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Characterization of NPY receptors controlling lipolysis and leptin secretion in human adipocytes

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Abstract In order to characterize neuropeptide Y (NPY) receptors present in human adipocytes, we used selective ligands together with specific molecular probes able to recognize the different NPY receptor subtypes. RT-PCR experiments revealed the presence of Y_1 receptor transcripts with Y_4 and Y_5 and absence of Y_2 signals. Binding studies, using selective radioiodinated ligands, detected a high number ($B_{max} = 497 \pm 124$ fmol/mg protein) of a high affinity binding site only with [^{125}I]peptide YY (PYY) and [^{125}I](Leu^{31},Pro^{34})PYY. These sites exhibited a typical Y_1 profile as indicated by the rank order of affinity of NPY analogs and the high affinity of two selective NPY receptor antagonists, SR120819A and BIBP3226. In [^{35}S]GTPγS binding experiments, PYY activation was totally inhibited by SR120819A and BIBP3226. Both compounds antagonized, with similar efficiency, the antilipolytic effect exerted by NPY in isolated adipocytes. Finally, PYY and Y_1 ligands enhanced adipocyte leptin secretion, an effect totally prevented by SR120819A. Thus, highly expressed in human adipocytes, the Y_1 receptor sustains the strong antilipolytic effect of NPY and exerts a positive action on leptin secretion. **© 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Neuropeptide Y; Peptide YY; Human adipocyte; Lipolysis; Neuropeptide Y Y_1 receptor; Leptin

1. Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide which belongs to a family of neuropeptides including NPY, peptide YY (PYY) and pancreatic polypeptide (PP). NPY is widely distributed throughout the central and peripheral nervous systems in mammals where it is co-stored with norepinephrine in the sympathetic nerve terminals [1]. Related to its widespread distribution, NPY exerts a variety of biological effects especially on cardiovascular regulation, food intake, metabolism, behavioral and endocrine functions via several receptor subtypes. Five distinct NPY receptors have been cloned belonging to the seven transmembrane G protein-coupled receptor family: Y_1, Y_2, Y_4, Y_5, Y_6 and a putative Y_3 receptor which remains to be elucidated [2]. Among various actions, NPY and PYY exert a strong, direct antilipolytic effect on adipocytes through receptors previously named PYY-preferring receptors [3,4]. These receptors also seem to be expressed in other cell types such as rat small intestine [5] and kidney proximal tubule cells [6] and are distinguished by a higher binding affinity for PYY than for NPY. PYY-preferring receptors are negatively coupled to adenylyl cyclase and their inhibitory effect is sensitive to the pertussis toxin. This type of receptor appears to be a 62 kDa protein in adipocytes and a 44 kDa glycoprotein in the intestine. To date, PYY-preferring receptors have not been successfully cloned or clearly identified [3,4,7].

In an attempt to precisely characterize the NPY/PYY receptors present in mature human adipocytes, we used new selective peptide and non-peptide NPY ligands together with specific molecular probes able to recognize the different NPY receptor subtypes. Functional characterization of the NPY receptor involved in the antilipolytic effect of NPY was performed by studying [^{35}S]GTPγS binding activation and by measuring fat cell lipolysis in the presence of selective NPY Y_1 receptor antagonists. We clearly demonstrated that a typical Y_1 receptor is highly expressed in human adipocytes and supports the antilipolytic effect of NPY and PYY. We also showed that these peptides are able to regulate leptin production via NPY Y_1 receptor activation.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals. The two selective NPY Y_1 receptor antagonists SR120819A [8] and BIBP3226 [9] were synthesized in Sanofi-Synthelabo (Toulouse, France). They were dissolved in DMSO at 10^{-2} M and then diluted in the appropriate test solvent. NPY, PYY and fragments were from Neosystems (Strasbourg, France). Bovine serum albumin was from Biosepra (Paris, France). Collagenase and enzyme for glycerol assays came from Boehringer Mannheim (Mannheim, Germany). EDTA and HEPES were purchased from Merck-Clevenot (Nogent sur Marne, France). The radioligands, [^{125}I]PYY (human), [^{125}I](Leu^{31},Pro^{34})PYY (human), [^{125}I](3–36)-PYY (human), [^{125}I]PP (human) and [^{125}I]PP (rat) (2000 Ci/mmol) and [^{35}S]GTPγS (1250 Ci/mmol) were from New England Nuclear, Life Sciences (Les Ulis, France). Radioimmunoassay (RIA) kits for leptin were from R&D Systems Europe Ltd. (Abingdon, UK).

