β-Adrenergic regulation of lipolysis and blood flow in human skeletal muscle in vivo

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Hagström-Toft, Eva, Staffan Enoksson, Erik Moberg, Jan Bolinder, and Peter Arner. β-Adrenergic regulation of lipolysis and blood flow in human skeletal muscle. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E909–E916, 1998.—Little is known about the regulation of catecholamine-stimulated lipolysis in human skeletal muscle. Therefore, β-adrenergic regulation of lipolysis and blood flow was investigated in healthy subjects in vivo by use of microdialysis of the gastrocnemius muscle. First, during a hypoglycemic, hyperinsulinemic clamp, which induces a lipolytic response in skeletal muscle tissue, the muscle was locally perfused with β-adrenoceptor blocking agents. Perfusion with nonselective (propranolol) and β2-selective (ICI-118551) blocking agents counteracted the hypoglycemia-induced lipolysis (P < 0.01), but perfusion with metoprolol (β1-blocker) did not affect the glycerol response. Second, selective β-adrenoceptor agonists were perfused in situ into skeletal muscle during resting conditions. β2-Adrenoceptor stimulation with terbutaline induced a concentration-dependent increase in skeletal muscle glycerol levels and in tissue blood flow, whereas perfusion with β1- or β3-adrenoceptor agonists (dobutamine or CGP-12177) did not influence the glycerol concentration or blood flow. In conclusion, in skeletal muscle tissue, only the β2 subtype is of importance among β-adrenoceptors for regulation of lipolysis and blood flow. This is in contrast to adipose tissue, where β1- and β3-adrenoceptors are also involved.

β-adrenoceptors; insulin; glycerol; microdialysis; hypoglycemia

MOBILIZATION OF LIPIDS from the fat stores is a key event in the regulation of endogenous energy supply to the body. Several factors are rate limiting for lipid mobilization, in particular, the rate of hydrolysis (lipolysis) of triglycerides (TG) to the end products glycerol and free fatty acids (FFA) and the blood flow in lipolytically active tissues. Skeletal muscle is the major site for utilization of FFA during exercise. Although most of these FFA originate from the circulation, it has been calculated that as much as 50% of the FFA oxidized during exercise is derived from hydrolysis (lipolysis) of intracellular TG in muscle (see Refs. 14 and 30 for reviews). Indeed, muscle tissue contains significant amounts of TG, between 10 and 50 µmol/g muscle in humans (14).

However, our knowledge about the regulation of TG metabolism in muscle is very limited. It has been observed that rodent diaphragm muscle and rodent heart muscle can release the two end products of lipolysis, FFA and glycerol (14, 30). In rat and human skeletal muscle, a lipase very similar to the adipose tissue hormone-sensitive lipase has been found (11, 21). These data indicate that a lipolytic process is present in skeletal muscle. Recent studies suggest that this process can be stimulated by catecholamines (8, 15) and inhibited by insulin (11, 15) in vivo in humans, thus hormonally regulated in the same fashion as in adipose tissue. In fat cells, catecholamines accelerate lipolysis through three β-adrenoceptor subtypes: β1, β2, and β3 (see Refs. 1, 9, 26 for reviews). Concerning the function of β-adrenoceptor subtypes in human skeletal muscle, no information is available. However, radioligand binding studies have demonstrated a dominant expression of the β2-subtype in a tissue homogenate that could be of both muscular and vascular origin (27).

The present study was undertaken to characterize the β-adrenoceptor subtypes involved in the in vivo regulation of lipolysis in muscle by use of the microdialysis technique. The major question was whether they differ from those of adipose tissue regulation. In one set of experiments, skeletal muscle was microdialyzed with selective β-adrenoceptor blockers before and during endogenous catecholamine activation by hypoglycemia. In another set of experiments, the muscle tissue was locally stimulated with receptor subtype-selective β-adrenergic agonists. Local tissue blood flow is an important regulatory factor for lipolysis in adipose tissue (1), and it has been suggested that vascular factors may modify the metabolic events in skeletal muscle (10). We therefore added a flow marker (ethanol) to the microdialysis solvent to monitor changes in tissue nutritive blood flow during the experiments (19). We report on β-adrenoceptor regulation of skeletal muscle lipolysis, which is markedly different from that of adipose tissue lipolysis.

