The lipid-mobilizing effect of atrial natriuretic peptide is unrelated to sympathetic nervous system activation or obesity in young men

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Abstract **We recently demonstrated that natriuretic peptides and especially the atrial natriuretic peptide (ANP) are powerful lipolytic agents on isolated human fat cells. To search for a possible influence of obesity on ANP responsiveness, we compared the lipolytic effects of human ANP (h-ANP) on isolated subcutaneous abdominal adipose tissue (SCAAT) fat cells from young healthy lean and obese men. The lipid-mobilizing effects of an intravenous infusion of h-ANP was studied, as well as various metabolic and cardiovascular parameters that were compared in the same subjects. h-ANP (50 ng/min/kg) was infused iv for 60 min. Microdialysis probes were inserted in SCAAT to measure modifications of the extracellular glycerol concentrations during h-ANP infusion. Spectral analysis of blood pressure and heart rate oscillations that were recorded using digital photoplethysmography were used to assess changes in autonomic nervous system activity. h-ANP induced a marked and similar increase in glycerol and nonesterified fatty acids, and a weak increase in insulin plasma levels in lean and obese men. Plasma norepinephrine concentrations rose similarly during h-ANP infusion in lean and obese men. The effects of h-ANP infusion on the autonomic nervous system were similar in both groups, with an increase in the spectral energy of the low-frequency band of systolic blood pressure variability and a decrease in the spectral energy of the high-frequency band of heart rate. In SCAAT, h-ANP infusion increased extracellular glycerol concentration and decreased blood flow similarly in both groups. The increase in extracellular glycerol observed during h-ANP infusion was not modified when 0.1 mM propranolol was added to the microdialysis probe perfusate to prevent β-adrenoceptor activation. These data show that ANP is a potent lipolytic hormone independent of the activation of the sympathetic nervous system, and that obesity did not modify the lipid-mobilizing effect of ANP in young obese subjects.**—Galitzky, J., C. Sengenès, C. Thalamas, M. A. Marques, J-M. Senard, M. Lafontan, and M. Berlan. **The lipid-mobilizing effect of atrial natriuretic peptide is unrelated to sympathetic nervous system activation or obesity in young men.** ***J. Lipid Res.*** **2001.** 42: **536–544.**

Supplementary key words adipocyte • ANP • microdialysis • human fat cells

In 1981, de Bold et al. (1) showed that infusion of atrial tissue extracts in rats induced strong natriuresis. The first member of the natriuretic family was discovered: atrial natriuretic peptide (ANP). Subsequently, two other natriuretic peptides (NP) were isolated: brain natriuretic peptide (BNP) (2) and C-type natriuretic peptide (CNP) (3). Their biological activities are mediated by specific receptors bearing a guanylyl cyclase activity (NPr-A, NPr-B) or not (NPr-C). NPr-A and NPr-B are the active receptors, whereas NPr-C seems to be involved in clearance of the peptide. NP are involved in the regulation of blood pressure and blood volume. They inhibit renin, vasopressin, and aldosterone release. They markedly stimulate diuresis and natriuresis, and are potent vasodilators.

NPr have been identified in various tissues including rat fat cells (4–7). Furthermore, a study has shown that human adipose tissue expresses NPr mRNA (8). However, in spite of the presence of NPr in rodent fat cells, and ANP-induced cGMP production (4–7, 9), no biological responses have yet been reported for fat cells. We recently demonstrated that NP are a new pathway controlling human adipose tissue lipolysis (10). They operate via a cGMP-dependent pathway that does not involve phosphodiesterase-3B inhibition and adenosine 3′,5′-cyclic monophosphate (cAMP) production, and ANP was the most potent compound among the three NP (ANP, BNP, and CNP) (10).

The aim of the present work was to evaluate in vitro (on isolated fat cells) and in vivo (during iv h-ANP infusion) the lipolytic effect of h-ANP in young healthy lean and obese subjects. The local lipolytic effect of h-ANP on subcutaneous abdominal adipose tissue (SCAAT) was evaluated using

Abbreviations: SCAAT, subcutaneous abdominal adipose tissue; ANP, atrial natriuretic peptide; NEFA, nonesterified fatty acids.

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the microdialysis method. The cardiovascular effects, as well as the action of h-ANP on the sympathetic nervous system, were also compared in the two groups. It was shown that h-ANP is a powerful lipolytic agent in human isolated fat cells from lean and obese subjects; no difference was observed between the two groups. Administration of h-ANP (iv infusion) induced a potent lipid-mobilizing effect that was largely independent of sympathetic nervous system activation. Finally, the various h-ANP-induced metabolic effects [i.e., local increase of extracellular glycerol concentration in SCAAT, and increase in plasma glycerol, nonesterified fatty acids (NEFA), norepinephrine, and insulin levels] were similar in lean and obese subjects.

