In Vivo $\alpha_1$-Adrenergic Lipolytic Activity in Subcutaneous Adipose Tissue of Obese Subjects

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ABSTRACT

The role of $\alpha_1$-adrenoceptors in lipid mobilization and blood flow was investigated in situ using microdialysis of subcutaneous adipose tissue in severely obese subjects. The lipolysis rate was assessed by determination of interstitial glycerol concentration. The $\alpha_1$-adrenoceptor agonist norepinephrine caused an increase in glycerol level in adipose tissue that was similar to that observed with the physiologic $\alpha_1,2\beta_1$-adrenoceptor agonist norepinephrine, whereas the $\alpha_1$-adrenoceptor antagonist urapidil showed no effect on basal lipolysis rate. However, the enhanced glycerol concentration due to norepinephrine and norepinephrine was suppressed in the presence of urapidil. The $\beta$-adrenoceptor antagonist propranolol showed no effect on norepinephrine-stimulated glycerol output. Blood flow was assessed using the ethanol escape technique. Perfusion with norepinephrine decreased blood flow, whereas urapidil enhanced blood flow significantly. Despite the increase in blood flow, the basal interstitial glycerol concentration remained unchanged. Although norepinephrine at high concentrations could inhibit the urapidil-induced increase in blood flow, the norepinephrine-induced glycerol output was not affected. These results demonstrate that $\alpha_1$-adrenoceptors are involved in regulation of lipolysis rate and microcirculation of adipose tissue. However, the observed changes in local blood flow were not related to glycerol output.

Human adipocytes express $\alpha$- and $\beta$-adrenoceptors involved in the regulation of lipolysis. $\alpha$-Receptors have been identified as $\alpha_1$- and $\alpha_2$-subtypes, and both have different effector mechanisms and implications in fat cells (Arner, 1992). $\alpha_1$-Adrenoceptors mediate an increase of intracellular Ca$^{2+}$ and protein kinase C through phosphoinositide hydrolysis, and $\alpha_2$-adrenoceptors mediate inhibition of adenylate cyclase and cyclic AMP synthesis through Gi-protein coupling (Arner, 1992). Several studies have investigated the role of $\alpha_2$-adrenoceptors on lipolysis in human fat cells in vivo (Arner et al., 1990; Galitzky et al., 1993; Stich et al., 1999), whereas the function of the $\alpha_1$-adrenoceptor remains unclear (Fain and Garcia-Sainz, 1983). Previous studies in rat adipocytes in vitro have provided evidence that the $\alpha_1$-adrenoceptor is involved in the control of glycogenolysis and lactate production (Faintrenie and Géloën, 1996; Lawrence and Liner, 1977), but no effect on lipolysis was observed (Faintrenie and Géloën, 1996).

Clinical applications of $\alpha_1$-adrenoceptor agonists are used to treat hypotension by elevation of peripheral resistance (Hengstmann, 1986), whereas $\alpha_1$-adrenoceptor antagonists are used to treat hypertension (Lardinois and Neuman, 1988). The receptor antagonists also have a favorable influence on lipid metabolism (Lardinois and Neuman, 1988), such as lowering serum triglyceride levels, presumably by enhancing very low-density lipoprotein catabolism (Leren et al., 1980). Adipose tissue is the sole source for delivery of nonesterified fatty acids into the plasma and has an important role in regulating hepatic triacylglycerol secretion (Frayn and Summers, 1998). As a consequence, in subjects with enlarged adipose tissue deposits, stimulation of $\alpha_1$-adrenoceptors may contribute to alterations in lipid metabolism and blood pressure.

We therefore investigated the metabolic and vascular effects of $\alpha_1$-adrenergic agents in human subcutaneous adipose tissue in vivo. The microdialysis technique was used to measure glycerol output and blood flow after application of urapidil ($\alpha_1$-antagonist) and norepinephrine ($\alpha_1$-agonist) on fat tissue. The effect of stimulation of $\alpha_1$-adrenoceptors by norepinephrine was compared with that of the physiological catecholamine norepinephrine ($\alpha_1,2\beta_1$-agonist).

In this article, we present results demonstrating that lipolysis and blood flow are independently modulated by $\alpha_1$-adrenergic agents in subcutaneous adipose tissue.