MATERIALS AND METHODS

Subjects

Twenty-four healthy, drug-free volunteers were investigated (11 men, 13 women, 29 ± 1 yr; body mass index, 23.4 ± 0.5 kg/m²). Each subject participated in only one of the three studies except for one subject who participated, on separate occasions (4 mo apart), in both one of the clamp studies and the β-adrenoceptor agonist study. All of them performed regular physical activity but not at an athletic level. The study was approved by the Ethics Committee of Karolinska Institute, and the subjects were given a detailed description of the study before their informed consent was obtained.
Microdialysis Device

The principles of the microdialysis technique for lipolysis studies (25) and the microdialysis device (32) have been described in detail. The microdialysis catheter consists of a semipermeable polyamide membrane (30 × 0.62 mm, molecular weight cutoff 20,000), which is glued to the end of a double-lumen polyurethane tube. The microdialysis catheter is inserted percutaneously and continuously perfused. The perfusate solution enters the device through the outer lumen and streams by the membrane, where an exchange of metabolites takes place. The dialysate then leaves the catheter through the inner lumen, and samples are collected.

Estimates of blood flow variations can be made qualitatively by adding a flow marker (e.g., ethanol) to the perfusate solution (26). Ethanol is not locally degraded and does not affect tissue metabolism. The ethanol concentration is determined in the in- and outgoing perfusate, respectively, and changes in the ethanol concentration ratio (out- vs. ingoing ethanol concentration) will reflect changes in the local blood flow. This technique has been validated against the 133Xe clearance technique in skeletal muscle (19), and it can detect changes in local blood flow. This technique has been validated against the 133Xe clearance technique in skeletal muscle (19), and it can detect the changes in blood flow that occur in the area immediately surrounding the microdialysis catheter.

Study Protocol

All subjects were investigated in the supine position after an overnight fast. See the schematic figure of the study procedure (Fig. 1).

Hypoglycemic, hyperinsulinemic clamp (n=8+8)

-30 0 60
Equilibration Baseline Hypoglycemia Recovery 180 min
Insertion of microdialysis catheters
Arterialization of venous blood
Start of sampling
Perfusate: a) n=8, b) n=8
  1. Ringer 2. Propranolol 3. Ringer (0.3 µl/min)

Beta-adrenoceptor agonist stimulation, n=9

-30 0 90 180 min
Equilibration Baseline Perfusion with beta-adrenoceptor agonists
Insertion of microdialysis catheters
Agonist 10^-6 mol/l

Infusion of insulin and glucose. After superficial skin anesthesia (EMLA, Astra, Södertälje, Sweden), four microdialysis catheters were inserted percutaneously with a steel guide cannula in the medial portion of the gastrocnemius muscle, 1–2 in each leg. The penetration of the muscle fascia could easily be recognized during insertion, and the intramuscular location was additionally proved by the presence of involuntary muscular twitches during insertion. The distance between two catheters always exceeded 30 mm. The microdialysis catheters were continuously perfused with Ringer solution (147 mmol/l Na⁺, 4 mmol/l K⁺, 2.20 mmol/l Ca²⁺, 156 mmol/l Cl⁻) with or without the addition of adrenoceptor blocking agents in two subsets of experiments. First, in eight subjects, the catheters were perfused with Ringer solution without (catheter 1) or with the addition of the nonselective β-adrenoceptor blocking agent propranolol (10^-4 mol/l; catheter 2), respectively. In the second set of experiments, in eight other subjects, the perfusate was Ringer solution without (catheter 1) or with the addition of either the β₁-selective adrenoceptor blocker metoprolol (10^-8 mol/l; catheter 2) or the β₂-selective blocker ICI-118551 (10^-8 mol/l; catheter 3). These concentrations of metoprolol and ICI-118551 have, in previous microdialysis experiments on human adipose tissue, been shown to block effectively and selectively the designated receptors (2), whereas a stronger concentration would lead to a mixed blockade of more than one receptor. Furthermore, all perfusate solutions contained ethanol (50 mmol/l) for the estimation of tissue blood flow variations. The perfusate flow rate was 2 µl/min. In addition, one microdialysis catheter was perfused at 0.3 µl/min with Ringer solution alone to determine the true tissue levels of glycerol during the experiment. At this flow rate and with the presently used dialysis membrane length, recovery of glycerol has recently been shown to exceed 90%, which means that the almost true tissue levels are measured (15). The perfusate was collected in 15-min fractions.