MATERIALS AND METHODS

Subjects

Seven healthy, young, lean men 22–32 years of age (mean ± SEM: 28.7 ± 1.9) with a body mass index (BMI) ranging from 20.2 to 24.9 (mean ± SEM: 22.2 ± 0.7) and seven healthy, young, obese men 22–34 years of age (mean ± SEM: 25.5 ± 1.9) with a BMI ranging from 29.2 to 36.3 (mean ± SEM: 31.5 ± 1.4) who had not been submitted to any pharmacological or nutritional protocol prior to the study were recruited. All had a stable weight during the previous 3 months. Selection of the subjects was based on a screening evaluation of detailed medical history, physical examination, complete blood count, urine analysis, resting electrocardiogram, blood pressure measurements, and several blood chemistry analyses. To avoid diabetic subjects, a glucose tolerance test was performed 1 week before the experimentation on overnight-fasting subjects with 75 g oral glucose. Measurement of plasma insulin and glucose concentration was performed 60, 90, and 120 min after glucose load. Plasma samples for glucose and insulin levels were taken before and 120 min after glucose ingestion. The study was approved by the Ethical Committee of Toulouse Hospital. All the subjects gave their informed consent for the experimental conditions after detailed explanation. The investigations were carried out in the Center of Clinical Investigation of Toulouse University Hospital.

Experimental protocol

Subjects entered in the Center of Clinical Investigation of the University Hospital at 8:00 AM and were maintained in the supine position during the experimental period. An indwelling polyethylene catheter was inserted into the antecubital vein of each arm. At 8:30 AM, a needle microbiopsy (100–200 mg) of adipose tissue was performed under local anesthesia 10–15 cm from the umbilicus. The h-ANP infusion was performed through the intraveinous catheter placed in the right arm, using an Auto-Syringe infusion pump (Becton Dickinson, France). A suitable rate of h-ANP administration was achieved by an appropriate infusion rate and concentration. Blood samples were withdrawn from a catheter placed in the left arm. Resting baseline measurements were performed during the first 40 min. Immediately after the 40-min baseline period, h-ANP, with isotonic saline as vehicle, was first administered as a bolus (25 μg) followed by an infusion at a constant rate of 50 ng/kg/min for 60 min. The total volume infused was <40 ml. Appropriate doses of h-ANP were selected on the basis of changes in cardiovascular parameters previously determined by other groups (11, 12). These doses have been shown to promote safe but significant physiological responses. During the baseline period and h-ANP infusion, the heart rate was continuously recorded with a standard three-lead electrocardiogram (Kontron Instruments, UK). Systolic and diastolic blood pressures were evaluated every 15 min with a Dynamap device (Laboratoire Baxter, France).

Microdialysis probes (Carnegie Medecin, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cutoff were inserted percutaneously after epidermal anesthesia (200 μl of 1% lidocaine, Roger-Bellon, Neuilly-s-Seine, France) into the abdominal SCAAT at a distance of 10 cm immediately to the right of the umbilicus. The probes were connected to a microinjection pump (Harvard apparatus; S.A.R.L., Les Ulis, France) and perfused with Ringer's solution (139 mM sodium, 2.7 mM potassium, 0.9 mM calcium, 140.5 mM chloride, 2.4 mM bicarbonate, and 5.6 mM glucose). In three subjects of each group, an additional probe was inserted in the abdominal SCAAT separated by at least 10 cm from the first one and perfused with Ringer's solution supplemented with 0.1 mM propranolol (β-adrenergic receptor antagonist). The perfusate solutions were supplemented with ethanol (1.7 g/l). Ethanol was added to the perfusate to estimate changes in the blood flow, as previously described (13–15). After a 30-min equilibration period, a 30-min fraction of dialysate was collected at a flow rate of 0.5 μl/min. Then, the infusion was set at 2.5 μl/min for the remaining experimental period. The calibration procedure using various infusion rates was applied for interstitial glycerol concentration determination in SCAAT, as previously described by our group (16, 17). A simplified but relevant and less time-consuming method was selected in this study. The estimated extracellular glycerol concentrations were calculated by plotting (after log transformation) the concentration of glycerol in the dialysate measured at 0.5 and 2.5 μl/min against the infusion rates (18).

After the calibration of the probes, three 15-min fractions of the outgoing dialysate were collected and then h-ANP was infused iv for 60 min. Dialysate samples were collected for each 15-min period during infusion and during the 60-min postinfusion period. Water intake was allowed ad libitum during the experimental periods.

Spectral analysis of systolic blood pressure and heart rate

Blood pressure (BP) and heart rate (HR) were measured with a Finapress device (model 2300; Ohmeda, Trappes, France) using a cuff placed on the second phalange of the third finger of the dominant hand. All subjects were instructed to keep the cuffed finger at the level of the heart. Recordings were taken at the end of the basal period and at 30 and 60 min during h-ANP infusion. BP and HR data were digitalized, and a series of at least 512 equidistant values sampled at 2 Hz without artifacts was stored in a PC for off-line analysis. Spectral analysis was performed using a fast Fourier transform algorithm (Anapres; Notocord Systems, Croissy-sur-Seine, France). The integration of spectral modulus values for the consecutive bands from 0.004 to 1 Hz was used to estimate the total spectral variability of whole spectra. The integration of spectral modulus values of midfrequency (MF) band of systolic BP and HR (70–130 mHz), reflecting baroreflex-dependent sympathetic activity, and of high-frequency (HF) band of HR (respiratory rate ± 50 mHz), reflecting parasympathetic drive to the heart, were also calculated. Results are presented as absolute values or in normalized units: (MF spectral modulus/total spectral modulus) × 100.

