Natriuretic peptide-dependent lipolysis in fat cells is a primate specificity

Coralie Sengenès, Alexia Zagaroff-Girard, Agnès Moulin, Michel Berlan, Anne Bouloümié, Max Lafontan, and Jean Galitzky
Laboratoire de Pharmacologie Médicale et Clinique, Faculté de Médecine, Institut National de la Santé et de la Recherche Médicale, Unité 317, 31073 Toulouse Cedex, France

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Until now (and unlike in most species), in man, lipolysis was thought to be mainly regulated by catecholamines in white adipocytes (10, 31), whereas peptide hormones such as ACTH, melanotropins (α- and β-MSH), β-endorphin, growth hormone, glucagon, thyroid-stimulating hormone, cholecystokinin, and parathyroid hormone have lipolytic effects on adipocytes from various other mammalian species (41, 55). We recently demonstrated (49) that natriuretic peptides (NPs), which are peptide hormones, are lipolytic in human adipocytes to the same extent as β-adrenergic receptor (AR) agonists. Moreover, the NP-induced lipolysis involved cGMP-dependent and cAMP-independent pathways.

NPs are a family of cyclic peptides found in various animal species from different phyla (1, 25, 47, 52) and consist of at least three distinct endogenous peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). NPs possess various biological effects including actions on natriuresis, diuresis, vasodilation, and inhibition of the renin-angiotensin-aldosterone and the sympathetic nervous systems (50, 51). ANP and BNP are mainly secreted by atrial and ventricular cardiomyocytes in response to mechanical stretches (33, 53), whereas CNP is expressed in the central nervous system and in vascular endothelial cells (13, 26). NPs exert effects via membrane-bound receptors. Two classes of NP receptors (NPRs) have been defined by molecular cloning (35, 43, 48). The first class includes types A and B membrane guanylyl cyclase receptors, which are defined as GC-A and GC-B, respectively. Stimulation of these receptors induces intracellular cGMP production. The second class of ANP binding sites is a nonguanylyl cyclase-linked receptor termed clearance receptor or NPR-C. Although devoid of a cytoplasmic domain, studies have implicated NPR-C in mediating signal transduction through the inhibition of adenyl cyclase or the activation of phospholipase C (2, 3, 42). NPRs have been found in various tissues including white and brown adipose tissues (16, 17, 20, 22, 45, 46, 54).

Address for reprint requests and other correspondence: C. Sengenès, INSERM U317, Laboratoire de Pharmacologie Médicale et Clinique, Faculté de Médecine, 37 Allées Jules Guesde, 31073 Toulouse Cedex, France (E-mail: corasengenes@yahoo.com).

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Many studies have pointed out species-specific variations in the adrenergic control of lipolysis (8, 12, 27, 28). To determine whether NP-induced lipolysis also occurs in other species, we compared the effects of NPs on fat cells from primates (humans, macaques) and from various other mammal species (rodents: rats, mice, guinea pigs, hamsters; and nonrodent mammals: dogs, rabbits). The observed species-specific differences were investigated to provide some mechanistic interpretations.

MATERIALS AND METHODS

Subjects

Human subcutaneous adipose tissue (1–2 g) was obtained from 16 normal or moderately overweight women who were undergoing plastic surgery. Their mean age was 45.7 ± 3.5 yr and their mean body mass index was 24.4 ± 1.3 kg/m². The investigation respected the guidelines of the Ethical Committee of Toulouse University Hospital.

Animals

White-fat depots were dissected from six male Wistar rats (260–300 g, 6–10 wk old; visceral and epididymal adipose tissue), six male C57BL/6 mice (20–25 g, 8–10 wk old; visceral and epididymal adipose tissue), five male Beagle-Harrier dogs (10–12 kg, 8–10 wk old; subcutaneous, visceral, and epididymal adipose tissue), six male Syrian hamsters (Mesocricetus auratus; 80–100 g, 7–9 wk old; visceral and epididymal adipose tissue), four female New Zealand rabbits (2.8–3.1 kg, 13–15 wk old; visceral and perivarian adipose tissue), and six young male adult macaques (Macaca fascicularis; 2–5 kg, subcutaneous visceral and epididymal adipose tissue). All the animals were fed ad libitum and had free access to water. All animal studies were in agreement with the guidelines for animal care and fully conformed to the Guiding Principles for Research Involving Animals and Human Beings (20).