2.1.2. Biological materials. Adipose tissue was obtained from healthy female donors (30–55 years old) undergoing abdominal plastic surgery for cosmetic reasons. Tissue collection was performed according to our national ethical rules. Six to 12 independent preparations

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Abbreviations: NPY, neuropeptide Y; PYY, peptide YY; ADA, adenosine deaminase

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were studied after adipocyte isolation by collagenase digestion. Ready-to-use cultured human differentiated adipocytes grown in 96 well format were shipped from Stratagene Inc. (La Jolla, CA, USA).

2.2. NPY receptor expression in human adipocytes

Quantification of mRNA encoding human β_2-microglobulin and human NPY gene receptors (NPY-r) was performed with the human adipocyte cDNA purchased from Clontech Laboratories, Inc. (Palo Alto, CA, USA). DNA amplifications were carried out in PCR buffer (PE Biosystem, Courtaboeuf, France) containing 6 μl of cDNA solution from dog cDNA preparation or 1 ng of cDNA from Clontech, 0.1 mM dNTP (Amersham Pharmacia Biotech, Orsay, France), 0.25 μM of each 5′ and 3′ primers and 0.5 U *Taq* polymerase (PE Biosystem,) in a final volume of 20 μl. The mixture was amplified with the GeneAmp PCR 9600 Thermal cycler (PE Biosystem, Courtaboeuf, France). The thermal reaction profile consisted of a denaturation step at 95°C for 20 s, annealing at 60°C for 30 s and an extension step at 75°C for 20 s. The reaction was performed for 40 cycles. After PCR, 5 μl loading buffer (Novex, San Diego, CA, USA) was mixed with 10 μl PCR products and electrophoresed on a 3% agarose gel in the presence of ethidium bromide with 123 bp DNA markers (Gibco BRL Life Technologies, Cergy Pontoise, France) as molecular weight controls. Gene-specific sense and antisense oligonucleotide primers used for PCR and internal oligonucleotide probes used for Southern blot hybridization were designed from published human NPY-r sequence data. The oligonucleotides used were purchased from Genset (Paris, France). All the primers were 20 residue oligonucleotides with 50% G+C content and lacking 3′ complementary between primer pairs. β_2-Microglobulin primers (as internal control) (1): *sense* 5′-GCTTACATGTCTCG-3′; *antisense* 5′-GATGCTGCTTACATGTCTCG-3′. NPY1-r primers: *sense* 5′-925/CATGATGGACAAGATGAGAG-3′; *antisense* 5′-1257/GTGGACATGGCTATTGTTTC-3′. NPY2 primers: *sense* 5′-599/GATCATCCCGGACTTTGAGA-3′, *antisense* 5′-781/GCTCCAGGACTGACATGGTT-3′. NPY4-r primers: *sense* 5′-441/GAGGCATCAGCTCATCATCA-3′, *antisense* 5′-627/GACCACCTTATCTGCCAGGA-3′. NPY5-r primers: *sense* 5′-564/TCTGTTCTCCCCTTCCAGTG-3′, *antisense* 5′-861/ACCTGAGGCCCACTCTTTTT-3′.

The expected size of the amplicons was 268 bp for β_2-microglobulin, 383 bp for NPY1-r, 183 bp for NPY2-r, 187 bp for NPY4-r and 298 bp for NPY5-r. The specificity of each primer pair was analyzed by running PCR with human whole brain cDNA (Clontech), and Southern analysis of the PCR products with the corresponding internal oligonucleotide probe (data not shown) as already described [10]. Total adipocyte RNA was extracted using the Qiagen RNeasy kit (Qiagen, Hilden, Germany) and stored at −80°C until used.

2.3. Isolation of human adipocytes and membrane preparation

Adipocytes were isolated using the Rodbell method [11] in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 0.5 mg/ml collagenase, 3.5% bovine serum albumin and 6 mM glucose as previously described [12]. Isolated adipocytes were used immediately either for lipolysis measurements or membrane preparations for binding studies. The preparation of adipocyte membranes was performed according to Castan et al. [4]. At the end of the washing procedure, crude adipocyte membranes were suspended in the lysing buffer at a final concentration of 0.5–2 mg/ml and stored as aliquots in liquid nitrogen until used. Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard [13].

2.4. Binding assays with various NPY radioiodinated ligands

Adipocyte membranes (0.005–0.02 mg/ml) were incubated for 60 min at 30°C in a Krebs–Ringer buffer (pH 7.4) containing 20 mM HEPES, 1% bovine serum albumin, 0.33 mg/ml bacitracin and 0.1 nM of radioligand ([^{125}I]hPYY, (Leu^{31},Pro^{34})-hPYY, (3–36)-hPYY, hPP or rPP) as previously described [8]. Non-specific binding was determined in the presence of 0.3 μM porcine NPY when using [^{125}I]PYY ligands or 0.3 μM human PP when rat and human [^{125}I]PP were used. Competition experiments were carried out in the presence of 0.1 nM [^{125}I]hPYY and various concentrations of the compound to be tested. For saturation experiments increasing concentrations (0.01–2 nM) of [^{125}I]hPYY and [^{125}I](Leu^{31},Pro^{34})-hPYY were used. All experiments were carried out in duplicate. Data from binding experiments (i.e. apparent equilibrium dissociation constant (K_d), maximum binding capacity (B_{max}), IC_{50} (concentration of inhibitor required to obtain 50% inhibition of the specific binding) and Hill coefficient (n_H)) were analyzed using a non-linear regression program [14].