Materials and Methods

The study subjects were 38 women aged 39.9 ± 12 years (five of the women were postmenopausal). All clinical characteristics are given as mean ± S.D. Body mass index was 44.5 ± 12 kg/m² and body fat was 44.0 ± 6.3%. Systolic blood pressure and diastolic blood pressure were 156.1 ± 24 and 93.0 ± 14 mm Hg, respectively. The

ABBREVIATION: ANOVA, analysis of variance.
subjects had no other known diseases and used no chronic medication. Fasting plasma glucose concentration was 109.4 ± 37 mg/dl and fasting plasma insulin concentration was 27 ± 24 μU/ml. Four different experiments were done with groups of 9 to 11 subjects. Between the groups there were no significant differences in the clinical characteristics. The Ethics Committee of Ulm approved the study; all participants were given a description of the study and their informed consent was obtained. Subjects were studied at 8:00 AM in the supine position after an overnight fast for 12 to 14 h. The last meal before the experiment was a mixed meal. Energy (20%) was derived from protein, 30% from fat, and 50% from carbohydrate. Body composition was measured by bioelectrical impedance analysis, and blood was taken from a cubital vein.

Microdialysis experiments were performed at rest for 120 or 240 min. Depending upon the type of experiment, two to four microdialysis probes (30 × 0.3 mm Cuprophane, 3000 mol wt. cut-off, glued to 50- and 100-mm-long sections of nylon tubing) were inserted without anesthesia into the abdominal subcutaneous adipose tissue. The 100-mm nylon tubing was connected to a microinjection pump (Perfusor VI; Braun, Melsungen, Germany) and was continuously perfused (2.5 μl/min) with isotonic saline. The following adrenergic agents were added as sterile solutions to the dialysis perfusate: urapidil (Byk Gulden Lomberg Chemische Fabrik GmbH, Konstanz, Germany), norepinefrine (Gödecke AG, Berlin, Germany), norfenefrine (Hoechst Marion Roussel, Bad Soden, Germany), and propranolol (Zeneca, Plankstadt, Germany).

For the blood flow measurements, the perfusate contained ethanol at a concentration of 100 mM. In each experiment, 15-min fractions of the dialysate were collected. The first three fractions were excluded due to a transient rise in the concentrations of metabolites in the outgoing dialysate after insertion of the microdialysis probes (Arner and Bålow, 1993). Glycerol concentrations were analyzed with a bioluminescence method (Björkholm et al., 1981). For ethanol measurement, two consecutive samples were combined. Ethanol concentrations were determined by gas-chromatography (Curry et al., 1966).

Statistics. Values are mean ± S.E.M., and statistical evaluation was performed with the SPSS, program (SPSS Inc., Chicago, IL). ANOVA for repeated measurements with Tukey’s honestly significant difference post hoc test and Wilcoxon’s paired test were used for comparison of glycerol levels and ethanol ratio, when appropriate. A value of p < 0.05 was considered significant.

**Results**

The effect of α₁-adrenergic agents on glycerol levels in adipose tissue was investigated in the experiments depicted in Fig. 1A. Three microdialysis catheters were inserted in each subject (n = 11) and perfused with the basal solution alone for 60 min. Thereafter, either the α₁-agonist norfenefrine (10⁻³ M), the α₁-antagonist urapidil (10⁻³ M), or the α₁,α₂-β-agonist norepinefrine (10⁻³ M) was added to the dialysis solvent. Both norepinefrine and norfenefrine caused a significant elevation of glycerol level over time (one-way ANOVA for repeated measurement: F = 18.8, p < 0.001, F = 11.3, p < 0.001, respectively), whereas the addition of urapidil had little effect on glycerol outflow (F = 1.1, p = 0.396). The kinetic profiles of glycerol concentration in the presence of norfenefrine and norepinefrine were similar. Maximal glycerol release was achieved after 1 h followed by a decline in concentration. Comparison of the two curves by two-way ANOVA for repeated measurement revealed F = 2.2, p = 0.152.

To monitor blood flow, the ethanol escape technique was used. Figure 1B shows a significant decrease of ethanol ratio after application of urapidil to adipose tissue (one-way ANOVA for repeated measurement: F = 5.5, p < 0.001), indicating a persistent enhancement of blood flow. In contrast, norepinefrine induced a significant increase in the ethanol ratio (F = 3.9, p < 0.05), which signified a decrease in blood flow. There was also a decline in blood flow in the presence of norepinefrine, although the change did not reach significance (F = 1.9, p = 0.06).