Adipocyte Isolation

Isolated adipocytes were obtained according to Rodbell’s method (44) by collagenase digestion of adipose tissue fragments in Krebs-Ringer bicarbonate buffer containing 3.5% bovine serum albumin (KRB) and 6 mmol/l glucose at pH 7.4 under gentle shaking at ~120 cycles/min at 37°C. Fat cells were filtered through a silk screen (250 μm) and washed with KRB buffer to eliminate collagenase.

Lipolysis Measurements

Isolated adipocytes were brought to a suitable dilution (2,000–3,000 cells/assay) in KRB buffer for lipolysis assays and were incubated with pharmacological agents at the indicated concentrations in a final volume of 100 μl for 90 min at 37°C. At the end of the incubation, 20- to 50-μl aliquots of the infranatant were taken for glycerol determination (9), which was used as the lipolytic index. Total lipid content was determined gravimetrically after solvent extraction. Rat ANP was used for rodent species, dog, and rabbit experiments, and human ANP was used for primate (human and nonhuman) species.

Determination of cGMP and cAMP Concentrations

Fat cells were incubated for 15 min at 37°C in the presence of 0.1 mmol/l isobutylmethylxanthine (IBMX, a nonspecific phosphodiesterase (PDE) inhibitor) and were then stimulated or not by 1 μmol/l ANP for 15 min. The reaction was stopped by addition of chloroform-methanol-1 N HCl (2 vol/1 vol/0.1 vol). After centrifugation (5,000 rpm for 5 min), the aqueous phase of each sample was freeze-dried and the cyclic nucleotide content was measured according to the kit manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI).

Real-Time Quantitative PCR Assay

Changes in mRNA levels of specific genes were quantified by real-time PCR. Total rat or human RNAs were extracted using the Qiagen RNeasy kit. Briefly, isolated mature adipocytes (2 or 5 ml of packed cells from rats or humans, respectively) were disrupted in the lysis buffer (vol/vol) furnished with the kit and stored at ~80°C. After thawing, the aqueous phase was delipidated by chloroform (vol/vol) and the mRNAs were extracted according to the manufacturer’s instructions. RNA concentrations were determined using a fluorimetric assay (Ribogreen). RNA (2 μg) was reverse-transcribed using the ThermoScript RT system (Life Technologies) according to the manufacturer’s instructions (random hexamers and dNTPs were also supplied by Life Technologies). Reverse transcription was also performed without ThermoScript enzyme on RNA samples to provide a control for contamination of samples with genomic DNA. PCR primers were designed using Primer Express software according to the recommendations of Applied Biosystems. Optimum primer concentrations were determined by performing PCR reactions with a range of primer concentrations and comparing the rates of product accumulation for human GC-A (forward: TGGAACCGAAGCTTTCAGAGG and reverse: CCATATCCCAGAGGAGAACTGCT), human NPR-C (forward: GGAAGACATCGTGCGGAATA and reverse: TGGCTCCGATGTTGTGACT), rat GC-A (forward: TCCTTCTCTGCGCCCTACATTCG and reverse: AACCTTAACCTCTTTCTGCTTTTCCTACA), and rat NPR-C (forward: GGAAGTCATTGTTGGATCTTGG and reverse: AGAGCCCAAGGATATTGGACA).

The amplification reaction was performed in duplicate on 20 ng of the cDNA sample (5 μl) in a final volume of 26 μl in 96-well optical reaction plates (Applied Biosystems) in a GeneAmp 5,700-sequence detection system. For GC-A and NPR-C (human and rat), the PCR mixture contained 8 μl of 900 nmol/l forward and reverse primer mix and 13 μl of SYBR Green PCR Master Mix (Applied Biosystems), which contains the fluorescent dye SYBR Green. The dye exhibits fluorescence enhancement upon binding to double-stranded DNA, and the enhancement of fluorescence is proportional to the initial concentration of the cDNA. For the ribosomal RNA control (18S rRNA), the PCR mixture contained 8 μl of primers and fluorigenic probe mix (Applied Biosystems) and 13 μl of TaqMan Universal PCR Master Mix (Applied Biosystems). All reactions were performed under the same conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Results were analyzed with GeneAmp 5700 software and all values were normalized to levels of the 18S rRNA control.