After 45 min of baseline sampling, an intravenous insulin infusion (0.15 U/kg body wt; Isohuman Rapid, Hoechst, Germany) was infused during 60 min. After ~25-~30 min, the
concentration of arterialized blood glucose had fallen to <2.5 mmol/l, and a variable glucose infusion (200 mg/ml) was started to maintain the blood glucose concentration at 2.5 mmol/l during 30 min. Then the insulin infusion was terminated, and blood glucose was allowed to recover gradually to the fasting level during the following 120 min. Blood glucose was monitored bedside for adjustments of the glucose infusion rate (HemoCue, Angelholm, Sweden). Plasma samples were drawn in the middle of each dialysate sampling period for the analysis of plasma glycerol. Insulin and catecholamine concentrations were determined at regular intervals during the procedure.

β-Adrenoceptor stimulation in situ. Four microdialysis catheters were inserted in the calf muscle of nine fasted subjects and perfused with the Ringer-ethanol solution as described in Hypoglycemic insulin clamp. Samples were collected in 15-min fractions, and the perfusate flow rate was 2.0 µl/min. After a baseline period of 45 min, β-adrenoceptor-specific agonists were added to the perfusate of three of the microdialysis devices: the β1-selective agonist dobutamine in one catheter, the β2-selective agonist terbutaline in a second catheter, and the β3-selective agonist CGP-12177 in the perfusate of a third catheter. The initial concentration was 10⁻⁷ mol/l. After 90 min, the concentration was increased to 10⁻⁶ mol/l, and sampling was continued for an additional 90-min period. The fourth microdialysis catheter served as a control and was perfused with Ringer-ethanol solution throughout the study period.

Chemical Analysis

Dialysate glycerol was determined with an enzymatic fluorometric method, with a tissue sample analyzer (CMA/60, CMA Microdialysis, Stockholm, Sweden). In uncharted experiments, this glycerol method gave almost identical values as a bioluminescence method (17). Dialysate ethanol was determined with an enzymatic spectrophotometric method (5), and the out- vs. ingoing perfusate ratio of ethanol was calculated. Plasma glycerol was determined with bioluminescence (17), insulin in serum was determined with a commercial RIA (Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (24), and catecholamines were determined by high-performance liquid chromatography (16). The coefficients of variation in the basal state (t = −22.5 min to −7.5 min) were 11.4 ± 3.5% for ethanol, 7 ± 2% for dialysate glycerol, 8.5 ± 3.5% for plasma glycerol, 6.3 ± 1.6% for insulin, and 11.7 ± 2.7% for norepinephrine. The basal concentrations for epinephrine were below the method's limit of detection for the majority of the subjects.

Statistics

Data are presented as means ± SE. Comparisons between the local glycerol responses to the different drugs were performed over time, with a two-factor analysis of variance (ANOVA). Differences between time segments were evaluated by comparing the areas under the glycerol curves (AUC) with the Student’s paired t-test with Bonferroni correction of the P value. For statistical evaluation of changes in the ethanol outflow vs. inflow ratio variations over time in the individual, one-factor ANOVA for repeated measurements was used. The different time points were compared with post hoc analysis of the one-factor ANOVA (Fisher’s protected least significant difference test). A software package was used for all statistical calculations (Statview SE + Graphics V. 1.05, Abacus Concepts, Berkeley, CA).

