Role of Phosphodiesterase III in the Antilipolytic Effect of Insulin In Vivo

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The effect of three types of phosphodiesterase (PDE) inhibitors on in vivo antilipolysis was investigated in healthy subjects using a 2-h euglycemic, hyperinsulinemic (40 mU · m^{-2} · min) clamp together with microdialysis of abdominal subcutaneous adipose tissue. During hyperinsulinemia (~330 pmol/l), the circulating glycerol concentration was reduced to ~50% of the basal level of 53.2 ± 3.6 μmol/l, indicating an antilipolytic effect. The decrease in adipose tissue dialysate glycerol, which mirrors the change in interstitial glycerol concentration, was about 40% during hyperinsulinemia when Ringer's solution alone was perfused. Local perfusion with a selective PDE IV inhibitor, rolipram (10^{-4} mol/l), did not influence the insulin-induced decrease in dialysate glycerol ($F = 0.8$ vs. perfusion with Ringer's solution by two-factor analysis of variance [ANOVA]), although rolipram increased the dialysate glycerol level by 144 ± 7% of the baseline value. However, local perfusion with a selective PDE III inhibitor, amrinone (10^{-3} mol/l), or a nonselective PDE inhibitor, theophylline (10^{-2} mol/l), abolished the ability of insulin to lower dialysate glycerol ($F = 16.5, P < 0.01$ and $F = 8.5, P < 0.01$, respectively, as compared with perfusion with Ringer's solution). The findings could not be explained by changes in the local blood flow (as measured by a microdialysis–ethanol escape technique), which was not affected by hyperinsulinemia in the presence or the absence of PDE inhibitors in the dialysis solvent. We conclude that PDEs play an important role in mediating the antilipolytic effect of insulin in vivo and that PDE III is the dominant isoenzyme modulating this effect. *Diabetes* 44:1170–1175, 1995

Insulin and catecholamines play a major role in the acute regulation of human adipose tissue lipolysis (1). Whereas the stepwise action of catecholamine-induced lipolysis is well delineated, the mechanisms for the antilipolytic effect of insulin are not fully known. When insulin is bound to the receptor, an activation of tyrosine kinase in the receptor occurs (2), leading to an activation of phosphatidylinositol 3-kinase (3), an enzyme recently thought to be involved in the mediation of insulin-induced glucose uptake and antilipolysis in rat adipocytes (4). Although the course is not completely understood, one important effect of the insulin signal is a reduction in the intracellular cAMP content, leading to a reduction in activated hormone-sensitive lipase. This is accomplished by inhibition of the adenylate cyclase (5,6), downregulation of β-adrenoceptors (7), or perhaps most importantly, activation of cAMP phosphodiesterase (PDE) (8). There are at least five major families of PDE isoenzymes (9), but during recent years, mainly PDE type III (cGMP-inhibited, low K_m, cAMP-PDE) has been suggested as a mediator of the antilipolytic effect of insulin in adipose tissue (10,11). Knowledge about the PDE effects on adipose tissue lipolysis has hitherto been based on in vitro findings. However, we have recently presented a report on the effects on lipolysis in human adipose tissue in vivo of nonselective and selective (PDE III) inhibition of PDE (12) found using a microdialysis method. It has been shown that PDE III inhibition with amrinone stimulates lipolysis, but not to the same extent as theophylline, the nonselective PDE inhibitor. This indicates that other PDE isoenzymes should be involved in the regulation of lipolysis. Both drugs were also shown to affect adipose tissue blood flow, a finding which could be important for the rate of lipolysis (13). Regarding the antilipolytic effect of insulin, it has been shown in vitro that it can be abolished by inhibition of the nonselective PDE inhibitors theophylline (14) and isobutylmethylxanthine (15). This was also noted for the selective inhibitor of PDE isoenzyme III (10,11,16), whereas the inhibition of PDE isoenzyme IV did not inhibit antilipolysis by insulin (17,18).

The present study was undertaken to examine the in vivo role of PDE in the antilipolytic effect of insulin. Subcutaneous adipose tissue of healthy subjects was microdialyzed with solvents containing nonselective or selective PDE inhibitors (acting on the type III or type IV isoforms) before and during intravenous infusion of insulin under euglycemic conditions. A unique role of PDE III was demonstrated.

RESEARCH DESIGN AND METHODS

Subjects. The study group consisted of nine healthy and drug-free volunteers (five men and four women), aged 26–51 years (mean 41 years). They were not obese and had body mass indexes ranging from 21.5 to 25.6 kg/m^2 (mean 23.8 ± 0.5 kg/m^2). The study was approved by the Ethics Committee of Karolinska Institute. The subjects were given a detailed description of the experiments, and their consent was obtained.