2.5. [^{35}S]GTPγS binding

Crude adipocyte membranes (0.030 mg/ml) were incubated in binding buffer with [^{35}S]GTPγS in the presence of the appropriate concentration of agonist, hPYY (100 nM), and GDP 10^{-6} M for 45 min at 25°C. Non-specific binding was determined in the presence of 10^{-5} M unlabelled GTPγS. The reaction was stopped by filtration through Whatman GF/B fiber filters. The radioactivity bound to filters was measured by scintillation counting. All results are expressed as percentage of the basal activity. Values are the mean ± S.E.M. of three to four experiments.

2.6. Lipolysis measurements

The lipolytic activity was analyzed on isolated adipocytes obtained after collagenase digestion. A fat cell suspension (1000–2000 cells) in a Krebs–Ringer bicarbonate buffer, pH 7.4, was incubated in plastic vials with gentle shaking at 37°C. After 90 min, the incubation tubes were placed in an ice bath, the adipocytes were separated from the buffer, and 20–50 μl of the infranatant removed for the radioenzymatic determination of glycerol according to the method of Bradley and Kaslow [15]. The glycerol produced by the cells was taken as an index of lipolysis. Pharmacological agents were added just before the beginning of the incubation in 10 μl portions in vehicle to obtain a suitable final concentration. Adenosine deaminase (ADA) was included in the incubation medium to prevent inhibition due to adenosine released by the fat cells; as previously reported, this procedure allows a more accurate definition of the antilipolytic effects. The lipolytic activity of all isolated fat cell batches was checked using 1 μM isoproterenol. All experiments were performed in duplicate. Values are given as means ± S.E.M. ($n = 6$). Student's paired t-test was used for statistical evaluation.

2.7. Leptin assays

By using cultured ready-to-use human differentiated adipocytes grown in 96 well plates, we measured the influence of various NPY analogs on leptin production. These mature adipocytes were obtained from subcutaneous preadipocytes cultured in a differentiation medium as previously described [16]. The batch of cells used was at the third passage and had been validated by the supplier using the adipocyte-specific genes: aP2 (fatty acid binding protein), peroxisome proliferator-activated receptor γ and leptin. They exhibited the typical morphology of mature adipocytes with multiple lipid vesicles (Fig. 5A). Culture medium was changed every 2 days. Cells were treated with or without 10^{-7} M NPY analogs (hPYY, (Leu^{31},Pro^{34})-hNPY and rPP) in the presence or absence of SR120819A (10^{-6} M). 100 μl aliquots of adipocyte culture medium were taken at various times for leptin measurements by RIA (22, 50 and 69 h after the beginning of the treatment) and replaced by 100 μl of medium containing the appropriate treatments. All compounds were dissolved in culture medium. Data are means ± S.E.M. ($n = 4$–6). Statistical analysis of data was performed by means of multifactorial analysis of variance (ANOVA), with repeated measurements. Post hoc comparisons to the respective control were performed with Dunnett's test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Expression of NPY receptor genes in human adipocytes

Using specific primers, quantification of human NPY gene receptors was performed from PCR analysis of mRNA extracted from either isolated human adipocytes or commercial human adipocyte cDNA. The presence of human β_2-microglobulin transcripts was also assessed as a control. As shown in Fig. 1, single bands of 383 bp (NPY Y_1), 187 bp (NPY Y_4), 298 bp (NPY Y_5) and 268 bp (β_2-microglobulin) were specifically generated with specific primers. No NPY Y_2-r transcript was detected. These PCR products have the sizes predicted from the genomic sequences. Similar bands were obtained using mRNA from either freshly isolated or cultured human

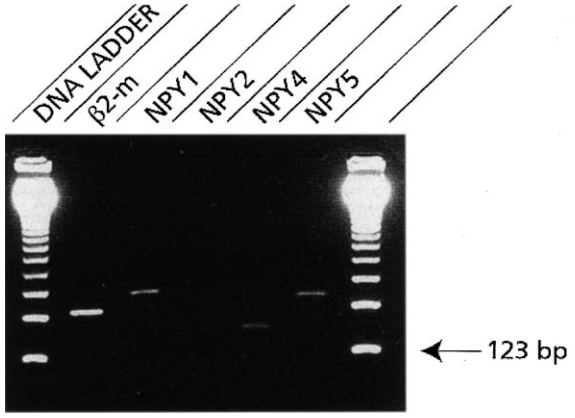

Fig. 1. NPY receptor gene expression in human adipocytes. NPY-r expressions were obtained after PCR amplification of cDNA from human differentiated adipocytes using specific primers. Negative control was performed with no cDNA. Internal control was obtained by PCR of β_2-microglobulin gene. The size of the amplified products is indicated in bp on the right. PCR products were separated by electrophoresis on 3% agarose gel in the presence of ethidium bromide with 123 bp DNA markers as molecular weight controls.

