Involvement of a cGMP-dependent Pathway in the Natriuretic Peptide-mediated Hormone-sensitive Lipase Phosphorylation in Human Adipocytes*

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Our previous studies have demonstrated that natriuretic peptides (NPs), peptide hormones with natriuretic, diuretic, and vasodilating properties, exert a potent control on the lipolysis in human adipocytes via the activation of the type A guanylyl cyclase receptor (1, 2). In the current study we investigated the intracellular mechanisms involved in the NP-stimulated lipolytic effect in human preadipocytes and adipocytes. We demonstrate that the atrial NP (ANP)-induced lipolysis in human adipocytes was associated with an enhanced serine phosphorylation of the hormone-sensitive lipase (HSL). Both ANP-mediated lipolysis and HSL phosphorylation were inhibited in the presence of increasing concentrations of the guanylyl cyclase inhibitor LY-83583. ANP did not modulate the activity of the cAMP-dependent protein kinase (PKA). Moreover, H-89, a PKA inhibitor, did not affect the ANP-induced lipolysis. On primary cultures of human preadipocytes, the ANP-mediated lipolytic effect was dependent on the differentiation process. On differentiated human preadipocytes, ANP-mediated lipolysis, associated with an increased phosphorylation of HSL and of perilipin A, was strongly decreased by treatment with the inhibitor of the cGMP-dependent protein kinase I (cGKI), Rp-8-pCPT-cGMPS. Thus, ANP-induced lipolysis in human adipocytes is a cGMP-dependent pathway that induces the phosphorylation of HSL and perilipin A via the activation of cGKI. The present study shows that lipolysis in human adipocytes can be controlled by an independent cGKI-mediated signaling as well as by the classical cAMP/PKA pathway.

Natriuretic peptides (NPs) are a family of polypeptide hormones (atrial NP (ANP), brain NP, and C-type NP) that regulate blood pressure, natriuresis, diuresis, together with renin and aldosterone release by direct effects on the kidney, the adrenal glands, and the systemic vasculature (3–5). NPs signaling operates through two groups of cell surface receptors: the guanylyl cyclase (GC) receptors named GC-A and GC-B (6, 7) and the clearance receptor or type C NP receptor (NPr-C) without intrinsic GC activity (8–10). The cGMP produced after the activation of GC-coupled receptors has multiple effectors within the cell, including the families of cGMP-dependent protein kinases (cGKI), cGMP-stimulated/cGMP-inhibited phosphodiesterases (PDEs), cGMP-gated cation channels, and, in some cases, cAMP-dependent protein kinase A (PKA) (11, 12).

In addition to their renal, adrenal, and vascular effects, NPs may affect the metabolism of human adipocytes. In previous studies, we have demonstrated that ANP and brain NP, but not C-type NP, are potent stimulators of human fat cell lipolysis (1, 2). Clinical studies have validated their action in vivo (13, 14). However, the intracellular pathway involved in the NP-stimulated lipolytic effect still remained to be characterized. Former studies on human adipocytes have shown the essential role of cAMP in the modulation of the lipolytic pathway. Indeed, the main lipolytic regulators in human fat cells, i.e. catecholamines and insulin, are known to exert their effects through the regulation of intracellular cAMP content. On one hand, catecholamines induce their lipolytic effect through their interaction with β-adrenergic receptors. The consecutive increase in intracellular cAMP concentration leads to the activation of PKA which phosphorolyses the hormone-sensitive lipase (HSL). Activated HSL hydrolyzes the triglycerides into nonesterified fatty acids and glycerol (15, 16). On the other hand, the inhibition of lipolysis by insulin involves the activation of the main PDE of the adipocyte, the PDE-3B, which promotes a reduction in the intracellular levels of cAMP (17).

Our previous studies have shown that the PDE-3B activity as well as levels of intracellular cAMP are not modified by ANP in human adipocytes and that the presence of a GC-A receptor is required to trigger the lipolytic effect of NPs (1, 2). For the first time, we demonstrate that the lipolytic pathway activated by NPs involves a separate pathway in human fat cells (i.e. an increase in intracellular cGMP content followed by the phosphorylation of HSL and perilipin mediated by the activation of cGKI). Moreover, ontogenesis of this new pathway is described during human preadipocyte differentiation.