Adipocyte preparation and lipolysis measurements

Isolated adipocytes were obtained according to the method of Rodbell (19) by collagenase digestion of adipose tissue fragments in Krebs Ringer bicarbonate-HEPES buffer containing albumin (KRBA; 3.5 g/100 ml) and glucose (5.6 mM) at pH 7.4 under gentle shaking at around 60 cycles/min at 37°C. Then, the fat cells were filtered through a silk screen (pore size: 250

μm) and washed three times with KRBA buffer to eliminate collagenase. Isolated adipocytes were brought to a suitable dilution in KRBA buffer for lipolysis assays and incubated with pharmacological agents in a final volume of 100 μl for 90 min at 37°C. The mean fat cell numbers incubated in lipolysis measurement were 1,276 ± 177 and 1,434 ± 152 in 100 μl for lean and obese, respectively. At the end of the incubation, 20- to 50-μl aliquots of the infranatant were taken for glycerol determination used as the lipolytic index.

Drugs and biochemical determinations

The following reagents were used for fat cell isolation and lipolysis measurement: bovine serum albumin; fraction V, fatty-acid free; collagenase from *Clostridium histolyticum*; dibutyryl-cAMP (Boehringer-Mannheim, Meylan, France); isoprenaline hydrochloride (Sigma, Saint Quentin Fallavier, France); and human synthetic h-ANP (Neosystem, Strasbourg, France). h-ANP Clinalfa for biomedical and clinical research that was perfused in subjects came from France Biochem, Meudon, France. Plasma ANP concentrations were determined using a radioimmunoassay kit from Phoenix Pharmaceuticals (Belmont). Plasma noradrenaline and adrenaline were assayed by high performance liquid chromatography using electrochemical (amperometric) detection, as previously described (17). The detection limit was 10 pg/sample for both catecholamines; day-to-day variability was 4% and within-run variability, 3%. Ethanol in dialysate and perfusate (5 μl) was determined with an enzymatic method (20); the intra-assay and interassay variabilities were 3.0% and 4.5%, respectively. Glycerol was determined in plasma and in dialysate using an ultrasensitive radiometric method (21); the intra-assay and interassay variabilities were 5.0% and 9.2%, respectively. Plasma glucose was assayed with a glucose oxidase technique (Biotrol, Paris, France); the intra-assay and interassay variabilities were 1.5% and 5.1%, respectively. NEFA were assayed with an enzymatic method (Unipath, Dardilly, France); the intra-assay and interassay variabilities were 1.1% and 1.6%, respectively. Plasma insulin was measured using a Bi-insulin IRMA kit from Sanofi Diagnostics Pasteur (Marne-La-Coquette, France); the intra-assay and interassay variabilities were 2.7% and 5.8%, respectively.

Statistical analysis

All the values are given as means ± SEM. A statistical comparison of the values was first performed using two-way analysis of variance (ANOVA) for repeated measures with subjects (lean vs. obese) and time as factors of the analysis. Subsequently, the effects of h-ANP were analyzed in each group using one-way ANOVA with the time as the factor of the analysis, followed by a Bonferroni-Dunett post hoc test, taking basal values as control. The plasma and extracellular response curves were analyzed using a nonpaired *t*-test on the total integrated changes over baseline values [area under the curves (AUC)] using the trapezoidal method. Values were considered statistically significant when $P < 0.05$. Statistical analyses were performed using software packages (Statview 4.5® and SuperAnova 1.11®, Abacus Concepts Inc., Berkeley, CA).

RESULTS

In vitro lipolytic effects of h-ANP and isoprenaline on isolated fat cells

In both groups, the lipolytic effects of h-ANP were compared with those of isoprenaline (β-adrenergic receptor agonist) taken as a reference for the lipolytic effect. Spontaneous glycerol release (basal lipolysis) was similar in both groups (0.45 ± 0.11 vs. 0.42 ± 0.09 μmol glycerol/10^6 cells for lean and obese, respectively). Moreover, the lipolytic effect initiated by dibutyryl cAMP, a drug acting at a postreceptor level, did not differ in fat cells from lean and obese subjects (2.04 ± 0.36 vs. 1.51 ± 0.19 μmol glycerol/10^6 cells, respectively; $P = 0.12$). The concentration-response increase in glycerol release by isolated adipocytes is depicted in **Fig. 1**. In comparison with isoprenaline, h-ANP induced a strong lipolytic effect; maximal lipolytic effects were obtained with 1 μM isoprenaline and 0.01 μM ANP. Calculated pD2 ($-\log EC_{50}$) for h-ANP effect was similar for lean and obese adipose tissue (9.07 ± 0.43 vs. 9.03 ± 0.31, respectively). Therefore, no significant difference in the lipolytic effect of isoprenaline or h-ANP was observed when comparing the responsiveness of the adipocytes from lean and obese subjects ($P = 0.24$ and 0.12, respectively).

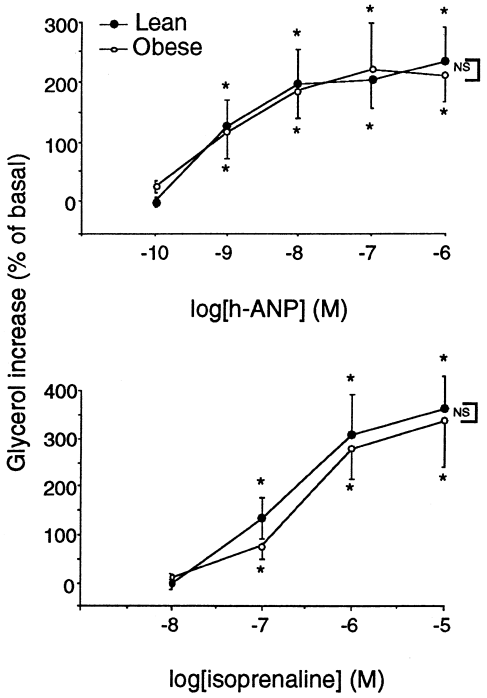

Fig. 1. Lipolytic effects of h-ANP and isoprenaline in abdominal subcutaneous isolated human fat cells from lean and obese subjects. Lipolysis is expressed as percentage of increase glycerol production. Data are expressed as means ± SEM of experiments carried out on adipose tissue from seven lean and seven obese subjects. * $P < 0.05$ when compared with basal values. NS, not significant; h-ANP, human ANP.

