Activation of $\alpha_2$-adrenergic receptors blunts epinephrine-induced lipolysis in subcutaneous adipose tissue during a hyperinsulinemic euglycemic clamp in men

**Vladimir Stich,1 Tereza Pelikanova,2 Petr Wohl,2 Coralie Sengenès,3 Alexia Zakaroff-Girard,3 Max Lafontan, and Michel Berlan.** Activation of $\alpha_2$-adrenergic receptors blunts epinephrine-induced lipolysis in subcutaneous adipose tissue during a hyperinsulinemic euglycemic clamp in men. *Am J Physiol Endocrinol Metab* 285: E599–E607, 2003; 10.1152/ajpendo.00502.2002.—The aim of this study was to investigate whether hyperinsulinemia modifies adrenergic control of lipolysis, with particular attention paid to the involvement of antilipolytic $\alpha_2$-adrenergic receptors (AR). Eight healthy male subjects (age: 23.9 ± 0.9 yr; body mass index: 23.8 ± 1.9) were investigated during a 6-h euglycemic-hyperinsulinemic clamp and in control conditions. Before and during the clamp, the effect of graded perfusions of isoproterenol (0.1 and 1 μM) or epinephrine (1 and 10 μM) on the extracellular glycerol concentration in subcutaneous abdominal adipose tissue was evaluated by using the microdialysis method. Both isoproterenol and epinephrine induced a dose-dependent increase in extracellular glycerol concentration when infused for 60 min through the microdialysis probes before and during hours 3 and 6 of the clamp. The catecholamine-induced increase was significantly lower during the clamp than before it, with the inhibition being more pronounced in hour 6 of the clamp. Isoproterenol (1 μM)-induced lipolysis was reduced by 28% and 44% during hours 3 and 6 of the clamp, respectively, whereas the reduction of epinephrine (100 μM)-induced lipolysis was significantly greater (by 63 and 70%, $P<0.01$ and $P<0.04$, respectively) during the same time intervals. When epinephrine was infused in combination with 100 μM phentolamine (a nonselective $\alpha$-AR antagonist), the inhibition of epinephrine (10 μM)-induced lipolysis was only of 19% and 40% during hours 3 and 6 of the clamp, respectively. The results demonstrate that, in situ, insulin counteracts the epinephrine-induced lipolysis in adipose tissue. The effect involves 1) reduction of lipolysis stimulation mediated by the $\beta$-adrenergic pathway and 2) the antilipolytic component of epinephrine action mediated by $\alpha_2$-ARs.

Microdialysis; glycerol; isoproterenol; blood flow; $\alpha_2$-adrenergic receptor antagonist


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concentration of the catecholamines. Simultaneously, insulin can be delivered intravenously during a hyperinsulinemic-euglycemic clamp with no changes of catecholamine concentration in plasma or in dialysate from adipose tissue or muscle (23, 26). Animal studies have shown a decrease of the lipolytic response to norepinephrine during a hyperinsulinemic-euglycemic clamp in adipose tissue (8). From additional human investigations, it has been concluded that the β-adrenergic lipolytic effect in adipose tissue is counteracted by the action of insulin (14, 18). Until now, no study has taken into account the role of the antilipolytic α2-adrenergic pathway in the in situ adrenergic response when plasma insulin levels are increased.

The first objective of the present study on the basis of in situ microdialysis use was to investigate the counteraction of hyperinsulinemia on the lipolytic effect of isoproterenol (a selective β-AR agonist) or epinephrine (a catecholamine exhibiting both α2- and β-AR agonist properties) in adipose tissue. The second objective was to delineate the contribution of the antilipolytic α2-AR pathway to the blunted adrenergic lipolytic response in adipose tissue during hyperinsulinemic-euglycemic clamp.

