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In situ assessment of the role of the β_1-, β_2- and β_3-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue

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1 The involvment of β_1-, β_2- and β_3-adrenoceptors in the control of lipolysis and nutritive blood flow was investigated in abdominal subcutaneous adipose tissue of healthy young adults by use of an *in situ* microdialysis technique.

2 Dialysis probes were infused either with isoprenaline (non-selective β-adrenoceptor agonist), CGP 12,177 (selective β_3-adrenoceptor agonist having β_1-/β_2-antagonist properties), dobutamine (selective β_1-adrenoceptor agonist) or terbutaline (selective β_2-adrenoceptor agonist). The recovery of each probe used for perfusion was calculated by an *in vivo* calibration method. The local blood flow was estimated through the measurement of the escape of ethanol infused simultaneously with the drugs included in the probe.

3 Isoprenaline infusion at 0.01 μM had a weak effect while higher concentrations of isoprenaline (0.1 and 1 μM) caused a rapid, sustained and concentration-dependent increase of glycerol outflow; the maximum increase was $306 \pm 34\%$ with 1 μM. Isoprenaline also increased the nutritive blood flow in adipose tissue; a significant effect appeared at 0.1 μM isoprenaline and was greater at 1 μM.

4 CGP 12,177 (10 and 100 μM) increased the glycerol concentration in the dialysate (128 ± 8 and $149 \pm 12\%$, respectively) and nutritive blood flow. Terbutaline and dobutamine (100 μM) both provoked rapid and similar increases in glycerol outflow (252 ± 18 and $249 \pm 18\%$, respectively). Both, terbutaline and dobutamine increased nutritive blood flow.

5 It is concluded that β_1- and β_2-adrenoceptor subtypes are both mainly involved in the mobilization of lipids and in the control of nutritive blood flow. β_3-Adrenoceptors play a weaker role in the control of lipolysis and nutritive blood flow in human subcutaneous abdominal adipose tissue.

Keywords: Lipolysis; blood flow; human subcutaneous adipose tissue; glycerol; microdialysis; β_1- β_2- and β_3-adrenoceptors; isoprenaline; terbutaline; dobutamine; CGP 12,177

Introduction

Catecholamines control lipolysis in mammal fat cells through the activation of β-adrenoceptors (Lafontan & Berlan, 1993; Coppack *et al.*, 1994). Thirty years ago, their effects were mainly defined in terms of β_1- and β_2-adrenoceptors according to the classification of Lands *et al.* (1967). More recent investigations have shown that, in fat cells from various mammals, β-adrenoceptor-dependent effects can also be generated through the activation of a third β-adrenoceptor subtype named the β_3-adrenoceptor (for reviews see Zaagsma & Nahorski, 1990; Lafontan & Berlan, 1993; Giacobino, 1995). Earlier and more recent *in vitro* studies have revealed a lack of β_3-adrenoceptor-mediated lipolysis in human (Langin *et al.*, 1991; Rosenbaum *et al.*, 1993; Van Liefde *et al.*, 1994) and non-human primate (Bousquet-Mélou *et al.*, 1994) fat cells. Nevertheless other authors have suggested that lipolysis can also be stimulated in human fat cells through stimulation of a β_3-adrenoceptor. The results were based on the effects of BRL 37,344 (initially claimed to be a selective β_3–adrenoceptor agonist) and CGP 12,177 (a non-selective β_1- and β_2–adrenoceptor antagonist having β_3-adrenoceptor agonist activity) which increased lipolysis in human isolated adipocytes (Hollenga *et al.*, 1990; Lönnqvist *et al.*, 1993). In fact, it has been recently demonstrated that BRL 37,344 displays significant adrenoceptor agonist activity (Dolan *et al.*, 1994). Furthermore, controversies still exist concerning the presence of β_3-adrenoceptor mRNAs in human adipose tissue. Investigators have shown that β_3-adrenoceptor mRNAs can either be found (Lönnqvist *et al.*, 1993; Krief *et al.*, 1993; Revelli *et al.*, 1993) or not (Thomas & Liggett, 1993) in human adipose tissues.

