Neuropeptide Y and Peptide YY Inhibit Lipolysis in Human and Dog Fat Cells through a Pertussis Toxin-sensitive G Protein

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Abstract

Neuropeptide Y (NPY) and peptide YY (PYY) are regulatory peptides that have considerable sequence homology with pancreatic polypeptide. Because (*a*) NPY has been shown to be colocalized with noradrenaline in peripheral as well as central catecholaminergic neurons, and (*b*) alpha2-adrenergic receptors of adipocytes play a major role in the regulation of lipolysis, we investigated the effect of NPY and PYY on isolated fat cells.

In human fat cells NPY and PYY promoted a dose-dependent inhibition of lipolysis elicited by 2 μg/ml adenosine deaminase (removal of adenosine) whatever the lipolytic index used (glycerol or nonesterified fatty acids). In dog fat cells NPY and PYY inhibited adenosine deaminase-, isoproterenol- and forskolin-induced lipolysis. In humans and dogs the effects of NPY or PYY were abolished by treatment of cells with *Bordetella pertussis* toxin, clearly indicating the involvement of a Gi protein in the antilipolytic effects.

This study indicates that, in addition to alpha2-adrenergic agonists, NPY and PYY are also involved in the regulation of lipolysis in human and dog adipose tissue as powerful antilipolytic agents. Further studies are needed to characterize the pharmacological nature of the receptor mediating the inhibitory effect of NPY and PYY in fat cells. (*J. Clin. Invest.* 1990. 85:291–295.) adipocyte • G protein • lipolysis • neuropeptide Y • peptide YY

Introduction

Neuropeptide Y (NPY)[1] is a 36-amino acid tyrosine-rich peptide that has considerable sequence homology with regulatory peptides from the gut such as peptide YY (PYY) and pancreatic polypeptide (1). In contrast to PYY and pancreatic polypeptide, NPY is strictly a neuropeptide. NPY has been shown to be colocalized with NE in peripheral noradrenergic as well as central catecholaminergic neurons. Apart from the vascular smooth muscle sympathetic innervation where colocalization of NE and NPY have clearly been revealed, NE and NPY-containing fibers also innervate the smooth muscle of the urino-genital tract, including the vas deferens and uterus. Very recently, it has also been shown by immunocytochemistry that NPY is costored with NE in the perivascular fibers, whereas it is absent in the parenchyma of brown adipose tissue. It was suggested that blood vessels and adipocytes in brown fat have separate innervation by subpopulations of autonomic neurons (2). As far as we know, no data are available in white fat, which has a more limited sympathetic innervation, with 95–98% of the fat cells not directly innervated.

Although not all the physiological functions of NPY have been completely elucidated, the peptide produces several responses when it is injected centrally or systemically. The responses include inhibition of the evoked release of NE from peripheral noradrenergic neurons, vasoconstriction after in vivo administration, and potentiation of the contractile response promoted by epinephrine after in vitro administration. Various other physiological effects have also been described after central administration of NPY or stimulation of sympathetic nerves (3).

The contractile effect of NPY is attributed to reduced accumulation of cAMP in cerebral blood vessel cells. Moreover, NPY and PYY have been found to inhibit vasoactive, intestinal peptide–stimulated cAMP production in epithelial cells isolated from rat small intestine (4). In rat cerebral cortex NPY was also found to inhibit NE-stimulated adenylate cyclase activity. Furthermore, NPY inhibited isoproterenol-stimulated adenylate cyclase activity in cultured atrial cells as well as in atrial membranes (5).

The observed colocalization of NPY and NE in many central and peripheral neurons has certain physiological relevance. NPY and NE are released together upon sympathetic activation during physiological exercise in man (6). Nevertheless, in the rat a different pattern of NPY and NE response depending on the stressful conditions used may also suggest differences in the process of release of the two neurotransmitters or in the kinetics of their spillover into the blood flow (7).

Since there still has not been any demonstration of NPY effects on tissues involved in the regulation of metabolism such as liver and white adipose tissue, which are highly sensitive to activation by NE, the present study attempts to characterize a possible action of NPY on the lipolytic function in fat

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1. *Abbreviations used in this paper:* ADA, adenosine deaminase; NEFA, nonesterified fatty acids; NPY, neuropeptide Y; PYY, peptide YY.

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cells. PYY was also used and a comparison with NPY effects was made because there is a strong structural homology between both peptides. Moreover, NPY and PYY have been described as sharing a common receptor site on small intestinal epithelial cells (4). Both agents have similar effects on the gut: PYY and NPY cause hydroelectric absorption and inhibit vasoactive intestinal peptide and PGE_2-induced secretion in the small intestine both in vivo and in vitro.

