Atrial natriuretic peptide stimulates lipid mobilization during repeated bouts of endurance exercise

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1Franco-Czeck Laboratory for Clinical Research on Obesity, French Institute of Health and Medical Research (INSERM U586) and 2Department of Sports Medicine and Obesity Unit, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 3Obesity Research Unit, INSERM UPS U586, Louis Bugnard Institute, Toulouse Hospital, Paul Sabatier University; and 4Laboratory of Medical and Clinical Pharmacology, Faculty of Medicine, Toulouse, France

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Moro, Cédric, Jan Polak, Jindra Hejnova, Eva Klimcakova, François Crampes, Vladimir Stich, Max Lafontan, and Michel Berlan. Atrial natriuretic peptide stimulates lipid mobilization during repeated bouts of endurance exercise. Am J Physiol Endocrinol Metab 290: E864–E869, 2006. First published November 15, 2005; doi:10.1152/ajpendo.00348.2005.—Atrial natriuretic peptide (ANP) controls lipolysis in human adipocytes. Lipid mobilization is increased during repeated bouts of exercise, but the underlying mechanisms involved in this process have not yet been delineated. The relative involvement of catecholamine- and ANP-dependent pathways in the control of lipid mobilization during repeated bouts of exercise was thus investigated in subcutaneous adipose tissue (SCAT) by microdialysis. The study was performed in healthy males. Subjects performed two 45-min exercise bouts (E1 and E2) at 50% of their maximal oxygen uptake separated by a 60-min rest period. Extracellular glycerol concentration (EGC), reflecting SCAT lipolysis, was measured in a control probe perfused with Ringer solution and in two other probes perfused with either Ringer plus phentolamine (α1-AR antagonist) or Ringer plus both phentolamine and propranolol (β-AR antagonist). Plasma epinephrine, plasma glycerol, and EGC were 1.7-, 1.6-, and 1.2-fold higher in E2 than in E1, respectively. Phentolamine potentiated exercise-induced EGC increase during E2 only. Propranolol reduced the lipolytic rate during both E1 and E2 compared with the probe with phenolamine. Plasma ANP concentration increased more during E2 than during E1 and was correlated with the increase in EGC in the probe containing phenolamine plus propranolol. The results suggest that ANP is involved in the control of lipolysis during exercise and that it contributes to stimulation of lipolysis during repeated bouts of exercise.

microdialysis; insulin; epinephrine; guanosine 5′,5′-cyclic monophosphate

UNTIL RECENTLY, the regulation of human adipose tissue lipolysis during exercise was mainly attributed to both the increase in catecholamine levels and the concomitant decrease in plasma insulin concentration. Catecholamines activate lipolysis through β1- and β2-adrenergic receptors (AR) and inhibit it through α2-AR stimulation. The coordinated activation of both receptors modulates the intracellular cAMP concentration, which activates cAMP-dependent protein kinase, leading to the phosphorylation and activation of hormone-sensitive lipase (HSL) (2, 22). The recent discovery that natriuretic peptides (NP) are potent activators of cGMP, which activate a cGMP-dependent protein kinase (PKG). PKG-dependent phosphorylation of perilipin and HSL stimulate lipolysis (27, 28). NP are potent lipolytic agents when they act on isolated fat cells from subcutaneous adipose tissue (SCAT), specifically in primates (29). Intravenous infusion of pharmacological doses of hANP (human atrial natriuretic peptide) in humans promotes a strong lipid-mobilizing effect independently of reflex activation of the sympathetic nervous system (SNS) (12). A similar response occurred when ANP was perfused through a microdialysis probe inserted in SCAT (27). Moreover, within a physiological range, ANP stimulates adipose tissue lipolysis and fat oxidation and increases circulating levels of nonesterified fatty acids (NEFA) (7). Circulating ANP concentrations rise during short-term exercise of increasing intensity; the maximal concentrations of ANP increase two- to threefold (25).