Plasma ANP, NEFA, and glycerol levels during h-ANP infusion

During the baseline period, plasma concentrations of ANP were similar in obese and lean subjects (46 ± 7 vs. 36 ± 5 pg/ml, respectively). During ANP infusion, plasma ANP concentration values were 1,662 ± 119 vs. 1,249 ± 126 pg/ml and 1,721 ± 153 vs. 1,247 ± 142 pg/ml after 30- and 60-min ANP infusion, respectively. Values were slightly higher in obese subjects, but the difference was not significant. During the baseline period, plasma glyc-

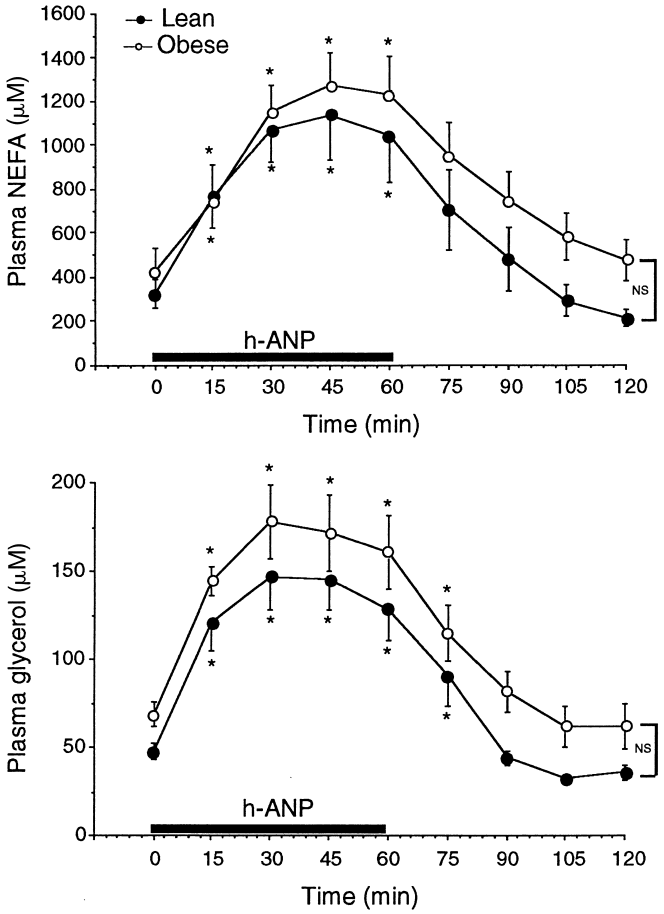

Fig. 2. Changes in plasma NEFA and glycerol concentrations in lean and obese subjects during the iv infusion of h-ANP. The effects of h-ANP were analyzed in each group using a one-way analysis of variance (ANOVA) for repeated measures with time as factor of the analysis, followed by a Bonferroni-Dunett post hoc test. Data are expressed as means ± SEM of seven lean and seven obese subjects. * $P < 0.05$ when compared with preinfusion values.