Radioligand Binding Assay

Isolated adipocytes were broken in a hypotonic lysing medium (5 mmol/l Tris-HCl, pH 7.4, 5 mmol/l EDTA) that contained several protease inhibitors (100 μmol/l phenylmethylsulfonyl fluoride, 0.5 mg/ml bacitracin, 1 μmol/l aprotinin, 10 μmol/l thiorphan). Crude adipocyte membranes were obtained by centrifugation of the lysate (48,000 g for 20 min at 4°C). The pellet was washed twice with 10 ml of binding buffer (50 mmol/l Tris-HCl, pH 7.4, 5 mmol/l MgCl₂,
0.1% bovine serum albumin, and 0.5 mg/ml bacitracin, 1 μmol/l apronitin, 10 μmol/l thiorphan) and finally resuspended in the same buffer at a final concentration of 1–2 mg protein/ml and immediately used for binding experiments. Assays were performed in a final volume of 200 μl containing 50 μl of membrane suspension and 50 μl of [125I]-ANP. Non-specific binding was defined in the presence of 1 μmol/l of unlabeled ANP. Saturation studies were performed with increasing concentrations (from 50 to 600 nmol/l) of [125I]-ANP. In competition studies, 250 nmol/l of [125I]-ANP and the specified concentrations of competitive analogs were used. Saturation experiments and competition studies were carried out under constant shaking for 45 min at 25°C. The incubation was stopped by centrifugation (13,000 for 10 min). The pellet was washed twice with 500 μl of binding buffer and the radioactivity was counted in a gamma counter.

Guanylyl Cyclase Assay

The guanylyl cyclase assay was performed according to the Domino method with some slight changes (18). Briefly, isolated adipocytes were broken up in a hypotonic lysing medium (5 mmol/l Tris-HCl, 5 mmol/l EDTA, 250 mmol/l sucrose, pH 7.4). Crude adipocyte membranes were obtained by centrifugation of the lysate (48,000 for 20 min at 4°C). The pellet was resuspended with 2 ml of washing buffer (20 mmol/l Tris, 3 mmol/l MgCl2 containing an antiprotease cocktail) and pelleted by centrifugation (20,000 for 20 min at 4°C). The pellet was resuspended with 1.5 ml of a buffer consisting of 20 mmol/l Tris-HCl, 5 mmol/l MgCl2, 100 mmol/l NaCl, 0.1% Triton X-100, 0.5% bovine serum albumin, 0.1 mmol/l Na3VO4, 18 mmol/l creatine phosphate, 0.25 mg/ml creatine kinase, 1 mmol/l ATP, and 1 mmol/l cGMP. Assays were performed in a final volume of 100 μl, and the reaction was started by addition of 1 mmol/l of [α-32P]GTP (100,000–500,000 cpm assay). Guanylyl cyclase assays were performed at 37°C, and the homogenate membrane preparation was incubated in the presence of 1 μmol/l ANP or 10 μmol/l isoproterenol. At increasing times (0, 10, 15, 30 min), the reaction was stopped by addition of 500 μl of 120 mmol/l zinc acetate and 600 μl of 144 mmol/l sodium carbonate. Tubes were frozen for 30 min at −80°C and then thawed and centrifuged for 10 min at 2,000 g and 4°C. Samples were poured over neutral alumina columns and [32P]cGMP was eluted with 2 ml of 100 mmol/l Tris-HCl, pH 7.5. Radioactivity was counted in a gamma counter.

Data Analysis

Values are given as means ± SE of n separate experiments. Student’s paired t-tests were used for comparisons between matched pairs. Differences were considered significant when P < 0.05. The concentration-response curves were fitted by nonlinear regression, the EC50 (half-maximal effect) was counted in a gamma counter. Prism software (San Diego, CA). Experiments were calculated and analyzed using GraphPad Prism software (San Diego, CA).

