Demonstration of an In Vivo Functional β_3-Adrenoceptor in Man

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Abstract

Although it is well established in several mammalian species that β_3-adrenoceptors play a major role in regulating lipolysis and thermogenesis in adipose tissue, the functional existence and role of this receptor subtype in man has been controversial. We investigated whether the β_3-adrenoceptor functionally co-exists with β_1- and β_2-adrenoceptors in vivo in human adipose tissue. Subcutaneous abdominal adipose tissue of healthy non-obese subjects was microdialyzed with equimolar concentrations of dobutamine (selective β_1-adrenoceptor agonist), terbutaline (selective β_2-adrenoceptor agonist), or CGP 12177 (selective β_3-adrenoceptor agonist). All three agents caused a rapid, sustained, concentration-dependent and significant elevation of the glycerol level in the microdialysate (lipolysis index). However, only terbutaline stimulated the nutritive blood flow in adipose tissue, as measured by an ethanol escape technique. Dobutamine and CGP 12177 was equally effective in elevating the glycerol level (maximum effect 150% above baseline). Terbutaline was significantly more effective than the other two β-agonists (maximum effect 200% above baseline). When adipose tissue was pretreated with the β_1/β_2-selective adrenoceptor blocker propranolol the glycerol increasing effect of dobutamine or terbutaline was inhibited by 80–85% but the glycerol response to CGP 12177 was not influenced. It is concluded that a functional β_3-adrenoceptor is present in vivo in man. It co-exists with β_1- and β_2-adrenoceptors in adipose tissue and may therefore play a role in lipolysis regulation. It appears, however, that the β_2-adrenoceptor is the most important β-adrenoceptor subtype for the mobilization of lipids from abdominal subcutaneous adipose tissue because of its concomitant stimulatory effect on lipolysis and blood flow. (*J. Clin. Invest.* 1995. 95:2239–2245.) Key words: lipolysis • adipose tissue • catecholamines • glycerol • blood flow

Introduction

Catecholamines were initially thought to act through two β-adrenoceptor subtypes, defined as β_1- and β_2-adrenoceptors (1). However, the introduction of an increasing number of selective β-adrenoceptor ligands has led to the conclusion that this early classification is insufficient. Recently a third atypical β-adrenoceptor subtype has been isolated by molecular cloning (2–5) and the role of this receptor in catecholamine action should also be considered. Studies on laboratory animals have now established that a number of tissues express a third β-adrenoceptor subtype. For example, the thermogenic brown adipocyte β-adrenoceptor can be differentiated from classical β_1- and β_2-adrenoceptors (6). Furthermore, lipolysis in white adipocytes of several species is predominantly mediated by the β_3-adrenoceptor (7, 8). In man, the presence of a biologically active β_3-adrenoceptor has been controversial. In spite of this a number of β_3-adrenoceptor agonists have been developed and their potential role for treating obesity has been tested in clinical trials (9).

Earlier in vitro studies of lipolysis in isolated human subcutaneous fat cells or tissue have revealed little or no lipolytic activity mediated by the β_3-adrenoceptor (10–13). However, several groups have clearly demonstrated that β_3-adrenoceptor mRNA is expressed in human fat cells obtained from living donors (14–16). Furthermore, the existence of a functional β_3-adrenoceptor involved in the regulation of lipolysis in human omental fat cells has recently been demonstrated in vitro (17). The latter study showed that CGP 12177, but not the "classical" β_3-adrenoceptor agent BRL 37344 used in the earlier human studies, is a selective β_3-adrenoceptor agonist in isolated human fat cells. CGP 12177 is a useful tool in β_3-receptor studies, since it is not only a non-selective β_1- and β_2-adrenoceptor blocker but also has β_3-agonist activity (9). Since a β_3-adrenoceptor may be involved in the pathophysiology of obesity and diabetes it is necessary to find a functional role for this receptor subtype in vivo in order to establish firmly its importance in man.

In the present study, we have used the microdialysis technique (18) to investigate β-adrenoceptor subtype function in human adipose tissue in vivo. The microdialysis method is based on continuous monitoring of glycerol (lipolysis index) in the extracellular water space of adipose tissue. The nutritive blood flow in the tissue surrounding the microdialysis probe can also be measured by the clearance of ethanol from the perfusion media (19, 20). It is important to consider blood flow in lipolysis experiments because of its potential effects on lipid mobilization from adipose tissue (20, 21). With the microdialysis method we compared the effects of the selective adrenergic agonists dobutamine (β_1-selective), terbutaline (β_2-selective), and CGP 12177 (β_3-selective) on lipolysis and nutritive blood flow in situ in subcutaneous abdominal adipose tissue in non-obese healthy subjects.

Methods

Subjects. The study group undergoing microdialysis comprised healthy and drug-free non-obese volunteers (6 men and 14 women) who were aged 19 to 57 years (mean 34 yr). Some were investigated two or three times, but with a different agonist each time. Body mass index (kg/

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m^2) ranged from 18.4 to 24.8 (mean 21.2). They were investigated the morning after an overnight fast. Some methodological in vitro studies on isolated fat cells were also performed. For this purpose, subjects undergoing elective abdominal surgery were investigated. None of them had jaundice, metabolic disorder, or malignant disease. No attempt was made to select the latter subjects on the basis of age, sex, or body weight. No one had received any form of regular pharmacotherapy during the 6 mo prior to the study. All subjects undergoing elective surgery had fasted overnight. Biopsies of abdominal subcutaneous adipose tissue were obtained during the operation, which was performed in the morning. General anesthesia was induced by a short-acting barbiturate and maintained by fentanyl and a mixture of oxygen and nitrous oxide. Only saline was given intravenously until the tissue specimen had been removed. The study was approved by the Ethics Committee at the hospital. The subjects were given a detailed description of the experiments and their consent was obtained.