The present experiments were performed on human and dog fat cells, two cell models having functional similarities since they both possess a well-defined adrenergic responsiveness controlled by alpha2- and beta adenoceptors and are devoid of responsiveness to lipolytic peptides (glucagon, corticotropin), unlike the fat cells of various other species (hamster, rat, rabbit). Moreover, the dog used for studies on the regulation of lipomobilization in the laboratory offers easier standardization and homogeneity than the human for further studies on regulating events in the area of NPY and PYY. We demonstrate that NPY and PYY are powerful antilipolytic agents. The antilipolytic effect is suppressed by treatment of the fat cells with pertussis toxin.

Methods

Human studies

Seven sedentary male volunteers (mean age, 28±4 yr; range, 24–35 yr) were included in this study. None of these subjects had any metabolic disorder, received any pharmacological treatment, or performed any bout of exercise or training. All subjects were Caucasians and gave informed consent.

Biopsy procedure. Using a 2.3-mm-diam needle mounted on a syringe containing 2 ml of 9% NaCl, after anesthesia with 0.5% lidocaine solution without epinephrine, 200 mg of subcutaneous adipose tissue was drawn 5 cm from the navel in the right half of the abdomen. On all subjects this biopsy was performed between 10 a.m. and midday.

Adipocyte isolation. Adipocytes were isolated using the Rodbell method (8) in KRB (pH 7.4) containing 0.5 mg/ml collagenase, 4% human albumin, and 90 mg/100 ml glucose. After 50 min of digestion at 37°C and filtration isolated adipocytes were obtained and suspended in a volume so that the final concentration was the same in all subjects.

Measurements of adipocyte lipolysis. As an indicator of adipocyte lipolysis, the quantity of glycerol and nonesterified fatty acids (NEFA) liberated in the medium was measured. 50-ml aliquots of the cell suspension were placed in 1.5-ml conical tubes. 10 of these tubes were used for cell counting and sizing. 10 others provided an evaluation of the initial quantity of glycerol and NEFA contained in the medium. Agents for lipolysis stimulation or inhibition (10 μl) in solution at various concentrations were added to the remaining tubes. For each concentration, three identical tubes were prepared.

After 3 h at 37°C all the tubes were placed in a water bath at 95°C for 7 min to stop the incubation. Afterward, the Kather technique (9), based on the transformation of glycerol into 3-phosphoglycerol, allowed the reduction of NAD to NADH. The latter was measured by bioluminescence with a luciferase solution using a Wallac luminometer (model 1251; LKB Instruments, Inc., Gaithersburg, MD). NEFA levels were also determined with a luminometric technique (10). The amounts of glycerol and NEFA were taken as the average of the quantities obtained from the three tubes of incubation.

Estimating the quantity of lipids. To find out the quantity of lipids incubated in each tube, the total volume of incubated cells was multiplied by the average density (0.915, density of triolein). The total cell volume incubated was obtained by multiplying the average number of adipocytes contained in the tube by the average cell volume. The average number of adipocytes was determined by counting. To do this, the 50 μl contained in each counted microtube was diluted with 450 μl KRB and albumin. A 10-μl portion of this suspension was placed dropwise on a slide of plastic material and the adipocytes were counted with a microscope. The average number of adipocytes per 10 μl represents the number of adipocytes per microliter of the initial suspension.

The average cell volume was calculated after measurement of adipocytes. This was done with a micrometer scale placed in the eyepiece. Each division corresponded to 13 μm, so the adipocytes were divided into classes by multiples of 13 μm. Cells with diameters < 39 μm were not counted. For each category of cells the total volume was obtained by multiplying the average volume of the class (calculated from an average diameter, assimilating the adipocyte to a sphere) by the number of cells in that class. By adding the total cellular volume of each class and dividing by the number of cells measured, the average adipocyte volume of the subject (in nanoliters) was obtained.

Animal studies

Preparation of dog adipocyte membranes. Adipose tissue was obtained from six beagle dogs, weighing 15–20 kg. Omental adipose tissue was obtained after an overnight fast; a biopsy was taken immediately after the induction of general anesthesia by 30 $mg \cdot kg^{-1}$ i.v. pentobarbitone. The isolated adipocytes were obtained as previously described (11).

