Autoreceptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by \( Y_2 \) receptors

XIAOLI CHEN, DEBORA A. DI MAGGIO, SONG PING HAN, AND THOMAS C. WESTFALL

Department of Pharmacological and Physiological Science, Saint Louis University
Health Sciences Center, St. Louis, Missouri 63104

**Chen, Xiaoli, Debora A. DiMaggio, Song Ping Han, and Thomas C. Westfall.** Autoreceptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by \( Y_2 \) receptors. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1737–H1744, 1997.—Pheochromocytoma (PC)-12 cells express \( Y_1 \), \( Y_2 \), and \( Y_3 \) neuropeptide Y (NPY) receptors when differentiated with nerve growth factor (NGF). The present work evaluated NGF-differentiated PC-12 cells as a model system to study modulation of NPY release by NPY autoreceptors. We demonstrated that both \( K^+ \) and nicotine stimulated concomitant release of NPY and dopamine from differentiated PC-12 cells. We also showed in this study that NPY release from PC-12 cells was attenuated in a concentration-dependent manner by peptide YY (PYY)-(13–36), a selective agonist for the \( Y_2 \) type of NPY receptors. This result demonstrated that NPY release could be modulated by NPY autoreceptors of the \( Y_2 \) subtype. The inhibitory action of PYY-(13–36) may be mediated at least in part by inhibition of N-type \( Ca^{2+} \) channels, because PYY-(13–36) could not produce further inhibitory effects in the presence of a maximum effective concentration of \( w \)-conotoxin, an N-type \( Ca^{2+} \)-channel blocker. The inhibition by PYY-(13–36) could be blocked by pretreatment of cells with pertussis toxin, suggesting that an inhibitory GTP-binding protein was involved. Furthermore, the function of NPY autoreceptors could be modulated by other receptors such as \( \beta \)-adrenergic and ATP receptors. The evoked release of NPY was also attenuated by ATP and adenosine, which have been shown to be colocalized and coreleased with NPY from sympathetic nerve terminals. These results suggest that PC-12 cells differentiated with NGF may be an ideal model to study regulatory mechanisms of NPY release and that autoreceptor-mediated regulation of NPY release appears to act through the \( Y_2 \) subtype of the NPY receptor.

take catecholamines; dopamine; adenosine 5'-triphosphate; adenosine

**NEUROPEPTIDE Y (NPY)** is a tyrosine-rich 36-amino acid peptide initially isolated from porcine brain by Tate-moto et al. (35). NPY belongs to a pancreatic polypeptide family of peptides that includes peptide YY (PYY), to which it has \(-70\%\) homology, and pancreatic polypeptide (PPY), to which it has \(-50\%\) homology. NPY has a wide distribution in the central and peripheral nervous systems. It is colocalized with catecholamines in certain populations of central adrenergic and noradrenergic neurons as well as in the peripheral sympathetic nerves and the adrenal medulla (9). NPY is also coreleased with norepinephrine from perivascular sympathetic nerves (21).

Although initially NPY receptors were classified according to differences in binding and rank order of the pharmacological potency of NPY fragments and analogs (41), at present, at least five distinct NPY receptors have been established by molecular cloning approaches. These include \( Y_1 \) (12, 19, 20, 29), \( Y_2 \) (11, 32), \( Y_4 \) (12, 22), \( Y_5 \) (12), and \( Y_6 \) (13, 37). Another potential subtype, the \( Y_3 \) receptor, has not yet been cloned but has been suggested based on functional and biochemical studies (24, 25, 27). Of these receptor subtypes, the pharmacological profiles for \( Y_1 \), \( Y_2 \), and \( Y_3 \) are the best established, whereas those for types \( Y_4 \), \( Y_5 \), and \( Y_6 \) need further investigation.