Microdialysis protocol. The microdialysis probe and the details of the microdialysis experiments have been described previously (22, 23). The probe consists of a dialysis tubing (10 × 0.5 mm; 20 kD molecular weight cut-off), which is glued to the end of a double-lumen steel cannula. An ethanol based perfusion solvent enters the probe through the inner cannula, streams upwards in the space between the inner cannula and the outer dialysis membrane and leaves the probe through the outer cannula. Glycerol and ethanol are determined in the perfusate. The former is used as an index of lipolysis and the ratio of ethanol out/in is used for the evaluation of nutritive blood flow, as discussed (18). The subjects were investigated in the supine position at 8 a.m. after an overnight fast. The dialysis probe was inserted percutaneously without anesthesia into the abdominal s.c. adipose tissue immediately to the right or left of the umbilicus. Two or three dialysis probes were used simultaneously in each experiment. The distance between adjoining probes was ~ 30 mm. The probe was connected to a microinjection pump and was continuously perfused (1.5 μl/min) with Ringer's solution (sodium 1.5×10^{-1} mol/liter; potassium 4×10^{-3} mol/liter, calcium 2.3×10^{-3} mol/liter; chloride 1.6×10^{-1} mol/liter) supplemented with 50 mmol/liter of ethanol. The following agents in a sterile solution were added to the basal dialysis solvent after 1 h of microdialysis: terbutaline (selective β_2-adrenoceptor or agonist), dobutamine (selective β_1-adrenoceptor agonist), and CGP 12177 (selective β_3-adrenoceptor agonist).

In each experiment, 15-min fractions of the outgoing dialysate were collected, unless otherwise stated. The first fraction was excluded because there is a transient rise in the concentration of metabolites in the outgoing dialysate during the first part of dialysis, which probably reflects the initial trauma when the dialysis probe is inserted into the adipose tissue, as discussed in detail elsewhere (18). In the present microdialysis conditions, the in vivo recovery of glycerol is ~ 10% (24).

Previous experiments have clearly demonstrated that the glycerol levels in the dialysate and the ethanol ratio are constant for several hours when microdialysis is performed under basal conditions in the supine position at rest (20, 23, 24).

Studies on isolated fat cells. Adipose tissue obtained during surgery was immediately transported to the laboratory in saline. The fat cells were isolated by Rodbell's method (25) and used for a lipolysis assay that has previously been described in detail (26). Briefly, isolated fat cells were incubated at a final concentration of 2% (vol/vol) in Krebs-Ringer phosphate buffer (pH 7.4). The medium contained 20 g/l bovine serum albumin, 1 gram/liter glucose and 0.1 gram/liter ascorbic acid as well as various concentrations of β-adrenergic ligands (dobutamine, terbutaline, CGP 12177, the nonselective β-adrenergic agonist isoprenaline, the β_1-β_2-selective antagonist propranolol and the β_1-selective antagonist metoprolol). All ligands were added simultaneously at the start of the incubation. Each incubation was run in duplicate for 2 h at 37°C, with air as the gas phase. The release of glycerol to the medium at the end of incubation was used as an index of lipolysis and was expressed per gram of lipid. The total lipid content of the incubated fat cells was measured gravimetrically after organic extraction (27). The concentration of agonists that produced a half-maximum effect (EC_{50}) was calculated from linear conversion of the concentration–response curve, as described previously (28), and expressed as pD_2 (-log mmol/liter for EC_{50}). Antagonist potencies were evaluated by calculating their pA_2 values (-log mol/liter). Concentration-response curves for agonists were constructed from experiments made in the absence or the presence of 3 or 4 different concentrations of each antagonist, and dose ratios for each antagonist concentration were calculated from the EC_{50} values thus obtained. Schild plots were constructed by the method of Arunlakshana and Schild (29). The slopes of Schild plots determined by linear regression analysis did not differ from 1 ($P > 0.05$, Student's t test). The slopes were constrained to 1 for estimation of pA_2 values (30). The maximum lipolytic effect of each agonist was calculated and related to the maximum effect of the full agonist isoprenaline. This relation is termed intrinsic activity. Isoprenaline caused a 5–6-fold elevation of the basal rate of lipolysis at the maximum effective concentration.

Table I. Pharmacological Properties of Selective β-Adrenoceptor Agonists in Abdominal Subcutaneous Fat Cells

Agonist	pD_2	IA	Metoprolol pA_2	Propranolol pA_2
Dobutamine	7.1±0.3	1.06±0.11	7.9±0.2§	8.6±0.5
	(n = 14)		(n = 8)	(n = 5)
Terbutaline	6.7±0.2	1.06±0.15	6.4±0.2*	8.9±0.2
	(n = 12)		(n = 6)	(n = 5)
CGP 12177	7.0±0.2	0.34±0.08‡	5.7±0.2‡	6.2±0.1‡
	(n = 14)		(n = 8)	(n = 6)
ANOVA	NS	<0.001	<0.001	<0.001

Values (mean±SE) were obtained from in vitro lipolysis experiments, which were conducted as described in Methods. n = number of experiments. * $P < 0.05$ compared with CGP 12177, ‡ $P < 0.001$ compared with dobutamine and terbutaline, § $P < 0.001$ compared with terbutaline. *IA*, intrinsic activity relative to the maximum effective lipolytic action of isoprenaline, which was determined from experiments performed in parallel with the experiments with selective agonists. In the whole material (n = 14) maximum isoprenaline effect was 587±61% stimulation of the basal rate of glycerol release.

Analysis of glycerol. 15 μl of perfusate or 50 μl of fat cell incubation medium were used for the analysis of glycerol. An automatic ultrasensitive kinetic bioluminescent assay for glycerol (31) and a bioluminescent analyzer were used for the assay.