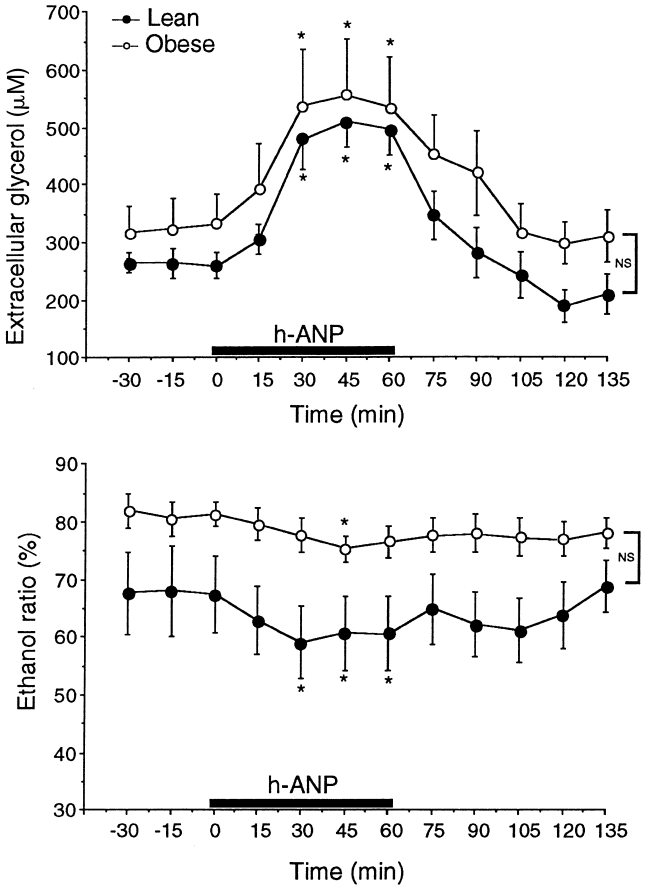

Fig. 3. Changes in extracellular glycerol concentrations and ethanol ratio (ethanol dialysate concentration/ethanol perfusate level × 100) in subcutaneous adipose tissue during the iv infusion of h-ANP. The probes were perfused with Ringer's solution. After a calibration period, a 2.5-μl/min flow rate was maintained for 120 min, and dialysate fractions were collected at 15-min intervals. After 45 min (basal period), h-ANP was infused to the subjects. The effects of h-ANP were analyzed in each group using a one-way ANOVA for repeated measures with time as factor of the analysis, followed by a Bonferroni-Dunett post hoc test. Data are expressed as means ± SEM of seven lean and seven obese subjects. * $P < 0.05$ when compared with preinfusion values.

erol concentrations were higher in obese subjects (48 ± 5 and 69 ± 7 μM, respectively; $P < 0.02$). However, no difference was found in plasma NEFA levels (322 ± 67 and 422 ± 107 μM, respectively). In both groups, plasma NEFA and glycerol concentration increased significantly after 15 min of h-ANP infusion and reached a maximal value at t = 30 min (**Fig. 2**). Twenty minutes after stopping the infusion, plasma concentrations returned to the basal values. The calculated AUC for the plasma NEFA response was not different for lean and obese subjects (35,322 ± 5,255 and 34,317 ± 5,621 μM/60 min, respectively; $P = 0.43$). The calculated AUC for the plasma glycerol response was also not different for lean and obese subjects (4,654 ± 702 and 5,036 ± 599 μM/60 min, respectively; $P = 0.35$).

Extracellular glycerol concentration in SCAAT during h-ANP infusion

At rest, the baseline extracellular glycerol concentration in SCAAT did not differ in lean and in obese subjects (259 ± 21 and 331 ± 52 μM, respectively). Adipose tissue glycerol levels at rest were 4–5 times higher than those in venous plasma in both groups of subjects. During h-ANP infusion, the extracellular glycerol concentration increased in both groups, the increase being significant from the 30th min of infusion (**Fig. 3**). The maximal h-ANP-induced increase of glycerol was obtained 45 min after the beginning of infusion and was 216 ± 41 and 213 ± 60 μM in lean and obese subjects, respectively. The calculated average AUC for h-ANP-induced glycerol increase over 60 min was not significantly different in the two groups (9,440 ± 1,415 vs. 8,860 ± 2,602 μM/60 min in lean and obese, respectively; $P = 0.44$).

Because infusion of h-ANP (at twice the rate) has been suspected to promote a lipid-mobilizing effect via sympathetic nervous system activation (11,12), we evaluated in situ lipolysis during h-ANP infusion with a probe perfused with a high concentration of propranolol (0.1 mM), a β-adrenergic receptor antagonist, to prevent any putative β-adrenergic effect of catecholamines on adipose tissue

(**Fig. 4**). In these conditions, it was found that propranolol did not modify the increase in extracellular glycerol concentration in response to h-ANP infusion (calculated AUC were 6,805 ± 1,266 vs. 9,103 ± 2,284 μM/60 min for the control probe and for the probe with added propranolol, respectively; $P = 0.28$).

Subcutaneous adipose tissue blood flow during h-ANP infusion

Adipose tissue blood flow was assessed by ethanol outflow/inflow ratios (ethanol concentration measured in the dialysate divided by the ethanol concentration measured in the perfusate × 100) from the probes and the results are reported in Fig. 3. Before and during h-ANP infusion, the ethanol outflow/inflow ratio was higher in obese than in lean subjects ($P < 0.03$). A significant decrease of the ethanol outflow/inflow ratio was observed in lean subjects starting from t = 30 min of h-ANP infusion, whereas the decrease became significant at t = 45 min in obese subjects (Fig. 3). However, the corresponding AUC for h-ANP-induced vasodilation were not different between the two groups ($P = 0.12$). In the comparative study performed to evaluate the effect of propranolol (Fig. 4), the decrease in the ethanol outflow/inflow ratio was not significant during h-ANP infusion in the two microdialysis probes, and the corresponding AUC were not different between the two groups ($P = 0.17$).