Microdialysis probe. The microdialysis equipment has been described in detail elsewhere (19). Briefly, a tubular dialysis membrane (0.5 × 10 mm, 20,000 MW cutoff) is glued to the end of a double-lumen steel cannula. The inlet tubing is connected to a microinfusion pump (CMA/100 Microinjection Pump, CMA Microdialysis AB, Stockholm, Sweden) and continuously perfused (1.5 μl/min). The dialysis perfusion fluid enters the probe through the inner cannula, streams upward in the space between the inner cannula and the outer dialysis membrane, and leaves the probe through the outer cannula. Timed fractions of dialysate fluid are collected for the analysis of glycerol (lipolysis index) and ethanol (blood flow index).

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ANOVA, analysis of variance; CV, coefficient of variation; PDE, phosphodiesterase.

Experimental protocol. The subjects were examined in the supine position in the morning after an overnight fast. After local skin anesthesia (EMLA, Astra, Södertälje, Sweden), the microdialysis probes (3 or 4 per experiment) were inserted percutaneously into the abdominal subcutaneous adipose tissue immediately to the right or the left of the umbilicus. The distance between two probes was always 30 mm. The basal dialysate perfusion fluid was Ringer's solution (sodium 1.5×10^{-1} mol/l, potassium 4×10^{-3} mol/l, calcium 2.3×10^{-3} mol/l, chloride 1.6×10^{-1} mol/l) with 50×10^{-3} mol/l ethanol (Vin & Spritcentralen, Stockholm, Sweden). To this was added 10^{-2} mol/l of theophylline (Draco, Lund, Sweden), 10^{-3} mol/l of amrinone (Sterling-Winthrop, New York, NY), or 10^{-4} mol/l of rolipram (a gift from Schering AG, Berlin, Germany) in sterile solution. The concentrations of amrinone and theophylline presently used have previously been shown to induce a maximal lipolytic effect in microdialysis experiments (12). Separate dose-response experiments were performed in order to find the correct concentration of rolipram to use in the study. The dialysate was collected in 15-min samples.

Plasma samples were drawn every 15 min, in the middle of each dialysate sampling period, from a polyethylene catheter (Venflon) inserted into a superficial hand vein. The hand was placed in a heated box (60–65°C) for arterialization of venous blood (20). Arterialization (≥94% O_2 saturation) was confirmed by blood-gas analysis. A second catheter was introduced into an antecubital vein of the ipsilateral arm for the infusion of glucose and insulin in accordance with the hyperinsulinemic euglycemic clamp technique previously described (21).

After 60 min of baseline microdialysate sampling and two baseline plasma samples (at −10 and 0 min), insulin was infused at 40 mU · m^2 · min during 120 min, of which the first 8 min was a priming infusion. Arterialized blood glucose was checked every 5th minute (HemoCue, HemoCue AB, Ängelholm, Sweden), and varying amounts of 20% glucose were infused to keep blood glucose levels constant at the fasting level.

Analyses of glucose and insulin. Arterialized plasma glucose concentration was determined by a routine enzymatic method (22). Plasma insulin was analyzed using a commercial radioimmunoassay kit (Pharmacia Insulin RIA, Pharmacia, Uppsala, Sweden).

Analysis of glycerol. Ten microliters of plasma or dialysate was used for the analysis of glycerol. An automatic ultrasensitive kinetic bioluminescence assay of glycerol (23) and a bioluminescent analyzer were used for the assay.

Analysis of ethanol. Ten microliters of dialysate fluid or perfusate solvent was used to measure the ethanol concentration with an enzymatic fluorometric method (24). The ratio of ethanol concentration in the outgoing and ingoing dialysate fluids was determined to evaluate changes in blood flow. Alterations in this ratio reflect changes in the blood flow surrounding the microdialysis probe (25).

Statistical analysis. Values are means ± SE. Coefficient of variation (CV) was calculated as the standard deviation divided by the mean × 100 (%). Analysis of variance (ANOVA) for repeated measures and, when applicable, Student's t test were used for statistical comparisons.

RESULTS

Plasma glucose, glycerol, and insulin. The arterialized plasma glucose concentration was stable at the fasting level throughout the 2-h clamp, the CV being 6.1 ± 0.7% (Fig. 1*A*). Plasma free insulin increased rapidly during the priming infusion to a peak of 565 ± 29 pmol/l at 7.5 min. Thereafter, a stable insulin concentration of ~330 pmol/l was obtained during the clamp (CV 9.9 ± 2%) (Fig. 1*A*). The circulating concentration of glycerol is shown in Fig. 2*B*. The fasting arterialized glycerol concentration was 53.2 ± 3.6 μmol/l. After initiation of the insulin infusion, plasma glycerol concentrations decreased continuously to about 50% of the basal level during the 2nd hour of the clamp.