Drugs and Chemicals

The nonselective β-AR agonist (−)-isoproterenol hydrochloride, the specific A1-adenosine receptor agonist phenylisopropyladenosine (R-PIA), the α2-AR agonist UK-14304, the nonselective PDE inhibitor IBMX, bovine serum albumin (fraction V), forskolin, bacitracin, apronitin, thiorphan, and neutral alumina were from Sigma-Aldrich (Saint Quentin Fallavier, France). Crude collagenase, enzymes for glycerol assays, and Complete Mini tablets of protease inhibitors were from Boehringer Mannheim (Mannheim, Germany). Human α-ANP (1–28), rat α-ANP (1–28), and CNP were from Neosystem Laboratories (Strasbourg, France). Human BNP (1–32) came from Novabiochem (France Biochem, Meudon). Bromo-cGMP (Br-cGMP) was from Alexis Biochemicals (Ceger, Paris, France). Human [3-125I]iodotyrosyl-28-α-ANP was from Amersham France (Les Ulis). [8-3H]guanosine-3’,5’-cyclic phosphate ammonium salt came from Amersham (Orsay, France). [α-32P]-guanosine-5’-triphosphate and [γ-32P]adenosine-5’-triphosphate were from NEN (Paris, France). LY-83,583 came from Alexis Biochemicals (Cger). Ribogreen was from Molecular Probes (Leiden, The Netherlands). SYBR Green chemistry or TaqMan PCR detection were from Applied Biosystems (Courtaboeuf, France).

RESULTS

Compared Lipolytic Activity of Isoproterenol and ANP in Human, Nonhuman Primate, Rodent, and Nonrodent Mammal Fat Cells

Fat cells from rats, mice, hamsters, rabbits, dogs, macaques, and humans were incubated with increasing concentrations of isoproterenol (from 10−10 to 10−5 mol/l) and ANP (from 10−11 to 10−6 mol/l). Spontaneous glycerol release (basal lipolysis) was similar from one species to another (0.31 ± 0.03 μmol glycerol·100 mg lipid·1·90 min−1). As depicted in Fig. 1A, isoproterenol was a strong activator of lipolysis in all of the species studied. However, under our experimental conditions, the analysis of the maximum lipolytic effect and apparent affinity (pD2) values revealed species-specific differences that appeared to reflect species differences in the affinity of the β-AR agonist (Table 1). The highest isoproterenol efficacy was found in mouse adipocytes (5.49 ± 0.5 μmol glycerol·100 mg lipid·1·90 min−1) and the best potency was observed in human fat cells (pD2 = 7.70 ± 0.07). The effects of ANP on adipocyte lipolysis were compared with those of isoproterenol taken as a reference. As shown in Fig. 1B, ANP only induced a lipolytic effect in primate fat cells and was devoid of any lipolytic activity in rat, mouse, hamster, rabbit, or dog isolated adipocytes. Furthermore, female fat cells from macaques (n = 2) and rats (n = 2) were exposed to increasing concentrations of ANP. As for human adipocytes (19, 49), the responses of male and female macaque fat cells to ANP stimulation were comparable, and rat female fat cells were insensitive to increasing concentrations of ANP. Adipocytes from young rats 22–26 wk old (51–75 g) were stimulated with increasing concentrations of ANP, and no ANP-induced lipolysis was observed. Finally, NPs are well known to be degraded by neutral endopeptidase; however, addition of 10 μmol/l thiorphan, a neutral endopeptidase inhibitor, did not reveal a NP lipolytic activity in the nonprimate species.

Characterization of NP Lipolytic Response in Human Primate Fat Cells

To compare the NP-induced lipolysis between humans and primates, the lipolytic response initiated by NP and the rank order of potency were characterized in macaque fat cells. Isolated macaque adipocytes were
Fig. 1. Comparison of lipolytic effects of isoproterenol (A) and atrial natriuretic peptide (ANP; B) on isolated adipocytes from human (○), macaque (+), rat (●), dog (□), hamster (▲), and rabbit (×). Lipolysis is expressed in percent of maximum isoproterenol effect. Values are means of 16 experiments for humans; 6 experiments for macaques, rats, mice, hamsters, and dogs; and 4 experiments for rabbits. SE values (<5%) are not shown for clarity. [Isoproterenol], concentration of isoproterenol; [ANP], concentration of ANP.