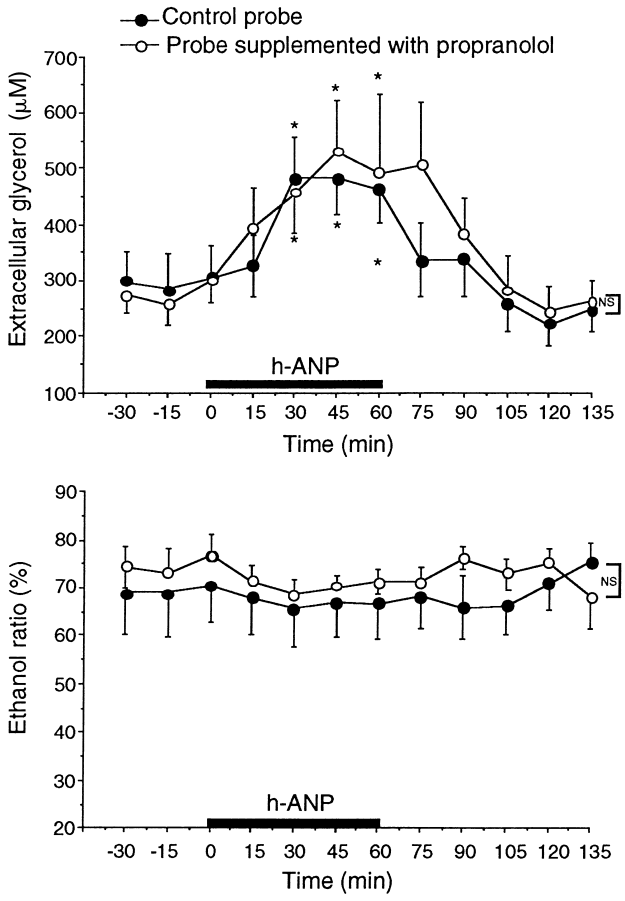

Fig. 4. Effect of propranolol on the changes in extracellular glycerol concentrations and ethanol ratio (ethanol dialysate concentration/ethanol perfusate level × 100) in subcutaneous adipose tissue during the iv infusion of h-ANP. Propranolol (β-adrenergic receptor antagonist, 0.1 mM) was added to one probe. After a calibration period, a 2.5-μl/min flow rate was maintained for 120 min, and dialysate fractions were collected at 15-min intervals. After 45 min (basal period), h-ANP was infused to the subjects. The effects of h-ANP were analyzed in each group using a one-way ANOVA for repeated measures with time as factor of the analysis, followed by a Bonferroni-Dunett post hoc test. Data are expressed as means ± SEM of six subjects (three lean and three obese). * $P < 0.05$ when compared with preinfusion values.

Plasma glucose and insulin concentrations during h-ANP infusion

During the baseline period, the plasma concentration of glucose was similar in both groups (4.76 ± 0.24 and 4.95 ± 0.15 mM in lean and in obese subjects, respectively), whereas the plasma insulin level was higher in obese subjects (3.3 ± 0.6 and 5.7 ± 1.1 μU/ml in lean and in obese subjects, respectively; $P < 0.05$). No significant variations of plasma glucose level were observed in either group during the h-ANP infusion. A weak but not significant increase in plasma insulin was observed during h-ANP infusion (**Table 1**). The calculated AUC for insulin variations in the plasma during the h-ANP infusion did not show significant differences between the two groups (66.6 ± 20.5 vs. 54.0 ± 35.7 $\mu U \cdot ml^{-1} \cdot 60\ min^{-1}$ in lean and in obese subjects, respectively; $P = 0.38$).

Plasma catecholamine concentrations and cardiovascular responses to the h-ANP infusion

At rest, plasma norepinephrine and epinephrine concentrations were similar in the two groups. Plasma norepinephrine concentration increased significantly after 30 min of h-ANP infusion and reached a plateau for the subsequent 30 min of infusion (**Table 2**). The plasma epinephrine concentrations did not change during h-ANP infusion. The AUC calculated for the h-ANP-induced increase in norepinephrine (4,168 ± 1,031 vs. 5,691 ± 1,001 $pg \cdot ml^{-1} \cdot 60\ min^{-1}$ in lean and obese, respectively) showed no significant differences between the two groups of subjects ($P = 0.22$). h-ANP infusion induced a discrete

TABLE 1. Effect of h-ANP infusion on plasma insulin and glucose concentrations in lean and obese subjects

		h-ANP Infusion (50 ng/min/kg)		
	Basal	30 Min	60 Min	*P*
Plasma insulin (μU/ml)				
Lean	3.3 ± 0.6	4.7 ± 0.8	4.8 ± 0.8	
Obese	5.7 ± 1.1	6.5 ± 0.9	7.6 ± 1.2	NS
Plasma glucose (mM)				
Lean	4.7 ± 0.2	4.9 ± 0.1	5.1 ± 0.2	
Obese	4.8 ± 0.1	5.2 ± 0.2	5.2 ± 0.2	NS

Values are means ± SEM. A statistical comparison of the values was first performed by two-way analysis of variance (ANOVA) for repeated measures with subjects (lean vs. obese) and time as factors of the analysis. Subsequently, the effects of h-ANP were analyzed in each group by one-way ANOVA with time as the factor of the analysis, followed by a Bonferroni-Dunett post hoc test, taking basal values as control. hANP, human ANP; NS, not significant.

TABLE 2. Effect of h-ANP infusion on plasma norepinephrine concentration, systolic and diastolic blood pressure, and heart rate in lean and obese subjects

		h-ANP Infusion (50 ng/min/kg)		
	Basal	30 Min	60 Min	*P*
Plasma norepinephrine (pg/ml)				
Lean	93 ± 15	186 ± 28[a]	175 ± 24[a]	
Obese	122 ± 21	226 ± 31[a]	236 ± 31[a]	NS
Plasma epinephrine (pg/ml)				
Lean	48 ± 21	55 ± 18	46 ± 19	
Obese	35 ± 9	50 ± 16	63 ± 24	NS
Heart rate (beats/min)				
Lean	57 ± 5	60 ± 5	60 ± 5	
Obese	60 ± 3	68 ± 3[a]	74 ± 5[a]	$P < 0.04$
Systolic blood pressure (mmHg)				
Lean	115 ± 2	113 ± 8	123 ± 8	
Obese	128 ± 14	111 ± 6	118 ± 8	NS
Diastolic blood pressure (mmHg)				
Lean	62 ± 2	62 ± 2	75 ± 7	
Obese	70 ± 7	67 ± 5	67 ± 6	NS

Values are means ± SEM. A statistical comparison of the values was first performed by two-way ANOVA for repeated measures with subjects (lean vs. obese) and time as factors of the analysis. Subsequently, the effects of h-ANP were analyzed in each group by one-way ANOVA with time as the factor of the analysis, followed by a Bonferroni-Dunett post hoc test, taking basal values as control.

