very slow reversal of the inhibition, which was not completed after 30 min. (B) Dose–response relationship of [Leu^{31},Pro^{34}]NPY-induced inhibition: 1 n*M* caused an inhibition of 45%; maximum inhibition (55%) was reached at 10 n*M*; application of 100 n*M* did not increase the magnitude of the inhibition but caused a long-lasting inhibition. (C) Dose–response relationship of NPY(13–36): 1 n*M* did not inhibit secretion; 10 n*M* resulted in an inhibition of 35%; and 100 n*M* caused a long-lasting inhibition of 50%.

10 n*M*. Complete inhibition of the oscillations in all cells was achieved by 100 n*M* NPY(13–36) [Fig. 4(D), $N = 13$].

Effect of 8-Br-cAMP on Ca^{2+} Oscillations in Single Melanotropes

The cell-permeable cAMP analogue 8-Br-cAMP increased the frequency of spontaneous Ca^{2+} oscillations by 134 ± 12% when applied at 1 m*M* [Fig. 5(A), $N = 33$]. 8-Br-cAMP at 1 m*M* induced oscillations in six out of eight nonoscillating cells [Fig. 5(B)]. Under NPY-inhibited conditions (1 nM), 8-Br-cAMP induced Ca^{2+} oscillations in 35 out of 41 cells [Fig. 5(C)].

Effect of NPY on cAMP Release From Neurointermediate Lobes

When 1 μM NPY was added to the superfusion medium in the presence of IBMX, α-MSH secretion was reduced by 60 ± 5%. Parallel to this inhibition, an inhibition of cAMP secretion of 55 ± 13% was found (Fig. 6). In the same experiment, a pulse of dopamine was given as this is known to exert a well-characterized effect on cAMP production (8). Dopamine produced 55 ± 8% inhibition of α-MSH secretion and 40 ± 6% inhibition of cAMP secretion. The dopamine-induced inhibition had fully disappeared within 30 min following dopamine removal (two superfusion fractions), whereas the inhibition of NPY was long-lasting: even after 1 h (four superfusion fractions) the release of α-MSH and cAMP had not returned to basal levels.

DISCUSSION

In the present study, we investigated the action mechanism by which NPY inhibits secretory activity of *Xenopus* melanotropes. The finding that NPY is a blocker of secretion from melanotropes in neurointermediate lobes is consistent with earlier studies (32). From the studies on the action of NPY analogues on α-MSH secretion from neurointermediate lobes, showing that NPY, PYY, and [Leu^{31},Pro^{34}]NPY inhibit secretion whereas NPY(13–36) does not, we conclude that a Y_1-like receptor subtype is involved. The finding that PYY is slightly more potent