Analysis of ethanol. 5 μl of dialysis solvent or perfusate were employed to measure ethanol, as described (20). The ratio of ethanol concentration in the outgoing and ingoing dialysis solvents was determined whereby the nutritive blood flow could be evaluated. It has been shown that changes in this ratio indirectly reflect changes in the nutritive blood flow of the tissue surrounding the microdialysis probe (19, 20).

Drugs and chemicals. Bovine serum albumin (fraction V) and crude collagenase (Clostridium histolyticum type I) were from Armour Pharmaceutical Company (Eastbourne, UK) and Sigma Chemical Company (St. Louis, MO), respectively. Ethanol and Ringer's solution were obtained from the Central Wine and Spirits Company (Stockholm, Sweden) and Pharmacia, respectively. Dobutamine and terbutaline were obtained from Lilly (Indianapolis, Indiana) and Draco (Lund, Sweden), respectively. The following drugs were gratefully received as gifts: propranolol (Zeneca, MacCesfield, UK), CGP 12177 (SmithKline Beecham, Epsom, UK), metoprolol (Hässle, Mölndal, Sweden), and isoprenaline (Draco, Lund, Sweden). The adrenergic agents used in the microdialysis experiments were stored as stock-solutions at −70°C. The same batches were used throughout the study.

Statistical analysis. Values are mean±SE. The Student's t test or

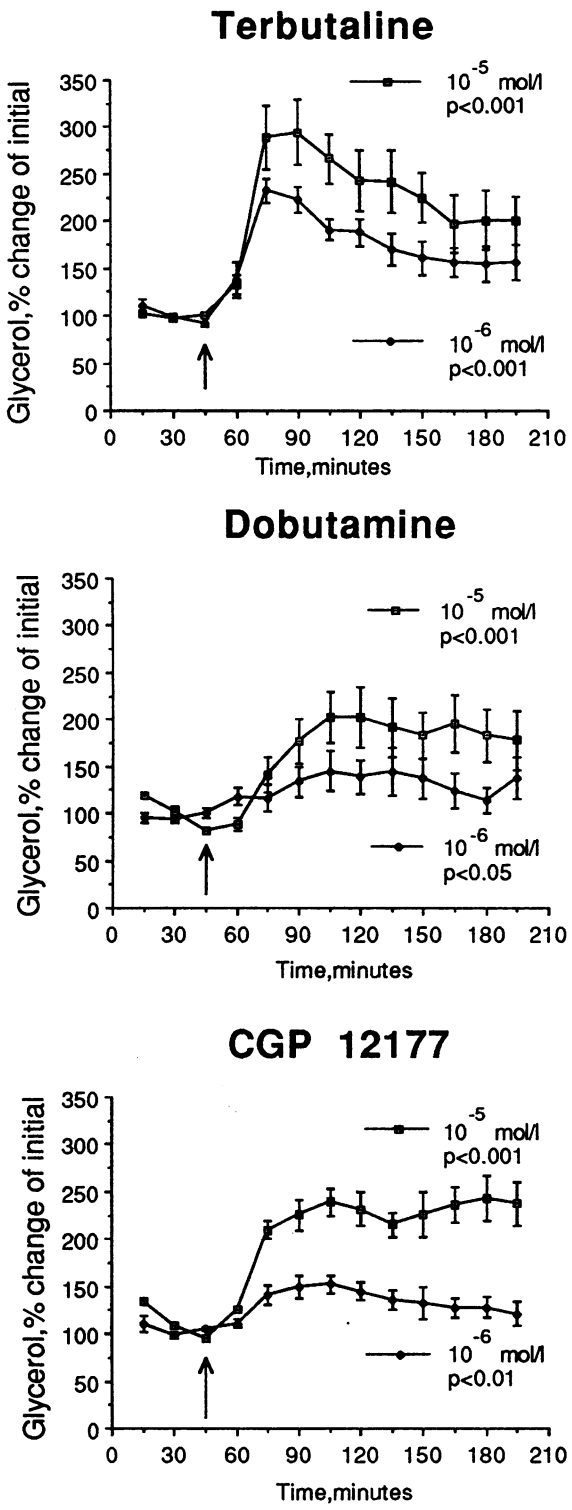

Figure 1. Effect of the selective β-adrenoceptor agonists dobutamine terbutaline, and CGP 12177 on the glycerol level in adipose tissue. Adipose tissue was microdialyzed for 195 min and glycerol was determined in the perfusate which was collected at 15-min intervals. 10^{-6} mol/liter or 10^{-5} mol/liter of the agonist was added after 45 min, indicated by an arrow. The mean glycerol level during the 45-min pretreatment period was calculated. All individual fractions were expressed as a percentage of this value: 22±3 μmol/liter in the experiments with 10^{-6} mol/liter of dobutamine (n = 7); 19±3 mmol/liter in he experiments with 10^{-5} mol/liter of dobutamine (n = 6); 23±2 μmol/liter in the experiments with 10^{-6} mol/liter of terbutaline (n = 6); 26±3 μmol/liter in the experiments with 10^{-5} mol/liter of terbutaline (n = 6); 33±4 μmol/liter in the experiments with 10^{-6} mol/liter of CGP 12177 (n = 7); and 27±2 μmol in the experiments with 10^{-5} mol/liter of CGP 12177 (n = 7). These values did not differ significantly. The change in glycerol over the whole experimental period was evaluated by one way ANOVA for each agonist concentration using the absolute glycerol values. The statistical results and symbols are given in the graph. A statistical comparison of the two agonist concentrations was also made on values for the whole time course (testing dose vs. time with ANOVA). The concentration dependency was statistically significant ($P < 0.001$) for all three agonists. Values are mean±SE.

analysis of variance (ANOVA) with Fisher's PLSD test for posthoc analysis were used for the statistical comparisons.