MATERIALS AND METHODS

Subjects

For the study, eight healthy young men aged 23.9 ± 0.9 yr (range: 22–25 yr) with a body mass index ranging from 21.4 to 27.0 (mean ± SD: 23.8 ± 1.9), who had not been submitted to any pharmacological or nutritional protocol before the study, were recruited. All had a stable weight during the previous 3 mo. Selection of the subjects was based on a screening evaluation of detailed medical history, a physical examination, and several blood chemistry analyses. The Ethical Committee of Third Faculty of Medicine, Charles University, approved the study. All subjects gave their informed consent for the experimental conditions after detailed explanation. The investigations were carried out in the Diabetes Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Experimental Protocol

Studies were performed in random order, and the eight subjects were studied on three separate occasions (twice with a clamp and once without). These three examinations were separated by a 5- to 8-day period, and patients were instructed to keep their nutritional and physical activity habits during that time. The subjects entered the hospital at 8:00 AM and were maintained in the supine position during the experimental period. An indwelling polyethylene catheter was inserted into the antecubital vein of each arm. At 8:30 AM, microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cutoff were inserted percutaneously into the abdominal SCAAT after epidural anesthesia (200 μl of 1% lidocaine; Roger-Bellon, Neuilly-s-Seine, France). When two probes were used, they were separated by at least 10 cm and inserted at a distance of 10 cm to the right of the umbilicus. When necessary, a third control probe was inserted in the contralateral side at a similar distance from umbilicus. The probes were connected to a microperfusion pump (Harvard Apparatus, SARL, Les Ulis, France) and infused with Ringer solution (in mM: 139 sodium, 2.7 potassium, 0.9 calcium, 140.5 chloride, 2.4 bicarbonate, and 5.6 glucose). Ethanol was added to the perfusate to estimate changes in the blood flow, as previously described (12, 13). The ethanol ratio was calculated as ethanol ratio (%) = (ethanol concentration in outgoing dialysate/ethanol concentration in ingoing perfusate) × 100. The variations of the ethanol ratio were taken as an index of variations of adipose tissue blood flow (ATBF). After a 30-min equilibration period, a 30-min fraction of dialysate was collected at a flow rate of 0.5 μl/min. Then, the perfusion flow rate was set at 2.5 μl/min for the remaining experimental period. This simplified but relevant and less time-consuming method was selected in this long-lasting study (24, 30). The estimated extracellular glycerol concentration (EGC) was calculated by plotting (after log transformation) the concentration of glycerol in the dialysate measured at 0.5 and 2.5 μl/min against the perfusion rates. The EGC found in the present study fits with previous determinations performed in lean subjects (16, 17). The average recovery of probes was calculated as the ratio of the concentration of glycerol in dialysate at the perfusion rate of 2.5 μl/min and the calculated EGC. The mean recovery was 29 ± 5% (SD), and the range was from 27 to 33%. Study 1. After calibration of the probe, two 15-min fractions of the outgoing dialysate were collected in all probes. Thereafter, one probe was infused with two graded epinephrine concentrations (1 and 10 μM in Ringer solution) for 30 min each, and a second probe was infused with two graded isoproterenol concentrations (0.1 and 1 μM in Ringer solution) for 30 min each. The concentrations of isoproterenol used were 10-fold lower than those of epinephrine because this agonist is 10-fold more potent than epinephrine toward β-AR. A third probe was infused with Ringer solution throughout the whole experimental period. After the end of the above-mentioned perfusions, a hyperinsulinemic-euglycemic clamp was started and performed for 6 h. During hours 3 and 6 of the clamp, the perfusions using epinephrine (probe 1) and isoproterenol (probe 2) were performed with identical concentrations to those before the clamp. During the time between the above-mentioned perfusions, the probes were infused with Ringer solution. Dialysate samples were collected for each 10-min period during the specific agent perfusions and for each 15 min during the rest of the time. Study 2. The experimental protocol was identical to that of study 1, except that, in probe 2, the graded concentrations of epinephrine (1 and 10 μM, i.e., the same concentrations as in probe 1) were infused each for 30 min with 100 μM phentolamine.

Control study (8 subjects). One probe was inserted into SCAAT, and the repeated perfusions, which used the same concentrations of epinephrine and the same time schedule as those described in the experimental protocol, were performed, except that the hyperinsulinemic-euglycemic clamp was not realized (Fig. 1). Control assays were also performed with phentolamine in combination with epinephrine without insulin administration in six subjects.