Adipose tissue glycerol. Figure 2 shows the effect of systemic hyperinsulinemia and in situ PDE inhibition on adipose tissue glycerol levels. During the hyperinsulinemic clamp in situ, perfusion with Ringer's solution resulted in a 40% decrease of the dialysate glycerol concentration compared with the baseline level of 25.5 ± 3.6 μmol/l (Fig. 2*A*). When the selective PDE IV inhibitor rolipram was added to the perfusate solution (n = 7), both the baseline dialysate glycerol levels and the decrease in glycerol during insulin infusion were similar to those of perfusion with Ringer's solution alone (Fig. 2*B*). On the other hand, the addition of the selective PDE III inhibitor amrinone (n = 9) resulted in only a transient, though not significant, initial decrease in the dialysate glycerol levels, which thereafter increased slightly above the baseline during systemic hyperinsulinemia (Fig. 2*C*). The baseline level of glycerol (25 ± 2.8 μmol/l) in the amrinone experiment did not differ from the corresponding level in the control experiment. Lastly, in situ perfusion with the nonselective PDE inhibitor theophylline (n = 9) increased the basal levels of dialysate glycerol significantly, as compared with levels in Ringer's perfusion (61.9 ± 9.3 vs. 25.5 ± 3.6 μmol/l, $P < 0.001$). During insulin infusion, there was a small and transient, but significant, decrease in dialysate glycerol at 15–45 min after the start of the insulin infusion; thereafter, there was no change in the dialysate glycerol concentration as compared with the baseline (Fig. 2*D*). When data were expressed as a percentage change from basal dialysate glycerol levels, statistical comparison of Ringer perfusion and perfusion with the various PDE inhibitors using two-way ANOVA showed no difference between Ringer's and the selective inhibitor of PDE IV rolipram (F = 0.8, NS). On the contrary, perfusion with amrinone and theophylline almost completely abolished the glycerol-lowering effect of hyperinsulinemia (Ringer's vs. amrinone F = 16.5, $P < 0.001$; Ringer's vs. theophylline F = 8.5, $P < 0.001$).

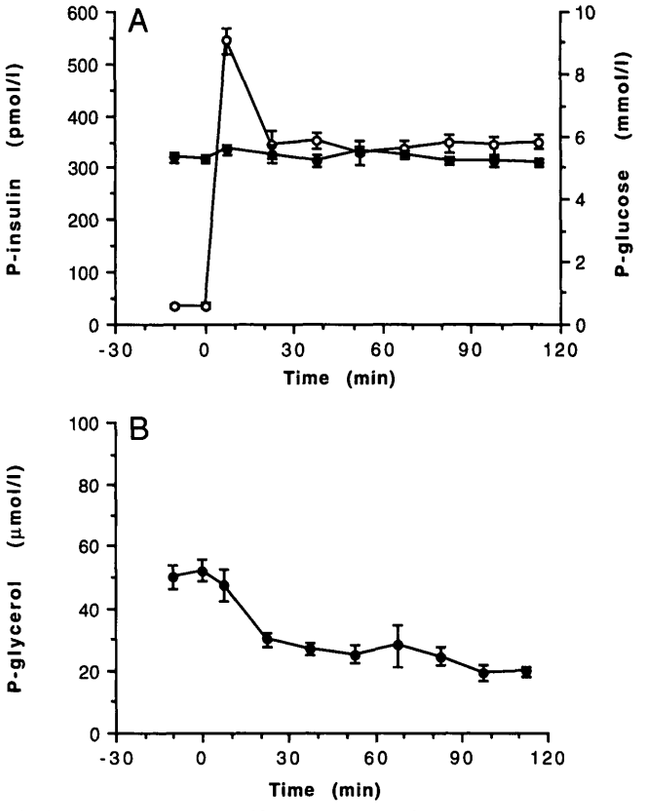

FIG. 1. The plasma glucose (●), free insulin (○) (*A*), and glycerol (*B*) levels in the fasted state and during a 2-h insulin clamp are shown. A euglycemic hyperinsulinemic clamp was performed in nine healthy subjects in the morning after an overnight fast. At time 0, a priming, followed by a continuous (40 mU · m^2 · min) insulin infusion, was started. A variable glucose infusion was used to maintain the circulating glucose at the fasting level. Arterialized plasma samples were drawn in the basal state and then every 15 min. Values are means ± SE.