At the sympathetic neuroeffector junction, the \( Y_1 \) subtype of NPY receptors is primarily located on the postsynaptic membrane of vascular smooth muscle cells. Activation of the \( Y_1 \) receptor with NPY or selective \( Y_1 \) agonist \([\text{Leu}^{31}, \text{Pro}^{34}]\text{NPY}\) produces potentiation of the contraction of a variety of vasoactive agents in vitro (29, 45) and marked increases in systemic blood pressure in vivo (17). The excitatory action of \( Y_1 \)-receptor activation is usually accompanied by an increase in the intracellular calcium concentration in vascular muscle and other cells (33, 34). \( Y_2 \) receptors are primarily located on the presynaptic membrane of sympathetic nerve terminals and have been shown to have inhibitory actions on norepinephrine release. \( Y_2 \) receptors are also located on other neurons such as the dorsal root ganglion. In this preparation, \( Y_2 \) agonists attenuate the evoked-release substance P and calcium influx (36). A pertussis toxin (PTX)-sensitive GTP-binding protein appears to couple the \( Y_2 \) receptor to the intracellular effects in this preparation. The action of NPY on neurotransmitter or hormone release, however, appears to vary with the tissue preparations used. For example, NPY potentiates the stimulatory effect of luteinizing hormone releasing hormone on luteinizing hormone release from rat anterior pituitary cells (4), whereas the \( Y_2 \) agonist decreases glutaminergic transmission in rat hippocampal neurons (3). Catecholamine release is enhanced by NPY in the perfused bovine adrenal gland (17), whereas it is attenuated in chromaffin cells (18) and in pheochromocytoma (PC)-12 cells (6, 7). These differences may be due to the differential distribution of \( Y_1 \), \( Y_2 \), or \( Y_3 \) receptors as well as by the heterogeneity of the cell population in these tissues. These variations in action may be reduced if a cell line with controlled \( Y_1 \), \( Y_2 \), or \( Y_3 \) receptor expression is used in release studies.

The regulation of NPY release by various neurotransmitter receptors has been previously studied in several laboratories. Unfortunately, most of these studies have been carried out in tissue preparations containing more than one cell type. Therefore, it is sometimes difficult to demonstrate the direct action of the neurotransmitter on NPY-containing neurons. There are advantages to having a homogeneous cell population that both releases NPY and has receptors for the modulatory
Y3-receptor expression can be induced by NGF. The release in the presence of a high-K
ried out to examine the time course of NPY immunoreactivity with appropriate drugs. Preliminary experiments were car-
MATERIALS AND METHODS
is not clear which subtype of NPY receptor mediates a mechanism for autoregulation of NPY release. It
release from pig kidney was attenuated by PYY, suggest-
Our previous work (6, 7) has established PC-12 cells as a model system in which to study the regulation of catecholamine release. The phenotype of PC-12 cells is altered by treatment with nerve growth factor (NGF) or glucocorticoid. Whereas the undifferentiated PC-12 cell does not have functional NPY receptors, treatment with either dexamethasone or NGF results in expression of such receptors. Dexamethasone induces functional Y1-receptor expression, whereas Y2- and Y3-receptor expression can be induced by NGF. The purpose of the present study was threefold: 1) to establish PC-12 cells as a model system in which to study NPY release, 2) to determine whether NPY release is subject to inhibitory autoreceptor inhibition, and 3) to examine the effects after activation of other transmitters during NPY release and the interaction between these receptors and NPY autoreceptors during NPY release.

MATERIALS AND METHODS

Cell Cultures

PC-12 cells with a low passage number (<30) were a generous gift from Dr. Steven Sabol (National Institutes of Health, Bethesda, MD). PC-12 cells were cultured in cell culture inserts (25 mm, 0.45-µm pore size, Falcon Plastics, Oxnard, CA), housed in six-well plates (Costar, Cambridge, MA), and grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum and 10% heat-inactivated horse serum and incubated in a humidified atmosphere containing 5% CO2 in air at 37°C for 5–7 days. PC-12 cells were also differentiated with NGF (50 ng/ml).

NPY-Immunoreactivity Release Study

PC-12 cells were grown on cell culture inserts with NGF for 5–7 days. NPY (and also dopamine)-release experiments were performed according to the method described by DiMaggio et al. (6, 7). The inserts were placed into wells containing low (2.5 mM) or high (50 mM) K+ Krebs bicarbonate buffer with appropriate drugs. Preliminary experiments were carried out to examine the time course of NPY immunoreactivity (ir; NPYir) release in the presence of a high-K+ buffer. Samples were collected at 3, 5, 10, 15, and 20 min of exposure to the high-K+ (50 mM) buffer and measured for NPYir. It was observed that the peak release of NPYir occurred after incubation with a high-K+ buffer for 15 min. A 20-min incubation showed no further increase. Therefore, we decided to carry out all additional experiments with a 15-min incubation period. After a 15-min incubation period, the inserts were then removed from the wells. Aliquots of the release medium were assayed immediately for NPYir. Cells were still intact at the end of the incubation period, and cells on the inserts were extracted with acid for determining total NPYir.