Euglycemic-Hyperinsulinemic Clamp

The hyperinsulinemic-euglycemic clamp was performed by DeFronzo's method (9). A catheter for insulin and glucose infusion was inserted into an antecubital vein, and a second catheter for blood sampling was placed in a dorsal vein of the ipsilateral hand. The hand was kept in a warm box (60°C) to provide arterialization of venous blood. Priming plus continuous infusion of crystalline human insulin (1 mU·kg⁻¹·min⁻¹; Actrapid Human, Novo, Bagsvaerd, Denmark) was given for 6 h. Euglycemia (the fasting blood glucose concen-
tration: 4.34 ± 0.07 mM) was maintained by a variable 15% glucose perfusion. The perfusion rate was determined by measurement of arterialized plasma glucose (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA) every 5 min. Plasma concentrations of glucose, glycerol, and free insulin were analyzed in the basal state (mean value of 3 samples obtained 70, 65, and 60 min before the start of the clamp) and every 60 min during the clamp.

**Drugs and Biochemical Determinations**

The following drugs were used: isoproterenol hydrochloride (Isuprel, Winthrop), epinephrine hydrochloride (Epinphrine, Braun), and phentolamine methanesulfonate (Regitine, Ciba-Geigy, Rueil-Malmaison, France). Ethanol in dialysate and perfusate was determined with an enzymatic method (5); the intra-assay and interassay variabilities were 3.0 and 4.5%, respectively. Glycerol was determined in plasma and in dialysate by using an ultrasensitive radiometric method (6); the intra-assay and interassay variabilities were 5.0 and 9.2%, respectively. Plasma glucose was assayed with a glucose oxidase technique (Biotrol, Paris, France); the intra-assay and interassay variabilities were 1.5 and 5.1%, respectively. Nonesterified fatty acids were assayed with an enzymatic method (Unipath, Dardilly, France); the intra-assay and interassay variabilities were 2.7 and 5.8%, respectively.

**Data Analysis**

All values are given as means ± SE. A statistical comparison of the values was performed by using two-way ANOVA for repeated measures with experimental conditions (control vs. euglycemic-hyperinsulinemic clamp) as factors of the analysis. Subsequently, the effects of drug perfusions were analyzed in each experimental condition by using two-way ANOVA with time as the factor of the analysis and followed by a Bonferroni-Dunnett post hoc test. The extracellular response curves were analyzed by using a paired t-test on the total integrated changes over baseline values (area under the curves (AUC)) by using the trapezoidal method. Values were considered statistically significant when P < 0.05. Statistical analyses were performed by using software packages (Statview 4.5 and SuperAnova 1.11, Abacus Concepts, Berkeley, CA).

**RESULTS**

The fasting plasma free insulin concentration was 6.2 ± 1.2 mU/l. Insulin infusion induced a steady-state insulin level of 83.5 ± 1.4 mU/l that remained stable during the time of the clamp with an average individual insulin level variation being 5.0 ± 2.0%. The fast-
ing arterial plasma glucose level was 4.34 ± 0.07 mM. The subjects were clamped at their individual fasting glucose levels. The coefficients of variation for the glucose levels during hours 3 and 6 of the clamp were 4.36 ± 0.45 and 2.30 ± 0.47%, respectively. The glucose consumptions were calculated from the exogenous glucose infusion rates during hours 3 and 6 of the clamp, and the values were 9.44 ± 0.39 and 10.21 ± 0.26 mg · kg⁻¹ · min⁻¹, respectively.

*Lipolytic Response to Graded Epinephrine Perfusion in Subcutaneous Adipose Tissue: Control Study*

After calibration of the probes, the two basal values of the EGC were similar. Addition of epinephrine to the perfusate caused a rapid dose-dependent increase in the EGC (Fig. 1). As soon as epinephrine was removed from the perfusate, EGC progressively decreased and returned to preperfusion values within 90 min. In the control investigation, i.e., without euglycemic-hyperinsulinemic clamp, the three successive epinephrine perfusion procedures at the two concentrations led to increases in EGC that were not different (Fig. 1A). The calculated AUC values were not significantly different among the three successive perfusions regardless of the epinephrine concentration used (Fig. 1B). Blood samples were taken before the start of each local perfusion for plasma glycerol concentration determination. No significant differences were found among the three values (48.4 ± 11.4, 48.0 ± 15.1, and 65.7 ± 11.4 μM, respectively). In six subjects, addition of phentolamine into the perfusate did not significantly modify the maximal increase of EGC in response to epinephrine perfusion during all three perfusions mentioned above (not shown).