To assess whether the observed lipolytic effect of the α₁-adrenergic agonist norfenefrine could be counteracted by the α₁-adrenergic antagonist urapidil, both agents were applied to adipose tissue in combination (Fig. 2A). Two dial-

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**Fig. 1.** Effect of α₁-adrenoceptor agonist norfenefrine (△), α₁-adrenoceptor antagonist urapidil (○), and α₁,α₂-β-adrenoceptor agonist norepinefrine (□) on glycerol output and local blood flow in subcutaneous adipose tissue of 11 obese subjects. The perfusate was supplemented with ethanol, 100 mM. After a 45-min equilibrium period, dialysate was collected at 15-min intervals for 3 h. A, glycerol output. After basal measurement for 60 min, the agents were added to the perfusion solution (indicated by an arrow). The change in glycerol levels over the entire experimental period, as assessed by one-way ANOVA for repeated measurement for each agonist, was significant for norfenefrine (F = 11.3, p < 0.001) and norepinefrine (F = 18.8, p < 0.001), but not for urapidil (F = 1.1, p = 0.396). Statistical comparison of the three curves as assessed by two-way ANOVA for repeated measurement was performed with the agents and time as factors in the analysis followed by Tukey’s honestly significant difference post hoc test. The three curves were different (F = 7.02, p < 0.001); the effect of urapidil was different from the effects of norfenefrine (p < 0.001) and norepinefrine (p < 0.001); the norfenefrine and norepinefrine curves were not different. B, ethanol clearance. The percentage of ethanol in dialysate versus perfusate was determined. A low ratio indicates an escape of ethanol from the dialysate, which, in turn, reflects a rise in local blood flow. Norfenefrine increased the ethanol ratio (F = 3.9, p < 0.05), urapidil decreased the ethanol ratio (F = 5.5, p < 0.001), and norepinefrine showed no significant effect (F = 1.9, p = 0.06). All three curves were different as assessed by two-way ANOVA for repeated measurement and post hoc analysis (F = 7.3, p = 0.003). Values are mean ± S.E.M.
In the first catheter, where increasing norfenefrine were added to the perfusate. Norfenefrine increased glycerol concentration as analyzed using one-way ANOVA for repeated measurement with time as the factor of the analysis ($F = 19.4$, $p < 0.001$). The increase was significant from the 75-min fraction ($p < 0.001$). Comparison of the two curves by two-way ANOVA for repeated measurement revealed a significant reduction of glycerol concentration in the presence of urapidil ($F = 17.0$, $p < 0.001$). Blood flow decreased in the presence of norfenefrine at the highest dose ($10^{-3}$ M) compared with basal (Wilcoxon's paired test, $p < 0.05$). The effect of urapidil on increasing blood flow (one-way ANOVA for repeated measures, time 0–60 min ($F = 7.1$, $p < 0.01$), was abolished by norfenefrine ($10^{-7}$, $10^{-5}$ M) (Wilcoxon’s paired test, $p < 0.05$, time 120–150 min). Values are mean ± S.E.M.

When urapidil was perfused in combination with the different norfenefrine concentrations, there was a significant reduction in the norfenefrine-induced glycerol elevation (two-way ANOVA for repeated measures from time 60 to 240 min; $F = 19.4$, $p < 0.001$). The maximal norfenefrine effect was attained by $10^{-4}$ M (145% above basal), and this high glycerol level was sustained until the end of the experiment, including the period when $10^{-3}$ M norfenefrine was added.

In the second proba, where urapidil ($10^{-3}$ M) was applied, the $\alpha_1$-adrenoceptor antagonist increased blood flow steadily from the beginning of the experiment (0 min). This effect was sustained in the presence of norfenefrine at the lowest concentration ($10^{-8}$ M). However, the effect was abolished when norfenefrine at higher concentrations ($10^{-4}$, $10^{-3}$ M) was present. The norfenefrine-induced change in blood flow was significant ($p < 0.05$, Wilcoxon’s paired test, time 120–150 min).

