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 norfenefrine caused an increase in glycerol level in adipose tissue that was similar to that observed with the physiologic $\alpha_1,\beta_2$-adrenoceptor agonist norepinephrine, whereas the $\alpha_1$-adrenoceptor antagonist urapidil showed no effect on basal lipolysis rate. However, the enhanced glycerol concentration due to norfenefrine and norepinephrine was suppressed in the presence of urapidil. The $\beta$-adrenoceptor antagonist propranolol showed no effect on norfenefrine-stimulated glycerol outflow. Blood flow was assessed using the ethanol escape technique. Perfusion with norfenefrine decreased blood flow, whereas urapidil enhanced blood flow significantly. Despite the increase in blood flow, the basal interstitial glycerol concentration remained unchanged. Although norfenefrine at high concentrations could inhibit the urapidil-induced increase in blood flow, the norfenefrine-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 adenylyl cyclase and cyclic AMP synthesis through G$\gamma$-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 García-Sáinz, 1983). Previous studies in rat adipocytes in vitro have provided evidence that the $\alpha_2$-adrenoceptor is involved in the control of glycogenolysis and lactate production (Faintreinie and Géloën, 1996; Lawrence and Larner, 1977), but no effect on lipolysis was observed (Faintreinie and Géloën, 1996). Clinical applications of $\alpha_2$-adrenoceptor agonists are used to treat hypertension by elevation of peripheral resistance (Hengstmann, 1986), whereas $\alpha_2$-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 depots, 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 norfenefrine ($\alpha_1$-agonist) on fat tissue. The effect of stimulation of $\alpha_1$-adrenoceptors by norfenefrine was compared with that of the physiological catecholamine norepinephrine ($\alpha_1,\alpha_2\beta$-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/m$^2$ 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 ml/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), norfenefrine (Gödecke AG, Berlin, Germany), norepinephrine (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 because of 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 norepinephrine (10−3 M) was added to the dialysis solvent. Both norepinephrine 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 norepinephrine 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, norfenefrine 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 norepinephrine, although the change did not reach significance (F = 1.9, p = 0.06).

To assess whether the observed lipolytic effect of the α1-adreceptor agonist norfenefrine could be counteracted by the α1-adreceptor antagonist urapidil, both agents were applied to adipose tissue in combination (Fig. 2A). Two dial-
Fig. 2. Effect of perfusion of increasing concentrations of norfenefrine (□) (10⁻⁶, 10⁻⁴, and 10⁻³ M) and norfenefrine (10⁻⁶, 10⁻⁴, and 10⁻³ M) plus urapidil (10⁻³ M) (●) 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 = 19.4, p < 0.001). The increase was significant from the 75-min fraction (Wilcoxon’s paired test, p < 0.05). Urapidil in combination with norfenefrine increased glycerol concentration (F = 11.7, p < 0.001). The increase was significant from the 165-min fraction (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 = 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 (10⁻⁶, 10⁻³ M) (Wilcoxon’s paired test, p < 0.05, time 120–150 min).

Values are mean ± S.E.M.

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, 10⁻⁸, 10⁻⁷ 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

Time (min)

Glycerol concentration in dialysate above basal

Fig. 3. Increase in glycerol concentration in dialysate above basal after stimulation of lipolysis with norfenefrine (10⁻⁶ M), and norfenefrine (10⁻³ M) plus propranolol (10⁻⁵, 10⁻⁴, and 10⁻³ M). Data are mean values over 1 h. The differences between the bars were not significant (F = 0.035) as judged by one-way ANOVA. Values are mean ± S.E.M.
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\[ \text{Discussion} \]

In the present study the effect of \( \alpha_1 \)-adrenergic receptor regula-

tion 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 \( \alpha_1 \)-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 \( \alpha_1 \)-adrenergic agonist norfrenefrine increased the glycerol concentration in the dialysate. This is an intriguing finding since previous studies have identified the \( \beta \)-type adrenergic receptor as the type responsible for elevating lipolysis (Lafontan and Berlan, 1993). \( \alpha_1 \)-Adrenoceptors have been detected in human pro-

eritoneal (Burns et al., 1981) and omental adipocyte membranes (Seydoux et al., 1996). They activate the phosphoinositide pathway and increase \( \text{Ca}^{2+} \) concentration. However, the physiological role of \( \text{Ca}^{2+} \) in regulation of adipocyte lipolysis is unclear. \( \alpha_1 \)-Adrenoceptors 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

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\[ \text{significantly at higher doses (} \]

\[ \text{one-way ANOVA for repeated measurement: } F = 23.6, p < 0.001. \]

In the second probe, increasing concentrations of norepinephrine (10\(^{-9}\), 10\(^{-6}\), and 10\(^{-3}\) M) were added to the perfusate in the presence of urapidil (10\(^{-3}\) M). The \( \alpha_1 \)-antagonist reduced the norepinephrine-induced increase in glycerol output (two-way ANOVA for repeated measurements: \( F = 7.5, p < 0.02, \) time 120–240 min).

Results for adipose tissue blood flow measurements are shown in Fig. 4B. Norepinephrine showed no influence on blood flow at all concentrations used. In addition, norepinephrine had no effect on the vasodilatory effect of urapidil.

Discussion

In the present study the effect of \( \alpha_1 \)-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 \( \alpha_1 \)-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).

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brown fat cells in subcutaneous adipose tissue in adult humans (Henny et al., 1988; Cinti, 1999), but whether the increase in lipolysis observed in the present study is due to the localized presence of brown fat cells remains to be determined.

Exposure of the extracellular space of adipose tissue to the selective \( \alpha_1 \)-agonist norfrenefrine produced an increment of 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 and lipolytic 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 norfrenefrine is similar to that for norepinephrine may suggest a potential role for \( \alpha_1 \)-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 norfrenefrine (10\(^{-3}\) M) application was due to both the release of glycerol from fat cells and changes in blood flow. Norfrenefrine at high concentration (10\(^{-3}\) M) exerted a modest vasocostrictive 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 norfrenefrine was administered in increasing concentrations to adipose tissue, there was significant augmentation of glycerol outflow at a norfrenefrine concentration of 10\(^{-6}\) 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 \( \alpha_1 \)-antagonist 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
were insufficient to explain the $\alpha_1$-agonist-induced increase in interstitial glycerol concentration.

To gain more insight into the interaction between glycerol release and blood flow after $\alpha_1$-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 $\alpha_1$-adrenoceptors, but also $\beta$-adrenoceptors, which mediate vasodilation. 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 increases in subcutaneous adipose tissue. Local blood flow was altered in subcutaneous adipose tissue. 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 $\alpha_1$-adrenoceptors, but also $\beta$-adrenoceptors, which mediate vasodilation. 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.

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