*Lipolytic Response to Graded Epinephrine or Isoproterenol Perfusion in Subcutaneous Adipose Tissue During Hyperinsulinemic-Euglycemic Clamp: Study 1*

Immediately after the end of the first two graded epinephrine or isoproterenol perfusions in the probes, the hyperinsulinemic-euglycemic clamp was started. Before the euglycemic-hyperinsulinemic clamp, perfusion with addition of 1 and 10 μM epinephrine promoted an EGC increase (Fig. 2). Identical epinephrine perfusion during *hour 3* of the clamp induced an EGC increase that was markedly lower (*P = 0.05*) than that before the clamp (Fig. 2A). Finally, the reduction of the epinephrine-induced increase in EGC was even more pronounced during *hour 6* of the clamp (*P < 0.05*; Fig. 2B). From the calculated AUC values, it appeared that the effect of 1 and 10 μM epinephrine perfusion was reduced by 72 and 63%, respectively, during *hour 3* of the clamp and by 81 and 70% during *hour 6* of the clamp.

In the same protocol, graded concentrations of isoproterenol (0.1 and 1 μM) were infused in the second probe at the same time intervals (Fig. 2). Before the clamp, isoproterenol induced an increase in the EGC values. The maximal EGC values were close to those observed with the 10-fold higher concentration of epinephrine. During *hour 3* of the clamp, isoproterenol-induced EGC increase was lower than before the clamp (*P < 0.05*). Finally, the reduction of the isoproterenol-induced increase in EGC was more pronounced during *hour 6* of the clamp (*P < 0.05*). When the calculated AUC values were used (Fig. 2B), the effect of the 0.1 and 1 μM isoproterenol perfusion was reduced by 41 and 28%, respectively, during *hour 3* of the clamp and by 48 and 44%, respectively, during *hour 6* of the clamp. The reductions of the isoproterenol-induced increases in EGC were lower when compared with the reductions for epinephrine-induced increases at the same time interval: the effect of 0.1 μM isoproterenol was compared with that of 1 μM epinephrine (*P < 0.05 at hour 3* and *P < 0.01 at hour 6) and that of 1 μM isoproterenol with 10 μM epinephrine (*P < 0.01 at hour 3* and *P < 0.04 at hour 6).

*Comparison of the Lipolytic Response to Graded Perfusion of Epinephrine Alone or Associated with Phentolamine During Hyperinsulinemic-Euglycemic Clamp: Study 2*

Two probes were used; both were supplemented with epinephrine solutions that were identical in concentrations and time schedule of administration to those of study, but in probe 2, 100 μM phentolamine was added to all the epinephrine solutions. Before the euglycemic-hyperinsulinemic clamp, addition of phentolamine into the perfusate did not significantly modify the maximal increase of EGC in response to epinephrine perfusion (Fig. 3). During *hour 3* of the euglycemic-hyperinsulinemic clamp, the epinephrine perfusion resulted in a markedly decreased response when compared with that before the clamp. During the same time, the reduction of the lipolytic response was lower in the phentolamine-containing probe. The same situation also occurred during *hour 6* of the clamp. When the responses were evaluated by using AUC (Fig. 3B), it appeared that, during *hour 3* of the clamp, the epinephrine effect was reduced by 67 and 59% in the probe containing 1 and 10 μM epinephrine, respectively. It was less reduced by 35 and 47% in the probe containing the phentolamine in addition to epinephrine. The difference between the responses in the two probes was significant. The same difference was shown during *hour 6* of the clamp, where the effect of the sole epinephrine perfusion was reduced by 75 and 67% and the effect in the phentolamine-containing probe was reduced by only 19 and 40%, respectively. The difference between the responses in the two probes remained significant. Thus, during the euglycemic-hyperinsulinemic clamp, the responses of EGCs to epinephrine perfusion were significantly less reduced in the presence of phentolamine than in control probes containing epinephrine alone.