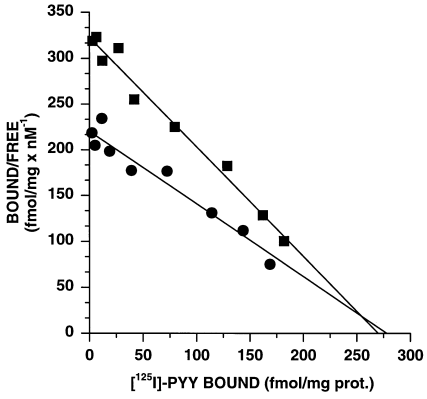

Fig. 2. Saturation of (■) [^{125}I]hPYY- and (●) [^{125}I](Leu^{31},Pro^{34})-hPYY-specific binding to human isolated adipocyte membranes. Incubations were carried out as described in Section 2 in the presence of increasing concentrations of ^{125}I ligands with membranes (12 μg protein) for 1 h at 30°C. Each point is the mean calculated from a typical experiment performed in duplicate and repeated three times without noticeable changes.

adipocytes as well as from commercially available human adipocyte cDNA (not shown).

3.2. Biochemical characterization of human adipocyte NPY receptors

Preliminary binding studies, performed on human adipocyte membranes in the presence of various receptor subtype-selective radioiodinated NPY ligands, evidenced high amounts of specific labelling when using the natural hormone, [^{125}I]hPYY, and (Leu^{31},Pro^{34})-hPYY, known as a Y_1, Y_4 and Y_5 ligand. Very low or no specific binding (even at a high adipocyte membrane concentration) was detected using either [^{125}I]hPYY(3–36), a Y_2 and Y_5 probe, [^{125}I]hPP, a Y_4 and Y_5 ligand, or [^{125}I]rPP, a specific Y_4 receptor radioligand (not shown). These data are consistent with a majority of NPY Y_1 receptors in our preparation. Saturation binding experiments performed on human adipocyte membranes with [^{125}I]hPYY and [^{125}I](Leu^{31},Pro^{34})-hPYY indicated that the specific binding observed was saturable. As shown in Fig. 2, Scatchard analysis of these data gave linear plots consistent with the presence of a single class of high affinity binding sites having apparent K_ds of 0.81 ± 0.11 and 1.26 ± 0.14 nM for [^{125}I]hPYY and [^{125}I](Leu^{31},Pro^{34})-hPYY, respectively. It is important to note that both ligands identified a similar binding capacity when used simultaneously on the same adipocyte preparation (for example, see Fig. 2). [^{125}I]hPYY binding demonstrated very high binding capacity with a mean B_{max} value of 497 ± 127 fmol/mg protein (i.e. approximately 50 000 sites/adipocyte). Significant variations were observed with individual tissue donors (from 174 to 780 fmol/mg protein which corresponds to 15 000–75 000 sites/fat cell).

In competition binding experiments, the relative affinities of several reference peptide and non-peptide NPY compounds were also studied to further characterize [^{125}I]PYY receptors expressed in human adipocytes. As shown in Table 1, the labelled receptor exhibited a typical Y_1 profile, as defined in a recent classification [2]: Y_4 (rat and human PP), Y_5 (D^{32}Tryp-NPY, (3–36)-hPYY and hPP) and Y_2 ((13–36)-

Table 1
Inhibition of [^{125}I]PYY binding to human adipocyte membranes by reference peptide and non-peptide NPY ligands

	IC_{50} (nM)
Peptides	
hPYY	0.89 ± 0.09
hNPY	1.09 ± 0.22
(Leu^{31},Pro^{34})-hNPY	1.75 ± 0.65
(3–36)-hPYY	121 ± 47
(13–36)-pNPY	373 ± 214
rPP	> 1000
hPP	105 ± 12
D^{32}Tryp-hNPY	> 1000
(Pro^{30},Tyr^{32},Leu^{34})-NPY (28–36)	17.7 ± 5.2
PYX1	> 1000
PYX2	> 1000
Non-peptides	
BIBP3226	10 ± 6
SR120819A	27 ± 7

Half maximum specific binding (IC_{50}) was calculated according to [14]. Values are means ± S.D. of three to six independent determinations performed in duplicate.