Quantitation of NPYir

NPYir was determined directly in acid extracts of tissue and release medium by radioimmunoassay using a specific antiserum that was raised in rabbits against porcine NPY, as described by DiMaggio and co-workers (6, 7). Radioimmunoas-
say was performed using a 5-day disequilibrium method. Duplicated samples were incubated with the NPY antiserum. Twenty-four hours later, 125I-NPY was added to each tube. After a 72-h incubation period (at 4°C), antibody-bound 125I-NPY was separated from free 125I-NPY by centrifugation after addition of a second antibody (goat anti-rabbit serum) and polyethylene glycol, and radioactivity was measured in a γ-counter. The antiserum was examined for cross-reactivity with homologous peptides and peptide fragments by incubating antiseras with several dilutions of unlabeled NPY-(1—36) or the appropriate peptide. It was observed that the NPY antiseras did not recognize heterologous and homologous peptide sequences including rat β-endorphin, PYY, PPY and the COOH-terminal hexapeptide of human PPY, as well as a variety of COOH-terminal fragments including NPY-(14—36), NPY-(18—36), NPY-(13—36), and peptide YY2 (PYX2). The NPYir in acid extracts of tissue and release medium was characterized and compared with authentic rat and porcine NPY-(1—36) by passage over a high-performance liquid chromatography (HPLC) column followed by assay. The elution profiles of NPYir were compared with profiles of authentic NPY eluted under similar conditions. It was observed that NPYir material eluted as a single peak and coeluted with synthetic human NPY (6, 7). The antiseras recognized mature NPY-(1—36).

Quantitation of Dopamine

When the inserts were removed from the wells containing buffer and agents, perchloric acid (0.4 N) containing 0.1% cysteine was added to the aliquots of the release medium. The cells on the inserts were extracted with acid for determining total dopamine content. Dopamine was determined by HPLC column and quantified by electrochemical detection (23). The HPLC system consists of a Varian (model 2510) solvent delivery system and an autosampler (model 9090). The separation of dopamine was performed isocratically using a filtered and degassed mobile phase consisting of 10% methanol, 0.1 M sodium phosphate, 0.2 mM sodium octyl sulfate, and 0.1 mM EDTA, adjusted to pH 2.8 with phosphoric acid. The HPLC system was coupled to a 386 SX computer with which chromatograms were recorded and analyzed with Varian Star workstation software.

Statistics

Data were expressed as means ± SE of the percentage of fractional release, which was the amount of NPYir released in the buffer divided by the amount of NPYir present in the tissue before depolarization stimulation times 100. Statistical analyses were carried out using two-way analysis of variance followed by Newman-Keuls multiple-range test.

Materials

Porcine NPY, PYY-(13—36), and PYX2 were purchased from Peninsula Laboratories (Belmont, CA). Benextramine,
isoproterenol, clonidine, ATP, α,β-methylene ATP, nifedipine, and ω-conotoxin were obtained from Sigma Chemical (St. Louis, MO). [125]NPy was purchased from Amersham (Arlington Heights, IL). Goat anti-rabbit serum was obtained from Linco Research (St. Louis, MO). PEG-8000 was obtained from Fisher Scientific (St. Louis, MO). NGF was purchased from Collaborator Biomedical Products (Bedford, MA). DMEM, fetal bovine serum, and horse serum were purchased from J RH Biosciences (Lenexa, KS).

RESULTS

Differentiated PC-12 Cell as a Sympathetic Neuronal Model to Study NPY Release

Release of NPYir and dopamine from NGF-differentiated PC-12 cells. PC-12 cells in inserts were incubated in the buffer containing stimulants for 15 min. Aliquots of the same sample were then measured simultaneously for NPYir and dopamine by radioimmunoassay and HPLC-electrochemical detection, respectively (see METHODS). The basal release of NPYir and dopamine from NGF-treated PC-12 cells was 0.04 ± 0.003 and 2.04 ± 0.06 ng/well, respectively. The percent basal fractional release of NPYir and dopamine was 7.03 ± 0.09 and 2.08 ± 0.08%, respectively. KCl (50 mM) or nicotine (100 µM) produced significant increases in release of NPYir and dopamine from NGF-treated PC-12 cells (Fig. 1).