Lipolysis measurements. The lipolytic activity was analyzed on isolated adipocytes prepared according to the technique of Rodbell (8) with minor modifications. KRB containing BSA (3.5%) and glucose (6 mM), adjusted to pH 7.4 with 1 N NaOH just before use, was used as previously described (12). After collagenase action (1 mg/ml KRB), isolated fat cells were washed three times and the packed cells were brought to a suitable dilution in KRB. The cells were incubated in plastic vials (1 ml incubation medium) with gentle shaking in a water bath under an air phase at 37°C. After 90 min the incubation tubes were placed in an ice bath, the adipocytes were separated from the buffer, and 200 μl of the infranatant were removed for the enzymatic determination of glycerol which was taken as an index of the lipolytic rate. Total lipid was evaluated gravimetrically after extraction with the method of Dole and Meinertz (13). Pharmacological agents were added just before the beginning of the incubation in 10-μl portions in vehicle to obtain a suitable final concentration. The lipolytic activity of all isolated fat cell batches was controlled using 1 μM isoproterenol (a nonselective beta agonist).

Treatment of human and dog fat cells with pertussis toxin was performed as described by Murayama and Ui (14).

Data analysis. All experiments were performed in duplicate. Values are means±SEM. Wilcoxon's test was used for comparisons between matched pairs, differences being considered significant when $P < 0.05$.

Drugs and chemicals. The following reagents were used: human albumin ORHA 20/21 (Behring); isoproterenol and *Bordetella pertussis* toxin (Sigma Chemical Co., St. Louis, MO); and tetradecyl aldehyde (Aldrich Chemical Co., Milwaukee, WI). Collagenase, BSA, luciferase from *Photobacterium fischeri*, and enzymes for glycerol assays came from Boehringer Mannheim GmbH (Mannheim, FRG). All other chemicals and organic solvents were of reagent grade. NPY and PYY were of human origin and purchased from Peninsula Laboratories, Inc. (Belmont, CA).

Results

This study was designed to assess whether NPY and PYY have a functional impact on human and dog fat cell responsiveness. The lipolytic activity was measured under various conditions. Since there is no direct correlation between changes in cAMP levels and lipolysis within certain concentration ranges of lipolytic agents, it was felt that lipolysis measurements were a better index for exploration of a putative physiological impact of such peptides than cAMP or adenylate cyclase measurements.

NPY and PYY effects on human fat cells. Experiments were carried out on isolated human fat cells incubated in the presence of increasing concentrations of NPY and PYY. Neither agent had any lipolytic effect on isolated fat cells. Thus, the investigations were focused on the exploration of the putative antilipolytic potencies of these peptides requiring specific experimental conditions leading to the activation of the rate of fat cell lipolysis. However, the increment of this rate must be controlled since activation of lipolysis, promoted higher than necessary by strong lipolytic agents, cannot be counteracted by inhibitory agents (15). As previously reported, the basal lipolytic rate of the isolated fat cell can be severely restrained by endogenous adenosine released or resulting from cell breakage (9). Therefore, the lipolytic activity of human fat cells was activated by removing adenosine by addition of 2 μg/ml adenosine deaminase (ADA) in the incubation medium. Under these particular conditions spontaneous lipolysis was 0.84±0.2 μmol/100 mg of lipid after 3 h of incubation, while spontaneous lipolysis (in the absence of ADA) was only 0.41±0.07 μmol/100 mg lipid.

NPY promoted a dose-dependent inhibition of lipolysis regardless of which lipolytic index was used (glycerol or NEFA) (Fig. 1). PYY was more potent than NPY in human fat cells.

The inhibiting concentrations of NPY appear somewhat higher under our assay conditions than those of PYY. However, similar half-effective concentrations (EC_{50}) have already been described in rat intestinal epithelial cells (4) and rat atrial cells (5). Nevertheless, local concentrations of NPY could exist in the adipocyte vascular bed and may achieve such high levels. The maximal inhibition obtained at higher doses of peptides was slightly weaker than that defined with 1 μM epinephrine associated with 10 μM propranolol (beta-adrenergic antagonist) or 1 μM UK 14,304 (full alpha2-adrenergic agonist) tested under similar conditions.

Effects of PYY and NPY in dog fat cells. To extend the study of such an observation to an animal model the dog was selected, since, as previously defined by our group, the fat cell of the dog possesses an adrenergic responsiveness that is very similar to that described in the human fat cell (beta1-, beta2- and alpha2 adrenoceptors coexist in human and dog fat cell membranes; 16, 17). Moreover, as opposed to various commonly used animal models such as rat, hamster, and rabbit, the fat cells of man and dog do not possess receptors for lipolytic peptides that are positively coupled with adenylate cyclase (18).