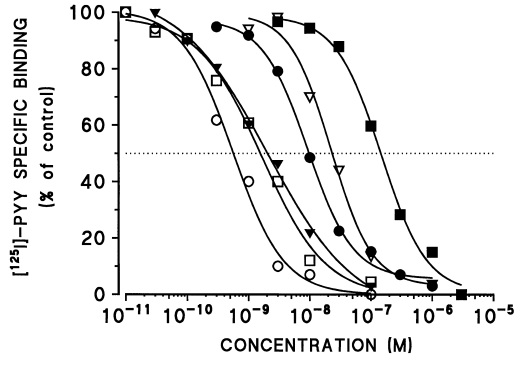

Fig. 3. Inhibition of [^{125}I]PYY-specific binding to membranes of isolated human adipocytes by reference peptide and non-peptide NPY ligands. Incubations were carried out in the presence of 0.1 nM [^{125}I]hPYY and increasing concentrations of the compound to be tested: hPYY (○), hNPY (□), (Leu^{31},Pro^{34})-hPYY (▼), BIBP3226 (●), SR120819A (▽) and (3–36)-hPYY (■). Data are the mean of duplicate determinations (n = 3–6).

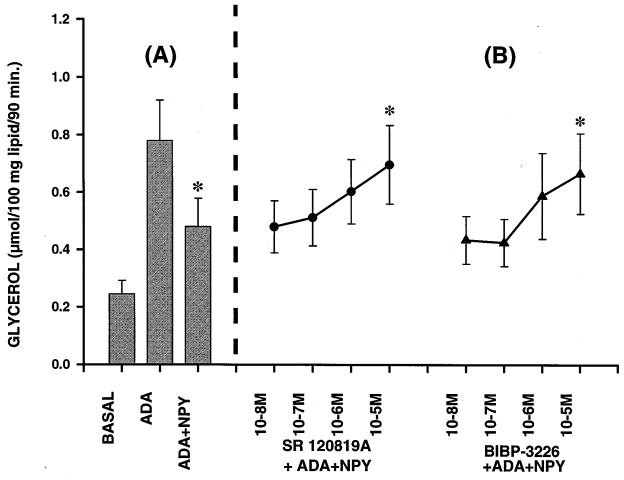

Fig. 4. Effect of the selective NPY Y_1 receptor antagonists BIBP3226 and SR120819A on NPY-induced lipolysis inhibition in human isolated fat cells. A: Effect of hNPY (100 nM) on ADA (4 μg/ml)-stimulated lipolysis. Spontaneous basal lipolysis corresponds to 0.243 ± 0.047 μmol/100 mg lipid/90 min glycerol release. Results are means ± S.E.M. of six separate experiments performed in duplicate. hNPY significantly antagonized the lipolytic activity measured in the presence of ADA. Comparison (ADA+NPY vs. ADA) was performed using Student's paired *t*-test: $*P < 0.05$. B: Dose-dependent antagonism of SR120819A and BIBP3226 on 100 nM hNPY antilipolytic effect measured in the presence of ADA. Results are means ± S.E.M. of six separate experiments performed in duplicate. Comparison with the control (ADA+NPY) was performed using Student's paired *t*-test: $*P < 0.05$.

pNPY and (3–36)-hPYY) ligands had low affinities whereas (Leu^{31},Pro^{34})-hPYY, a Y_1 probe, and the selective non-peptide Y_1 receptor antagonists BIBP3226 and SR120819A displayed high binding affinities. As shown in Fig. 3, these latter molecules displaced [^{125}I]hPYY dose-dependently. In every case the proportion of BIBP3226- or SR21819A-insensitive binding sites accounted for less than 5% of the specific binding demonstrating that the great majority of sites labelled by [^{125}I]hPYY are Y_1 receptors. Dose–response curves for all compounds tested gave a linear Hill plot and pseudo Hill coefficient (n_H) near unity (not shown).

3.3. Functional studies in isolated human adipocytes

3.3.1. [^{35}S]GTPγS binding. NPY receptors are coupled to heterotrimeric, pertussis toxin-sensitive G proteins and agonist activation leads to the inhibition of adenylyl cyclase. We used [^{35}S]GTPγS binding as a functional measure of NPY receptor activation in human adipocytes. Human PYY stimulated [^{35}S]GTPγS binding to adipocyte membranes with an EC_{50} value of 16 ± 7 nM. Additionally (Leu^{31},Pro^{34})-hNPY but neither (3–36)-hPYY nor human and rat PP increased [^{35}S]GTPγS binding. BIBP3226 and SR120819A, known as selective Y_1 receptor blockers, dose-dependently antagonized hPYY (100 nM)-stimulated [^{35}S]GTPγS binding with K_i values of 6 ± 3 and 27 ± 3 nM ($n = 3$), respectively. These results are consistent with the binding data (Table 1).