The large discrepancies revealed in *in vitro* studies led us to investigate *in situ*, using the microdialysis technique, the role played by the various β-adrenoceptor subtypes in the control of lipolysis in the subcutaneous abdominal adipose tissue of healthy young adults. Special attention was also paid to the changes affecting nutritive blood flow using a recently described method (Galitzky *et al.*, 1993). Microdialysis probes were perfused either with the non-selective β-adrenoceptor agonist, isoprenaline, or with the selective β_1- and β_2-adrenoceptor agonists, dobutamine and terbutaline respectively. CGP 12,177, which has been shown to exert lipolytic effects attributed to β_3-adrenoceptors in human omental and subcutaneous fat cells (Lönnqvist *et al.*, 1993), was used for stimulation of β_3-adrenoceptors. During the completion of the present study a paper dealing with a similar approach has been published (Enocksson *et al.*, 1995). Several points of our results will be discussed in relation to those reported in this paper.

Methods

Subjects

Sixteen healthy young adults (9 men, 7 women) from 20 to 28 years old (mean ± s.e.mean: 24 ± 1 years) who had not been

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submitted to any nutritional or pharmacological protocol prior to the study were selected for these investigations. Their body weight and height were 63±5 kg (range from 54 to 78 kg) and 171±2 cm (range from 158 to 186 cm) respectively. They were all drug-free. All were weight stable during the preceding 3 months and their body mass index was 21.5±1.7 kg m^{-2} (range from 18.7 to 24.7 kg m^{-2}). None was obese: their fat mass, assessed by the skinfold thickness method (Durnin *et al.*, 1974), was 19±2% of body weight (ranged from 13 to 27%). The investigation protocol was approved by the local Ethical Committee of the Hospital.

Experimental protocols

Subjects were studied at 08 h 00 min in the supine position after an overnight fast. A biopsy of abdominal subcutaneous adipose tissue was performed at 08 h 30 min (after superficial anaesthesia with 0.5% lignocaine solution without adrenaline) with a 2.3-mm-diameter needle. By successive suctions, approximately 200 mg of adipose tissue were drawn into a syringe. Adipocytes were immediately isolated by the method of Rodbell (1964) in a Krebs-Ringer bicarbonate HEPES solution (pH = 7.4) containing 2% bovine serum albumin, 6 mM glucose (KRBHA) and 0.5 mg ml^{-1} collagenase. Isolated adipocytes were washed three times and the cells were used for lipolysis measurements in KRBHA. All pharmacological compounds were added to a 5 μl volume at the start of the incubation performed with 2,000–3,000 fat cells in a final volume of 100 μl KRBHA. The incubation was run for 90 min, and 30 μl of infranatant was removed for the determination of glycerol. Lipolytic activity was expressed as μmol of glycerol released per 100 mg lipid (determined gravimetrically). The intrinsic activity of each β-adrenoceptor agonist was calculated by dividing its maximal lipolytic effect by the maximal lipolytic effect of the full β-adrenoceptor agonist isoprenaline (intrinsic activity = 1). The pD_2 values were determined by computer-fitting analysis of concentration-response curves obtained with the various β-adrenoceptor agonists.

Adipose tissue microdialysis was performed with 20 × 0.5 mm probes having a 20,000 dalton molecular weight cut-off (Carnegie Medicine, Stockholm, Sweden). The probes were inserted percutaneously after light intradermal anaesthesia (100 μl of 1% lignocaine, Roger-Bellon, France) into the abdominal subcutaneous adipose tissue at a distance of 100 mm to the right and/or the left of the umbilicus. The probes were connected to a multichannel microinjection pump (Harvard Apparatus, Southnatick, Mass, U.S.A.) and continuously perfused with a sterile Ringer solution (sodium 154 nmol l^{-1}, potassium 4 mmol l^{-1}, calcium 2.5 mmol l^{-1}, chloride 160 mmol l^{-1}) supplemented with ethanol (1.7 g l^{-1}) at 0.8 μl min^{-1}. No collection of the outgoing dialysate was made during the first 30 min after implantation. Then, in each set of experiments, the *in vivo* recovery rate at 2.5 μl min^{-1} was evaluated for each probe using the measurement of dialysate glycerol concentrations at various perfusion rates in agreement with a previously described method (Stahle *et al.*, 1991). Briefly, the probes were perfused at 4 successive rates (0.8 μl min^{-1}, 1.5 μl min^{-1}, 3.5 μl min^{-1} and 2.5 μl min^{-1}, respectively), and the glycerol concentrations were determined in the dialysate for each perfusion rate at the steady state. These concentrations were plotted (after log-transformation) against the perfusion rates. Regression analysis was used to calculate the glycerol concentration at 'zero flow', corresponding to the interstitial glycerol concentration. The ratio (dialysate glycerol concentration at 2.5 μl min^{-1})/(interstitial glycerol concentration) × 100 expressed the % recovery rate at 2.5 μl min^{-1} of the probe.