The microdialysis method is useful to monitor local lipid mobilization and changes in cAMP or cAMP release in SCAT. It is the most suitable method available to perform mechanistic explorations of adipose tissue function in vivo (1, 3). Exercise is a prerequisite for both SNS activation (11, 15) and NP release (5, 26). We (25) demonstrated that physiological ANP release during acute endurance exercise contributes to the stimulation of lipid mobilization and NEFA supply for the working muscle.

A previous study showed that hormonal and metabolic responses differed during two repeated exercise bouts of similar intensities (31). A higher secretion of epinephrine and increased lipid mobilization were found in SCAT during the second exercise bout. The hormonal partners responsible for the enhanced lipolysis have not been delineated so far.

The aim of the present study was to examine the relative contributions of catecholamines and ANP in the control of lipolysis during sequential periods of exercise. To do so, healthy male subjects fasted overnight and then performed two exercise bouts (E1 and E2) for 45 min each at 50% of their maximal oxygen uptake (VO2max) separated by a 60-min rest period. To assess the specific role of ANP in the control of SCAT lipolysis, glycerol release was measured under local α- and β-blockade during two repeated bouts of exercise.

SUBJECTS AND METHODS

Subjects. Eight healthy young men aged 23 ± 0.6 yr (range 22–27 yr) with a mean body mass index of 24 ± 0.7 kg/m2 (range 21.6–24.9

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Table 1. Plasma norepinephrine, epinephrine, glucose, insulin, GH, and ANP concentrations during E1 and E2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Rest</th>
<th>Recovery 135</th>
<th>E1</th>
<th>Recovery 180</th>
<th>E2</th>
<th>Recovery 210</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>75</td>
<td></td>
<td>165</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>294±28</td>
<td>827±65†</td>
<td>901±75†</td>
<td>332±34</td>
<td>987±58†</td>
<td>992±45†</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>62±4</td>
<td>87±5†</td>
<td>109±9†</td>
<td>65±2</td>
<td>141±28†</td>
<td>185±38†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.9±0.2</td>
<td>5.1±0.2</td>
<td>4.8±0.2</td>
<td>4.9±0.4</td>
<td>4.5±0.2†</td>
<td>4.4±0.2†</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>11.5±1.1</td>
<td>9.3±0.8</td>
<td>8.4±0.7†</td>
<td>12.6±1.3</td>
<td>10.4±0.8</td>
<td>7.0±0.4†</td>
</tr>
<tr>
<td>GH, ng/ml</td>
<td>4.8±0.7</td>
<td>14.8±5.3†</td>
<td>3.9±1.1</td>
<td></td>
<td>7.1±0.5†</td>
<td></td>
</tr>
<tr>
<td>ANP, pg/ml</td>
<td>60.9±3.5</td>
<td>83.1±2.6†</td>
<td>65.5±3.8</td>
<td></td>
<td>92.7±3.4†</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. E1 and E2, first and second exercise; GH, growth hormone; ANP, atrial natriuretic peptide. †P < 0.05 compared with values measured after rest or after 60 min of recovery. *P < 0.05 compared with corresponding values measured during E1.
norepinephrine concentration kinetics during the second exercise bout. At the end of period E2, the plasma norepinephrine concentration rose significantly to $992 \pm 45$ pg/ml. Plasma epinephrine concentration rose significantly from $62 \pm 4$ to $109 \pm 9$ pg/ml at the end of E1. During recovery, epinephrine concentration decreased toward a value that did not differ from that measured in basal conditions (time 0 min). During E2, the plasma epinephrine concentrations rose dramatically to values ($185 \pm 38$ pg/ml) significantly higher than those measured during E1. Plasma GH concentrations increased during E1 and E2 (Table 1). Exercise-induced GH release was about twofold lower during E2 ($P < 0.05$).

**Plasma glucose, insulin, and ANP concentrations.** No significant changes in plasma glucose level were observed during E1. A significant decrease in plasma glucose concentration was observed at the end of E2 and during the second recovery period. Plasma insulin concentration decreased during E1 (Table 1). During recovery, insulin concentration returned to values that did not differ from those measured in basal conditions (time 30 and 135 min). At the end of period E2, plasma insulin concentrations decreased to values significantly lower than those measured during E1 ($P < 0.001$). At the end of E1, the plasma ANP concentration rose significantly. At the end of the recovery period, ANP concentrations decreased to a value that did not differ from that measured in basal conditions. At the end of E2, the plasma ANP concentration rose significantly towards values higher than during E1 (Table 1).