Concentration-dependent effect of PYY-(13—36) on K+-evoked NPYir release from NGF-treated PC-12 cells. K+ (50 mM) increased NPYir release about one- to twofold over that of basal. The selective Y2 agonist PYY-(13—36) (10^-8-10^-7 M) inhibited K+-evoked NPYir release in a concentration-dependent manner (Fig. 2). The maximum inhibitory effect was achieved by 10^-7 M PYY-(13—36) (35% inhibition).

Mechanisms Underlying NPY Autoreceptor-Mediated Inhibition During NPYir Release

Antagonism of PYY-(13—36)-induced inhibition of NPYir release. PYX2 is a synthetic decapeptide amide and NPY analog that has been shown to specifically inhibit the binding of [3H]-labeled NPY to its receptors (35) and to inhibit the NPY-induced increases in intracellular calcium in human erythrocyte cells. In the present study, as shown in Fig. 3, the inhibition of K+-evoked NPYir release induced by PYY-(13—36) (200 nM) was abolished in the presence of PYX2 (200 nM), suggesting that the effect of PYY-(13—36) is mediated by NPY receptors.

Benextramine, an α-adrenoceptor antagonist, has been shown to inhibit [3H]-labeled NPY specific binding in rat brain membranes and irreversibly binds to what appears to be the Y2 receptor in the bovine hippocampus (8). Benextramine (1 µM) also attenuated the PYY-(13—36)-induced inhibition of the K+-evoked NPYir release in the present study (Fig. 4).

Effect of PTX on PYY-(13—36)-induced inhibition of NPYir release. Pretreatment of PC-12 cells with PTX (50 ng/ml) for 18 h did not significantly change basal or
K⁺-evoked NPYir release. However, the PYY-(13—36)-induced inhibition of K⁺-evoked NPYir release was abolished by PTX pretreatment (Fig. 5).

Effect of ω-conotoxin on NPYir release and PYY-(13—36)-induced inhibition of NPYir release. We examined the effect of ω-conotoxin on the K⁺-evoked release of NPYir. The fractional basal release of NPYir was 25 ± 3% and in the presence of ω-conotoxin at maximum effective concentration, 10⁻⁷ M did not produce an additional inhibitory effect on NPYir release (Fig. 6).

Action of Cotransmitters on NPYir Release and Interactions Between Regulatory Transmitter Receptors and NPY Autoreceptors

Effect of adenosine and ATP during NPY release. It has been shown that the adenosine analog cyclohex-

Fig. 5. Effect of pertussis toxin (PTX) on inhibitory effect of PYY-(13—36). Pretreatment of PC-12 cells with PTX (50 ng/ml, hatched bars) for 18 h did not significantly change basal and K⁺-evoked NPY release. Results are expressed as %FR. All data are expressed as means ± SE (n = 5–6). Significant difference as indicated: *P < 0.05.
Effect of isoproterenol on NPY release and PYY-(13—36)-induced inhibition of NPYir release. Isoproterenol, a β-adrenergic receptor agonist, has been shown to enhance nerve stimulation-evoked NPYir and norepinephrine release from the pithed guinea pig (5). In the present study, isoproterenol (200 nM) was shown to block the inhibitory effect of PYY-(13—36) on the K⁺-evoked NPYir release, although it did not significantly change the K⁺-evoked NPYir release (Fig. 9).

Effect of clonidine on the PYY 13–36-induced inhibition of NPY release. Clonidine, an α₂-adrenergic receptor agonist (10 µM), failed to alter the K⁺-evoked release of NPYir (Fig. 10). Higher concentrations also failed to inhibit K⁺-evoked NPYir release (data not shown). In addition, clonidine did not significantly change the inhibitory effect of PYY-(13—36) on the K⁺-evoked NPYir release.

Fig. 8. Effect of ATP on inhibitory effect of PYY (13—36) ATP (3 mM) blocked inhibitory effect of PYY (13—36) (200 nM) on K⁺ (50 mM)-evoked NPY release. This effect of ATP was antagonized by α₂mATP (100 nM). Results are expressed as %FR. All data are expressed as means ± SE (n = 5–6). Significant difference as indicated: *P < 0.05, **P < 0.01.