The perfusion flow rate was maintained at 2.5 μl min^{-1}, after the calibration period, in order to evaluate the effects of the pharmacological agents. Ten-minute fractions of the outgoing dialysate were collected. Glycerol was assayed in each collected fraction as lipolysis index. The ethanol level was measured in each fraction to assess the changes occurring in the nutritive blood flow of the adipose tissue, using the ethanol outflow/inflow ratio, as previously described (Hickner *et al.*, 1991; Galitzky *et al.*, 1993). Four to six fractions were collected to evaluate basal glycerol and ethanol levels before adding the various β-adrenoceptor agonists in the perfusate.

In the first set of experiments, a microdialysis probe was implanted for the evaluation of the effects of increasing concentrations of isoprenaline (0.01, 0.1 and 1 μM). Each concentration was applied for 40 min. A venous blood sample, for fasting plasma glycerol assay, was drawn from an indwelling polyethylene catheter in the cubital vein before the dialysis experiments. In a second protocol, two probes were implanted symmetrically on each side of the navel, the first one was perfused with two concentrations of CGP 12,177 (10 and 100 μM), while the other, control probe was perfused without any active drug over the same period to check the spontaneous changes which could occur along the perfusion period. Finally, in a third experimental protocol, two probes were implanted in the same way to evaluate the effects of 100 μM terbutaline or dobutamine.

Drugs and analytical methods

CGP 12,177 (4-[3-*t*-butylamino-2-hydroxypropoxy] benzimidazol-2-one) came from Ciba-Geigy (Basle, Switzerland). Isoprenaline hydrochloride (Isuprel) was obtained from Winthrop (Clichy, France). Dobutamine hydrochloride (Dobutrex) was obtained from Lilly (Saint-Cloud, France) and terbutaline sulphate (Bricanyl) from Astra (Nanterre, France).

Glycerol was determined by an ultrasensitive radiometric method, in 10 μl fractions of dialysate or plasma (Bradley & Kaslow, 1989); the intraassay and interassay variabilities were 5.0% and 9.2%, respectively. Ethanol in dialysate and perfusate was determined in 5 μl fractions by an enzymatic method (Bernst & Gutman, 1974); the intraassay and interassay variabilities were 3.0% and 4.5%, respectively.

Statistical analysis

All the values are given as means±s.e.mean. ANOVA and Wilcoxon's paired test were used for comparisons of glycerol levels and ethanol ratio, when appropriate. $P<0.05$ was considered as being statistically significant. All statistical comparisons were performed by means of a statistical software package (Statview II, Abacus Concepts Inc., Berkeley, CA, U.S.A.).

Results

In vitro *lipolysis*

Isoprenaline, terbutaline, dobutamine and CGP 12,177 caused a concentration-dependent stimulation of glycerol release from isolated fat cells (Figure 1). The maximal lipolytic effects of the three selective β-adrenoceptor agonists were significantly less than that of isoprenaline, and the concentration-response curves were to the right of that to isoprenaline. Thus the intrinsic activities relative to isoprenaline were: 0.69±0.1, 0.65±0.07 and 0.17±0.05, and the pD_2 values were 6.14±0.19, 6.16±0.14 and 5.89±0.16 for terbutaline, dobutamine and CGP 12,177, respectively. The pD_2 values for these three β-adrenoceptor agonists were not significantly different from each other, but all were significantly different from that of isoprenaline (8.22±0.28, $P<0.001$). Under these conditions therefore, the selective β-adrenoceptor agonists were 50–90 fold weaker than isoprenaline at human adipocyte β-adrenoceptors.

Dialysis experiments

In the first experiment, the post-absorptive plasma concentrations of glycerol were 38±7 μmol l^{-1}. Using the calibration method based on the determination of glycerol levels

in the outgoing dialysate with different perfusion rates, the corresponding calculated extracellular concentration of glycerol in abdominal subcutaneous adipose tissue of our subjects was significantly higher: 127±9 μM ($P<0.001$) and the *in vivo* recovery of the probes at 2.5 μl min^{-1} was 35±3%.