EXPERIMENTAL PROCEDURES

Subjects—Human subcutaneous adipose tissue (1–2 g) was obtained from 16 normal or moderately overweight women undergoing plastic...
surgery. Their mean age was 48.4 ± 3.8 years, and their mean body mass index was 26.3 ± 0.9 kg/m². The study was approved by the ethical committees of Toulouse and Frankfurt am Main University Hospitals.

**Human Mature Adipocyte Preparation**—Isolated adipocytes were obtained according to the method of Rodbell (18) by collagenase digestion of adipose fragments in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g/100 ml) and glucose (6 mmol/liter) at pH 7.4 and under gentle shaking at around 60 cycles/min at 37 °C. Then the fat cells were filtered through a silk screen and washed three times with Krebs-Ringer bicarbonate buffer to eliminate collagenase.

**Human Preadipocyte Preparation and Culture**—The isolation of human fat cell precursors was performed as described by Hauner et al. (19). All of the visible fibrous material and blood vessels were discarded from adipose tissue samples. The remaining adipose tissue was cut into small pieces and digested in phosphate-buffered saline containing 0.15% collagenase and 2% bovine serum albumin under gentle shaking at 120 cycles/min at 37 °C. After centrifugation, the stromal cell fraction was resuspended and incubated in an erythrolysis-lysing buffer (155 mmol/liter NH₄Cl, 5.7 mmol/liter K₂HPO₄, 0.1 mmol/liter EDTA) and then filtered through a nylon mesh (150 μm). After additional centrifugation, washing, and filtration steps, stromal cells were suspended in 0.15% collagenase and 2% bovine serum albumin under gentle shaking at around 60 cycles/min at 37 °C. Then the fat cells were filtered through a silk screen and washed three times with Krebs-Ringer bicarbonate buffer to eliminate collagenase.

**Real Time Quantitative PCR Assay**—RNA (2 μg) was reverse transcribed using the ThermoScript RT system (Invitrogen) according to the manufacturer’s instructions (Random Hexamers and dNTPs were also supplied by Invitrogen). 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. Optimized primer concentrations were determined by performing PCR with a range of primer concentrations and comparing the rate of product accumulation (Table II).

**Lipolysis Measurement in Human Preadipocytes or Mature Adipocytes**—Isolated mature adipocytes were incubated for 6 h in basal medium at 37 °C and, for the first 3 days, 1 g/ml gentamycin (basal medium) in the presence of 66 μmol/liter insulin, 1 mmol/liter triiodothyronine, 100 μmol/liter cortisol, and, for the first 3 days, 1 μg/ml cigitazone. The medium was changed every 2 days. Before the experiments, the cells were placed in basal medium for 24 h, washed with phosphate-buffered saline, and either lysed for Western blot analysis or treated for lipolysis experiments.

**PKA Activity Measurement**—Human mature adipocytes were incubated in both the absence and presence of ANP (1 μmol/liter) or forskolin (0.9 μmol/liter) for 5 min. The cells were lysed at 4 °C in lysis buffer containing 1% Nonidet P-40, 20 mmol/liter Tris-HCl (pH 7.5), 150 mmol/liter NaCl, 1 mmol/liter CaCl₂, 1 mmol/liter MgCl₂, 10% glycerol, 1 mmol/liter Na₂VO₄, and anti-protease mixture. The protein lysates were centrifuged (10 min, 10,000 g, at 4 °C) followed by 40 cycles of centrifugation (Table I).

**Immunoprecipitation**—Human mature fat cells or preadipocytes were lysed at 4 °C in lysis buffer. The lysates were centrifuged (10 min, 10,000 × g, at 4 °C), and the supernatant was analyzed by Western blot.
loading of the lanes. The membranes were blocked overnight at 4 °C with a blocking buffer (50 mmol/liter Tris-HCl pH 7.5), 200 mmol/liter NaCl, 0.05% polyethylene-sorbitan monolaurate (Tweeen), 3% bovine serum albumin, and 10% horse serum), incubated with a primary antibody for 90 min, rinsed, blocked for 40 min, and incubated with secondary antibody for 60 min. The immunocomplexes were visualized using the chemiluminescence reagent kit. The autoradiographs were scanned by an imaging densitometer and quantified using the National Institutes of Health Image program.