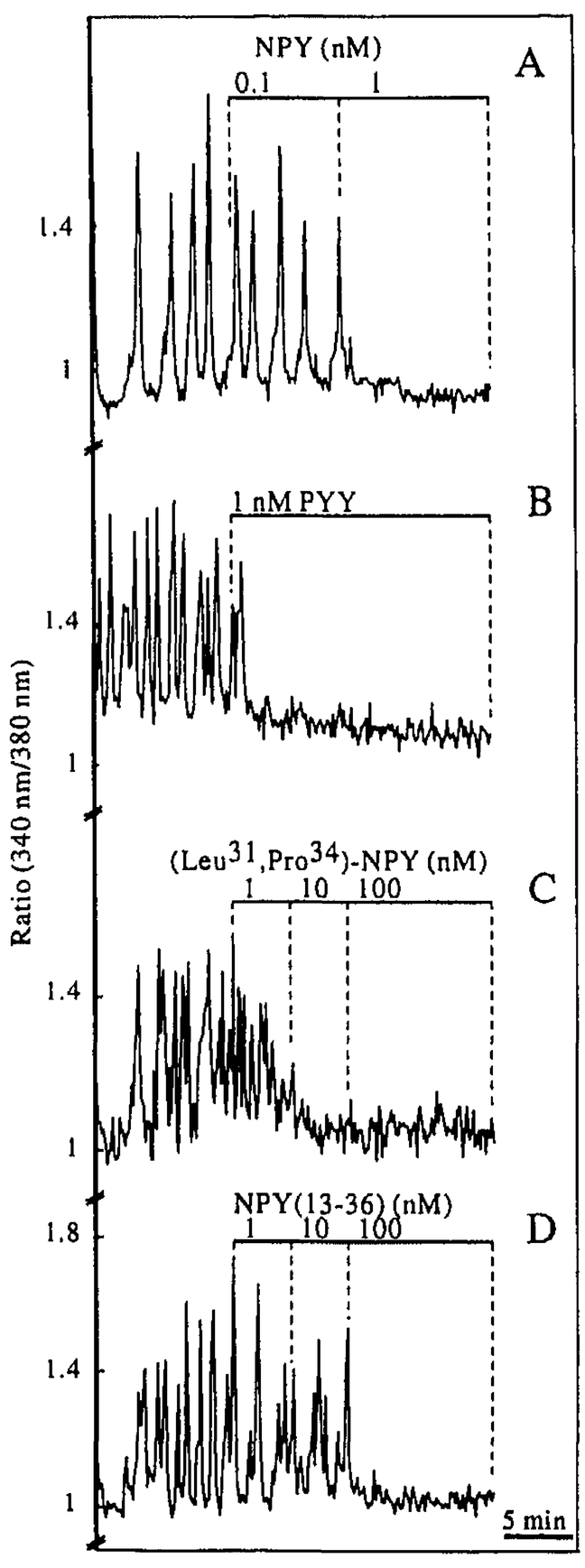

FIG. 4. Effects of NPY, PYY, and [Leu^{31},Pro^{34}]NPY on spontaneous Ca^{2+} oscillations. (A) NPY inhibits spontaneous oscillations in all cells studied at a concentration of 1 n*M*. (B) PYY inhibits the oscillations in all cells at 1 n*M*. (C) [Leu^{31},Pro^{34}]NPY inhibits the oscillations in 22% of the cells at 1 n*M*. To achieve an inhibition in all cells a concentration of 10 n*M* had to be applied. (D) NPY(13–36) inhibits oscillations in all cells at a concentration of 100 n*M*. At 10 n*M* oscillations were blocked in 5 out of 13 cells.