3.3.2. Lipolysis measurements. On human isolated adipocytes, NPY and PYY promoted a dose-dependent inhibition of lipolysis prestimulated by ADA [12]. As shown in Fig. 4A, hNPY (100 nM) significantly antagonized the lipolytic activity observed in the presence of ADA (4 μg/ml) by about 40%, consistent with previous results. In this model, the glycerol production was taken as an index of the lipolytic rate and the validity of each preparation was controlled on the basis of its isoproterenol response. Tested under similar experimental conditions, SR120819A and BIBP3226 (10^{-9}–10^{-6} M) dose-dependently antagonized NPY inhibition with a similar potency (Fig. 4B). The highly selective profile of these molecules further supports the involvement of NPY Y_1 receptors in the antilipolytic effect of NPY on human adipocytes.

3.3.3. Leptin production in differentiated human adipocytes. The cultured human adipocytes used demonstrated differentiated adipocyte-specific physiology as evidenced, in particular, by their capacity for leptin production and as shown by their typical morphology with multiple intracellular lipid vesicles, in agreement with the mature adipocyte phenotype (Fig. 5A). Moreover, we detected significant amounts of specific binding with [^{125}I]hPYY and [^{125}I](Leu^{31},Pro^{34})-hPYY but no specific labeling with [^{125}I]hPYY(3–36) or [^{125}I]hPP as previously seen with isolated adipocyte membranes (not shown). Cell pretreatment of these cells with 100 nM hPYY and (Leu^{31},Pro^{34})-hNPY enhanced leptin release at the different times studied as compared with the vehicle-treated control (Fig. 5B). This effect was prevented by pretreating these cells with the selective Y_1 receptor antagonist SR120819A (1 μM). The Y_4, Y_5 ligand, hPP, had no effect on leptin production (not shown). All these data are in agreement with a specific effect of NPY/PYY on peripheral leptin secretion via a Y_1 receptor-mediated pathway.

4. Discussion

The present study characterized the nature and the role of the NPY receptors expressed in human differentiated adipocytes. Using selective molecular probes directed against the different human NPY receptors and specific ligands in binding and functional studies, we clearly demonstrated that the Y_1 receptor is expressed in high amounts by human adipocytes and is responsible for the antilipolytic effect of NPY/PYY on fat cells. Stimulation of the Y_1 receptor is also able to control leptin production in these cells.

In a first set of experiments, the molecular expression of the different NPY receptor transcripts in human adipocytes was studied by PCR analysis using selective primers for NPY Y_1, Y_2, Y_4 and Y_5 receptors. NPY Y_1 transcripts together with significant amounts of NPY Y_4 and NPY Y_5 receptor mRNAs were detected, whereas no Y_2 signal was found in this tissue.

Binding experiments performed on isolated human adipocyte membranes clearly identified sites exhibiting a classical Y_1 profile as referred to in the recently published NPY classification [2]. Firstly, the identification performed with subtype-selective radioiodinated NPY ligands showed the absence of specific binding with [^{125}I]hPYY(3–36) (a Y_2 and Y_5 probe), [^{125}I]hPP (a Y_4 and Y_5 ligand) and [^{125}I]rPP (a specific Y_4 probe), whereas only the natural hormone [^{125}I]hPYY and (Leu^{31},Pro^{34})-hPYY (Y_1, Y_4, Y_5) identified a single class of high affinity binding sites with a similar binding capacity, in accordance with a unique Y_1 receptor population in our preparation. Secondly, competition binding studies performed with selective peptide and non-peptide ligands showed a high affinity for PYY and confirmed the Y_1 nature of the site identified based upon the following rank order of affinity: hPYY ≥ hNPY ≈ (Leu^{31},Pro^{34})-hNPY > BIBP3226 ≈ SR-