Fig. 9. Effect of isoproterenol (Iso) on inhibitory effect of PYY (13—36). Iso (200 nM), a β-adrenoceptor agonist, blocked inhibitory effect of PYY (13—36) (200 nM) on K⁺-evoked NPY release, although it did not significantly change K⁺-evoked NPYir release. Results are expressed as %FR. All data are expressed as means ± SE. Significant difference as indicated: *P < 0.05, **P < 0.01.

Fig. 10. Effect of clonidine (Clon) on inhibitory effect of PYY 13–36 on NPY release. Clon (10 µM), an α₂-adrenoceptor agonist, did not significantly change inhibitory effect of PYY (13—36) on K⁺-evoked NPY release. Results are expressed as %FR. All data are expressed as means ± SE. Significant difference as indicated: *P < 0.05.

DISCUSSION

PC-12 cells have an immature adrenal chromaffin cell phenotype, and they synthesize and store both catecholamines and NPY (6, 7). Undifferentiated PC-12 cells express only the Y₁ subtype of NPY receptors (2, 6, 7). The phenotype of PC-12 cells can be altered to resemble sympathetic neurons by differentiation with NGF. Previous studies from our laboratory (2, 6, 7) have shown that differentiated PC-12 cells express both functional Y₁ and Y₂ subtypes of NPY receptors. Recent studies also suggest the presence of Y₃ receptors in NGF-differentiated PC-12 cells (24, 25). Activation of both Y₁ and Y₂ receptors decreases evoked adenosine 3',5'-cyclic monophosphate (cAMP) accumulation. In addition, there is evidence that activation of both Y₁ and Y₂ receptors may also inhibit calcium channels, resulting in a decrease in intracellular calcium levels in various preparations (2, 26). We previously observed (2) that activation of Y₂ but not Y₁ receptors in NGF-differentiated PC-12 cells resulted in an inhibition of calcium entry and simultaneous decrease in dopamine release. Because NPY is also synthesized and stored in PC-12 cells, the present study was designed to further examine PC-12 cells differentiated with NGF as a sympathetic neuronal model to study NPYir release and its regulation by NPY receptors and by other neurotransmitter receptors.

As a cell membrane depolarizing agent, 50 mM K⁺ induced a significant increase in NPYir release that was also accompanied by a significant increase in dopamine release. This observation, together with our previous fura 2 study in differentiated PC-12 cells (2), suggests that an immediate increase in intracellular calcium transient appears to be responsible for the stimulatory effect of K⁺ on NPYir and dopamine release. We also obtained results showing that nicotine increased the release of both NPYir and dopamine. Numerous studies have demonstrated that nicotine stimulates neurotransmitter release by activating nico-
tion of protein kinase C, and an increase in cAMP production (15).

Activation of Y2 receptors by the Y2-type selective agonist NPY-(13—36) has been shown to attenuate the release of neurotransmitters, such as catecholamine and substance P (2, 6, 7, 36). NPY receptor activation also attenuated the release of NPY from sympathetic nerves in the pig kidney (28). Results obtained in the present study, in which we observed that the Y2-selective ligand PYY-(13—36) inhibited the evoked release of NPYir, are consistent with these observations. Thus the induced release of NPYir as well as the presence of functional Y2 receptors in the differentiated PC-12 cells provided an opportunity to study the modulation of NPYir release in a homogeneous cell population.

The inhibitory action of PYY-(13—36) on the K+-evoked NPYir release was completely abolished by PYX2. This decapeptide amide is an NPY analog that has been reported to displace [3H]NPY binding from its receptors (35). The antagonism of the inhibitory effect of PYY-(13—36) on NPYir release by PYX2 suggests therefore that the inhibition of PYY-(13—36) on NPYir release is a receptor-mediated process.

The NPY autoreceptor present in PC-12 cells differentiated with NGF seems to be of the Y2 subtype based on the following observations: 1) the Y2-selective ligand PYY-(13—36) attenuated the K+-induced NPYir release; 2) NPY-(13—36), another selective Y2-receptor agonist, attenuated the K+-induced calcium influx in NGF-differentiated PC-12 cells (2); and 3) NPY-(13—36) did not affect the K+-induced calcium influx in dexamethasone-differentiated PC-12 cells (2), which only express the Y1 subtype of NPY receptors (6, 7).