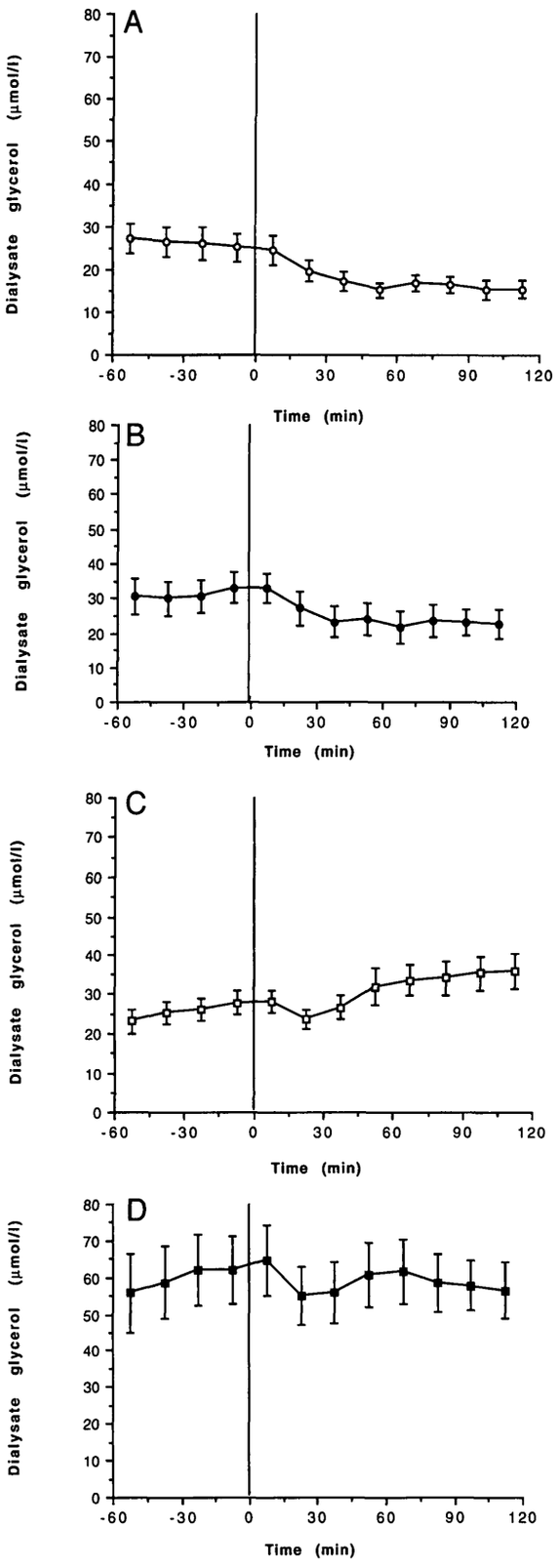

FIG. 2. The adipose tissue dialysate glycerol levels during a 2-h insulin clamp. Microdialysis probes (0.5 × 10 mm) were inserted into the abdominal subcutaneous adipose tissue and continuously perfused (1.5 μl/min) with Ringer's solution (n = 9) (*A*) with the addition of 10^{-4} mol/l rolipram (n = 7) (*B*), 10^{-3} mol/l amrinone (n = 9) (*C*), and 10^{-2} mol/l theophylline (n = 9) (*4*). Fractions of dialysate were sampled every 15 min. After 60 min of baseline sampling, the continuous insulin infusion was started (time 0). Statistical calculations performed with ANOVA repeated measurements are as follows: F = 11.47, P < 0.001; F = 7.60, P < 0.001; F = 7.64, P < 0.001; and F = 2.46, P < 0.05 for *A–D*, respectively. Values are means ± SE.

Similar results were found when the maximum glycerol-lowering effect of insulin (%) was determined for the situations with and without PDE inhibitors; there was no difference between the perfusion with rolipram and the perfusion with Ringer's solution alone (about 40% decrease for both). On the other hand, amrinone and theophylline markedly attenuated the maximum glycerol-lowering effect of the insulin infusion (~15% decrease for both). These values differed compared with the control situation (P < 0.001).

The concentration-response relationship for theophylline- and amrinone-induced changes in adipose tissue glycerol levels have been reported recently (12). In the present study, the same relationship was examined for rolipram using the same experimental protocol as described previously (12). Microdialysis was performed without (baseline) or with either 10^{-6} mol/l or 10^{-4} mol/l of rolipram in the dialysis solvent (n = 3). A concentration-dependent increase in dialysate glycerol was obtained with rolipram. The values as percentage of baseline were 123 ± 4 for 10^{-6} mol/l and 144 ± 7 for 10^{-4} mol/l. There were no significant effects of these concentrations of rolipram on the ethanol ratio.