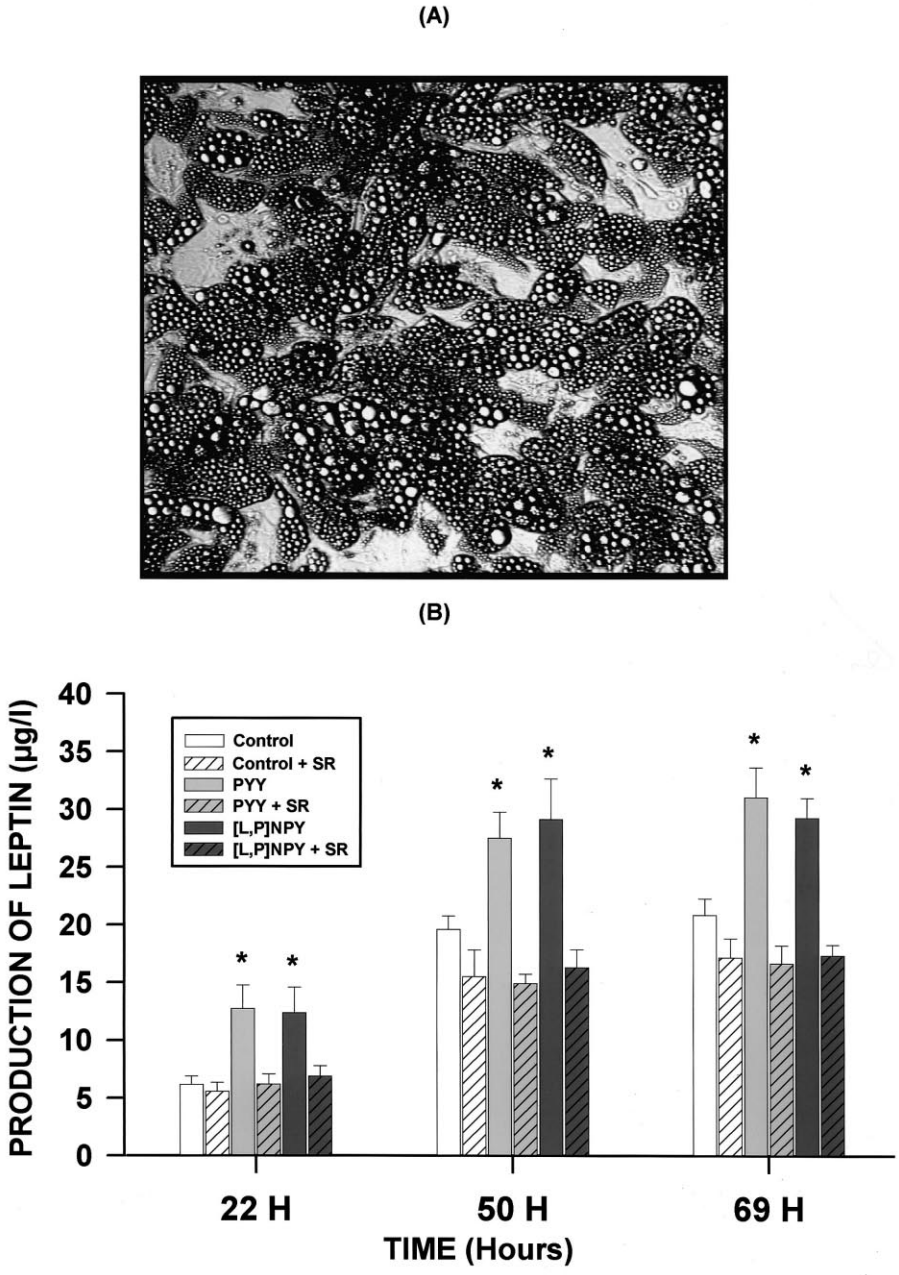

Fig. 5. Effect of NPY analogs in the presence or absence of SR120819A on leptin secretion in cultured differentiated human adipocytes. A: Morphology of differentiated human adipocytes seeded in 96 well plates. B: Kinetics of leptin production after adding 100 nM hPYY, (Leu^{31},Pro^{34})-hNPY or culture medium vehicle in the presence (hatched) and in the absence (open) of 1 μM SR120819A (SR). Values are means ± S.E.M. of four (treated) to six (control) determinations. Differences were considered significant when $P < 0.05$.

120819A > hPP ≈ (3–36)-hPYY ≈ (13–36)-pNPY > rPP ≈ D-^{32}Tryp- hNPY. Of note, the two well-known selective Y_1 receptor antagonists, BIBP3226 and SR120819A, completely displaced radioligand binding with high affinity. Thirdly, there is a strict correlation between the binding characteristics and the biological responses observed in human adipocytes: PYY and the Y_1 ligand, (Leu^{31},Pro^{34})-hNPY, equally activated [^{35}S]GTPγS binding, which was totally blocked by the selective Y_1 receptor antagonists SR120819A and BIBP3226. These two antagonists also blocked the antilipolytic effect evoked by hPYY in human isolated adipocytes with a similar potency. In these functional studies, we were unable to find any role for potential Y_4 and/or Y_5 receptors present in these fat cells. We can hypothesize that even if the Y_4 and Y_5 mRNAs were detected in human differentiated adipocytes, the proteins are not or poorly expressed. Discrepancies between mRNA existence and receptor expression have already been reported in several systems and could be explained by the highly sensitive PCR method able to amplify low abundance mRNAs. A low level or absence of transcription together with a loss of these sites during the experiments cannot be excluded. It is also questionable if there is any role of Y_4 and Y_5 receptors in early stages of adipocyte maturation and/or differentiation.