**Plasma NEFA and glycerol levels.** Plasma NEFA concentration was unchanged throughout E1 (Fig. 1); then, after the first 15 min of the recovery period, it increased ($P < 0.01$) and finally decreased toward values similar to those found in basal conditions at time 0 min. During E2, the plasma NEFA concentration increased significantly compared with values measured during E1. Plasma glycerol increased progressively during E1 and then, during the recovery period, returned to values that were not significantly different from those determined before exercise (time 0 min). During E2, a significant increase in plasma glycerol concentration was observed compared with E1.

**Changes in EGC during the two exercise bouts.** At rest, before E1, the mean basal EGC did not differ between the control probe (160.6 $\pm$ 16.9 mol/l) and the probe perfused with phentolamine (■), and the probe perfused with phenolamine + propranolol (gray square). Changes were calculated by the difference between the mean values obtained during exercise (E1 or E2) and the value measured before exercise E1 or E2. Data are expressed as means $\pm$ SE. *$P < 0.05$ compared with E1. NS, not significant.

Fig. 1. Plasma nonesterified fatty acid (NEFA) and glycerol concentrations during repeated bouts of 45-min cycle ergometer exercise (E1 and E2). Data are expressed as means $\pm$ SE. *$P < 0.05$ compared with values measured before E1 or E2. $^*$ $P < 0.05$ compared with corresponding values measured during E1.

Fig. 2. **A:** changes in extracellular glycerol concentrations (EGC) during E1 and E2 in the control probe (■), the probe perfused with phentolamine (●), and the probe perfused with phenolamine + propranolol (gray square). Changes were calculated by the difference between the mean values obtained during exercise (E1 or E2) and the value measured before exercise E1 or E2. Data are expressed as means $\pm$ SE. *$P < 0.05$, probe with phentolamine compared with control probe; ¥ $P < 0.05$, probe with phenolamine + propranolol compared with control probe; #$P < 0.05$, probe with phenolamine + propranolol compared with probe with phenolamine.
phentolamine plus propranolol, the EGC were lower than in the control and also lower than in the probe with phentolamine alone during E1. During E2, the EGC were lower compared with the phentolamine probe but not with the control probe. Figure 2A represents the differences in exercise-induced responses during E1 compared with E2. Regardless of the presence of α-blockade or combined α- and β-blockade, the increase in EGC was more pronounced during E2 than during E1. EGC was significantly increased during E2 in the probe perfused with phentolamine but not during E1. EGC was ~35% lower in the phentolamine-plus-propranolol probe during E1 than in the control probe. This effect did not reach significance during E2.

Finally, a correlation was found between the increase in plasma ANP and the increase in EGC evaluated from the probe containing phentolamine plus propranolol (Fig. 3).

**Changes in extracellular cAMP and cGMP levels during the two exercise bouts.** We determined that extracellular cAMP and cGMP levels in SCAT do discriminate between the relative contribution of catecholamines and ANP during exercise-induced lipolysis. These levels were determined from the pooled dialysates obtained at rest and during periods E1 and E2 in the probes with phentolamine alone or associated with propranolol. The determinations were performed in six subjects. The results, depicted in Fig. 4, show that extracellular cGMP concentration increased significantly ($P < 0.04$) and similarly in each probe during E1. Extracellular cGMP tended to increase more during E2 than during E1, but the difference did not reach significance ($P < 0.09$). Extracellular cAMP concentration increased significantly during E1 ($P < 0.04$) regardless of the presence of α-blockade or combined α- and β-blockade, and cAMP increase tended to be higher during E2 than during E1. In addition, it was observed that the level of cAMP was reduced in the probe containing phentolamine plus propranolol at rest and during E1 and E2 compared with the probe with phentolamine alone.