Results

HSL Phosphorylation after ANP Exposure on Human Adipocyte—Because the serine phosphorylation of HSL is the limiting step of HSL activation and lipolysis, we first studied the effects of treatments for increasing times (5, 10, and 30 min, respectively) with 10 nmol/liter ANP or 100 nmol/liter isoproterenol (β-adrenergic receptor agonist) on the serine phosphorylation rate of HSL in human mature adipocytes. The selected concentrations are known to promote stimulation of lipolysis to a similar extent. As shown in Fig. 1A, Western blot analysis using an antibody directed against human HSL, performed on immunoprecipitates obtained with an antibody directed against phosphoserine residues, evidenced one band, the molecular mass of which corresponded to HSL (88 kDa). ANP, as well as isoproterenol, induced a time-dependent phosphorylation of HSL on serine residues (Fig. 1A). The kinetics of HSL phosphorylation observed for the both lipolytic agents exhibited statistically significant differences. Although exposure to isoproterenol was associated with a transient phosphorylated state of HSL, ANP-induced HSL phosphorylation, which was maximal after 10 min of incubation (3-fold increase compared with control), remained elevated for the following 20 min (p < 0.05, n = 6) (Fig. 1B). Moreover, the same experiments performed on rat adipocytes, known as a nonresponsive species regarding ANP-mediated lipolysis (2), showed that ANP was unable to promote HSL phosphorylation (data not shown).

Role of cGMP in ANP-induced Lipolysis on Human Fat Cells—To further characterize the intracellular pathway involved in the ANP-mediated HSL phosphorylation, human fat cells were treated with increasing concentrations (1–100 μmol/liter) of the GC inhibitor, LY-83583. As shown in Fig. 2A, LY-83583 decreased in a concentration-dependent manner lipolysis stimulated by 10 nmol/liter ANP. Maximal inhibition was observed with 100 μmol/liter LY-83583 (from 1.48 ± 0.02 μmol of glycerol/100 mg of lipid for ANP alone to 0.07 ± 0.01 μmol of glycerol/100 mg of lipid for ANP plus LY-83583, p < 0.001, n = 5). Isoproterenol-induced lipolysis was not statistically modified by LY-83583 exposure whatever the concentration used (data not shown). 10 μmol/liter LY-83583, which was responsible for 71 ± 14% inhibition of
the ANP-mediated lipolysis, strongly inhibited the ANP-induced cGMP production (from 330 ± 60 pmol cGMP/100 mg lipid for ANP alone to 48 ± 12 pmol cGMP/100 mg lipid for ANP plus LY-83583 corresponding to 85 ± 4% inhibition, p < 0.001, n = 6) (Fig. 2A, inset). Finally, 10 μmol/liter LY83583, devoid of any effect on basal HSL phosphorylation, markedly inhibited the ANP-induced HSL phosphorylation as evidenced by the Western blot analysis using anti-human HSL antibody performed on immunoprecipitates obtained with anti-phosphoserine (Fig. 2B).

Role of PKA in the ANP-induced Lipolysis on Human Fat Cells—To rule out possible cross-talk between the cGMP- and the cAMP-dependent pathways, we measured the effect of ANP on PKA activity in human mature adipocytes. As depicted in Fig. 3A, ANP, even at high concentration (1 μmol/liter), did not affect the activity of PKA in human mature fat cells. However, 0.1 mmol/liter forskolin, a direct activator of adenylyl cyclase, led to a 7.5-fold increase of the PKA activity compared with untreated cells (p < 0.001, n = 6). In addition, pretreatments of the human mature adipocytes for 30 min with increasing concentrations (0.5–10 μmol/liter) of the PKA inhibitor H89 did not affect the lipolysis stimulated by 10 nmol/liter ANP, whereas it inhibited in a concentration-dependent manner the lipolysis induced by isoproterenol (from 0.95 ± 0.02 μmol glycerol/100 mg lipid (isoproterenol alone) to 0.74 ± 0.03 μmol glycerol/100 mg lipid (isoproterenol plus H-89), p < 0.001, n = 6).