The effects of increasing concentrations of isoprenaline on dialysate glycerol concentrations and ethanol ratio in subcutaneous adipose tissue are depicted in Figure 2. Addition of the lowest concentration (0.01 μM) of isoprenaline slightly increased the dialysate concentrations of glycerol when compared to the last pre-perfusion value. This increase became significant after 30 min of administration. Higher concentrations of isoprenaline induced a significant and concentration-dependent increase in dialysate glycerol levels (199±13% and 306±34% of the baseline with 0.1 μM and 1 μM, respectively). Simultaneously, isoprenaline promoted a significant decrease of the ethanol outflow/inflow ratio (Figure 2), indicating that the nutritive blood flow in adipose tissue was increased (vasodilatation).

The effects of 10 and 100 μM of CGP 12,177 were investigated (Figure 3). Since the *in vitro* CGP 12,177-induced lipolysis was found to be weaker (higher pD_2 value and lower intrinsic activity) than that of isoprenaline, a second microdialysis probe, perfused with the Ringer solution (supplemented with ethanol), was inserted in the same subject as a control, in order to check any spontaneous modification of lipolysis or blood flow. The *in vivo* recovery rates were not different in the two probes (34±3% *versus* 33±2%). Dialysate glycerol concentrations were unchanged when comparing the first four fractions of each probe ($F=0.45$, $P=0.71$) and the two curves for this basal period ($F=1.99$, $P=0.18$). CGP 12,177 (10 μM) increased weakly but significantly (128±8% of the basal value) the dialysate glycerol concentrations after 20 min perfusion (Figure 3). Perfusion with the higher concentration (100 μM) led to a 149±12% increment over the basal value after 20 min perfusion. The effect was not significantly different from that induced by the lower concentration of CGP 12,177 (ANOVA with repeated measures from 60 to 140 min: $F=1.23$, $P=0.30$) suggesting that maximal effects were obtained. During the experimental period, glycerol concentrations were stable in the control probe. The difference between this

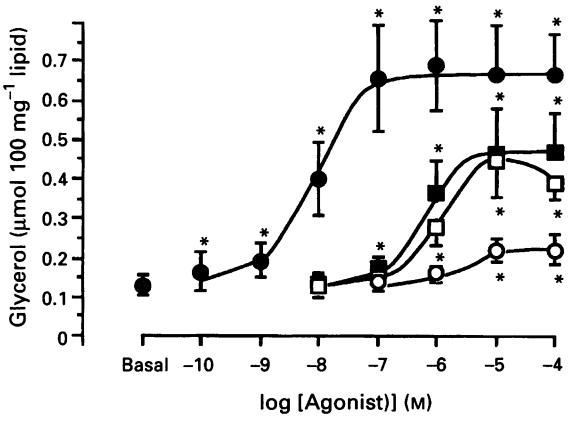

Figure 1 Concentration-response curves of β-adrenoceptor agonists in abdominal subcutaneous adipocytes of 8 normal subjects. Lipolysis induced *in vitro* by terbutaline (■), dobutamine (□) or CGP 12,177 (○), selective β_2-, β_1 and β_3-adrenoceptor agonists, respectively, were compared to the lipolytic effect of isoprenaline (●), a non-selective β-adrenoceptor agonist. *$P<0.05$ when compared to the basal values (Wilcoxon's paired test).

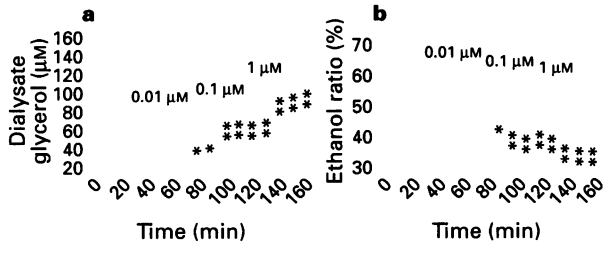

Figure 2 Effect of isoprenaline on the dialysate concentration of glycerol (a) and on the ethanol ratio (b) in the subcutaneous adipose tissue of 8 normal subjects. The probe was implanted in the abdominal region and perfused with Ringer solution supplemented with 1.7 g l^{-1} ethanol. After a calibration period with different perfusion rates, the probe was continuously perfused at 2.5 μl min^{-1} and the dialysate was collected at 10 min intervals. After a 40 min basal period, isoprenaline was added to the perfusate at the concentrations indicated with the arrows. The ethanol ratio (ethanol in the outgoing dialysate/ethanol in the ingoing dialysate × 100) is calculated from the concentrations measured in the ingoing and outgoing dialysate. The glycerol concentration and the ethanol ratio in the last fraction preceding the addition of the drug (40 min) were used as basal values. Values are mean±s.e.mean *$P<0.05$, **$P<0.01$ when compared to the basal values (Wilcoxon's paired test).