*Extracellular and Plasma Glycerol Concentrations During the Euglycemic-Hyperinsulinemic Clamp*

The changes of EGC during the euglycemic-hyperinsulinemic clamp were evaluated in a probe that was...
infused with Ringer solution throughout the experimental protocol (Fig. 2). Dialysate samples were taken at the same time intervals as in the other probes. Blood samples were taken 5 min before the clamp and at minutes 180 and 360 of the clamp for plasma glycerol concentration determination. We observed a progressive decrease of EGC (246 ± 16.3, 194 ± 36, and 150 ± 37 μM) and plasma glycerol concentrations (34.8 ± 3.9, 9.7 ± 2.5, and 9.2 ± 2.1 μM) during the euglycemic-hyperinsulinemic clamp.

Fig. 2. In situ effects of 3 successive perfusions of graded concentrations of Epi (1 and 10 μM) or isoproterenol (Iso; 0.1 and 1 μM) on EGC in subcutaneous abdominal adipose tissue during euglycemic-hyperinsulinemic clamp. Epi or Iso perfusion was applied through the microdialysis probes at each concentration during 30 min, and dialysate samples were collected for each 10-min period. The successive graded Epi or Iso perfusions were separated by 2 h, and dialysate samples were collected for each 15-min period. The third probe was perfused with Ringer solution during the entire protocol. A: absolute values of EGC. The intravenous infusion of insulin was started immediately after Epi or Iso was removed from the probe, and euglycemic-hyperinsulinemic clamp was maintained until the end of the experimental period. B: AUC (μM/30 min) of the variation of the EGC induced by Epi or Iso during euglycemic-hyperinsulinemic clamp. Values are means ± SE. **P < 0.02 compared with values obtained during the first graded perfusion of Iso.

Modifications of Regional ATBF

The changes in the local adipose tissue microcirculation were evaluated by using the method based on the measurement of ethanol washout. Ethanol ratio was calculated as reported in METHODS and taken as an index of ethanol washout. The higher ethanol ratio corresponds to the lower ethanol washout, and this reflects the lower regional ATBF. The average ethanol ratios measured during the last 10-min period of each
graded perfusion of catecholamines are presented in Fig. 4. In the control study, there were no differences in the epinephrine-induced increase (at 10 μM) in the ethanol ratio during the course of the protocol despite the fact that the increase did not reach a level of significance during the third perfusion (Fig. 4). In the study with clamp (probe 2), the epinephrine-induced increase in the ethanol ratio (10 μM) before the clamp was not different from increases during the clamp. Both isoproterenol perfusion (probe 1) and the perfusion of epinephrine combined with phentolamine (probe 2) did not induce a significant decrease in ethanol ratios during the clamp.

**DISCUSSION**

The present study, using the microdialysis technique, reveals the impacts of hyperinsulinemia on the adrenergic regulation of lipolysis in subcutaneous adipose tissue in situ. The lipolytic effects of epinephrine and of β-AR adrenergic agonist isoproterenol were reduced during a euglycemic-hyperinsulinemic clamp. This suggests that the β-adrenergic component of epinephrine action was reduced during hyperinsulinemia. In addition to that, the greater reduction of the lipolytic effect of epinephrine (when compared with isoproterenol) might be explained by the involvement of the antilipolytic component of epinephrine action mediated by α2-ARs. This conclusion is supported by the finding that the lipolytic effect of epinephrine was enhanced by addition of the α2-AR antagonist phentolamine during hyperinsulinemia; the EGC increase thus becomes similar to that initiated by isoproterenol.