Table 1. Maximal lipolytic effect of isoproterenol in isolated fat cells from human, macaque, dog, rat, mouse, hamster, and rabbit

<table>
<thead>
<tr>
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<th>µmol Glycerol/100 mg Lipid·1·90 min⁻¹</th>
<th>pD₂</th>
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<tbody>
<tr>
<td>n</td>
<td>Basal lipolysis</td>
<td>Isoproterenol (10 µmol/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>16</td>
<td>1.48 ± 0.20</td>
</tr>
<tr>
<td>Macaque</td>
<td>6</td>
<td>1.43 ± 0.40</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
<td>3.38 ± 0.48</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4</td>
<td>1.59 ± 0.10</td>
</tr>
<tr>
<td>Hamster</td>
<td>6</td>
<td>1.42 ± 0.18</td>
</tr>
<tr>
<td>Rat</td>
<td>6</td>
<td>2.57 ± 0.30</td>
</tr>
<tr>
<td>Mouse</td>
<td>6</td>
<td>5.49 ± 0.50</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 experiments; *P < 0.05 compared to values obtained for human isoproterenol pD₂. pD₂, −log of concentration of agonist that induces half-maximal effect.

adipocytes, the following relative rank order of lipolytic potency of NPs can be proposed for macaque fat cells: ANP > BNP > CNP.

cGMP is classically considered to be the second messenger generated after NPR-A or NPR-B (GC-A or GC-B) activation. In macaque fat cells, Br-cGMP (a membrane-permeable cGMP analog) increased lipolysis. Lipolysis induced by 10 µmol/l Br-cGMP was similar between macaque and human fat cells (Table 2).

The activity of type 3B phosphodiesterase (PDE-3B), the main enzyme involved in cAMP degradation in adipocytes, is known to be inhibited by cGMP in acellular systems (15); therefore, we studied the role of PDE-3B in NP-induced lipolysis in macaque fat cells as in our previous work on human adipocytes (49). Isolated macaque fat cells were preincubated with 1 µmol/l UK-14304 and 1 µmol/l PIA. In that context of low cAMP levels obtained by potent inhibition of adenylyl cyclase activity, PDE-3B activity was expected to be strongly decreased due to a poor substrate availability. In isolated macaque adipocytes, isoproterenol-stimulated lipolysis (0.1 µmol/l) was completely suppressed by the “inhibitory cocktail,” which confirmed the low cAMP content, whereas ANP-induced lipolysis (10 nmol/l) was not modified (data not shown).

Table 2. Effect of Br-cGMP in human, macaque, and rat fat cells

<table>
<thead>
<tr>
<th>Fat Cells</th>
<th>Human</th>
<th>Macaque</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal lipolysis</td>
<td>0.42 ± 0.02</td>
<td>0.60 ± 0.15</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>Br-cGMP (10 µmol/l)</td>
<td>1.28 ± 0.03*</td>
<td>1.80 ± 0.26*</td>
<td>0.94 ± 0.02*</td>
</tr>
<tr>
<td>Fold increase</td>
<td>3.07 ± 0.02</td>
<td>2.94 ± 0.36</td>
<td>1.99 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 experiments (expressed in µmol glycerol/100 mg lipid·1·90 min⁻¹); *P < 0.01, significantly different from respective basal lipolysis values; †P < 0.01, significantly different from basal lipolysis stimulation (fold increase) in human fat cells.
Comparative Analysis Between ANP-Responsive Human Adipocytes Versus ANP-Nonresponsive Rat Adipocytes

Effect of Br-cGMP. To determine whether cGMP was lipolytic in rat adipocytes, rat fat cells were exposed to 10 mmol/l Br-cGMP. As can be seen in Table 2, in rat adipocytes, 10 mmol/l Br-cGMP stimulated basal lipolysis by approximately twofold. Compared with human fat cells, Br-cGMP-induced lipolysis was significantly lower in rat than macaque or human adipocytes (P < 0.001).