To limit possible $\beta$-adrenergic interactions on norfenefrine-stimulated lipolysis, microdialysis was performed under $\beta$-blockade. In a group of nine obese subjects, four microdialysis catheters were inserted and perfused simultaneously. In three catheters each, propranolol was perfused in different concentrations ($10^{-9}$, $10^{-6}$, and $10^{-3}$ M) from the beginning of the experiment. After a 60-min period, norfenefrine ($10^{-6}$ M) was added and both agents were perfused in combination for 1 h. One catheter was perfused with norfenefrine ($10^{-6}$ M) only after a basal period of 60 min.

Norfenefrine alone and in combination with propranolol ($10^{-9}$, $10^{-6}$, $10^{-3}$ M) induced a significant increase in glycerol outflow (one-way ANOVA for repeated measures; $F = 4.2–6.7$, $p < 0.01$, time 60–120 min). Figure 3 shows that there was no significant effect of propranolol on norfenefrine-stimulated lipolysis.

The results of an investigation of the possible influence of urapidil on norepinephrine-stimulated lipolysis are depicted in Fig. 4A. Two microdialysis probes were inserted in each of nine subjects. After basal measurement for 60 min, norepinephrine in increasing concentrations ($10^{-9}$, $10^{-6}$, $10^{-3}$ M, 60 min each) was added to the perfusate. The result shows that glycerol outflow was unchanged in the presence of the lowest catecholamine concentration ($10^{-9}$ M) but increased.

**Fig. 2.** Effect of perfusion of increasing concentrations of norfenefrine ($\square$) ($10^{-6}$, $10^{-4}$, and $10^{-3}$ M) and norfenefrine ($10^{-6}$, $10^{-4}$, and $10^{-3}$ M) plus urapidil ($10^{-3}$ M) ($\bullet$) on glycerol levels in dialysate (panel A) and ethanol ratio (panel B) in human subcutaneous adipose tissue. Every 60 min increasing concentrations of norfenefrine were added to the perfusate. Norfenefrine increased glycerol concentration as analyzed using one-way ANOVA for repeated measurement with time as the factor of the analysis ($F = 9.2$, $p < 0.001$). The increase was significant from the 165-min fraction ($p < 0.05$). The effect of urapidil on increasing blood flow (one-way ANOVA for repeated measures, time 0–60 min ($F = 7.1$, $p < 0.01$), was abolished by norfenefrine ($10^{-7}$, $10^{-5}$ M) (Wilcoxon’s paired test, $p < 0.05$, time 120–150 min). Values are mean ± S.E.M.
Data suggest that it may be possible to activate thermogenesis in brown fat cells. A primary role of 1-adrenergic agents is thought to be activation of a localized presence of brown fat cells remains to be determined.

Exposure of the extracellular space of adipose tissue to the selective 1-agonist norfenefrine produced an increase in glycerol concentration in the dialysate, which was in the same range as that produced by the physiological catecholamine, norepinephrine. Both agents showed a similar kinetic response in glycerol output throughout the experiment when one single concentration was applied. Previous studies have reported that the lipolytic response to norepinephrine resulted in an increase in glycerol outflow to a peak level, followed by a decline (Arner et al., 1991). The observation that the glycerol kinetic response for norfenefrine is similar to that for norepinephrine may suggest a potential role for 1-adrenergic agents in catecholamine-stimulated lipolysis.

The concentration of glycerol in the interstitial compartment depends on fat cell metabolism and on the delivery and removal of glycerol by the microcirculation (Enoksson et al., 1995). The high level of glycerol concentration observed after norfenefrine (10^-3 M) application was due to both the release of glycerol from fat cells and changes in blood flow. Norfenefrine at high concentration (10^-3 M) exerted a modest vasoconstrictive effect, and this may have led to glycerol accumulation in the interstitial space. However, it is not clear to what extent blood flow changes were related to glycerol changes. In these experiments, when norfenefrine was administered in increasing concentrations to adipose tissue, there was significant augmentation of glycerol outflow at a norfenefrine concentration of 10^-3 M, although blood flow remained unchanged. On the other hand, an opposing effect, of increased blood flow without change in glycerol level, was observed in these experiments when the 1-agonist urapidil was applied to adipose tissue. The observation of enhanced blood flow would lead to an expectation of decreased glycerol concentration in the interstitial space. Our data indicate that the small changes in adipose tissue blood flow

**Discussion**

In the present study the effect of 1-adrenergic receptor regulation of lipolysis and blood flow was demonstrated in human subcutaneous adipose tissue in situ. This investigation was performed by continuously monitoring glycerol levels in the extracellular fluid of adipose tissue before and after administration of 1-adrenergic agents, using the microdialysis technique. Adipose tissue blood flow was assessed indirectly by measuring the ethanol escape ratio (Hickner et al., 1991; Felländer et al., 1996).