The inhibitory Y2 receptor seems to be coupled to intracellular events through an inhibitory GTP-binding protein of the Gi or G0 type, because the inhibitory effect on NPYir release was prevented by pretreatment with PTX. A similar PTX sensitivity was seen for the Y2-mediated inhibition of the nicotine-induced release of dopamine (2).

Numerous studies have demonstrated calcium-dependent release of NPY in a variety of preparations. Our previous studies measured intracellular calcium transients using fura 2 spectrofluorometry as well as dopamine release from differentiated PC-12 cells. These studies showed that Y2-receptor agonists inhibited K+-induced increases in intracellular calcium concentration by reducing the calcium influx with simultaneous inhibition of the evoked catecholamine release (2). NPY Y2 autoreceptor-induced inhibition of NPYir release seen in the present study may also be mediated by the inhibition during calcium influx. Therefore, the effects of specific calcium channel blockers on NPYir release and PYY (13—36)-induced inhibition of NPYir release was examined. ω-Conotoxin, an N-type of calcium channel blocker, was shown to mimic the inhibitory effect of PYY-(13—36) on NPYir release. Moreover, in the presence of the maximum effective concentration of ω-conotoxin, PYY-(13—36) did not produce further inhibition on NPYir release. The lack of synergism or additivity between PYY-(13—36) and ω-conotoxin in inhibiting NPYir release suggests that the PYY-(13—36)-attenuated NPYir release may be mediated through an inhibition of N-type calcium channels, although inhibition of other types of Ca2+ channels may also occur.

Norepinephrine, epinephrine, ATP, and NPY are colocalized in the adrenal gland. In the present study, we observed that NPYir release from PC-12 cells can be modulated by ATP as well as adenosine. The adenosine-induced inhibition of the evoked release of NPYir is consistent with the observation in the guinea pig heart that the adenosine analog cyclohexyladenosine significantly reduced the stimulated overflow of NPY (14). Adenosine and ATP are well known to reduce the electrical field stimulation-induced overflow of norepinephrine in rat vas deferens (16) and in blood vessels (38). In isolated myocytes obtained from the right ventricles of ferrets, ATP decreased L-type calcium currents in a concentration-dependent manner (30). However, ATP has not previously been observed to inhibit NPYir release. Thus this appears to be the first demonstration that ATP and adenosine can modulate NPYir release. This is of particular interest because of the fact that ATP is colocalized and coreleased with NPY and catecholamines from sympathetic neurons and the adrenal medulla.

Isoproterenol has been shown to enhance nerve stimulation-evoked NPYir and norepinephrine release from the pithed guinea pig (5). In the present study, isoproterenol did not produce a significant effect on the K+-evoked release of NPYir. However, the inhibition by PYY-(13—36) on NPYir release was attenuated by isoproterenol. The blockade induced by isoproterenol therefore suggests an interaction between Y2 and β-adrenergic receptors. The interaction may occur at a receptor level or intracellular second messenger level. The existence of a catecholamine-NPY receptor interaction has previously been proposed in that NPY reduced the density of a2-adrenergic binding sites and clonidine decreased the binding of iodinated NPY in the medulla oblongata (16). This kind of reciprocal modulation may occur between NPY receptors and other types of receptors. As discussed above, ATP was shown to attenuate NPYir release from PC-12 cells; however, ATP did not enhance the inhibitory effect of PYY-(13—36) on NPYir release. In contrast, ATP blocked the inhibitory effect of PYY-(13—36), and this effect was reversed by desensitization of the ATP receptor with α,β-methylene ATP. These results suggest that there might be an interaction between the Y2 receptor and the ATP receptor. The possible interaction between autoreceptors and other transmitter receptors may represent a new mechanism regulating transmitter release. Clonidine has been shown to inhibit the release of NPY from sympathetic nerve terminals, whereas a2-antagonists enhance the evoked release (14). However, in this study, we did not observe any effect of clonidine on NPYir release. One possible explanation is suggested by the observation that adrenal chromaffin cells express imidazoline but not a2-adrenergic receptors (31).
In conclusion, this study has demonstrated, using NGF-differentiated PC-12 cells as a model, that NPYir release and its regulation mechanisms can be studied. NPYir release can be downregulated by its autoreceptors. The autoregulation of NPYir release may be modulated by purine receptors and PTX-sensitive GTP-binding protein. NPYir release can also be modulated by purine receptors and β-adrenergic receptors. The interaction may occur at the level of the receptor as well as at the level of intracellular signaling.

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