RESULTS

Hyperinsulinemic, Hypoglycemic Clamp

The plasma and near-true (i.e., >90% recovered) skeletal muscle tissue glycerol concentrations are shown in Fig. 2. In the basal, fasted state, the tissue glycerol concentration was almost twice that in plasma. During insulin infusion, there was an initial decrease in glycerol, followed by an increase starting after 30–45 min in plasma and muscle (ANOVA repeated measurement, P < 0.0001). The plasma glycerol concentration increased throughout the 3-h study, whereas the skeletal muscle tissue concentration peaked after 2 h and decreased toward basal levels at the end of the study. Throughout the experiment, the level of muscle tissue glycerol was higher than the respective plasma concentration (two-factor ANOVA, P = 0.0001). The plasma glucose and insulin concentrations and the catecholamine concentrations during the experiment are presented in Table 1. Plasma glucose concentrations de-
creased gradually during the first 30 min of the insulin infusion and remained at the hypoglycemic level during the infusion. After the insulin infusion was stopped, the glucose levels gradually returned to the initial concentrations during the remaining 2 h of the study. The peak value of norepinephrine and epinephrine occurred 50–70 min after the insulin infusion was started.

The effects of local β-adrenoceptor blockade are depicted in Figs. 3 and 4. The addition of propranolol to the perfusate largely prevented the glycerol increase after hypoglycemia, unlike in the control (no blocker in perfusate) (two-factor ANOVA, \( P = 0.007 \), Fig. 3A). The baseline glycerol levels in the experiments were 30 ± 4 and 31 ± 3 µmol/l (nonsignificant) for the perfusion with Ringer solution and propranolol, respectively. These values are much lower than those in Fig. 2 (~90 mmol/l). The difference is because of the perfusion rates.

Fig. 3. Effect of β-adrenoceptor blocking agents on skeletal muscle glycerol concentrations during insulin-induced hypoglycemia. Microdialysis catheters were implanted in gastrocnemius muscle and perfused at 2 µl/min during a 60-min hyperinsulinemic, hypoglycemic clamp. A: perfusion with Ringer solution alone (○) and nonselective β-adrenoceptor blocker propranolol (●). Data are % of baseline concentrations of glycerol (n = 8 subjects). Values are means ± SE. P value for 2-factor ANOVA analysis is depicted. B: effects of β1-adrenoceptor blocker metoprolol (□), β2-adrenoceptor blocker ICI-118551 (●), and perfusion with Ringer solution alone (○) are shown in 8 subjects. P value, Student’s paired t-test for AUC Ringer vs. AUC ICI-118551.

In the subset of experiments using selective β-adrenoceptor blocking agents, it was seen that the glycerol response when the β1-blocker metoprolol was added did not differ from the control (Ringer solution alone). However, when ICI-118551, the selective β2-blocker, was perfused, the increase in glycerol after hypoglycemia was markedly attenuated (AUC ICI 17,963 ± 1,412 µmol·l⁻¹·min vs. AUC Ringer 21,768 ± 1,846 µmol·l⁻¹·min, \( P = 0.02 \)) (Fig. 3B).
The ethanol clearance during hypoglycemia and \( \beta \)-adrenoceptor stimulation is shown in Fig. 4. With the use of this technique, the local skeletal muscle blood flow was found to be unaltered during hypoglycemia (\( P = 0.7–1.4; P = 0.2–0.7 \) by one-factor ANOVA), because the ethanol ratio was almost constant throughout the experiment in the control state (perfusion with Ringer solution alone). However, the addition of propranolol or ICI-118551 resulted in a significant increase in ethanol outflow vs. inflow ratio during and after hypoglycemia, indicating a decrease in blood flow (propranolol, \( P = 0.03 \); ICI-118551, \( P = 0.007 \)). Metoprolol did not alter the ethanol ratio (\( P = 0.98 \), Fig. 4, A and B).