Results

The selectivity of the β-adrenergic agonists was investigated on isolated fat cells from the abdominal subcutaneous region by determining their pD_2 values and the pA_2 values for the agonists versus β-adrenergic antagonists (Table I). These methodological experiments could not be performed using microdialysis because the pharmacological design required a vast number of combinations between agonists and antagonists. Dobutamine, terbutaline, and CGP 12177 caused concentration-dependent stimulation of glycerol release in vitro (graph not shown). The pD_2 values for all three agonists was ~ 7. The pA_2 values for propranolol on terbutaline- or dobutamine-induced lipolysis were high (~ 9) and did not differ, whereas the pA_2 values for propranolol on CGP 12177 were much lower (~ 6) and differed significantly from the values obtained with terbutaline or dobutamine ($P < 0.001$). This indicates that CGP 12177 acts on a receptor different from that of dobutamine or terbutaline. Metoprolol had a 1.5 log unit higher pA_2 value for dobutamine-induced lipolysis than terbutaline-induced lipolysis ($P < 0.001$) and a 0.7 log unit higher pA_2 value for terbutaline-induced lipolysis than CGP 12177-induced lipolysis ($P < 0.05$). Thus, terbutaline acts on a receptor different from that of dobutamine and CGP 12177. Terbutaline and dobutamine were full agonists relative to isoprenaline whereas CGP 12177 was a partial agonist, since the intrinsic activity of CGP 12177 was only 0.34. This confirms earlier data on fat cells from the same adipose region (17).

Fig. 1 depicts the results with microdialysis experiments where the action of the β-adrenoceptor selective agents dobutamine (β_1), terbutaline (β_2), and CGP 12177 (β_3) on the glycerol level in adipose tissue was investigated. When 10^{-6} or 10^{-5} mol/liter of these agents was added to the dialysate, a significant, rapid, concentration-dependent, and sustained increase in the perfusate glycerol level was observed. The maximum effect of the two concentrations was calculated in the various experiments. These effects occurred at slightly different times in the individual experiments. The mean glycerol level in the dialysate level during the 45-min pretreatment period was used as baseline value. At the lowest concentration dobutamine, terbutaline and CGP stimulated the glycerol level by 63±24, 137±11, and 60±10%, respectively. The terbutaline effect was significantly different from the effects obtained with dobutam-

ine or CGP 12177 ($P < 0.01$ by ANOVA). The corresponding values at the highest agonist concentration were 133±32, 206±38, and 152±22%. Again the terbutaline values differed from the values obtained with dobutamine and CGP 12177 ($P < 0.01$ by ANOVA). The values for the two different concentrations of dobutamine, terbutaline and CGP 12177 differed significantly ($P < 0.01$ or better using Student's t test).

In uncharted experiments it was observed that the ability of the adrenergic agonists to increase the glycerol level was reversible. Thus, when adrenergic agonists were removed from the dialysis solvent the glycerol level in the microdialysate gradually fell towards the baseline.

The results with blood flow are depicted in Fig. 2. Neither dobutamine nor CGP 12177 influenced the ethanol ratio. The same was true of the low concentration of terbutaline. At 10^{-5} mol/liter, however, terbutaline caused a rapid, marked and sustained decrease in the ethanol ratio, which was significant. The latter data indicate a stimulation of nutritive blood flow. The curves for ethanol ratio obtained with 10^{-6} and 10^{-5} mol/liter of terbutaline differed significantly.

To investigate further the selectivity of the action of CGP 12177 in vivo, adipose tissue was microdialyzed with β-adrenergic agonists after pretreatment of the tissue for 1 h with 10^{-5} mol/liter of propranolol (Fig. 3). CGP 12177 (10^{-5} mol/liter) caused a marked, rapid, significant and sustained increase in the glycerol level. The maximum glycerol increase over baseline induced by 10^{-5} mol/liter of CGP 12177 was 156±15% in the presence of propranolol. This value did not differ statistically from the corresponding value (152±22%) obtained with 10^{-5} mol/liter of CGP 12177 alone in the experiments depicted in Fig. 1. However, the glycerol increasing effect induced by 10^{-5} mol/liter of either dobutamine or terbutaline was almost completely blocked by propranolol. The maximum responses induced by dobutamine and terbutaline were 30±4 and 27±6%, respectively, as compared with the responses induced by these agents in the absence of propranolol, (from the experiments in Fig. 1), which were 133±28 and 206±36%, respectively. These results differed significantly ($P < 0.01$ using t test) when experiments with and without propranolol were compared. In the propranolol experiments the glycerol response to CGP 12177 was significantly different ($P < 0.01$) from that to dobutamine or terbutaline. The latter two glycerol responses did not differ statistically. The ethanol ratio was also determined in the propranolol experiments (graph not shown). Dobutamine, terbutaline or CGP 12177 did not alter the ethanol ratio, which varied between 65 and 70% throughout all experiments.

Discussion

This study presents direct in vivo evidence that a functional β_3-adrenoceptor exists in human subcutaneous adipose tissue. Its existence was shown by a new technique for in situ lipolysis investigations—microdialysis of the extracellular water space of intact adipose tissue. It is possible to deliver simultaneously pharmacologically active molecules to the tissue and sample molecules from the tissue through the microdialysis probe without disturbing the composition of the extracellular compartment, as reviewed in detail (18).