Subtype of cGK Expressed in Human Adipocytes—The most likely intracellular target of cGMP is the cGK. Because the presence of cGK has never been reported in adipocytes, we first characterized the expression of cGK in mature human fat cells. As shown in Fig. 4, the PCR product of 469 bp corresponding to cGKI was found in human mature adipocytes as well as in mouse heart and kidney (positive control); however, the PCR product of 726 bp corresponding to cGKII was detected in
mouse heart and kidney but was not detected in human fat cells.

Ontogenesis of the ANP-induced Lipolysis System in Primary Cultures of Human Preadipocytes—To precisely determine the contribution of cGKI in the ANP-mediated transduction pathway, experiments were further performed in an adipocyte model containing less lipid content, i.e. primary cultures of human preadipocytes differentiated in adipocytes. The presence of a large lipid vacuole in mature adipocytes limits the use of lipophilic pharmacological tools. Because the ontogenesis of the NP system was never described, the expression of several components of the ANP transduction pathway, i.e. NP receptors, cGKI, and HSL, was studied during the adipocyte differentiation process (days 0, 5, 10, and 15). The amounts of NP receptor mRNAs, GC-A, GC-B, and NPr-C, were determined using real time RT-PCR analysis. As depicted in Fig. 5A, GC-A mRNA levels were increased during the adipocyte differentiation with a maximal expression observed after 5 days (3-fold increase in mRNA levels in day 5 compared with day 0). GC-B or NPr-C mRNA levels were not statistically modified.

The expression of cGKI, evidenced by Western blot analysis (Fig. 5B), decreased with differentiation (a decrease of 37 ± 7% in the amount of cGKI protein was observed in day 15 versus day 0 (p < 0.05, n = 5)); however, the protein level of HSL, assessed via Western blot analysis (Fig. 5C), progressively increased (15 ± 2-fold increase in HSL protein amount in day 15 versus day 0). Finally, lipolysis challenges were performed during the differentiation process with 10 nmol/liter ANP (Fig. 5D). After 5 days, ANP significantly increased the release of glycerol in the extracellular medium (3-fold increase compared with basal value). The extent of the lipolytic response induced by ANP increased during the differentiation process (9- and 5-fold increases compared with basal value after 10 and 15 days, respectively). In parallel we performed lipolysis challenges in the presence of 10 μmol/liter thiorphan, a neutral endopeptidase inhibitor because NPs are known to be degraded by neutral endopeptidase. In that context, no significant improvement of the NP-induced lipolysis was found (data not shown).

ANP Induces the Serine Phosphorylation of HSL Together with Perilipin A in Differentiated Human Preadipocytes—HSL and perilipin are described to be concomitantly phosphorylated in response to lipolytic stimulation in intact adipocytes. Then we examined the effect of 10 nmol/liter ANP on the phosphorylation of HSL and perilipin in 10-day differentiated human preadipocytes. 10-day differentiated human preadipocytes were chosen because of their low lipid content but high cGKI and HSL protein levels and maximal ANP-induced lipolysis.

As shown in Fig. 6A, Western blot analysis using the anti-human HSL antibody, performed on immunoprecipitates obtained with the anti-phosphoserine antibody on protein extracts from cells treated with ANP for increasing amounts of time (0, 10, and 30 min, respectively), showed that ANP induced a time-dependent phosphorylation of HSL on serine residues with a time course similar to that described in mature human adipocytes (n = 5). The ANP-mediated serine phosphorylation of HSL in human preadipocytes was confirmed by Western blot analysis using the anti-phosphoserine antibody performed on immunoprecipitates obtained with the antibody against the human HSL on protein extracts from cells treated for 30 min with ANP (Fig. 6B). In parallel, we show that a 10-min incubation period of human preadipocytes with 100 nmol/liter isoproterenol also led to increased serine phosphorylation of HSL.

Our preliminary experiments have shown that perilipin A is the main perilipin isoform expressed in differentiated human preadipocytes. To determine whether ANP could stimulate the phosphorylation of perilipin A, Western blot analysis was performed using an antibody against perilipin A on protein extracts from 10-day differentiated human preadipocytes, which were either treated or not treated for 30 min with 10 nmol/liter ANP or for 10 min with 100 nmol/liter isoproterenol. As depicted in Fig. 6C, ANP treatment induced a marked shift in the migration of perilipin A, similar to that observed under stimulation with isoproterenol (n = 6).