Lipolysis in human fat cells is mainly controlled by insulin and catecholamine. Catecholamines (i.e., epinephrine and norepinephrine) promote the activation of both lipolytic β-ARs and antilipolytic α2-ARs. The occurrence of an antilipolytic potency of catecholamines, particularly epinephrine, which exhibits a higher affinity for the α2-AR, is noticeable in human subcutaneous adipocytes. Consequently, epinephrine is less efficient in stimulating lipolysis than a selective catecholamine that possesses only the β-AR potency. Moreover, the antilipolytic action of epinephrine is particularly expressed in subcutaneous adipocytes from obese subjects, and we have previously demonstrated that, in obese subjects, the activation of the α2-AR strongly reduces the lipolytic effect of physiological catecholamines during exercise (30). To assess the
hypothesis of a balanced activity of β- and α2-ARs (when the involvement of the β-AR pathway is lowered in adipose tissue, the antilipolytic α2-AR component is strengthened), we compared the effect of insulin administration on the lipolytic effect of epinephrine with isoproterenol explored in situ.

At the beginning of the present studies, we verified the absence of desensitization when catecholamine perfusions were performed. The interval between the various epinephrine perfusions has been selected to prevent (or limit) the incidence of desensitization and limit its interference with the effect of insulin. Repeated intravenous perfusions of epinephrine (28) or microdialysis perfusion (24) could lead to desensitization of catecholamine-induced lipolysis in human adipose tissue. This phenomenon was not found in rat adipose tissue when norepinephrine was infused into microdialysis probes at 1-h intervals (8). In fact, it occurs in human adipose tissue when catecholamine perfusions are applied at intervals of 1 h (24) or less (28). We verified the absence of desensitization when catecholamine perfusions were performed in an interval of 2 h. In the control study without euglycemic-hyperinsulinemic clamp, the repeated perfusions of epinephrine into the probes were realized, and they resulted in the same EGC increase for both epinephrine concentrations (Fig. 1).

It has previously been shown that the in vitro incubation of fat cells with insulin significantly reduces the lipolytic effect of isoproterenol (10). This effect is classically attributed to the insulin-dependent activation of phosphodiesterase, an intracellular enzyme that hydrolyzes cAMP. An additional explanation involves cross-regulation between insulin and β2-AR, i.e., growth factor tyrosine kinase receptors, like insulin receptor, directly phosphorylating a G protein linked to the β2-AR (3). This mechanism probably leads to internalization of β-AR into the cytoplasmic compartment since binding studies have revealed a decrease in β-AR in the plasma membrane of human fat cells treated with insulin (10). It has also been shown that insulin has no effect on the binding of α2-AR-selective radioligands in human fat cells (10) and on the antilipolytic effect of the selective α2-AR adrenergic agonist UK-14304 (personal data). So, in insulin-treated fat cells, the β/α2-AR balance is modified in favor of the α2-AR antilipolytic pathway in human fat cells.

At the concentration used, insulin inhibits spontaneous lipolysis in the SCAAT and lipid mobilization in the fasted subjects. Moreover, hyperinsulinemia reduced the isoproterenol-induced lipolysis (Fig. 2). Isoproterenol exhibits an ~10-fold higher affinity for β-ARs than epinephrine. Thus the chosen concentrations of epinephrine infused in the probes were 10-fold higher than those of isoproterenol to obtain a similar increase in EGC with both amines. In such conditions, before the euglycemic-hyperinsulinemic clamp, the increase in EGC was similar with the two catecholamines. The reduction of epinephrine-induced lipolysis during the clamp was significantly higher than that of isoproterenol-stimulated lipolysis for the corresponding concentrations. The blunting effect of insulin on the epinephrine-induced changes in subcutaneous EGC (an index of in situ lipolysis) could result from multiple mechanisms. The first one could be due to phosphodiesterase activation and/or phosphorylation of the G protein linked to the β-AR or the β-AR itself by insulin,
and the second one could result from the inhibition of the formation of cAMP through activation of fat cell α₂-ARs (7, 19). The latter mechanism could be involved in differences observed between the effects of hyperinsulinemia on the lipolytic effects of epinephrine alone and epinephrine coupled with phentolamine. When phentolamine (an α₁/α₂-AR antagonist) was infused in the probe together with epinephrine, the EGC response was higher during the clamp than with epinephrine alone, and, consequently, the reduction in lipolytic effect promoted by epinephrine, during the euglycemic-hyperinsulinemic clamp, was clearly less pronounced. It is noticeable that before the euglycemic-hyperinsulinemic clamp, phentolamine alone did not significantly change the epinephrine-induced increase in EGC. The latter observation suggests that, in the presence of low plasma insulin concentrations, the α₂-AR antilipolytic effect is not apparent. This is in disagreement with a previous report showing that phentolamine potentiates both spontaneous and norepinephrine-stimulated lipolysis (2) in a similar microdialysis protocol. However, in several other studies, the enhancing effect of phentolamine on spontaneous glycerol release has not been found (15, 21). In fact, it was shown that the potentiating effect of phentolamine resulting from α₂-AR blockade in SCAAT could be discerned clearly during exercise when the activity of the adrenergic system is increased (21).