**Modifications of adipose tissue blood flow in SCAT during exercise bouts.** Local changes in adipose tissue blood flow (ATBF) were evaluated using the ethanol washout method. The ethanol ratio was calculated in percent ratio of the ethanol concentration measured in the dialysate divided by the ethanol concentration measured in the perfusate $\times 100$ for the dialysate from each probe and taken as an index of ethanol washout. A high ethanol ratio corresponds to a lower ethanol washout and a lower regional ATBF. During the two resting periods, the ethanol outflow/inflow ratio was not different among the three probes. During the exercise bouts, a significant decrease in ethanol ratio was observed in the probe with phentolamine, the decrease being higher during E2 than during E1. No exercise-induced changes in ethanol ratio were observed in the control probe or in the probe combining the two AR antagonists.

**DISCUSSION**

This study reveals the major contribution of ANP in the stimulation of lipid mobilization from SCAT during repeated
bouts of endurance exercise. Numerous works have mentioned the role of epinephrine and insulin in this process, but the role of ANP has never been evoked. Sequential 45-min exercise bouts separated by a 60-min rest were used to promote SNS activation and ANP release. On the basis of the use of the microdialysis technique and local selective blockade of $\alpha_2$- and $\beta_{1,2}$-ARs within SCAT, we showed that more than 50% of the nonadrenergic lipolysis observed during a repeated bout of exercise is ANP dependent.

Catecholamines released under SNS activation during exercise were long considered to be the major agents controlling lipid mobilization from adipose tissue in humans (16, 34). We recently demonstrated (27) that NP act in a new pathway to control human fat cell lipolysis in vitro or when administered intravenously. Using the microdialysis method, we previously demonstrated that repeated bouts of exercise at moderate intensity ($50\% VO_{2\max}$) elicited a greater increase in lipid mobilization in SCAT of healthy men than exercise without repetition. Because when these experiments were performed the lipolytic effect of NP was unknown, the enhanced lipolytic response during the second exercise was attributed to a higher epinephrine response and reduced plasma levels of insulin (31).

We (25) previously verified that local infusion of propranolol was able to efficiently counteract the lipolytic effect of isoproterenol, a $\beta$-AR agonist, in SCAT. However, investigations have shown that, during exercise, addition of propranolol to the perfusion probe partly reduced the exercise-induced increase in glycerol levels in human SCAT (2, 15). During exercise, local $\alpha_2$-AR blockade enhances the glycerol output from SCAT (calculated from the probe perfused with phentolamine). This potentiating effect of the antagonist did not reach a significant level during E1 but became significant during the second exercise, E2. Plasma epinephrine concentrations were 1.7-fold higher during E2 than during E1, whereas the exercise-induced increase in plasma noradrenaline level was similar (Table 1). Thus the increased EGC in the presence of phentolamine had previously been attributed to the local suppression of the $\alpha_2$-AR-dependent antilipolytic effect of epinephrine on fat cells (32). In this study, in the presence of both propranolol and phentolamine, exercise-induced lipolysis in SCAT was only partly reduced ($\sim 35\%$ during both E1 and E2) compared with the probe containing phentolamine alone. The residual increment of EGC remaining after $\beta_{1,2}$ and $\alpha_2$-AR blockade suggests that nonadrenergic factors (possibly including the exercise-induced inhibition of insulin release and ANP release) contribute to the stimulation of lipolysis during exercise (Fig. 1).

The major evidence supporting the role of ANP in glycerol production is that the lipolytic rate (assessed by EGC) around the probe perfused with both phentolamine and propranolol was significantly higher during E2 than during E1 (during E1 and E2, the increase in EGC representing 65 and 74% of the increase found in the control probe, respectively; Fig. 1). In a previous study (25), we found that, during exercise, extracellular cGMP concentrations also increase, reflecting local ANP action within SCAT. In the present study, it was observed that the amount of extracellular cGMP was similar in the probe perfused with phentolamine alone or associated with propranolol (Fig. 2). In contrast, the concentration of cAMP was reduced in the probe combining the two pharmacological agents, so the lipid mobilization remaining after the local blockade of fat cell $\beta_{1,2}$ and $\alpha_2$-ARs has another origin. Nonadrenergic-dependent lipolysis is correlated with both plasma ANP (Fig. 3) and extracellular cGMP measured in SCAT. Thus the ANP-dependent lipolytic pathway appears to be involved in the enhancement of lipolysis in SCAT and responsible for the residual mobilization under adrenergic receptor blockade.