The microdialysis technique measures net changes in the metabolite concentrations in the extracellular water space of adipose tissue. No or little glycerol enters the adipose tissue via

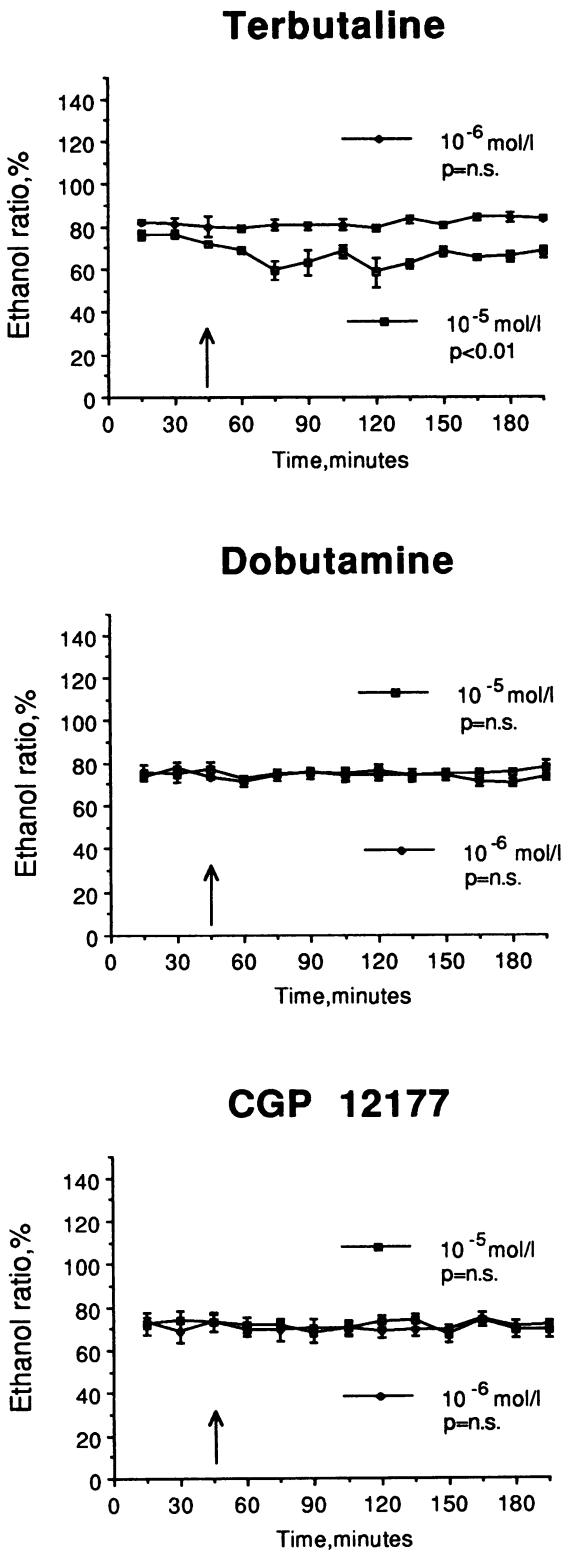

Figure 2. Effects of dobutamine, terbutaline or CGP 12177 on the ethanol ratio. Ethanol was determined in the ingoing and outgoing microdialysis solvents in the experiments described in the legend to Fig. 1. Mean±SE of the ratio in each dialysate fraction is given. The change over time was statistically evaluated by one-way ANOVA. The two curves in the terbutaline experiments differed significantly ($P < 0.01$ by two-way ANOVA). The two curves in the experiments with dobutamine or CGP 12177 did not differ statistically.

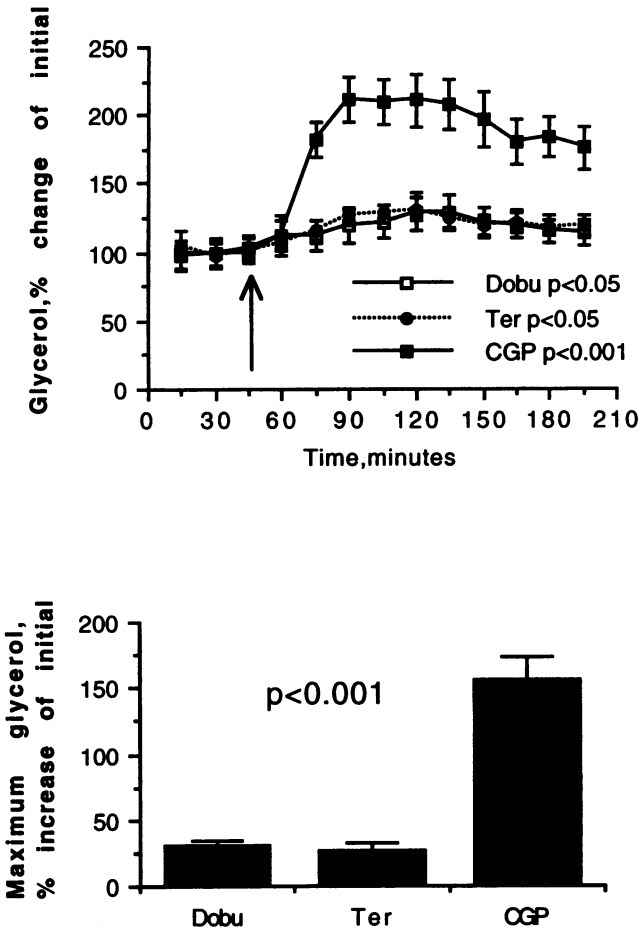

Figure 3. Effects of propranolol on agonist-induced changes of the adipose tissue glycerol level. Adipose tissue was microdialyzed with a solvent containing 10^{-5} mol/liter of propranolol. Dobutamine, terbutaline, or CGP 12177 were added after 45 min in a final concentration of 10^{-5} mol/liter. The upper graph shows the time course for the experiments which were conducted and evaluated as described in legend to Fig. 1. The mean pretreatment glycerol level was 35±4 mol/liter in the experiments with dobutamine (n = 8), 33±3 μmol/liter in the terbutaline experiments (n = 8), and 31±3 μmol/liter in the experiments with CGP 12177 (n = 8). The lower graph depicts the maximum increase in glycerol in the different experiments. ANOVA and posthoc analysis revealed that the data with CGP 12177 differed significantly from the data with dobutamine or terbutaline, whereas the data with the latter two agents did not differ statistically.