Involvement of cGK and Not of MAP Kinase in the Control of ANP-induced Lipolysis and HSL Phosphorylation—To examine the involvement of cGKI in the control of ANP-induced lipolysis and HSL phosphorylation, 10-day differentiated human preadipocytes were preincubated for 40-min periods with increasing concentrations (25 and 50 μmol/liter) of the cGK inhibitor Rp-8-pCPT-cGMPS and then stimulated with 10 nmol/liter ANP. As depicted in Fig. 7A, the pretreatment of the cells with Rp-8-pCPT-cGMPS led to a statistically significant decrease in the ANP-induced lipolysis (maximal decrease of 49 ± 11% of ANP-induced lipolysis in the presence of 50 μmol/liter Rp-8-pCPT-cGMPS compared with ANP alone). In parallel, Western blot analysis using the anti-human HSL antibody, performed on immunoprecipitates obtained with the anti-phosphoserine antibody, showed that Rp-8-pCPT-cGMPS pretreatment significantly diminished the ANP-induced HSL phosphorylation (Fig. 7B) (maximal decrease of 52 ± 5% of ANP-induced HSL phosphorylation in the presence of 50 μmol/liter Rp-8-pCPT-cGMPS compared with ANP alone).

To determine whether other serine/threonine kinases might be involved in the ANP-induced lipolysis, we examined the effect of ANP on the activation of the MAP kinases (ERK and p38). 10-day differentiated human preadipocytes were exposed to 10 nmol/liter ANP or to 100 nmol/liter isoproterenol for increasing periods of time (0, 2, 5, 10, and 30 min, respectively), and the activation of ERK and p38 MAP kinase were assessed by Western blot analysis performed using specific antibodies directed against the phosphorylated forms of ERK and p38. In parallel, Western blot analysis using anti-ERK or anti-p38 MAP kinase antibodies was performed on the same protein extracts. Irrespective of the time period of treatment, ANP or isoproterenol modified neither ERK nor p38 MAP kinase phosphorylation (n = 3, data not shown). Moreover, the preincubation of 10-day differentiated human preadipocytes for 90 min either SB203580 (0.2 and 2 μmol/liter), inhibitor of p38 MAP kinase or U0126 (5 μmol/liter) inhibitor of ERK, did not affect...
the HSL phosphorylation induced by 10 nmol/liter ANP for 30 min or 100 nmol/liter isoproterenol for 10 min, as assessed by Western blot analysis using anti-human HSL antibody performed on anti-phosphoserine immunoprecipitates ($n$ = 3, data not shown).

**DISCUSSION**

We have already shown that NPs, ANP and brain NP but not C-type NP, are strong activators of lipolysis in human fat cells (1, 2). The present study was undertaken to further elucidate the mechanisms underlying ANP-induced lipolysis in human adipocytes. We demonstrate that ANP-induced lipolysis is mediated by cGMP with the activation of cGKI, identified for the first time in human fat cells, which leads to the phosphorylation of HSL and perilipin A. Moreover, we describe ontogenesis of the ANP-dependent transduction pathway during the differentiation of human preadipocytes into adipocytes.

In humans, the hormonal control of lipolysis has always been related to insulin (inhibition of lipolysis) and catecholamines...
stimulation of lipolysis (21, 22). It is generally accepted that the modulation of intracellular cAMP levels (increased under β-adrenergic receptor stimulation or decreased by insulin) is the crucial point in the control of lipolysis because it determines the activation of PKA, which stimulates HSL activity by phosphorylation of HSL on serine residues. HSL catalyzes the first and rate-limiting step of hydrolysis of stored triglycerides and is thereby a key enzyme in the mobilization of free fatty acids from adipose tissue (23). The hallmark of HSL, which distinguishes this enzyme from all other known triacylglycerol lipases, is the control of its activity through phosphorylation (24). Thus, the ultimate rate of lipolysis in adipose tissue is dependent on the phosphorylation state of HSL (23). We demonstrated that the ANP-induced lipolysis is associated with an increase in the serine phosphorylation of HSL in human mature adipocytes but not in rat mature adipocytes, which is known to be a function of PKA (25). However, the inhibition of PKA by H-89 did not affect the ANP-induced lipolysis, but it did decrease the isoproterenol-stimulated lipolysis. Taken together, both results demonstrate that ANP-mediated lipolysis did not involve cross-talk between cGMP and PKA.