Ethanol escape. The effects of PDE inhibition and hyperinsulinemia on adipose tissue blood flow (as measured by ethanol escape) were investigated with an ethanol perfusion technique. The results are shown in Fig. 3. The ethanol outflow-to-inflow ratio reflects changes in microcirculation insofar as a reduced ratio indicates an increased loss of ethanol from the interstitial fluid, caused by enhanced blood flow (12,25,26). The ratio was not affected by the insulin infusion when the tissue was perfused with Ringer's solution or the PDE inhibitors. It should be mentioned that perfusion with rolipram induced a transient, but not significant, decrease in the ratio late in the 1st hour of the insulin infusion (F = 1.60, P = 0.12, using ANOVA for repeated measurements). However, when the ratios of Ringer's and rolipram perfusion were compared, the difference did not reach significance (F = 1.93, P = 0.054 by two-factor ANOVA).

DISCUSSION

These data show for the first time an in vivo interaction between insulin and PDE in humans. The results indicate that PDE III is the principal phosphodiesterase isoenzyme mediating the antilipolytic effect of insulin. The insulin levels obtained during the euglycemic insulin clamp in this study were in the upper physiological range and comparable with insulin concentrations reached in the postprandial state. In adipose tissue, these insulin levels resulted in a 40% decrease in glycerol, although the circulating insulin level increased to 350 pmol/l. It is possible that maximal antilipolysis was not obtained at this insulin level. However, it has been shown in vitro that the antilipolytic effect is the most sensitive of the insulin effects, e.g., antilipolysis occurs at lower insulin levels than glucose metabolism (27,28). In the circulation, there was a slightly more pronounced glycerol-lowering effect of insulin (about 50%). This might be explained by the existence of regional differences in lipolysis, which have been demonstrated in vitro (29,30) and in vivo (31,32), because the circulating levels of glycerol reflect the sum of glycerol release and uptake in the whole body.

The dialysis fluid obtained with microdialysis of adipose tissue mirrors the interstitial fluid. Thus, the dialysate glyc-

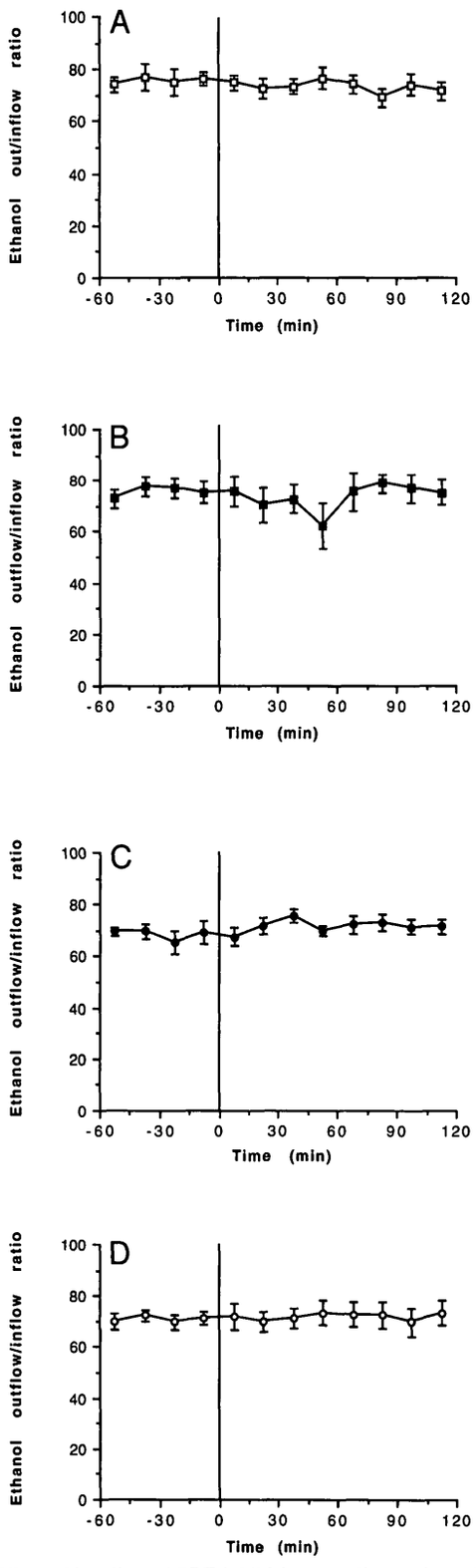

FIG. 3. Effect of insulin and PDE inhibition on adipose tissue blood flow. Microdialysis probes were inserted into the abdominal subcutaneous adipose tissue and perfused with Ringer's solution ± PDE inhibitors, as described in the legend to Fig. 2. In addition, 50 mmol/l ethanol was added to the perfusion fluid. The ethanol concentration was determined in the outgoing and ingoing solvents in time fractions, as described above. The ratio (%) was calculated.