Pharmacological characterization of NPRs. Radioligand binding studies using 125I-ANP as the ligand were performed to quantify NPRs in rat and human membrane adipocytes. Nonspecific binding, defined in the presence of 1 μmol/l cold ANP [rat ANP (r-ANP) or human ANP (h-ANP)], represented ~10% of the total bound radioactivity at the equilibrium. Specific binding of 125I-ANP was saturable and of high affinity in both species (Fig. 2A). Equilibrium dissociation constants (Kd), 147.7 ± 64 and 138.6 ± 38.4 pmol/l for human and rat membranes, respectively, were similar between the two species (Fig. 2B). The ligand used for binding assays did not allow delineation of NPR subtypes. The membrane receptor densities (Bmax values) were 274 ± 135 and 510 ± 123 fmol/mg protein for human and rat adipocyte membranes, respectively. The number of 125I-ANP binding sites was significantly lower in human than rat fat cell membranes (P = 0.02).

To determine more precisely the NPR subtypes expressed in both species, displacement of 125I-ANP binding was performed on human and rat fat cells using increasing concentrations of ANP, CNP (GC-B and NPR-C agonist), and c-ANP4–23 (a specific NPR-C agonist). For human adipocytes, 100% of bound 125I-ANP was displaced by the unlabeled ANP with an IC50 value of 0.46 ± 0.09 nmol/l (Fig. 3A). For c-ANP4–23 and CNP, there was an incomplete displacement of bound 125I-ANP (41.5 ± 7.7% and 25.4 ± 9.4% of bound 125I-ANP, respectively), which indicates the presence of a low NPR-C expression in human adipocytes. On the other hand, in rat fat cells, displacement-curve profiles were dramatically different: both c-ANP4–23
and CNP were as potent as unlabeled ANP to displace 100% of bound [125I]-ANP (Fig. 3B). Taken together, these data suggest an opposite NPR pattern of expression between human and rat fat cells.

**NPR gene expression.** To quantify GC-A and NPR-C mRNA expression in human and rat fat cells, real-time quantitative PCR assay was used. The mRNA GC-A/ NPR-C ratios were different from one species to another and were 0.017 ± 0.003 in rat fat cells and 1.21 ± 0.31 in human adipocytes, which confirms the opposite NPR subtype expression pattern in these two species revealed by binding assays. GC-A predominates in human fat cells.

**Guanylyl cyclase activity.** To assess the functionality of ANP receptors identified in rat and human fat cell membranes, guanylyl cyclase activities stimulated by ANP were studied. Guanylyl cyclase activity was measured after 5-, 10-, 15-, and 30-min stimulation with 1 μmol/l ANP or 10 μmol/l isoproterenol. ANP at 10⁻⁶ mol/l rapidly increased membrane guanylyl cyclase activity, and stimulation was maximized after 10 min (increase of 12.8 ± 3.7) in human fat cells and after 15 min (increase of 3.6 ± 0.8) in rat adipocytes (Fig. 4A). On the other hand, 10 μmol/l isoproterenol did not stimulate guanylyl cyclase activity in either species (data not shown). Moreover, 10 μmol/l LY-83,583, a guanylyl cyclase blocker, was used to inhibit ANP guanylyl cyclase activity stimulation. In human adipocytes, at 10 min the stimulation was shifted from an increase of 12.8 ± 3.7 to an increase of 4.4 ± 0.1 in the presence of 10 μmol/l LY-83,583. In rat fat cells, at 15 min the stimulation was increased 3.6 ± 0.8 versus 2.7 ± 0.6 in the absence or presence of 10 μmol/l LY-83,583, respectively (Fig. 4A).

**cGMP levels under ANP stimulation.** Finally, we compared cGMP formation under ANP stimulation in human and rat fat cells in the presence of IBMX (0.1 mmol/l). Basal intracellular cGMP levels were 1.2 ± 0.1 pmol·100 mg lipid⁻¹·15 min⁻¹ in human adipocytes and 9.75 ± 0.5 pmol·100 mg lipid⁻¹·15 min⁻¹ in rat fat cells. Figure 4B shows cGMP production induced by 1 μmol/l of r-ANP for rat adipocytes and h-ANP for human adipocytes. h-ANP and r-ANP increased by 258- and 2.8-fold basal cGMP production in human and in rat fat cells, respectively. cAMP production was not modified under ANP stimulation either in human fat cells or in rat adipocytes (data not shown).