NPs are known to modulate some cGMP-gated channels like the L-type calcium channels, inducing a Ca\(^{2+}\) increase in cardiac muscles and vascular smooth muscle cells (26–28). Previous observations point to a putative positive regulatory role of calcium in adipocyte lipolytic cascade (29–31). However, recent reports have brought some contradictory results showing the association of the increase in intracellular Ca\(^{2+}\) concentrations with inhibition of cGMP/cGKI Involvement in NP-mediated HSL Phosphorylation

**Fig. 6.** ANP and isoproterenol-induced HSL phosphorylation in 10-day differentiated human preadipocytes. A, 10-day differentiated human preadipocytes were exposed to 10 nmol/liter ANP, and HSL phosphorylation profile was analyzed by immunoprecipitation using an antibody directed against phosphoserine followed by a Western blot analysis using an antibody directed against human HSL. A representative blot of five independent experiments for ANP is shown. B, 10-day differentiated human preadipocytes were exposed to 10 nmol/liter ANP or 100 nmol/liter isoproterenol (ISO). Western blot analysis using the anti-phosphoserine antibody were performed on immunoprecipitates obtained with the antibody against human HSL on protein extracts from cells treated for 30 min with ANP and 10 min with isoproterenol. A representative blot of three independent experiments is shown. The blots were subsequently stripped and probed with antibodies directed against human HSL to assess the level of HSL in each lane. A representative blot of three independent experiments is presented. C, 10-day differentiated human preadipocytes were exposed for 30 min to 10 nmol/liter ANP or for 10 min to 100 nmol/liter isoproterenol (ISO), and homogenates were immunoblotted with anti-perilipin. The similar characteristic upward shift observed with ANP and ISO reveals the phosphorylation of perilipin A in stimulated cells (six and two independent experiments for ANP and ISO, respectively). *IP*, immunoprecipitation; *IB*, immunoblot.