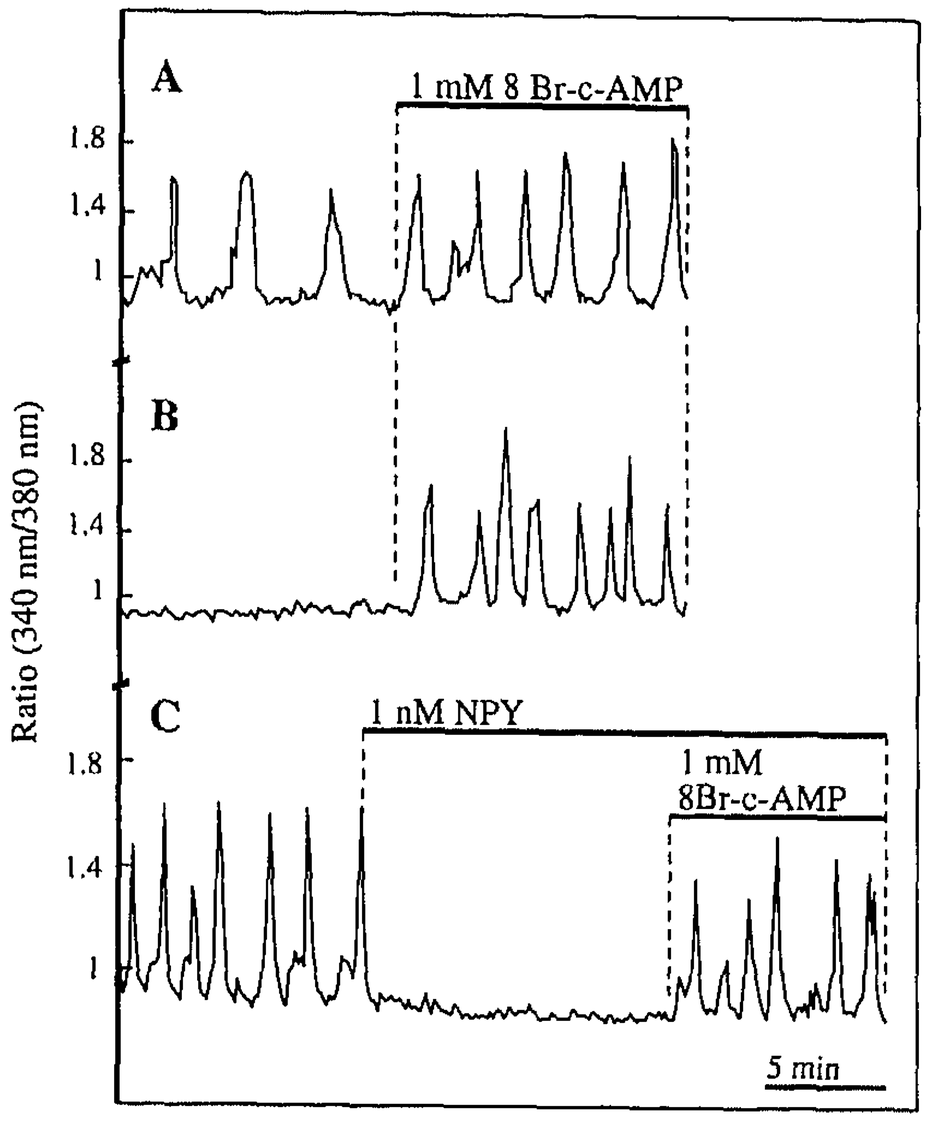

FIG. 5. Effects of 8-Br-cAMP on Ca^{2+} oscillations. (A) 8-Br-cAMP (1 m*M*) strongly increased the frequency of spontaneous oscillations (120%). (B) In six out of eight nonoscillating cells 1 m*M* 8-Br-cAMP induced oscillations. (C) 1 m*M* 8-Br-cAMP reversed the 1 n*M* NPY-induced inhibition of Ca^{2+} oscillations.

than NPY and [Leu^{31},Pro^{34}]NPY is slightly less potent than NPY is in agreement with the different potencies of these analogues to Y_1-receptors reported in other studies (3,18).

NPY acts with a long duration on *Xenopus* melanotropes as is clear from the fact that the recovery from inhibition by neurointermediate lobes and single melanotropes is slow. This action seems to be specific for NPY as it was not displayed by PYY. The most likely explanation for this phenomenon is that the binding of NPY to its receptor is less reversible than that of PYY.

The pharmacological characteristics of the NPY receptor responses found in our secretory studies with cultured melanotropes differed from the strict Y_1 classification found for freshly dissected neurointermediate lobes. Although the Y_1 receptor agonist [Leu^{31},Pro^{34}]NPY was effective both in fresh tissue and cultured single melanotropes, the cultured cells also showed an inhibitory secretory response to NPY(13–36), a NPY analogue that specifically activates a Y_2 receptor subtype. In other tissues, a 100- to 1000-fold difference in potency between these Y_1 and Y_2 receptor agonists for the Y_1 receptor has been reported (3,18). The fact that, in our experiments with cultured *Xenopus* melanotropes, only a 10-fold difference was found might indicate that with culturing there is induction of a Y_2 receptor. Alternatively, the results could reflect changes in Y_1 receptor structure as a result of cell culture and/or trypsinization. Changes in receptor structure after trypsinization and subsequent changes in receptor affinity for ligands have been reported for IgG receptors in monocytes (30). Because the Y_1 receptor agonist is more potent than the Y_2 receptor agonist and because for fresh lobes no Y_2 effect was found, we conclude that a Y_1-like receptor is primarily involved in the inhibition of α-MSH secretion in vivo. We further suggest that the Y_2 receptor agonist activity, seen in the present study and also reported by Kongsamut et al. (16), reflects an artifact.

It was apparent that the secretory activity of cells cultured on poly-L-lysine-coated coverslips was 100 times more sensitive to the inhibitory action of NPY and its analogues than that of the freshly dissected tissue. Possible explanations for this phenomenon are: 1) an increase in density of receptors on cultured cells, and 2) a relatively poor accessibility of the melanotropes in intact lobes for exogenously applied NPY. This accessibility could be hampered by, for instance, a relatively poor penetration of NPY into the tissue, the uptake of NPY by glial cells, or extracellular enzymatic degradation of NPY. It should be noted that in vivo NPY reaches the melanotrope via specialized local synaptic contacts so that the peptide may locally reach relatively high concentrations (10).

Testing NPY and its analogues on intracellular Ca^{2+} dynamics showed that NPY as well as analogues are capable of completely inhibiting spontaneous Ca^{2+} oscillations. In secretion studies with isolated melanotropes, NPY induced only 60–70% inhibition of basal secretion of radiolabeled products, which seems to be contradictory to the complete inhibition of Ca^{2+} oscillations by NPY in such cells. However, in recent studies we found that about one-third of radiolabeled products released by the single melanotropes consists of amino acids not incorporated into peptides (Jenks, unpublished observation). Therefore, we propose that NPY is able to inhibit completely both peptide secretion and Ca^{2+} oscillations.