**DISCUSSION**

The adrenergic system, which is considered to be the main system that controls human fat cell lipolysis, has attracted much interest for the metabolic and pharmacological properties of the lipolytic processes. Studies performed on white adipose tissues from various species have shown large interspecies differences in the control of lipolysis by catecholamines (7, 11, 27, 30) and peptide hormones (41, 55). We have recently demonstrated that peptide hormones such as the NP family activate lipolysis in human fat cells to a similar extent as catecholamines (49). These observations led us to investigate and compare the NP control of lipolysis in fat cells from humans, nonhuman primates (macaques), rodents (rats, mice, hamsters), and nonrodent mammals (dogs, rabbits). In this study, we demonstrate that NP-induced lipolysis is a primate adipocyte
specificity, because NPs were devoid of any lipolytic effect in other species even though NPRs were present. The lipolytic responsiveness of subcutaneous (human, macaque, dog) and internal (rat, mouse, hamster, rabbit) fat cells was evaluated by measuring isoproterenol-induced lipolysis. The lipolytic efficacy and potency of isoproterenol on fat cells differed from one species to another. The highest efficacy of isoproterenol was found in mouse fat cells and the highest potency was found in human adipocytes. These observations are in agreement with previous studies (8, 12). Lipolytic responsiveness to NPs was then examined and compared. ANP did not modify lipolysis in adipocytes from rats, mice, hamsters, dogs, or rabbits, whereas it did activate lipolysis in adipocytes from humans and macaques. Gender (21, 32, 40), age (24, 34, 38), or fat-depot localization (abdominal vs. subcutaneous; 4, 5, 32, 39, 56) are known parameters that could influence fat cell responsiveness and metabolism. Thus the effect of gender on NP response was studied. As in human adipocytes (19, 49), the responses of male and female macaque fat cells to NP stimulation were comparable, whereas female rat fat cells were insensitive to increasing concentrations of ANP. The influence of age was tested on fat cells from young rats. Nevertheless, as in adults, no NP-induced lipolysis was observed. Moreover, our previous study performed on young healthy men (19) showed that the NP-induced lipolysis was not different from the data obtained in the present study with middle-aged women. Fat-depot localization has also been evaluated. Personal unpublished data from studies performed on omental and subcutaneous adipose tissue from the same subjects showed that ANP-induced lipolysis was not statistically different. Finally, rodent fat cell lipolysis performed on inguinal versus epididymal adipose tissue did not reveal any ANP-induced lipolysis in this species. Taken together, these data showed that gender, age, or fat cell depot localization are not important factors that could explain the difference observed between primate and rodent species in the NP control of lipolysis.

To define the NP response in primate fat cells, the effects of other NPs (BNP, CNP) were studied in macaque adipocytes. BNP exerted lipolytic effects, and its efficacy was similar to that of ANP. Compared with our previous work (49), ANP and BNP efficacy was found to be twofold higher in macaque than in human adipocytes. CNP also activated lipolysis in macaque adipocytes and represented 47.5% of the maximal isoproterenol lipolytic effect. Two major biochemically and functionally distinct classes of NPRs are known: guanylyl cyclase (GC-A and GC-B) and clearance (NPR-C) receptors. GC-A and GC-B structures are very close; the major difference is the extracellular binding domain where the identity of the amino acid sequence is only 40%. This could explain the specificity for ligand binding between these two receptor subtypes. GC-A binds ANP and BNP with high affinity, whereas GC-B only binds CNP with high affinity (35, 43, 48). In macaque fat cells, the rank order of potency showed that ANP had a higher potency than BNP, which reflects the involvement of a GC-A receptor. Because we have previously shown (49) that CNP had a very weak lipolytic effect in human fat cells compared with macaques, it can be proposed that the GC-B receptor could be expressed on macaque fat cells because of the higher CNP-induced lipolysis.

Lipolysis in adipocytes is thought to be regulated only by hormones that modulate adenyl cyclase activity, cAMP contents, and PKA activity, which results in phosphorylation and activation of HSL. PDE-3B, the main enzyme involved in the degradation of cAMP in the adipocyte, is known to be inhibited by cGMP in acellular assays (14, 15, 36, 37). In a previous work (49), we showed that ANP-induced lipolysis in human fat cells was a cGMP-dependent pathway that does not involve PDE-3B inhibition. In this study, we compared isoproterenol- and ANP-induced lipolysis in the presence of a cocktail of agonists for $\alpha_2$-AR and $\alpha_1$-adreno-sine receptors. Activation of these two potent antilipolytic pathways leads to inhibition of adenyl cyclase activity and reduction of cAMP formation and consequently reduces PDE-3B substrate availability. In that context, ANP lipolytic activity was preserved in macaque adipocytes, whereas the isoproterenol-induced lipolysis was strongly blunted. Thus these data demonstrate that in primate (human and nonhuman) fat cells, the NP-induced lipolysis pathway is a cGMP-dependent pathway that does not induce PDE-3B inhibition.