In this cell model, the experimental approach for the evaluation of NPY effects was conducted on fat cells whose lipolysis was prestimulated by various lipolytic agents acting through different mechanisms. ADA (removal of adenosine), isoproterenol (beta-adrenergic agonist), and forskolin (direct stimulator of the catalytic unit of adenylate cyclase) were used. The inhibitory effects of 10^{-7} M NPY were tested in three different situations reported in Fig. 2. NPY clearly counteracted the lipolytic effect initiated by ADA (4 μg/ml), isoproterenol (1 μM), and forskolin (10 μM). These data suggest that NPY does not interfere directly with stimulatory receptors (beta adrenoceptors) in decreasing isoproterenol-induced lipolysis, but rather controls the cAMP production system itself as shown by the counteraction of forskolin and ADA effects. In other experiments we demonstrated that these effects are dose dependent (not shown). Inhibition of the lipolytic activity observed in the presence of ADA was explored along the dose-response curve for NPY and PYY (Fig. 3).

PYY and NPY reduced lipolysis by ~ 95% at the highest doses used. However, NPY was apparently less efficient than PYY in dog fat cells (EC_{50} 0.72±0.22 and 8.95±2.71 μmol glycerol/100 mg lipids per 90 min for PYY and NPY, respectively) as already described in human fat cells.

Abolition of the effects of NPY and PYY by pertussis toxin. As regulation of lipolysis involves regulation of plasma membrane adenylate cyclase activity and changes in cAMP levels, a possible action of NPY and PYY at the adenylate cyclase step could be proposed. Since an inhibition of lipolysis was observed, an involvement of a Gi protein could be considered. Such a hypothesis could be verified by treatment of fat cells with pertussis toxin, which specifically ADP-ribosylates the Gi proteins involved in the inhibition of adenylate cyclase blocking the inhibiting process mediated. Thus, NPY and PYY responsiveness was studied after treatment of fat cells with pertussis toxin and a comparison was performed with alpha2-adrenergic agonists in human fat cells. In control human fat cells, epinephrine alone or UK 14,304 counteracted the lipolysis observed in the presence of ADA (2 μg/ml) as previously seen (15); the effect was slightly stronger than that observed with NPY and PYY (Fig. 4). After pertussis toxin treatment of

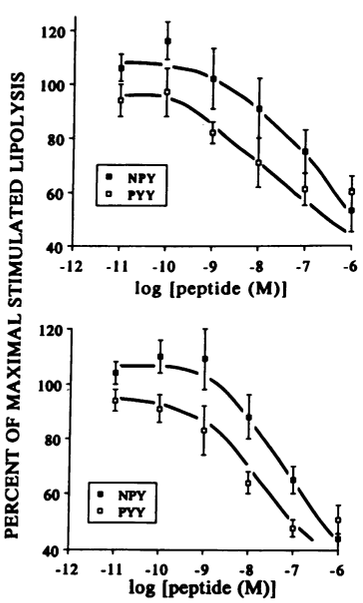

Figure 1. Dose-response curve of inhibition of ADA-stimulated lipolysis (2 μg/ml) by NPY (■) and PYY (□) in human adipocytes. The values of lipolysis are given by both glycerol and NEFA released in the medium and are given in the upper and lower panels, respectively. Results are mean±SE of seven separate experiments performed in triplicate.

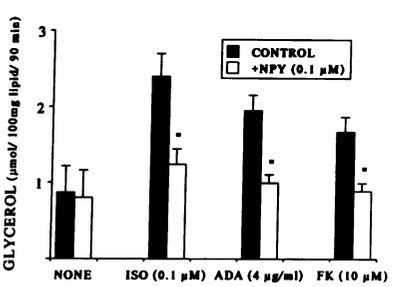

Figure 2. Effect of 10^{-7} M NPY (*open bars*) on isoproterenol (*ISO*)-, ADA-, or forskolin (*FK*)-stimulated lipolysis in dog fat cells. Values of lipolysis are expressed as μmol glycerol/100 mg lipid per 90 min. Spontaneous lipolysis was 0.87±0.35 μmol glycerol/100 mg lipid per 90 min. Results are mean±SE of six separate experiments performed in duplicate. *$P < 0.05$; Wilcoxon's test.