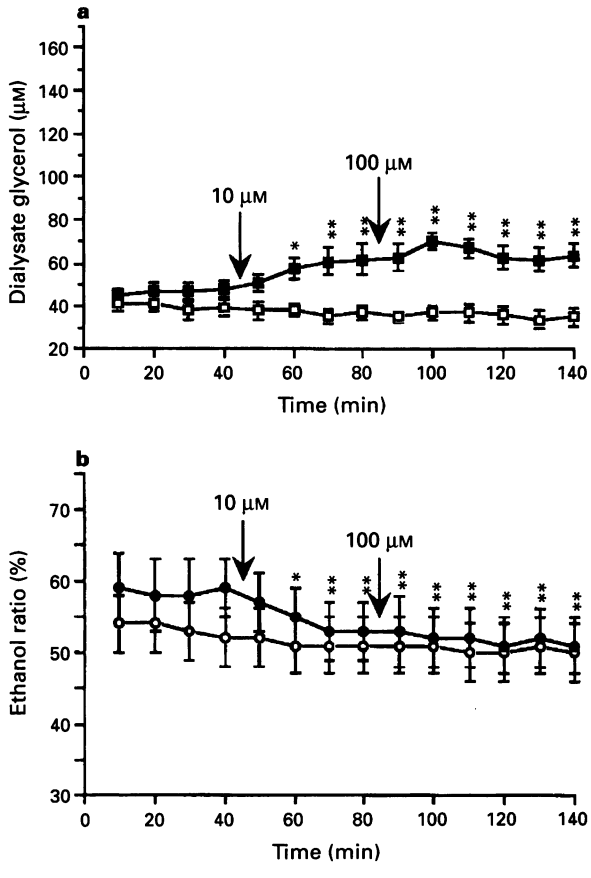

Figure 3 Effect of CGP 12,177 on the interstitial concentration of glycerol (a) and on the ethanol ratio (b) in the subcutaneous adipose tissue of 8 subjects. After a 40 min basal period, a first probe was perfused with CGP 12,177 at indicated concentrations (filled symbols), a second probe was perfused without any active drug (open symbols). For further details see legend to Figure 2. *$P<0.05$, **$P<0.01$ when compared to the basal values (Wilcoxon's paired test).

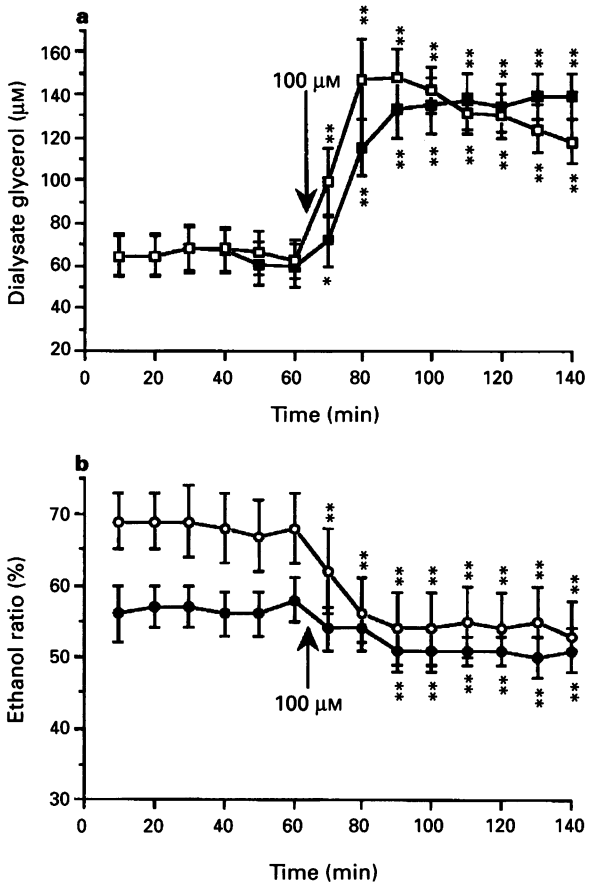

Figure 4 Effects of dobutamine and terbutaline (100 μM) on the extracellular concentration of glycerol (a) and on the ethanol ratio (b) in the subcutaneous adipose tissue of 8 subjects. After a 60 min basal period, a first probe was perfused with terbutaline (open symbols), and the second probe was perfused with dobutamine (filled symbols). For further details see legend to Figure 2. $^{*}P<0.05$, $^{**}P<0.01$ when compared to the basal values (Wilcoxon's paired test).