the blood stream, because blood contains 2–3 times less glycerol than adipose tissue reviewed (18). Since glycerol is not metabolized by adipose tissue to a significant extent (32), the contribution of fat cells to the glycerol level in the extracellular compartment is influenced only by the rate of production. Local blood flow may be of importance for substrate mobilization from adipose tissue (20), so that changes in blood flow may alter the glycerol level in the extracellular water space independently of lipolysis. Thus, it is necessary that changes in glycerol and tissue flow are considered together in the microdialysis experiment when lipolysis is examined. An indirect evaluation of tissue flow is possible using the ethanol escape technique (19, 20). The validity of this method has recently been investigated in detail (33, 34).

When microdialysis was performed with no active drug, steady-state levels of glycerol and the ethanol ratio during the 45-min pretreatment period, which confirms previous data (20, 23, 24). When, however, dobutamine, terbutaline or CGP 12177 were added, there was a concentration-dependent, rapid, and sustained increase in the tissue glycerol level without a concomitant change in the ethanol ratio, except with the highest concentration of terbutaline, which rapidly and continuously stimulated nutritive blood flow. This strongly suggests that all three selective β-adrenoceptor agonists caused a concentration-dependent stimulation of the lipolysis rate in situ. These effects were probably mediated through specific β-adrenoceptor subtype interactions. Methodological pA_2 experiments performed on isolated fat cells from the same region as the microdialysis was performed—abdominal subcutaneous adipose tissue—clearly revealed that dobutamine, terbutaline, and CGP 12177 were selective agonists for their assigned β-adrenoceptor subtype. The concentration of agonist leaving the probe is 10 times less than the concentration entering it, since in vivo recovery under the present conditions is ~ 10% (24). In addition, there is a further unknown dilution of the drug in the extracellular space until it reaches the fat cells. The in vitro data suggested that the potency of β_1-, β_2-, and β_3-adrenoceptors in human fat cells for the specific agonists is about 10^{-7} mol/liter (i.e., pD_2). The in situ concentration of dobutamine, terbutaline and CGP 12177 at the target tissue level should be less than 10^{-7} or 10^{-6} mol/liter, respectively, on the basis of the information discussed above. At these submaximum effective concentrations the action of receptor subtype specific agonists primarily reflects interactions with their assigned receptor subtype (35). Therefore, the findings with dobutamine, terbutaline and CGP 12177 most likely represent β-adrenergic receptor subtype specific interactions. In addition, it is almost impossible that CGP 12177 caused any lipolytic effects through the β_1- or β_2-adrenoceptors, since it is a non-selective blocker of β_1- and β_2-adrenoceptors in addition to its selective stimulatory effect on β_3-adrenoceptors (36). The present data with propranolol further support the notion that the agonist effect of CGP 12177 is β_3-adrenoceptor selective. This agent inhibited the lipolytic response to dobutamine and terbutaline almost completely bud did not influence the lipolytic action of CGP 12177.

The present data strongly suggest that a functional β_3-adrenoceptor seems to co-exist with β_1- and β_2-adrenoceptors in vivo in human subcutaneous adipose tissue and it is involved in lipolysis regulation. What is the relative importance of the three receptors subtypes for lipolysis regulation? We presently compared two equimolar agonist concentrations. Both doses were more effective using terbutaline (~ 200 and 150% stimulation, respectively) than using dobutamine or CGP 12177 (~ 150% and 50% stimulation, respectively). In addition, the highest concentration of terbutaline also stimulated local blood flow. The latter enhances the removal of glycerol from the extracellular space and consequently lowers the glycerol concentration in this compartment. In other words the difference in lipolytic potency between terbutaline and the other β-adrenoceptor agonists may be underestimated at the high agonist concentration. This indicates that β_2-adrenoceptors are more important than the β_1- and β_3-adrenoceptors for in vivo lipolysis stimulation. Whether the latter two receptors are equally effective remains to be established. CGP 12177 is only a partial agonist, whereas dobutamine is a full agonist. Thus, the true

lipolytic action of β_3-adrenoceptors relative to β_1-adrenoceptors may have been underestimated. A complete understanding of the relative importance of the three β-adrenoceptor subtypes for lipolysis in vivo can only be obtained when the true rate of glycerol mobilization (i.e., concentration times the rate of local tissue flow) is determined at submaximum and maximum effective concentrations of the different agonist alone or in different combinations. Unfortunately, such studies cannot be performed at present, since the ethanol escape technique only measures tissue flow in a semi-quantitative fashion.

We observed no effects of the β_1- or β_3-adrenoceptor agonists on nutritive tissue blood flow in the present experiments whereas the highest concentration of terbutaline stimulated this process. This suggests that only vasodilatory β_2-adrenoceptors are present in the vessels draining human subcutaneous adipose tissue. This is different from the case of dogs, where the vascular β-adrenoceptors of the subcutaneous adipose tissue seem to be of the β_1-subtype (37). However, previous studies in man show indirectly a predominance of vascular β_2-adrenoceptors (38, 39). When data with nutritive blood flow and lipolysis are considered together, they demonstrate the major role of β_2-adrenoceptors in stimulating lipid mobilization from the subcutaneous fat depots in man. Because of the combined stimulatory effects on lipolysis and blood flow, β_2-adrenoceptors can mobilize lipids from adipose tissue much more rapidly after catecholamine stimulation. This is similar to the inhibitory role of α_2-adrenergic receptors in lipid mobilization in situ. Recent microdialysis studies on subcutaneous adipose tissue have suggested that α_2-adrenoceptors inhibit lipid mobilization through a combination of antilipolytic and vasoconstrictor effects (20).