[a] $P < 0.05$ when compared with preinfusion values.

increase in HR, this effect being stronger in obese than in lean subjects. Systolic and diastolic BP were not significantly modified by h-ANP infusion.

The effects of h-ANP infusion on spectral variability of systolic BP and HR are depicted in **Table 3**. h-ANP infusion failed to significantly modify overall spectral variability of systolic BP. The relative energy of the MF band of systolic BP increased moderately in both groups during h-ANP infusion. Variance analysis with repeated measures showed that the changes in the MF band of systolic BP did not differ between the two groups. Conversely, the HF energy of the HR decreased during h-ANP infusion. The decrease in the relative energy of the HF band induced by h-ANP infusion was significantly more marked in obese than in lean subjects.

DISCUSSION

The findings of this study showed that *i*) synthetic h-ANP is a potent lipid-mobilizing agent in humans, confirming our previous findings on isolated human fat cells (10), *ii*) obesity per se did not modify the h-ANP-induced lipolysis in SCAAT or lipomobilization in toto, and *iii*) this lipid-mobilizing effect is mainly relevant to the specific lipolytic action of h-ANP on adipose tissue (the part of the lipid-mobilizing effect due to the increased sympathetic nervous activity probably being minor).

An increment of plasma NEFA levels under h-ANP infusion in humans has previously been described (11, 12). At the time, the authors attributed this effect to baroreflex-mediated activation of the sympathetic nervous system

TABLE 3. Effect of h-ANP infusion on spectral components of systolic blood pressure and heart rate variability in lean and obese subjects

		h-ANP Infusion (50 ng/min/kg)		
	Basal	30 min	60 min	*P*
% Low frequency systolic blood pressure				
Lean	18.1 ± 1.8	17.3 ± 2.2	19.4 ± 2.3	
Obese	19.9 ± 0.6	20.6 ± 0.9	19.7 ± 2.3	NS
% Low frequency heart rate				
Lean	17.9 ± 2.2	19.8 ± 1.6	19.4 ± 1.2	
Obese	19.7 ± 1.0	20.8 ± 0.9	20.6 ± 2.3	NS
% High frequency heart rate				
Lean	20.1 ± 2.1	17.2 ± 2.3	17.5 ± 2.8	
Obese	17.8 ± 2.3	13.2 ± 1.5[a]	10.4 ± 1.5[a]	$P < 0.03$

The values are as means ± SEM of spectral modulus for the overall variability of systolic blood pressure and heart rate and in normalized units (see Materials and Methods) for the midfrequency band. A statistical comparison of the values was first performed by two-way ANOVA for repeated measures with subjects (lean vs. obese) and time as factors of the analysis. Subsequently, the effects of h-ANP were analyzed in each group by one-way ANOVA with time as the factor of the analysis, followed by a Bonferroni-Dunett post hoc test, taking basal values as control.

[a] $P < 0.05$ when compared with preinfusion values.

and to the stimulation of fat cell β-adrenergic receptors. However, it cannot be excluded that a modest part of the lipid-mobilizing effect observed with h-ANP is relevant of a sympathetic nervous system activation. The present study suggests that this effect is mainly related to the direct action of h-ANP on fat cells. The first argument concerns the relationship between the level of sympathetic nervous system activation obtained with h-ANP infusion and the intensity of lipid mobilization. A comparison can be performed with both events initiated by orthostatism or by yohimbine administration: When sympathetic nervous system activation was achieved through orthostatism (i.e., passing from the supine to the upright position; 20 min), the plasma norepinephrine level rose about 117% from the basal value, and plasma NEFA and glycerol levels were increased only by about 26% and 58%, respectively (22). When sympathetic nervous system activation was induced by 0.2 mg/kg oral administration of yohimbine (an α2-adrenergic receptor antagonist), the plasma norepinephrine level rose about 165%, whereas plasma NEFA and glycerol levels increased only by 73% and 36%, respectively, 90 min after yohimbine intake (23). During h-ANP infusion, we observed that plasma norepinephrine levels increased less than during orsthostatism or yohimbine administration (i.e., 91% and 89% in lean and obese subjects, respectively), whereas plasma NEFA and glycerol levels were dramatically increased (250% and 200% for NEFA and 208% and 160% for glycerol in lean and obese subjects, respectively). Our results fit with those of Uehlinger et al. (12) and Weidmann et al. (11), who reported similar increases in plasma NEFA and norepinephrine plasma levels during h-ANP infusion in nonobese subjects. From this comparison, it is possible to conclude that a large part of the lipid-mobilizing effect of h-ANP is not related to sympathetic nervous system activation per se. A second important argument concerns the absence of propranolol effect on h-ANP-induced in situ lipolysis. Propranolol (added at high concentrations to the microdialysis perfusate) did not modify the increase in extracellular glycerol promoted by h-ANP infusion. It has been shown that at these concentrations, propranolol, which had no effect per se on basal glycerol release (24), could blunt the lipolytic effect of norepinephrine or epinephrine infused in the microdialysis probes (25). Moreover, Arner et al. (24) found that propranolol (0.1 mM) infused in the probe suppressed about 75% of catecholamine-induced lipolysis during exercise. The third argument is that we observed only marginal changes in spectral variability of blood pressure in the MF range. These changes in MF, a well-accepted index of the baroreflex-dependent sympathetic activity, together with the significant decrease in HF energy of the HR, indicate that h-ANP infusion elicited a deactivation of the baroreflex arch, which is due to a decrease in arterial and central venous pressure. Our data indicate, however, that h-ANP not only is a weak activator of the sympathetic nervous system, but may also have sympathoinhibitory effects. In fact, it was shown that even when ANP increases muscle sympathetic nerve activity (26), it blunts sympathetic activation during cold pressor test and lower body negative pressure (27). Thus, one can assume that most of the metabolic effects of h-ANP in this study are not related to a reflex increase in efferent sympathetic activity and to norepinephrine release.