During interpretation of lipolysis data based on glycerol concentrations in dialysate outflowing from the microdialysis probe, the regional ATBF must be taken into account. It has been demonstrated that the EGC in human adipose tissue is influenced by the local blood flow (4, 11, 13). In the present study, it was not possible to evaluate ATBF changes induced by the perfusion of pharmacological agents through the probe by the conventional technique, i.e., the 133Xe washout; the space of diffusion of the perfused agents, and consequently the volume in which the ATBF changes are produced in SCAAT are very narrow and different from the distribution volume of xenon injected directly into SCAAT. Consequently, the 133Xe-washout method reflects the blood flow in larger and not identical volumes of adipose tissue. Thus a method based on the evaluation of ethanol washout, measured by using ethanol ratio, was used for assessment of changes in adipose tissue microcirculation. The increase in ethanol ratio reflects a decrease in ATBF (12). The ethanol method does not enable the corrections of individual EGC for ATBF values, but, reflecting the variations in ATBF, it provides a possibility to assess whether and in which sense the changes in EGC were influenced by the changes in ATBF. In this study, epinephrine induced a decrease in ATBF that was significant at the concentration of 10 μM (Fig. 4). This finding could suggest that the epinephrine-induced increase in EGC is enhanced because of the reduction of local blood flow and, consequently, that the increase in the rate of lipolysis is over estimated. During the euglycemic-hyperinsulinemic clamp, the epinephrine effect on ATBF was not modified. This finding suggests that the reduction in epinephrine-induced increase of EGC, observed during euglycemic-hyperinsulinemic clamp, was not influenced by the changes in ATBF and thus reflects the true inhibition of lipolytic processes in adipose tissue. Insulin by itself, at the concentrations used, does not change ATBF as previously shown (29). When epinephrine was perfused with phentolamine, a tendency toward a nonsignificant increase in ethanol washout (i.e., an increase in local ATBF) was observed. Thus the potentiating effect of phentolamine on an epinephrine-induced increase in EGC during euglycemic-hyperinsulinemic clamp could be attributed to the blockade of fat cell α₂-ARs, and the slight phentolamine-induced vasodilatation could lead only to underestimation of potentiating action of phentolamine on epinephrine-induced lipolysis.

In summary, the present study demonstrates that hyperinsulinemia reduces the lipolytic effect of catecholamines in adipose tissue. The results suggest that several mechanisms are involved in the resulting insulin-induced blunting of the epinephrine-induced lipolysis in situ. First, the β-AR-dependent lipolytic pathway is reduced, presumably through the activation of phosphodiesterase-3B and by the desensitization of the β-AR-dependent mechanisms from insulin. These mechanisms could explain the reduction of the effect of isoproterenol. Second, the activation of the antilipolytic α₂-AR-dependent pathway by epinephrine could contribute to counteraction of the β-AR-dependent lipolytic pathway, which would explain the alterations that occur in the action of epinephrine. The present study suggests that hyperinsulinemia contributes to alterations of adipose tissue characteristics that were described in insulin resistance (such as upper body obesity) and suggests the mechanisms by which β- and α₂-AR-mediated pathways are involved in these alterations. It is not the only pathway related to hyperinsulinemia-induced actions, and its physiological importance, compared with other pathways, remains to be firmly established. Further studies investigating in vivo the relative contribution of β- and α₂-AR-mediated pathways in adipose tissue metabolism in conditions related to altered levels of plasma insulin (physiological: nutrition; pathological: obesity, diabetes) are warranted.

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DISCLOSURES

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