control curve and the curve during CGP 12,177 stimulation was highly significant during the 60–140 min period (ANOVA $F=24.2$, $P<0.001$).

Perfusion with 10 μM CGP 12,177 induced a significant decrease of the ethanol outflow/inflow ratio after 20 min of perfusion. As observed for glycerol outflow, the 100 μM concentration did not promote any additional effect. The ethanol outflow/inflow ratio in the control probe was stable during the experimental period.

The effects of 100 μM of the selective β_1- and β_2-adrenoceptor agonists, dobutamine and terbutaline were studied in the third set of experiments. This concentration was chosen since dobutamine and terbutaline exhibited lower pD_2 values than isoprenaline in *in vitro* lipolytic assays (Figure 1) and because our aim was to compare the maximal effect of the stimulation of the three β-adrenoceptor pathways. Two microdialysis probes were inserted in the same subject. The *in vivo* recovery rates were not different in the two probes (41±3% *versus* 42±5%). As seen in Figure 4, dobutamine and terbutaline promoted a strong increase of dialysate glycerol concentrations. The maximal effect was 241±18% of the basal value in the 100 min fraction with dobutamine and 252±18% in the 90 min fraction with terbutaline. The dobutamine effect on glycerol concentration was sustained during the perfusion period (ANOVA with repeated measures from 90 to 140 min: $F=0.34$, $P=0.84$). With terbutaline, there was a progressive decrease in effect over the perfusion period (ANOVA with repeated measures from 80 to 140 min: $F=3.53$, $P=0.01$) and the glycerol levels of the two last fractions were significantly lower than in the 80 and 90 min fractions ($P<0.05$). Both dobutamine and terbutaline significantly decreased the ethanol outflow/inflow ratio (Figure 4), which fell from basal value. This effect was significantly greater with terbutaline than with dobutamine (−15±1% *versus* −7±1%, respectively, $P<0.01$).

Discussion

The present investigation is an attempt to compare, in healthy young adult subjects, the *in situ* effects of the stimulation of the three different β-adrenoceptors involved in the control of both lipolysis and blood flow in human subcutaneous adipose tissue. The microdialysis technique, initially described in adipose tissue for metabolite disposal measurements (Lönnroth *et al.*, 1987; Jansson *et al.*, 1988) also provides an alternative approach for the study of the regulation of lipolysis and nutritive blood flow *in situ* (Arner *et al.*, 1990; Galitzky *et al.*, 1993). A study was recently performed by another group (Enocksson *et al.*, 1995), and while the results obtained on the regulation of glycerol outflow by β-adrenoceptor agonists were similar, there were some differences with blood flow results.

The value of the basal interstitial glycerol concentration in abdominal subcutaneous adipose tissue, calculated in the present study with the perfusion rate method, was approximately 3 fold higher than that found in the plasma, and quite similar to that defined using the no-net flow calibration procedure (Arner *et al.*, 1990; Jansson *et al.*, 1990; 1992). Since glycerol recovery did not differ between the probes used, a comparison of the absolute glycerol concentrations in dialysate was possible.

The relative involvement of the β_1-, β_2- and β_3-adrenoceptors in the control of lipolysis and nutritive blood flow was evaluated with dobutamine (selective β_1-agonist), terbutaline (selective β_2-agonist) and CGP 12,177, all of which are available for clinical studies. CGP 12,177 was used since this drug, which is a non-selective antagonist of β_1- and β_2-adrenoceptors without any identified partial agonism, has been reported to stimulate selectively β_3-adrenoceptors at concentrations higher than those required for β_1-/β_2-adrenoceptor antagonism. This original property has been demonstrated in fat cells from various species (Mohell & Dicker, 1989; Langin *et al.*, 1991) as well as in human fat cells (Lönnqvist *et al.*, 1993). β_1-/β_2-Antagonist properties of CGP 12,177 have previously been demonstrated in human subjects (Merlet *et al.*, 1993). Other β_3-adrenoceptor agonists available for human investigations, BRL 37,344 and CL 316,243, can exhibit β_1- and/or β_2-adrenoceptor agonist activity at high concentrations (Dolan *et al.*, 1994), and SR 58,611A exhibits a low affinity for β_3-adrenoceptors in the adipose tissue of various species, and is not active in human fat cells (Langin *et al.*, 1991; Bousquet-Mélou *et al.*, 1994).