Selective \( \beta \)-Adrenoceptor Stimulation in Situ

When terbutaline, the \( \beta_2 \)-selective adrenoceptor agonist, was perfused, there was a concentration-dependent increase in the skeletal muscle-derived glycerol concentration (one-factor ANOVA, \( P = 0.0001 \), Fig. 5). The glycerol concentration when perfusing at the lower agonist concentration was significantly lower (\( \approx 125\% \) increase over baseline) than at the higher agonist concentration (\( \approx 150\% \) increase above baseline; \( P < 0.05 \)). The tissue-derived glycerol when dobutamine or CGP-12177 was perfused did not differ from glycerol concentrations when Ringer solution alone was perfused. The glycerol concentrations in the experiment were also evaluated statistically, with AUC for the respective glycerol AUC when Ringer solution was fused. The glycerol concentrations when Ringer solution alone was perfused (AUC 0–180 min: dobutamine, 14,083 ± 602\%·min; CGP-12177, 15,414 ± 1,538\%·min; Ringer, 13,623 ± 620\%·min).

The data representing local tissue blood flow are shown in Fig. 6. The ethanol ratio showed a concentration-dependent decrease when terbutaline was added to the perfusate, indicating an increased tissue blood flow with increasing concentrations of terbutaline (one-factor ANOVA, \( P = 0.001, 10^{-6} \) vs. \( 10^{-5} \) mol/l, \( P < 0.05 \), with post hoc analysis). The \( \beta_1 \)- and \( \beta_3 \)-adrenoceptor agonists did not affect the ethanol ratio significantly compared with the control solvent in which ethanol ratio remained almost constant throughout the experiment.

DISCUSSION

In this study, the \( \beta \)-adrenergic regulation of lipolysis in skeletal muscle has been investigated for the first time in vivo. Two different methods have been used, both employing microdialysis, which is an excellent tool for pharmacological investigation of lipolysis regulation, as discussed in detail (25).

In the first type of experiment, endogenous catecholamine production was enhanced by hypoglycemia. When muscle tissue concentrations of glycerol were monitored with the high recovery catheter (>90%), a marked increase in tissue glycerol was observed after hypoglycemia and endogenous elevation of catecholamines. The glycerol concentrations were much higher than in blood (twice as high at rest and thrice as high at the peak response levels to hypoglycemia). These data confirm earlier experiments (15) and indicate a marked basal lipolytic activity in muscle that is stimulated by endogenous catecholamines. Muscle tissue lipolysis has been evidenced previously by the finding of reduced TG content after norepinephrine infusion and exercise (14, 30).

Fig. 5. Effect of \( \beta \)-adrenoceptor agonists on skeletal muscle tissue lipolysis. Four microdialysis catheters were implanted in gastrocnemius muscle in 9 healthy subjects and perfused at 2 \( \mu \)l/min with Ringer-ethanol solution. After 45 min of baseline sampling, increasing concentrations of selective \( \beta \)-adrenoceptor agonists were added to the perfusate: \( \beta_1 \)-agonist dobutamine (●), \( \beta_2 \)-agonist terbutaline (●), and \( \beta_3 \)-agonist CGP-12177 (○). Fourth catheter served as a control and was continuously perfused with Ringer-ethanol solution (○). Values are means ± SE. P values for the comparison of AUC terbutaline vs. AUC Ringer with Student’s paired t-test at 2 terbutaline concentrations are depicted.

Fig. 6. Effect of increasing concentrations of selective \( \beta \)-adrenoceptor agonists on variations in tissue blood flow, assessed by ethanol outflow-inflow ratio. Microdialysis catheters were implanted and perfused, as described in Fig. 5 legend. Relative changes in ethanol out-in ratio are shown during perfusion with Ringer solution alone (○), with \( \beta_1 \)-agonist dobutamine (●), the \( \beta_2 \)-agonist terbutaline (●), and \( \beta_3 \)-agonist CGP-12177 (○), respectively. P value for one-factor ANOVA analysis of variations in ethanol outflow-inflow ratio with terbutaline perfusion.
Glycerol is an end product of lipolysis. In theory, it could be formed by extracellular hydrolysis of TG through the action of lipoprotein lipase and the lipolysis of intracellular TG through the action of hormone-sensitive lipase (or it could even be derived from some other glycerol-containing molecule, such as phospholipids). It is very unlikely that extracellular lipolysis is of importance in the present experiments, because microdialysis data with glycerol in adipose tissue and skeletal muscle show similar kinetic patterns during the present type of hypoglycemic provocation (15). The initial decrease in tissue glycerol in response to insulin infusion was most prominent in adipose tissue, indicating a more pronounced antilipolytic effect of insulin in fat cells compared with muscle tissue. Consequently, the subsequent lipolytic effect of catecholamines appeared earlier in muscle than in adipose tissue but was also more transient (15).