In Ca^{2+} measurements, the Y_1 agonist was more potent than the Y_2 agonist in inhibiting the occurrence of spontaneous Ca^{2+} oscillations. The observation that the potency of the NPY analogues was equal for inhibiting single cell secretion and Ca^{2+} oscillations supports earlier arguments that spontaneous Ca^{2+} oscillations are the driving force for in vitro α-MSH secretion (25,27).

NPY receptors have been demonstrated to inhibit the adenylyl cyclase system in various cell types (1,18,19,29,34), but there are also reports that Y_1 receptor activation can lead to an increase in intracellular (Ca^{2+}) (1,7,29,33) and that the Y_2 receptor directly inhibits a (N-type) voltage-operated Ca^{2+} channel (2,4,5,12,33). To assess whether NPY receptor activation in *Xenopus* melanotropes leads to a decreased cAMP production, we investigated the effect of NPY on cAMP efflux. This efflux can be taken as

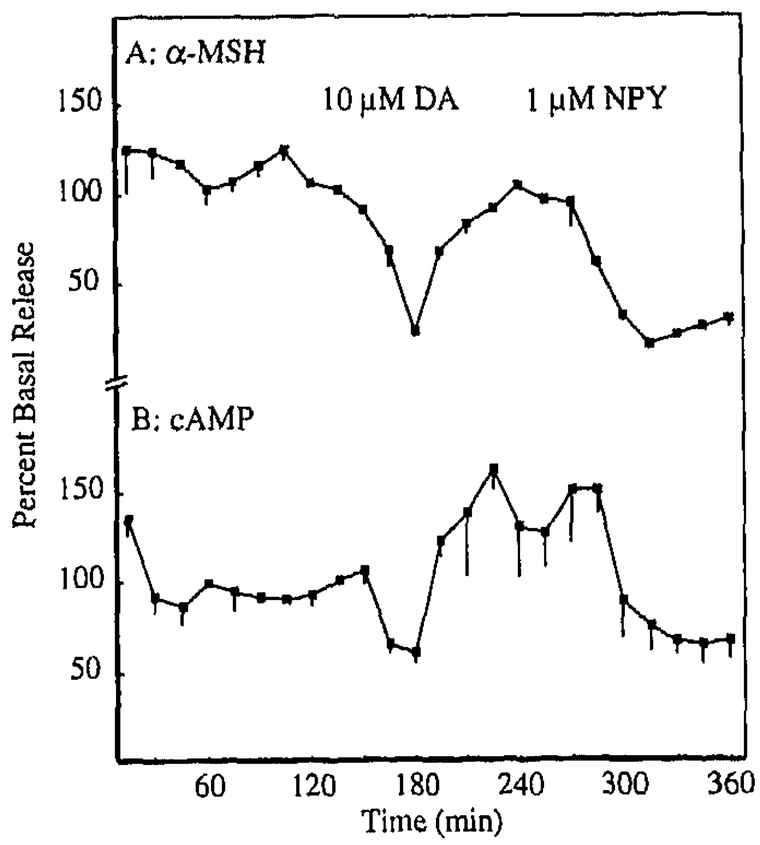

FIG. 6. Comparison of dopamine- and NPY-induced inhibition of the α-MSH secretion (A) and cAMP release (B) in neurointermediate lobes. A strong correlation between inhibition of α-MSH secretion and inhibition of cAMP efflux was noted for both factors.

a measure for the intracellular cAMP concentration (8). The results show that NPY decreases cAMP efflux from *Xenopus* melanotropes, indicating that NPY inhibits the production of cAMP. This finding is in agreement with the action of NPY in most cell systems studied.

For *Xenopus* melanotropes, it has been reported that inhibition of cAMP-dependent protein kinase leads to an abolishment of spontaneous Ca^{2+} oscillations (26). Our data, showing that a cell-permeable cAMP analogue can increase the frequency of spontaneously occurring Ca^{2+} oscillations and can induce oscillations in nonoscillating cells [see also (26)], support the idea that in *Xenopus* melanotropes a cAMP-dependent phosphorylation is involved in the induction of spontaneous Ca^{2+} oscillations. The observation that 8-Br-cAMP could reverse NPY-induced inhibition of Ca^{2+} oscillations is consistent with the conclusion that NPY inhibits *Xenopus* melanotropes through inhibition of adenylyl cyclase.

In summary, on the basis of the present findings we suggest that in *Xenopus* melanotropes NPY inhibits cAMP production via Y_1 receptors, leading to inhibition of spontaneous Ca^{2+} oscillations and subsequently to inhibition of α-MSH secretion.

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