The *in vitro* studies have shown that the potency of the three β-adrenoceptor agonists for human fat cell β-adrenoceptors was about 50 to 90 fold lower than that of isoprenaline, and that their maximal lipolytic effects were only obtained at 10 μM (Figure 1). Thus, in order to compare the relative lipolytic and vascular effects of the three agonists, a 10 fold higher concentration was used, since the concentration leaving the probe has been roughly estimated to be about 10 to 20% of that entering it (Arner *et al.*, 1988). In addition, before the drugs reach the adipocytes and the vascular bed an unknown further dilution will occur.

The infusion with 10 μM CGP 12,177 significantly increased the extracellular glycerol concentration, and a similar effect was observed with 100 μM CGP 12,177. This effect, also described by another group (Enocksson *et al.*, 1995), was shown to be resistant to propranolol-induced β_1-/β_2- antagonism. This action was considered to be β_3-adrenoceptor-dependent, although there is currently no selective β_3-adrenoceptor antagonist with which to establish this. Most experiments on fat cell β_3-adrenoceptors have been performed *in vitro* on isolated

adipocytes or tissue fragments. Using CGP 12,177 or other β_3-adrenoceptor agonists, Hollenga *et al.* (1990) and Lönnqvist *et al.* (1993) reported a weak but significant activation of lipolysis, whereas others failed to find any effect in fat cells from human subjects (Langin *et al.*, 1991; Rosenbaum *et al.*, 1993; Van Liefde *et al.*, 1994; Mauriège *et al.*, 1995) or non-human primates (Bousquet-Mélou *et al.*, 1994). Such discrepancies reported in *in vitro* assays could partly be explained by the selection of patients (age, sex and level of fatness), the nature of the fat deposits used for adipocyte isolation (abdominal, femoral, gluteal and omental) or differences in the assay system (heterogeneity of collagenase batches used for fat cell isolation or differences in albumin composition and fat cell density used in the assays). The *in vivo* approach, which largely gets round the technical problems introduced by the *in vitro* systems, suggests that β_3-adrenoceptors are weakly efficient in the subcutaneous adipose tissue of normal subjects. Nevertheless, the results do not exclude a more substantial role of β_3-adrenoceptors in the visceral fat, especially in obese subjects (Lönnqvist *et al.*, 1995). The present results argue for the existence of functional β_3-adrenoceptors in human subcutaneous adipose tissue. However, the effects of CGP 12,177 are weaker than those initiated by β_1- or β_2-adrenoceptor stimulation in either *in vitro* or *in vivo* situations. Our results are in agreement with those given by molecular approaches showing low levels of expression, in terms of levels of β_3-adrenoceptor mRNA in human adipose tissue (Granneman *et al.*, 1992; Lönnqvist *et al.*, 1993; Krief *et al.*, 1993; Revelli *et al.*, 1993).

Previous investigations have shown that regional differences in lipolysis in human adipose tissue are linked to variations of β_1-/β_2-adrenoceptor density in subcutaneous and visceral human fat cells (Hellmer *et al.*, 1992; Castan *et al.*, 1993). In the present study, both terbutaline and dobutamine strongly stimulated glycerol output (Figure 4), which supports a role for β_1- and β_2-adrenoceptors. However, since it is impossible to determine the concentration of the agents inside the tissue, it is not clear whether there is additionally some degree of β_3-adrenoceptor involvement in the actions of either of these agents. However, the *in vitro* studies reveal that, even at 10 μM, the drugs keep some subtype selectivity, since their respective effects differ from that of isoprenaline. The results on glycerol output agree with those of Enocksson *et al.* (1995) and the conclusions of previous *in vitro* studies carried out on human isolated adipocytes where the two β-adrenoceptor subtypes were characterized with binding studies, mRNA determinations, lipolytic assays (Arner, 1992; Lafontan & Berlan, 1993) or microdialysis (Arner *et al.*, 1991).