Some years ago, Valet et al. described a powerful antilipolytic effect of NPY/PYY in dog [3] and in human [12] adipo-

cytes mediated by PYY-preferring receptors. Atypical receptors exhibiting a preference for PYY versus NPY have also been reported in some systems such as the rat intestine [5] and in a mouse renal proximal tubule cell line where PYY was 2–10 times more potent than NPY [6]. Due to the limited number of specific pharmacological tools available at this time and to the high number of NPY receptor subtypes, a clear characterization of these sites was not possible. Marked species-specific differences have been observed for NPY receptors in terms of pharmacological properties and localization [17]. For example, rodent CNS is rich in Y_1 receptors whereas the human brain is almost devoid of Y_1 receptors [18]; similarly, prejunctional Y_2 receptors are present in the rat vas deferens whereas a Y_1 subtype has been identified in the rabbit [19]. Here, we report that functional Y_1 receptors are present in human adipocytes, in contrast to previous work showing that in dog adipocytes a Y_2 or Y_2-preferring subtype controls lipolysis [3]. In rat adipose tissue, dual control of lipolysis by NPY Y_1 and Y_2 receptor subtypes has been proposed [7]. In other species such as rabbit and guinea pig absent or very low antilipolytic responses to NPY/PYY have been reported [4].

It is also important to note the large number of Y_1 sites found in human adipocytes (B_{max}: 497 ± 124 fmol/mg protein, i.e. 50 000 sites/cell), close to that already reported for α2-adrenoceptors [3]. This value is more than 10 times higher than previously reported for putative PYY Y_1-preferring receptors in this tissue but the origin of adipose tissue and the experimental conditions likely explain these differences. In the present work we also observed significant variations in Y_1 receptor density according to the donors (15 000–75 000 sites/fat cell). It is questionable if there are any fat cell size-dependent differences that could explain this variability. Interestingly, it has been shown for α2 receptors in different species (hamster, rat, dog and human) that adipocyte hypertrophy is associated with a specific increase in α2 receptor number [20].

The potent antilipolytic effect described for the NPY/PYY system leads one to expect that Y_1 receptor antagonists could be very useful tools for regulating/decreasing lipid accumulation in human fat cells. Surprisingly, mice lacking the Y_1 receptor exhibit moderate obesity with increase in body fat without hyperphagia. These data are inconsistent with a prominent physiological role of Y_1 receptors in fat accumulation. However, the lipolytic role of Y_1 receptors in mice is not known and reduced locomotor activity observed in Y_1 knock-out animals might account for the decreased energy expenditure and subsequent increased fat deposits [21]. These observations in Y_1 knock-out mice are also inconsistent with results showing inhibition of feeding and weight reduction in mice treated with Y_1 receptor antagonists. Compensatory mechanisms involving other hormones or receptors are highly probable in these animals.

Finally, the present work establishes the role of NPY Y_1 receptors in the control of leptin production by human adipocytes. This protein is secreted into the blood stream by only mature white adipocytes. One function of leptin is to communicate the status of fat stores to the brain although a number of new functions have recently been revealed. We demonstrate that PYY and a Y_1 ligand enhanced leptin release, an effect which is totally blocked by a selective Y_1 receptor antagonist. NPY-related modulation of leptin release might represent a peripheral feedback mechanism in the control of leptin secretion. Thus, an increase in NPY/PYY plasma levels could enhance adipocyte leptin secretion. In turn, this hormone, via specific binding to hypothalamic receptors, inhibits the expression and synthesis in the hypothalamic arcuate nucleus of NPY, a strong orexigenic signal. Therefore, an increase in circulating leptin decreases NPY production and food intake and increases energy expenditure for protection from high fat diet-induced obesity and insulin resistance and to limit further adipocyte hypertrophy.

It has been shown that leptin expression and secretion is proportional to adipocyte size and under the control of cAMP. Agents that increase intracellular cAMP, such as β-adrenergic agonists or dibutyryl cAMP itself, have been shown to decrease leptin mRNA expression and leptin secretion [22,23]. As expected, NPY Y_1 receptor stimulation, which should reduce cAMP levels, is shown to have the opposite effects of β-agonists on leptin release.

In conclusion, the functional receptors involved in the antilipolytic effect of NPY/PYY in human adipocytes fulfill all the criteria of the recently cloned Y_1 receptor and corresponds to the PYY-preferring receptor proposed many years ago. In addition to the well-established central role of NPY as an hypothalamic neuropeptide controlling food intake, we propose a peripheral action for this hormone in regulating feeding behavior through modulation of leptin release from white adipocytes. The physiological relevance of this observation remains to be established in vivo.

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