erol concentration reflects the net changes in metabolic concentrations in the surrounding extracellular water. These changes are influenced mainly by the release of glycerol from adipose tissue and by changes in the local blood flow, increasing or reducing the removal of glycerol from the tissue. Glycerol is not reutilized in adipose tissue and the dialysate glycerol levels can thus be used as an index of lipolysis (33). Theoretically, the interstitial concentration of glycerol may be influenced by glycerol from extracellular triglycerides, hydrolyzed by lipoprotein lipase. However, this enzyme is stimulated by insulin and therefore should increase lipolysis, contrary to what is shown here. In addition, recent studies with a technique for measuring arteriovenous differences across the subcutaneous adipose tissue of the anterior abdominal wall have shown that only minor amounts of adipose tissue–derived glycerol originate from hydrolysis of lipoproteins in the fasting state (34). Furthermore, there should be no inflow of glycerol from the circulation to the adipose tissue interstitial fluid because the interstitial concentration of glycerol is at least three times higher than that of plasma, as shown previously using an in vivo equilibration technique (35,36). Unfortunately, this technique is too time-consuming in a study like the present one, and more importantly, it should not be necessary for understanding a kinetic study when comparing the effect of various drugs. In the present study, insulin was administered systemically for technical reasons because the pores of the dialysis membrane are too small to allow the penetration of insulin molecules. Membranes with a larger pore size have been used to study insulin kinetics in humans (37), but they are not easily usable in the kind of study we performed because much longer sampling times are needed for equilibration.

The data presented here show that the basal levels (before insulin infusion) of dialysate glycerol were similar when the tissue was perfused with Ringer's solution alone or with the addition of rolipram. When amrinone was added to the perfusate, the baseline increased with time, indicating a small lipolytic effect that confirms previous results obtained with microdialysis (12). When theophylline was perfused, the basal levels were greatly elevated because of the lipolytic effect of the drug. This finding is also in accordance with our previous data showing that theophylline is a stronger lipolytic agent than amrinone (12). During hyperinsulinemia, the antilipolytic (i.e., glycerol-reducing) effect of insulin was similar when the tissue was perfused with Ringer's solution and with rolipram (~40%), indicating that the inhibition of PDE IV does not affect antilipolysis, a finding which is in accordance with previous studies in vitro (17,18). On the other hand, in situ perfusion with amrinone, a selective inhibitor of PDE III, resulted in a complete inhibition of the antilipolytic effect of the insulin infusion, indicating the importance of this isoenzyme for the action of insulin in adipose tissue. It should be emphasized that the rolipram concentration used in the present study (10^{-4} mol/l) was in a concentration-response experiment shown to induce an increase in lipolysis (change from perfusion with Ringer's solution only) comparable with the maximum lipolytic effect obtained with amrinone in similar experiments—~140% of baseline (12). Thus, although 10^{-4} mol/l of rolipram stimulated lipolysis in vivo (presumably because of PDE IV inhibition), it did not counteract the antilipolytic effect of insulin. In situ theophylline perfusion also caused a reversal

of the antilipolytic effect of insulin. However, in contrast to amrinone, theophylline did not increase lipolysis further during the clamp. This difference is most probably due to the fact that maximal lipolysis was obtained with theophylline from the start, whereas perfusion with amrinone continuously increased lipolysis. Hence, these data suggest that PDE III is the principal isoenzyme mediating the antilipolytic effect of insulin in vivo—a finding in accordance with previous in vitro findings (10,11).

It has to be considered that the effects of theophylline on glycerol in the basal state could be partly explained by adenosine-receptor blocking. It has been shown that theophylline affects this receptor at much lower concentrations than those needed for PDE inhibition (38). However, the aim of the study was to investigate the antilipolytic action of insulin, and for this aspect, adenosine-receptor inhibition seems less important because it has been shown previously that insulin-induced inhibition of lipolysis in human fat cells is independent of adenosine (39). Regarding amrinone, adenosine receptor effects have been shown in vitro in rat hearts (40), but we have failed to find data on adipose tissue. Furthermore, the perfusate concentration of the drug used in this study (10^{-3} mol/l) leads to a tissue concentration that is much lower (probably, at most, one-tenth) because of an uncompleted equilibration of the perfusate and the interstitial fluid at the perfusate velocity and membrane length. Hence, available in vitro data indicate that adenosine effects occur at higher concentrations than those presently obtained (40), and antilipolysis induced by adenosine-receptor blocking should therefore be insignificant. The effect of rolipram on the adenosine receptor, so far as we know, is not known. However, another PDE IV blocking agent, RO-20-1724, has been shown not to affect the adenosine receptor (41).