In addition to measurement of glycerol concentration, it was important to have a semi-quantitative evaluation of the changes promoted in local blood flow by the same compounds. Potent stimulation of blood flow and adipose tissue drainage could be responsible for removal of glycerol from the extracellular space and a reduced glycerol output from the probe. The ethanol outflow/inflow ratio decreased after isoprenaline perfusion. This effect was concentration-dependent and indicated that β-adrenoceptor stimulation increased the local nutritive blood flow. A significant effect on lipolysis appeared with 0.01 μM isoprenaline only at the last step of infusion (Figure 2). To interpret this observation, it could be postulated that the vascular effect partly masks a positive action of the drug on lipolysis since an increment of glycerol drainage could be simultaneously promoted in the circulation. At higher concentrations, although the blood flow was increased, the lipolytic effect was still observed, since stronger activation of lipolysis was associated with the actions on blood flow. The fact that the ethanol outflow/inflow ratio was reduced during dobutamine, terbutaline or CGP 12,177 perfusion indicated that all the drugs induced vasodilatation in subcutaneous adipose tissue. β-Adrenoceptors have been reported to be involved in the control of vasodilatation of vascular bed in adipose tissue; they were considered to be mainly of the β_1- and β_3-subtypes in vessels of canine adipose tissue (Rosell & Belfrage, 1979; Shen *et al.*, 1994) and skin (Berlan *et al.*, 1994). In their present report, and using a similar microdialysis method, Enocksson *et al.* (1995) did not detect any effect of CGP 12,177 on the nutritive blood flow in human subcutaneous adipose tissue. There are various possible explanations for these discrepancies. The age of the subjects and the concentrations of the β-agonists differed in the two studies. Moreover, the methodological conditions were not similar. In our study, the dialysis probes were twice as long, they offered a higher diffusion area for ethanol escape and could improve evaluation of blood flow changes. Since CGP 12,177 has never been shown to exert any β_1- or β_2-agonist effects, the vasodilatation observed in the present study could be explained by the presence of β_3-adrenoceptors in the vascular bed of human subcutaneous adipose tissue; it will only be possible to assess this point when a selective β_3-antagonist becomes available.

Based on the effects of terbutaline alone and the differences between terbutaline- and dobutamine-mediated effects on blood flow, the results suggest that vasodilatory β_2-adrenoceptors are predominantly involved in the control of nutritive blood flow in human subcutaneous adipose tissue. This result agrees with previous observations (Linde *et al.*, 1981). Concomitantly with the establishment of vasodilatation, the concentration of glycerol in the dialysate reached a plateau, and then decreased progressively with terbutaline (Figure 4). This effect can be explained by the stronger vasodilatory effect of terbutaline which improves removal of glycerol from the extracellular space. However, another interpretation could also be proposed. Chronic terbutaline administration can induce a desensitization of fat cell β_2-adrenoceptors, since these receptors are known to be the most prone to desensitization in a number of tissues, including fat (Lafontan, 1994). A specific desensitization of β_2-adrenoceptors in rat fat cells has been recently demonstrated (Bousquet-Mélou *et al.*, 1995). Further studies are necessary to determine whether this phenomenon occurs in man under *in vivo* conditions. Finally, although a loss of β-adrenoceptor subtype selectivity of dobutamine and terbutaline could be suspected at the concentrations used, the differences observed in the β- effects suggest that the drugs still possess β-adrenoceptor subtype selectivity.

In conclusion, the present study, using *in situ* microdialysis, demonstrates a major role played by β_1- and β_2-adrenoceptors in the regulation of glycerol concentration in human adipose tissue. Stimulation of both fat cell β_2- and β_1-adrenoceptors activates lipolysis and glycerol outflow from adipose tissue. Concomitant stimulation of vascular β_2-adrenoceptors has also an important additional action on nutritive blood flow, which participates in the modulation of extracellular glycerol levels in adipose tissue. β_3-Adrenoceptors seem to play a minor role in the control of the function of abdominal subcutaneous adipose tissue in healthy young adults. Vascular effects could counteract metabolic actions and promote removal of glycerol from the extracellular space. The mechanism of the vasodilatation induced by CGP 12,177 requires further studies, but in the absence of selective β_3-antagonists it will be extremely difficult to ascertain whether this is mediated by β_3-adrenoceptors or by some other mechanism.

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