In vitro studies of experimental animals have pointed to β-adrenergic involvement in skeletal muscle lipolysis (14), but which β-adrenoceptor subtype is responsible for lipolysis in muscle after catecholamine stimulation? In the present investigation, this question was answered by performing microdialysis under conditions of local β-adrenoceptor blockade. We used selective or nonselective β-adrenoceptor blockers in concentrations known from previous microdialysis experiments in human muscle to block effectively the designated receptor(s) (2). Probably, the β2-adrenoceptor subtype is the only one of importance. First, a high concentration (10^-4 mol/l) of propranolol (nonselective β-blocker) almost completely prevented the lipolytic response, and second, when selective β-adrenoceptor blockade was used, the lipolytic response to hypoglycemia could be counteracted by a β2-adrenoceptor blocker (ICI-118551) but not by a β1-adrenoceptor blocker (metoprolol). These data clearly demonstrate the unique involvement of β2-adrenoceptors in the regulation of skeletal muscle lipolysis. Unfortunately, no selective β2-adrenoceptor blocker is available for human use in vivo. However, this receptor is probably absent in human skeletal muscle, because no mRNA for the β3-receptor gene has been found (22).

Is glycerol in the muscle tissue dialysate during rest and hypoglycemia derived from the muscle cells or from adipocytes in the muscle tissue? The time course for glycerol in muscle and blood differed markedly. During hypoglycemia, the difference in glycerol kinetics between skeletal muscle and blood (the latter compartment probably reflects overall lipolysis in both fat and muscle), respectively, strongly suggests that the myocyte is the real source of glycerol. It is very unlikely that glycerol kinetics in fat cells of muscle tissue differ from the kinetics in fat cells in adipose tissue, because glycerol is a water-soluble molecule that easily passes through tissues.

The involvement of β-adrenoceptor subtypes in muscle lipolysis regulation was further examined in the second type of experiments by perfusing the tissue with increasing concentrations of dobutamine (β1-adrenoceptor agonist), terbutaline (β2-adrenoceptor agonist), and CGP-12177 (β2-adrenoceptor agonist), respectively. These data show that only terbutaline influenced lipolysis, which occurred in a concentration-dependent fashion. The findings further support the view that the β2-adrenoceptor is the only receptor subtype of importance for the adrenergic regulation of lipolysis in skeletal muscle. This view is also strengthened by the previous investigations of radioligand binding to extracts from human skeletal muscle, showing a predominance of β2-receptors (27). The in vivo findings with agonists differ markedly from lipolytic microdialysis studies of human adipose tissue. With the use of the same agonists and antagonists as in the present study at the same concentrations, Arner et al. (2), Enoksson et al. (13), and others (4) found that all three β-adrenoceptor subtypes regulate lipolysis in fat. It can be ruled out that the tissue difference in response to β-adrenoceptor stimulation is because of bioavailability. In earlier adipose tissue experiments, the same type of microdialysis catheters were used as in the present microdialysis experiments. Furthermore, the drugs used mediate their action through cell surface receptors and do not have to enter the cells.