ANP and BNP represent a new potent pathway controlling human adipose tissue lipolysis, and their action involves a cGMP-dependent pathway that does not act via phosphodiesterase-3B inhibition and cAMP production (10). Previous studies have shown that obesity is associated with altered catecholamine-induced lipolytic responses of adipose tissue. This decreased catecholamine-induced lipolysis is attributable to both a reduced β-adrenergic effect and an increased α2-adrenergic antilipolytic effect (18, 28–30). Our present findings show that there is no difference in the in vitro lipolytic effect of h-ANP in adipocytes from obese and lean subjects. Spontaneous glycerol release (basal lipolysis), maximum values of lipolytic effects for isoprenaline and h-ANP, were similar in both groups. Calculated in terms of pD_2 values, h-ANP induces, as previously shown (10), a lipolytic effect at concentrations 100-fold lower than that of isoprenaline in fat cells from both groups. It is also noticeable that the β-adrenergic lipolytic effect was not reduced in the adipocytes from the obese subjects included in this study. In fact, the study was conducted for the first time on young obese subjects devoid of other pathologies. Finally, the lack of lipolytic difference found on isolated adipocytes is supported by the similar lipid-mobilizing action of h-ANP infused in the subjects, whatever the criterion chosen (plasma NEFA or glycerol increase). Because the adipocyte is a new target for ANP, these results fit with the only molecular study showing that the level of NPr-A mRNA expression in adipose tissue is reduced in obese hypertensive patients but not in obese nonhypertensive subjects (31). Another study clearly showed that h-ANP infusion induced a lower increase in NEFA level in hypertensive patients than in nonhypertensive subjects concomitant with a higher increase in plasma norepinephrine level (11). This suggests that obesity by itself did not change ANP sensitivity in adipose tissue. Alteration of ANP-induced cardiovascular and metabolic actions described in obese hypertensive patients seems to be related to hypertension. After low calorie diet that reduces weight and systolic and diastolic blood pressure, ANP effects are recovered and the injection of h-ANP induces a higher decrease in mean arterial blood pressure and a higher increase in plasma cGMP concentrations (32). However, from this study, it was difficult to assess whether these alterations of h-ANP effects were due to obesity alone or to the association of obesity plus hypertension. Our findings show no alteration of h-ANP-induced lipolysis or lipomobilization in nonhypertensive obese subjects.

The h-ANP-induced lipid mobilization could be influenced by other factors such as the plasma insulin level and the blood flow in adipose tissue. We found that h-ANP infusion had no significant effect on plasma insulin level. A significant increase was previously noted with a 2-fold higher infused dose of h-ANP than that used in the present study (12). It is possible that h-ANP directly affects

pancreatic β-cell function because cGMP is an important second messenger of ANP in various tissues. The presence of ANP receptors has not been actually demonstrated in pancreas, but some evidence exists for cGMP as a mediator of rat pancreatic islets function (33).

The modification of SCAAT blood flow during h-ANP infusion was assessed by ethanol clearance, a method previously validated. In our study, the absolute vasodilating action of h-ANP in adipose tissue was similar in lean and obese subjects, even if the basal blood flow was reduced in obese subjects, as shown by the elevated ethanol outflow/inflow ratio in basal conditions and as previously described (18, 34). It is known that an increment of lipolysis parallels an increase in adipose tisssue blood flow in physiological situations such as exercise. In the present study, ANP infusion decreases ethanol ratio, which reflects an increase in local blood flow in adipose tissue. It is noticeable that in the present study, ANP-induced plasma insulin changes are not observed. Thus, changes in insulin cannot be considered to interpret the present blood flow results.

Until now, most of the pharmacological approaches to the physiopathology of the human fat cell have focused their attention on the adrenergic control of this cell. This was based on the fact that noradrenaline and adrenaline are the two main hormones controlling human adipocyte lipolysis through different adrenergic receptor subtypes (16, 28). Our data show that lipid mobilization is due to a direct effect of ANP on the human adipocyte. Many studies have shown the possible dysregulation of the adrenergic control in adipose tissue lipolysis in obese subjects. However, our findings did not found any alteration in maximal h-ANP-induced lipolysis in obese subjects without associated pathologies. The demonstration of an effect of NP in the control of lipid mobilization raises the question about the physiological role of this new lipolytic pathway. It is noticeable that the lipid-mobilizing effect is observed with h-ANP plasma levels that are about 10-fold higher than those currently reported in various physiological situations. Moreover, the putative involvement of the ANP pathway in the development and pathogenesis of obesity associated with hypertension is provocative. Further studies are needed to answer these physiopathological questions.

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