To analyze the origin of the lack of lipolytic effect of NPs in rodent fat cells, we studied NPR expression and functionality. Because NPRs have already been described in rat adipocytes (20, 22), this species was chosen as a model. The binding of $^{125}$I-ANP to isolated adipocyte membranes from rats and humans demonstrated the presence of saturable, high-affinity binding sites. The $K_d$ values for human and rat adipocytes were similar to values reported for other tissues (48). Saturation experiments cannot give any information concerning NPR subtypes, but global $^{125}$I-ANP binding sites reflected by $B_{max}$ values were twofold higher in rat than human adipocytes. Previous studies have demonstrated that neither rat nor human adipocytes expressed GC-B (45, 54). This was confirmed by our previous study where CNP (GC-B and NPR-C agonist) had a very weak lipolytic effect on human fat cells (49) and was devoid of any lipolytic activity on rat adipocytes (personal data). To further characterize the subtype expression of NPRs in human and rat fat cells, on one hand, competition studies were performed with increasing concentrations of ANP, c-ANP$^{1–23}$, and CNP. Displacement-curve profiles were different from one species to another, which suggests a different pattern of NPR-subtype expression between human and rat adipocytes. In human fat cells, c-ANP$^{1–23}$ and CNP poorly displaced radiolabeled ANP thus revealing low NPR-C expression. Moreover, the similarity between c-ANP$^{1–23}$ and the CNP displacement-curve profile confirms the absence of the GC-B subtype in human adipocytes. On the contrary, in rat fat cells, c-ANP$^{1–23}$...
and CNP had equivalent potencies to displace radiolabeled ANP, which shows a predominance of NPR-C and a probably limited expression of GC-B receptors. On the other hand, the ratios of GC-A/NPR-C mRNA levels were compared between human and rat adipocytes using real-time quantitative PCR. Opposite patterns of expression for “biologically active” and “clearance” receptors in human adipocytes were equivalent, rat fat cells exhibited prevalence for the clearance receptor, which confirms competitive binding assays. These results are in accordance with previous studies on renal tissues (23) where variability in NPRs and a species-related variation of the relative density of clearance and biological receptors were observed. Our data also supported results obtained by Northern blot analysis, which shows that NPR-C mRNA levels were lower than GC-A levels in human fat pads (46), whereas the opposite was found in rat adipocytes (45, 54). Membrane guanylyl cyclase activities were measured in rat and in human adipocytes. The kinetics of activation were different in the two species with guanylyl cyclase activity being faster and more strongly stimulated in human than rat (10 min for human vs. 15 min for rat) fat cells. Finally, this was confirmed by cGMP production measurement under ANP stimulation between human and rat adipocytes. cGMP formation was stimulated in the two species but not to the same extent. In human adipocytes, ANP increased basal cGMP production ~300-fold, whereas it was only stimulated by threefold in rat adipocytes. Moreover, it has to be noticed that in rat adipocytes, cGMP determination was only possible in the presence of the PDE inhibitor IBMX, whereas it was not necessary in human fat cells (personal data). This observation could suggest that a higher total PDE activity exists in rat than human adipocytes.

In summary, our results clearly demonstrate that NP-induced lipolysis is a species-specific effect that only concerns primate fat cells. In this species, NP-induced lipolysis is mediated via the activation of a type A guanylyl cyclase receptor that increases intracellular cGMP content and is independent of the cAMP pathway. The lack of ANP-induced lipolysis that is observed in other species is explained by the opposite pattern of NPR distribution that favors clearance receptor expression. Knowledge of the signaling components of the NP system opens new insights into interesting and original mechanisms for the control of primate fat cell lipolysis. Nevertheless, the physiological or pathophysiological relevance of this pathway should now be delineated.

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