We also examined the adrenergic regulation of the nutritive blood flow in skeletal muscle tissue, because, in fat cells, it is evident that tissue blood flow is involved in the regulation of lipolysis (1). Vasomotor control is usually accompanied by a decreased rate of lipolysis in adipocytes, and the opposite is true for vasodilation. Catecholamine stimulation may also induce vasodilation via vasoactive lipolytic products and not only directly via adrenoceptors in the vessels (1). During the hypoglycemic experiment, propranolol and ICI-118551 inhibited β2-adrenoceptor-mediated vasodilation, leading to an increase in the ethanol outflow vs. inflow ratio above baseline, indicating vasoconstriction. On the contrary, metoprolol had no effect on the tissue flow. In the agonist experiments, terbutaline caused a concentration-dependent vasodilation, whereas dobutamine and CGP-12177 were ineffective regarding effects on blood flow. These data indicate that the β2-adrenoceptor is the major adrenoceptor subtype of importance for the regulation of blood flow in human skeletal muscle. On the other hand, it must be pointed out that the presently used ethanol-microdialysis technique cannot detect changes in muscle blood flow that are <50% (18). Thus some involvement of other β-adrenoceptor subtypes in the regulation of muscle blood flow cannot be excluded. The β-adrenoceptor-mediated changes in blood flow cannot explain the findings of attenuated glycerol during hypoglycemia, because restricted vasodilation would instead retain the glycerol in the tissue and, thus, increase the glycerol levels. It should be noted that α-adrenoceptors also might be of importance for the regulation of blood flow and lipolysis in skeletal muscle tissue, because in adipose tissue the hypoglycemia-induced lipolysis seems to be regulated by both α- and β-adrenoceptors (8, 20). However, the α-adrenoceptors were not the focus of this study. The importance of local blood flow for the tissue glycerol level is clarified by the findings in Figs. 5 and 6. When the terbutaline...
concentration was increased from $10^{-6}$ mol/l to $10^{-5}$ mol/l, there was only a small (but statistically significant) increase in the glycerol concentration from about 125% to about 150% of baseline, suggesting only a minor effect of a 10-fold increase in terbutaline concentration on the glycerol output from surrounding cells. However, at the same time, the blood flow rate was further increased by the change in terbutaline concentration. This increase in blood flow probably enhanced the removal of glycerol from the interstitial space of muscle tissue, as in the case of the effect of blood flow on glycerol levels in adipose tissue (12). Therefore, the true effect of increased terbutaline concentration on glycerol output is underestimated from mere examination of the changes in glycerol concentration.

The dual-stimulatory role of the $\beta_2$-adrenergic receptor in lipolysis and blood flow gives this receptor a particular role in the regulation of energy metabolism in muscle. During catecholamine stimulation, lipids can be rapidly mobilized in the muscle through $\beta$-adrenoceptor-mediated lipolysis, in combination with $\beta_1$-receptor-mediated vasodilation. With the microdialysis technique, it is unfortunately not possible to measure FFA because of their hydrophobic character. Hence, the present data cannot show the proportion of FFA mobilized from the tissue to that remaining for oxidation in the myocyte. It seems reasonable that FFA in some part are mobilized from the tissue in the nonexercising state (14, 30), but the role of this kind of energy mobilization from a tissue other than the major energy store, adipose tissue, remains unclear. It must, furthermore, be emphasized that our findings are based on examinations of the calf muscle. It is possible that lipolysis and blood flow are regulated differently in other muscle groups. For example, studies using the forearm muscle suggest involvement of $\beta_3$-adrenoceptors in blood flow regulation (6). Gender differences may also be of importance; however, the number of subjects in the present studies was too small for statistical evaluation of sex differences. Finally, the $\beta_2$-adrenergic receptor may also be involved in other catecholamine-regulated metabolic events in skeletal muscle, such as thermogenesis and glucose metabolism (3).

A number of studies suggest that the triglyceride content of muscle is regulated by catecholamines under physiological situations, such as exercise (14). A pathophysiological role of muscle TG has also been suggested, because there is an inverse relationship between the muscle TG content and the peripheral insulin sensitivity (31). The present study shows that the $\beta_2$-adrenergic stimulation of lipolysis in muscle (presumably because of hydrolysis of intracellular TG in myocytes) involves only $\beta_2$-adrenoceptors, as opposed to $\beta_1$-, $\beta_3$- and $\beta_3$-adrenoceptors in adipose tissue. Taken together, previous and present data point to an important role of lipolysis in muscle for energy metabolism in normal and pathophysiological conditions, which may be different from that in adipose tissue.

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