The fall in plasma insulin during exercise could also be involved in the enhancement of lipolysis due to the reduction of its antilipolytic effect. This decrease was more pronounced during E2. Insulin is an important factor controlling lipolysis in fat cells (14, 17, 24). The partial enhancement of extracellular cAMP (via a reduction of phosphodiesterase-3 activity) in the probe containing phentolamine plus propranolol suggests that insulin could be another candidate for the observed increase in lipolysis during exercise. To assess the contribution of insulin to exercise-induced lipolysis, it could be of interest to study the effect of two exercise bouts (E1 and E2) on lipid mobilization under conditions of stable insulinemia and glyceremia using euglycemic insulinemic clamp. Another hormonal stimulus possibly contributing to the exercise-induced lipolysis is GH, as its circulating levels increase during exercise (21, 31). Its mechanism of action involves adenylyl cyclase/Gs-Gi protein complex and cAMP production (35). However, GH cannot act in the probe perfused with both phentolamine and propranolol, because these two pharmacological drugs inhibit Gs and Gi-dependent pathways, respectively. Several other factors are known to modulate adipose tissue lipolysis. Among them, cortisol, parathyroid hormone (PTH), interleukin-6, and leptin are relevant candidates. Cortisol has a permissive effect on catecholamine-induced lipolysis, but an effect during short-term exercise is unlikely. PTH exerts a weak lipolytic effect in vitro in human fat cells (8) and stimulates lipid mobilization in humans; however, it acts at rather high concentrations that are clearly in an extraphysiological range. Interleukin-6 has been shown to stimulate lipid mobilization and oxidation in humans (33). Its effect appears at concentrations that are not encountered during endurance exercise, and a possible role of GH is not excluded (20). Leptin stimulates lipolysis in vitro in rodent fat cells but not in humans (30), and its plasma levels do not change during acute exercise (13). No data are available on a possible role of adiponectin in the regulation of lipolysis. Plasma adiponectin levels are not acutely regulated by exercise either (18). To sum up, a physiological lipolytic role of all the aforementioned factors seems unlikely during the acute bouts of endurance exercise used in this study.

For many years, exercise-induced lipolysis was attributed to catecholamines via the stimulation of fat cell $\beta$-AR. After the discovery of an antilipolytic action of catecholamines via the activation of $\alpha_2$-AR (4, 23), it was proposed that epinephrine, which exhibits a higher affinity for $\alpha_2$-AR than for $\beta$-AR, could exert a negative control on lipolysis. In vivo studies using microdialysis during exercise have revealed the physiological relevance of this pathway (15, 32). In this study, the lipolytic $\beta$-adrenergic responsiveness of catecholamines is totally counterbalanced by activation of $\alpha_2$-adrenergic antilipolytic receptors during E2. This effect could be related to the sustained rise in plasma epinephrine level during E2 in response to the decrease in plasma blood glucose concentration. Finally, after the discovery of the lipolytic effect and lipid-mobilizing action of NP (25, 27), it is proposed that the nonadrenergic lipolysis observed during the second exercise bout depends on ANP release. However, the lack of an ANP receptor antagonist limits reaching a final decision.
To conclude, this study raises the fundamental question of the role of ANP in the control of lipid mobilization during repeated bouts of endurance exercise. Besides epinephrine and insulin, ANP may act as a relevant physiological agent controlling exercise-induced lipolysis. The role played by unsuspected lipid-mobilizing factors like ANP in the control of lipolysis must be studied in greater detail under various physiological and pathophysiological conditions. Obese patients with or without insulin resistance should be included in these studies, as should highly trained subjects, in whom plasma ANP response to exercise or adipose tissue sensitivity to ANP could change.

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