Exposure of human white fat cells to the 1-adrenergic agonist norfenefrine increased the glycerol concentration in the dialysate. This is an intriguing finding since previous studies have identified the β-type adrenergic receptor as the type responsible for elevating lipolysis (LaFontan and Berlan, 1993). 1-Adreceptors have been detected in human preadipocytes (Burns et al., 1981) and omental adipocyte membranes (Seydoux et al., 1996). They activate the phosphoinositide pathway and increase Ca^{2+} concentration. However, the physiological role of Ca^{2+} in regulation of adipocyte lipolysis is unclear. 1-Adreceptors have been identified and extensively investigated in brown fat cells. A primary role of these cells is heat production (Arner, 1992). Human adults have comparatively few brown fat cells, which produce correspondingly minor effects on whole body thermogenesis (Lean, 1989). Data suggest that it may be possible to activate

**Fig. 4.** Effect of perfusion of increasing concentrations of norepinephrine (○) (10^-9, 10^-6, and 10^-3 M) and norepinephrine (10^-9, 10^-6, and 10^-3 M) plus urapidil (10^-9 M) (●) on glycerol levels in dialysate (A) and ethanol ratio (B) in human subcutaneous adipose tissue. Every 60 min, increasing concentrations of norepinephrine were added to the perfusate. Norepinephrine increased glycerol concentration as analyzed using one-way ANOVA for repeated measurement with time as the factor of the analysis (F = 23.6, p < 0.001). The increase was significant from the 35-min fraction (Wilcoxon’s paired test, p < 0.05). Comparison of the two curves by two-way ANOVA for repeated measurement revealed a significant reduction of glycerol concentration in the presence of urapidil (F = 7.5, p < 0.02, time 120–240 min). Values are mean ± S.E.M.
were insufficient to explain the α1-agonist-induced increase in interstitial glycerol concentration.

To gain more insight into the interaction between glycerol release and blood flow after α1-adrenergic stimulation, combination agonist-antagonist experiments were performed. In the presence of urapidil, which increased blood flow, norfenefrine at low concentrations showed no effect, but interesting effects were noted for the higher concentrations, at which norfenefrine compensated for the vasodilatory effect of urapidil. Blood flow returned to the basal level, but glycerol concentration was unaffected and continued to rise to near-maximal levels. In contrast, norepinephrine was unable to compensate for the urapidil-induced increase in blood flow. This may be due to the fact that norepinephrine activates not only α1-adrenoceptors, but also β-adrenoceptors, which mediate vasodilatation. Data from the combination experiments indicate that, under these experimental conditions, blood flow and glycerol concentration may be independently regulated, with glycerol concentration measured in the interstitial space reflecting lipolysis rather than adipose tissue blood flow.

Norepinephrine and norfenefrine differed in the absolute amount of glycerol released. The dose-response curve of glycerol production in response to stimulation with norfenefrine and norepinephrine shows that norepinephrine produced a greater glycerol release at a lower applied concentration. Norfenefrine is a selective α1-agonist, whereas norepinephrine exerts its effect via nonspecific α-adrenoceptor and β-adrenoceptor stimulation. Thus, each agent acts through different receptors, and different postreceptor mechanisms. It has been suggested that the stimulatory effect of catecholamines on lipolysis is strictly related to their effect on cAMP production (Honnor et al., 1985). Norfenefrine also may potentiate adenylyl cyclase activity because it has been shown recently that α1-adrenoceptor activation increased intracellular cAMP by an indirect mechanism (Schwinn et al., 1991; Perez et al., 1993). In conclusion, these results provide evidence that the α1-adrenoceptor agonist norfenefrine increases glycerol outflow in subcutaneous adipose tissue. Local blood flow was altered by α1-adrenoceptor agents, but this was apparently not coupled to lipolysis rate. The wide use of α1-adrenoceptor agents is based on their various effects on the cardiovascular system. However, investigation of the effects of α1-adrenoceptor agents on adipose tissue lipolysis is warranted to determine their possible role as therapeutic agents in obese subjects with the metabolic syndrome.

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References