It is of interest to compare the present in vivo and in vitro data with lipolysis, since the results were obtained from of the same adipose region. CGP 12177 was clearly less effective than both terbutaline and dobutamine in vitro as judged from the values for intrinsic activity. On the other hand, CGP 12177 and dobutamine were equally effective in vivo. Thus, some caution should be exercised when extrapolating from in vitro to in vivo when lipolysis is investigated in man.

The lipolytic effects of the investigated β-adrenergic agonist were rather stable over a long period of time. In a previous study, we observed only transient effects with dobutamine but stable effects with terbutaline (40). The differences in results are probably due to the use of different concentrations of agonist in the studies (10^{-8} mol/liter in the former study and $\geq 10^{-6}$ mol/liter presently).

We presently observed marked lipolytic effects of β_3-adrenoceptor stimulation in vitro in subcutaneous human fat cells, which is in contrast to earlier findings, showing no or small effects in vitro (10–13). Methodological reasons may explain the discrepancy in results. Unlike earlier investigation we are using a very sensitive lipolysis assay, based on dilute cell suspensions, which can detect lipolytic effects of physiological (i.e., circulating) concentrations of catecholamines—for example 1–10 nmol/liter of noradrenaline (41). In the previous studies BRL 37344 was used as β_3-adrenoceptor agonist. However, this agent is probably not a suitable β_3-adrenoceptor agonist in man since it has non-selective interactions with β-adrenoceptors in human fat cells (17). In a recent study the lipolytic action of CGP 12177 was investigated in vitro in subcutaneous human fat cells from the mammary region using a very sensitive lipolysis assay (42). The latter study showed marked and selective lipolytic effects of CGP 12177.

In summary, the present study demonstrates the existence of an in vivo functional β_3-adrenoceptor in man, which coexists with β_1- and β_2-adrenoceptors in adipose tissue and plays a role in lipolysis regulation. However, β_2-adrenoceptors seem to be of greater importance for catecholamine stimulation of the mobilization of lipids (at least from abdominal subcutaneous adipose tissue) because of the combined stimulation of lipolysis and nutritive blood flow by this receptor subtype.

Acknowledgments

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References

1. Lands, A. M., A. Arnold, A, J. P. McAuliff, F. P. Luduena, and G. Brown. 1967. Differentiation of receptor systems activated by sympathomimetic amines. *Nature (Lond.)*. 214:597–598.

2. Emorine, L. J., S. Marullo, M.-M. Briend-Sutren, G. Patey, K. Tate, C. Delavier-Klutchko, and A. D. Strosberg. 1989. Molecular characterization of the human β_3-adrenergic receptor. *Science (Wash. DC)*. 245:1118–1121.

3. Granneman, J. G., K. N. Lahners, and A. Chaudry. 1991. Molecular cloning and expression of the rat β_3-adrenergic receptor. *Mol. Pharmacol.* 40:895–899.

4. Muzzin, P., J. P. Revelli, F. Kuhne, J. D. Gocayne, W. R. McCombie, J. C. Venter, J. P. Giacobimo, and C. M. Fraser. 1991. An adipose tissue-specific β-adrenergic receptor. Molecular cloning and down-regulation in obesity. *J. Biol. Chem.* 266:24053–24058.

5. Nahmias, C., N. Blin, J.-M. Elalouf, M. G. Mattei, A. D. Strosberg, and L. J. Emorine. 1991. Molecular characterization of the mouse β_3-adrenergic receptor: relationship with the atypical receptor of adipocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3721–3727.

6. Arch, J. R. S. 1989. The brown adipocyte β-adrenoceptor. *Proc. Nutr. Soc.* 48:215–223.

7. Zaagsma, J., and S. R. Nahorski. 1990. Is the adipocyte β-adrenoceptor a prototype for the recently cloned atypical "β_3-adrenoceptor"? *Trends Pharmacol. Sci.* 11:3–7.

8. Lafontan, M., and M. Berlan. 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* 34:1057–1091.

9. Howe, R. 1993. β_3-adrenergic agonists. *Drugs of the Future.* 18:529–549.

10. Hollenga, C., F. Brouwer, and J. Zaagsma. 1991. Differences in functional cyclic AMP compartments mediating lipolysis by isoprenaline and BRL 37344 in four adipocyte types. *Eur. J. Pharmacol.* 200:325–330.

11. Langin, D., M. P. Portillo, J-S. Saulnier-Blache, and M. Lafontan. 1991. Co-existence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.* 199:291–301.

12. Rosenbaum, M., C. C. Malbon, J. Hirsch, and R. L. Leibel. 1993. Lack of β_3-adrenergic effect on lipolysis in human subcutaneous adipose tissue. *J. Clin. Endocrinol. Metab.* 77:352–355.

13. Hollenga, C., M. Haas, J. T. Deinum, and J. Zaagsma. 1990. Discrepancies in lipolytic activities induced by β-adrenoceptor agonists in human and rat adipocytes. *Horm. Metabol. Res.* 22:17–21.

14. Granneman, J. G., K. N. Lahners, and A. Chaudhry. 1993. Characterization of the human β_3-adrenergic receptor gene. *Mol. Pharmacol.* 44:264–270.

15. Krief, S., F. Lönnqvist, S. Raimbauld, B. Baude, A. van Spronsen, P. Arner, A. D. Strosberg, D. Ricquier, and L. J. Emorine. 1993. Tissue distribution of β_3-adrenergic receptor mRNA in man. *J. Clin. Invest.* 91:344–349.