As discussed previously, local blood flow may influence adipose tissue levels of glycerol. In this study, we measured blood flow with an ethanol-escape technique. We found that during hyperinsulinemia, neither perfusion with Ringer's solution alone nor perfusion with amrinone, rolipram, or theophylline added to the perfusate-induced changes in the local blood flow. This indicates that the changes observed in the glycerol concentration actually reflected lipolysis and were not due to an increase or decrease in the removal of glycerol. The ethanol-escape technique for indirect measurements of changes in blood flow was first introduced for use in muscle tissue (25) and was subsequently modified for use in adipose tissue (12). The technique has recently been validated by comparing it with the ^{133}Xe-clearance technique in muscle (26), and data showing similar accuracy for adipose tissue have been obtained in our laboratory (Fellländer G, Linde B, Bolinder J, unpublished observations). We could not use the ^{133}Xe-clearance technique in the present microdialysis study because the effects of various drugs perfused in situ on local blood flow were to be evaluated. It has been shown that xenon injected close to the microdialysis probe can be detected in the microdialysis fluid (42), but the area around each microdialysis catheter that is affected by a dialyzed drug would be too small to evaluate with the ^{133}Xe-clearance technique. It would also not be possible to distinguish the effect on xenon clearance of substances perfused in 2 or 3 different microdialysis probes in the same area.

In summary, this study shows that the antilipolytic effect of insulin in vivo can be abolished by PDE inhibition and it appears that PDE III is the major site of this action. The present results are based on findings in a single fat depot, the subcutaneous adipose tissue, and it remains to be established whether the present findings apply to adipose tissue in other regions, such as intra-abdominally located fat.

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REFERENCES

1. Arner P: Control of lipolysis and its relevance to development of obesity in man. *Diabetes Metab Rev* 4:507–515, 1988
2. Kahn CR: The molecular mechanism of insulin action. *Annu Rev Med* 36:429–451, 1985
3. Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S: Oncogenes and signal transduction. *Cell* 64:281–302, 1991
4. Okada T, Kawono Y, Takako S, Hazeki O, Ui M: Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. *J Biol Chem* 269:3568–3573, 1994
5. Iliano G, Cuatrecasas P: Modulation of adenylate cyclase activity in liver and fat cell membranes by insulin. *Science* 175:906–908, 1972
6. Houslay MD, Kilgour E: *Cyclic Nucleotide Phosphodiesterases: Structures, Regulation, and Drug Action.* Chichester, U.K., Wiley, 1990, p. 185–226
7. Engfeldt P, Hellmér J, Wahrenberg H, Arner P: Effects of insulin on adrenoceptor binding and the rate of catecholamine-induced lipolysis in isolated human fat cells. *J Biol Chem* 263:15553–15560, 1988
8. Manganiello VC, Vaughan M: An effect of insulin on cyclic adenosine 3′:5′ monophosphate phosphodiesterase activity in fat cells. *J Biol Chem* 248:7164–7171, 1973
9. Hall IP: Isoenzyme selective phosphodiesterase inhibitors: potential clinical uses. *Br J Clin Pharmacol* 35:1–7, 1993
10. Manganiello VC, Smith CJ, Degerman E, Vasta V, Törnqvist H, Belfrage P: Molecular mechanisms involved in the antilipolytic action of insulin: phosphorylation and activation of a particulate adipocyte cAMP phosphodiesterase. In *Molecular Biology and Physiology of Insulin and Insulin-Like Growth Factors.* Raizada MK, LeRoith D, Eds. New York, Plenum, 1991, p. 239–248
11. Manganiello VC, Degerman E, Smith CJ, Vasta V, Törnqvist H, Belfrage P: Mechanisms for activation of the rat adipocyte particulate cyclic-GMP-inhibited cyclic AMP phosphodiesterase and its importance in the antilipolytic action of insulin. In *Advances in Second Messenger and Phosphoprotein Research.* Vol. 25. Strada J, Hidaka H, Eds. New York, Raven, 1992, p. 147–164
12. Arner P, Hellmér J, Hagström-Toft E, Bolinder J: Effect of phosphodiesterase inhibition with amrinone or theophylline on lipolysis and blood flow in human adipose tissue in vivo, as measured by microdialysis. *J Lipid Res* 34:1737–1743, 1993
13. Rosell S, Belfrage P: Blood circulation in adipose tissue. *Physiol Rev* 59:1078–1104, 1979
14. Fain JN, Rosenberg L: Antilipolytic action of insulin on fat cells. *Diabetes* 21 (Suppl. 2):414–425, 1972
15. Makino H, Suzuki T, Kajinuma H, Yamazaki M, Ito H, Yoshida S: The role of insulin-sensitive phosphodiesterase in insulin action. In *Advances in Second Messenger and Phosphoprotein Research.* Vol. 25. Strada J, Hidaka H, Eds. New York, Raven, 1992, p. 185–199
16. Smith CJ, Vasta V, Degerman E, Belfrage P, Mangianello VC: Hormone-sensitive cyclic GMP-inhibited cyclic AMP phosphodiesterase in rat adipocytes. *J Biol Chem* 266:13385–13390, 1991
17. Shechter Y: Differential effects of two phosphodiesterase inhibitors on fat cell metabolism. *Endocrinology* 115:1787–1791, 1984
18. Gabbay RA, Lardy HA: The antilipolytic effect of insulin does not require adenylate cyclase or phosphodiesterase action. *FEBS Lett* 179:7–11, 1985
19. Tossman U, Ungerstedt U: Microdialysis in the study of extracellular levels of amino acids in the rat brain. *Acta Physiol Scand* 128:9–14, 1986
20. Abumrad NN, Rabin D, Diamond MP, Lacy WW: Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30:936–940, 1981
21. De Fronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979