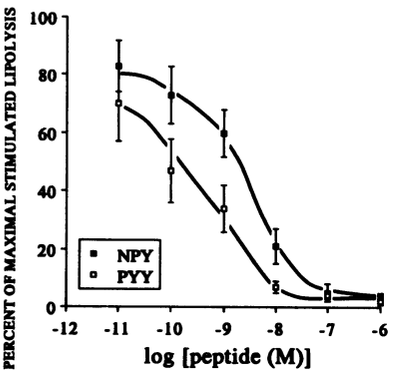

Figure 3. Dose-response curve of inhibition of ADA-stimulated lipolysis (4 μg/ml) by NPY (■) and PYY (□) in dog adipocytes. Values are expressed as percentage of maximal inhibition. Results are mean±SE of six separate experiments performed in duplicate.

the cells, a significant enhancement of basal lipolysis was observed and no extra effect was noticed with ADA. All the inhibitory effects observed with epinephrine, UK 14,304, NPY, and PYY were completely suppressed. This action was also confirmed in dog fat cells. Pertussis toxin treatment enhanced basal lipolysis and suppressed the antilipolytic effects of alpha2-adrenergic agonists, adenosine, PGE1, NPY, and PYY in dog fat cells (not shown).

To conclude, the inhibitory effect of NPY and PYY on lipolysis was abolished in human and dog fat cells, suggesting the involvement of a Gi protein.

Discussion

The original finding of this work was that NPY and its related analogue PYY can exert potent antilipolytic effects in human and dog fat cells.

NPY, a major neuropeptide, is costored with NE in sympathetic nerve terminals, while PYY is located in the endocrine cells of the intestinal mucosa from various species (19). In the intestine, which appears to be the most fully studied, PYY and NPY have been shown to have multiple interactions with different second messenger systems for reducing intestinal secretion. In the fat cell, the putative receptors for these peptides appear to be negatively coupled with adenylate cyclase since pertussis toxin, which is known to specifically ADP-ribosylate the two αi subunits of the GTP-binding regulatory proteins in human fat cells (20), uncouples the receptors from the αi subunits and blocks the inhibitory effects of NPY and PYY (Fig. 4) and also those of alpha2-adrenergic agonists. However, further studies which are considered to be out of the scope of the present paper are required to clearly delineate the mechanism(s).

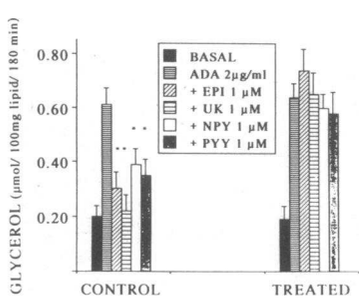

Figure 4. Effect of alpha2-adrenergic agonists (epinephrine [*EPI*], UK 14, 304 [*UK*]), NPY, and PYY on ADA-stimulated lipolysis before and after treatment of isolated fat cells with pertussis toxin. Human adipocytes were incubated for 90 min with (*treated*) or without (*control*) 10 μg/ml of pertussis toxin. Values of lipolysis are expressed as μmol glycerol/100 mg lipid per 90 min. Results are mean±SE of six experiments. $^*P < 0.05$; Wilcoxon's test.

Based on our results, we propose that NPY and PYY inhibit lipolysis through specific receptors coupled negatively with adenylate cyclase via a pertussis toxin–sensitive protein. In this regard, like alpha2 adrenoceptors, NPY sites are coupled to the inhibition of adenylate cyclase and lipolysis; their effect is also uncoupled by pertussis toxin treatment. Demonstration of the physiological relevance of NPY and PYY in the regulation of adipocyte function needs further study. However, several lines of evidence suggest its potential role on the fat cell. PYY-immunoreactive cells are numerous in the terminal ileum, colon, and rectum, whereas a limited number of these cells are found in the duodenum and jejunum. PYY is released into the blood in response to a meal or after intestinal perfusion with oleic acid (21, 22). The antilipolytic action described here fits reasonably with such physiological conditions. In contrast to PYY, NPY, which is strictly a neuropeptide, is found throughout all regions of the intestinal tract and brown fat. A modulating action (counteraction of beta-adrenergic effects) on fat cell lipolysis could be reasonably considered during the activation of the sympathetic nervous system since NE and NPY are colocalized in peripheral neurons, and the peptide and catecholamines may act as cotransmitters affecting the same target fat cell through similar mechanisms. It is noticeable that the receptors of the fat cell could be stimulated or costimulated by both endocrine (PYY) and neural (NPY) pathways. The detailed processes involved in the control of both transmitters as well as the mechanisms maintaining or controlling their spillover and degradation will probably require further extended studies to delineate the interest of such mechanisms in the control of fat cell function in physiological and pathological situations.

In conclusion, this work provides the first evidence for the existence of NPY- and PYY-dependent antilipolytic effects in fat cells. The action is probably mediated by adenylate cyclase inhibition. It is now necessary to define, with improved binding studies, the pharmacological nature of the receptor mediating the inhibitory effects of NPY and PYY in fat cells.

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