16. Revelli, J-P., P. Muzzin, A. Paoloni, M. Moinat, and J.-P. Giacobino. 1993. Expression of the β_3-adrenergic receptor in human white adipose tissue. *J. Mol. Endocrinol.* 10:193–197.

17. Lönnqvist, F., S. Krief, A. D. Strosberg, B. Nyberg, L. J. Emorine, and P. Arner. 1993. Evidence for a functional β_3-adrenergic receptor in man. *Br. J. Pharmacol.* 110:929–936.

18. Arner, P., and J. Bülow. 1993. Assessment of adipose tissue metabolism in man: comparison of Fick and microdialysis techniques. *Clin. Sci.* 85:247–256.

19. Hickner, R. C., H. Rosdahl, I. Borg, U. Ungerstedt, L. Jorfeldt, and J. Henriksson. 1991. Ethanol may be used with the microdialysis technique to moni-

tor blood flow changes in skeletal muscle: dialysate glucose concentration is blood flow-dependent. *Acta Physiol. Scand.* 143:355–356.

20. Galitsky, J., M. Lafontan, J. Nordenström, and P. Arner 1993. Role of vascular alpha$_2$-adrenoceptors in regulating lipid mobilization from human adipose tissue. *J. Clin. Invest.* 91:1997–2003.

21. Rosell, S., and E. Belfrage. 1979. Blood circulation in adipose tissue. *Physiol. Rev.* 59:1078–1104.

22. Tossman, U., and U. Ungerstedt. 1986. Microdialysis in the study of extracellular levels of amino acids in the rat brain. *Acta Physiol. Scand.* 128:9–14.

23. Arner, P., J. Hellmér, E. Hagström-Toft, and J. Bolinder 1993. Effect of phosphodiesterase inhibition with amrinone and theophylline on lipolysis and blood flow in human adipose tissue in vitro as measured with microdialysis. *J. Lipid. Res.* 34:1737–1743.

24. Arner, P., E. Kriegholm, P. Engfeldt, and J. Bolinder. 1990. Adrenergic regulation of lipolysis in situ at rest and during exercise. *J. Clin. Invest.* 85:893–898.

25. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* 239:375–380.

26. Lönnqvist, F., H. Nyberg, H. Wahrenberg, and P. Arner. 1990. Catecholamine-induced lipolysis in adipose tissue of the elderly. *J. Clin. Invest.* 85:1614–1621.

27. Dole, V. P., and H. Meinertz. 1960. Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* 235:2595–2599.

28. Östman, J., P. Arner, H. Kimura, H. Wahrenberg, and P. Engfeldt. 1984. Influence of fasting on lipolytic response to adrenergic agonist and adrenergic receptors in subcutaneous adipocytes. *Eur. J. Clin. Invest.* 14:383–391.

29. Arunlakshana, O., and H. O. Schild. 1959. Some quantitative use of drug antagonists. *Br. J. Pharmacol.* 14:48–58.

30. Tallarida, R. J., A. Cowan, and M. A. Adler. 1979. pA_2 and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.* 25:637–654.

31. Hellmér, J, P. Arner, and A. Lundin. 1989. Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal. Biochem.* 177:132–137.

32. Low, G. K., D. Steinberg, M. V. Vaughan, and S. Margolis. 1961. Studies of triglyceride biosynthesis in homogenates of adipose tissue. *J. Biol. Chem.* 236:1631–1637.

33. Hickner, R. C., H. Rosdahl, I. Borg, U. Ungerstedt, L. Jorfeldt, and J. Henriksson. 1992. The ethanol technique of monitoring blood flow changes in rat skeletal muscle: implications for microdialysis. *Acta. Physiol. Scand.* 146:87–97.

34. Hickner, R. C., D. Bone, U. Ungerstedt, L. Jorfeldt, and J. Henriksson. 1994. Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}Xe clearance. *Clin. Sci.* 86:15–25.

35. Kenakin, T. 1984. The classification of drugs and drug receptors in isolated tissue. *Pharm. Rev.* 36:165–214.

36. Van Lief, J., A. van Witzenburg, and G. Vauqguelin. 1993. Isoproterenol and selective agonists stimulate similar atypical beta-adrenoceptors in rat adipocytes. *Biochem. Pharmacol.* 45:974–977.

37. Belfrage, E. 1978. Comparison of β-adrenoceptors mediating vasodilatation in canine subcutaneous adipose tissue and skeletal muscle. *Acta Physiol. Scand.* 102:469–476.

38. Hjelmdahl, P., and B. Linde. 1983 Influence of circulating NE and Epi on adipose tissue vascular resistance and lipolysis in humans. *Am. J. Physiol.* 245:H447–H452.

39. Linde, B., P. Hjelmdahl, U. Freyschuss, and A. Juhlin-Dannfelt. 1981. Adipose tissue and skeletal muscle blood flow during mental stress. *Am. J. Physiol.* 256:E12–E18.

40. Arner, P., E. Kriegholm, and P. Engfeldt. 1991. In vivo interactions between beta$_1$- and beta$_2$-adrenoceptors regulate catecholamine tachyphylaxia in human adipose tissue. *J. Pharmacol. Exp. Ther.* 259:317–322.

41. Lönnqvist, F., H. Wahrenberg, L. Hellström, S. Reynisdottir, and P. Arner. 1992. Lipolytic catecholamine resistance due to decreased β_2-adrenoceptor expression in fat cells. *J. Clin. Invest.* 90:2175–2186.

42. Sennit, M. V., P. W. Young, D. Kikham, S. A.Smith, and M. A. Cawthorne. 1994. Multiple lipolytic β-adrenoceptor subtypes in human adipose tissue: demonstration of a functional β_3-adrenoceptor using CGP 12177. *Int. J. Obesity,* 19 (Suppl 2):19.