LY-83583 strongly inhibited the ANP-induced accumulation of cGMP, HSL phosphorylation, and lipolysis without a modification of isoproterenol-mediated lipolysis. This result clearly demonstrates that the signal transduction pathway stimulated by ANP to promote lipolysis in human adipocytes is strictly connected to an increase in intracellular cGMP concentrations. It is now recognized that there are four major classes of cGMP-regulated proteins: cGKs, cGMP-stimulated/cGMP-inhibited PDEs, cGMP-gated cation channels, and PKA (11, 12). Our previous study established that neither cAMP nor the PDE-3B (a cGMP-inhibited PDE), were involved in the NP-induced lipolysis. Because PKA could be activated by cGMP (25), we examined whether ANP could modify PKA activity in human adipocytes and found that ANP did not stimulate the activity of PKA. Moreover, the inhibition of PKA by H-89 did not affect the ANP-induced lipolysis, but it did decrease the isoproterenol-stimulated lipolysis. Taken together, both results demonstrate that ANP-mediated lipolysis did not involve crosstalk between cGMP and PKA. NPs are known to modulate some cGMP-gated channels like the L-type calcium channels, inducing a Ca\(^{2+}\) increase in cardiac muscles and vascular smooth muscle cells (26–28). Previous observations point to a putative positive regulatory role of calcium in adipocyte lipolytic cascade (29–31). However, recent reports have brought some contradictory results showing the association of the increase in intracellular Ca\(^{2+}\) concentrations with inhibition of cGMP/cGKI Involvement in NP-mediated HSL Phosphorylation
lipolysis (32, 33). Therefore, the involvement of Ca\textsuperscript{2+}/H\textsubscript{11001} in the NP-induced lipolysis may be ruled out. Finally, the potential involvement of a cGK, the most relevant target of cGMP, was considered. Two main forms of cGKs, encoded by distinct genes (34–36), have been identified and defined as type I and type II cGK. Type I is a cytosolic homodimeric protein, whereas the type II is a membrane-bound homodimeric protein. Smooth muscle cells, platelets, and cerebellum contain high concentrations of cGKI, whereas cGKII is highly concentrated in brain, lung, kidney, bone, and intestinal mucosa (12, 37). Although early observations pointed to the presence of a cGMP-binding protein at very low levels in rat adipose tissue (38), to our knowledge no information concerning the cGK isoform and its physiological role have ever been provided. The present study identified mRNAs of cGKI in human adipocyte but not of cGKII. To evaluate the contribution of cGKI in the NP-induced lipolysis, we used several specific cGK inhibitors such as KT5823, H\textsubscript{8}, or Rp-8-pCPT-cGMPS. Although these agents are known to inhibit the cGK-induced activation in many cell-free systems, we did not observe any effect on the NP-induced lipolysis in mature human adipocytes. The reason for the failure of such tools in adipose tissue may arise from the following problems: (i) difficulty of getting access to cGK in intact fat cells because of compartmentalization of the drug; (ii) requirement of longer incubation times (in comparison with broken cell systems) that are necessary to enable diffusion/transport of the drug through the cellular membrane, which is impossible for mature fat cells; and (iii) hydrophobicity of the compounds such that they are immediately trapped into the large lipid vacuole. This kind of problem has also been reported by groups working
on cGKs (12, 39). Please note that the quantitative analysis of cGK activation in intact cells is difficult because of the relative low expression of cGKs in most cell types, especially when compared with expression of other protein kinase such as PKA (12). Therefore, we shifted toward another adipocyte model and used the adipocytes that originate from primary culture of human preadipocytes and accumulate less lipid. Most preadipocyte cell lines currently available originate from mice and are not relevant for purposes of this study because we have demonstrated that the NP-induced lipolysis is unique to the primary fat cell (2). The ontogenesis of the different components involved in the transduction pathway of the NP-induced lipolysis was first investigated in the human preadipocytes during the differentiation process. As for mature adipocytes, human preadipocytes expressed the mRNAs of the three NPs receptor subtypes (GC-A, GC-B, and NP-C). However, we found only GC-A to be up-regulated during the differentiation process. The cGKI protein was found to be expressed in non-differentiated human preadipocytes, but its expression was slightly decreased with differentiation. However, prolonged cell culture is often associated with a down-regulation or a loss of cGK expression in many cell types (12). HSL expression was increased during the differentiation process, confirming its expression as a late marker of adipocyte differentiation (23). Finally, for the mature adipocyte, ANP was able to trigger a lipolytic effect that was markedly enhanced all along the differentiation process.

The ANP-induced lipolysis was associated with an increase in the serine phosphorylation of HSL assessed by Western blot analysis using either HSL or phosphoserine antibodies on immunoprecipitates obtained with phosphoserine or HSL antibodies, respectively. The hydrolysis of triglycerides is the result of an HSL activation by phosphorylation on serine residues followed by a translocation of HSL from the cytosol to the lipid droplets. The binding of phospho-HSL to the lipid droplet is only permitted when the perilipins (40), which are proteins that coat the surface of intracellular lipid droplets, are phosphorylated (41). Three perilipin isoforms (perilipins A, B, and C) have been described, and perilipin A is the most abundant isoform in adipose tissue (42). In differentiated human preadipocytes, we have previously described (40) and ANP induced a similar shift in perilipin A. Importantly, we observed that the lipolytic effect of ANPs is independent of the control by insulin (1). Moreover, the NP-induced lipolysis has a primate fat cell specificity, and it is not depot-, age-, or gender-dependent (2, 13, 14).

The present findings demonstrating a new control of HSL activity and lipolysis by natriuretic peptides, cGMP and especially cGKI, in human fat cells, raises a number of questions about the physiological role of this lipolytic pathway in comparison with the β-adrenergic control of human adipose tissue metabolism. A putative involvement in the development and in the pathogenesis of obesity cannot be excluded. Moreover, because elevated circulating NPs levels are found in several pathological states and are often associated to disease severity, this new pathway could play a decisive role in cachexia (48) and wasting diseases leading to adipose tissue regression.

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