22. Kaddish AH, Little RL, Sternberg JC: A new and rapid method for the determination of glucose by measurement of the rate of oxygen consumption. *Clin Chem* 14:116–131, 1968
23. Hellmér J, Arner P, Lundin A: Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal Biochem* 177:132–137, 1989
24. Galitsky J, Lafontan M, Nordenström J, Arner P: Role of vascular alpha2-adrenoceptors in regulating lipid mobilization from human adipose tissue. *J Clin Invest* 91:1997–2003, 1993
25. Hickner R. C, Rosdahl H, Borg I, Ungerstedt U, Jorfeldt L, Henriksson J: Ethanol may be used with the microdialysis technique to monitor blood flow changes in skeletal muscle: dialysate glucose concentration is blood flow-dependent. *Acta Physiol Scand* 143:355–356, 1991
26. Hickner RC, Bone D, Ungerstedt U, Jorfeldt, Henriksson J: Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}-Xe clearance. *Clin Sci* 86:15–25, 1994
27. Zieler KL, Rabinowitz B: Effect of very small concentrations of insulin on forearm metabolism: persistence of its action on potassium and free fatty acids without its effect on glucose. *J Clin Invest* 43:950–962, 1964
28. Schade DS, Eaton RP: Dose-response to insulin in man: differential effects on glucose and ketone body regulation. *J Clin Endocrinol Metab* 44:1038–1053, 1977
29. Ostman J, Arner P, Engfeldt P, Kager L: Regional differences in the control of lipolysis in human adipose tissue. *Metab Clin Exp* 28:1198–1205, 1979
30. Smith U, Hammarsten J, Björntorp P, Kral J: Regional differences and effect of weight reduction on human fat cell metabolism. *Eur J Clin Invest* 9:327–332, 1979
31. Arner P, Kriegholm E, Engfeldt P: In situ studies of catecholamine-induced lipolysis in human adipose tissue using microdialysis. *J Pharmacol Exp Ther* 254:284–288, 1990
32. Jansson P-A, Smith U, Lönnroth P: Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. *Am J Physiol* 258:E918–E922, 1990
33. Low GK, Steinberg D, Vaughan MV, Margolis S: Studies of triglycerides biosynthesis in homogenates of adipose tissue. *J Biol Chem* 236:1631–1637, 1961
34. Coppack SW, Fisher RM, Gibbons GF, Humphreys SM, McDonough MJ, Potts JL, Frayn KN: Postprandial substrate deposition in human forearm and adipose tissues in vivo. *Clin Sci* 79:339–348, 1990
35. Jansson P-A, Smith U, Lönnroth P: Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in man. *Am J Physiol* 258:E928–E922, 1990
36. Arner P, Kriegholm E, Engfeldt P, Bolinder J: Adrenergic regulation of lipolysis in situ at rest and during exercise. *J Clin Invest* 85:893–898, 1990
37. Jansson P-AE, Fowelin JP, von Schenck HP, Smith UP, Lönnroth PN: Measurements by microdialysis of the insulin concentration in subcutaneous interstitial fluid: importance of the endothelial barrier for insulin. *Diabetes* 42:1469–1473, 1993
38. Schwabe U, Ukena D, Lohse MJ: Xanthine derivates as antagonists at A_1 and A_2 adenosine receptors. *Arch Pharmacol* 330:212–221, 1985
39. Lönnqvist F, Wennlund A, Arner P: Antilipolytic effects of insulin and adenylate cyclase inhibitors on isolated human fat cells. *Int J Obes* 13:137–146, 1989
40. Earl CG, Linden J, Weglicki WB: Biochemical mechanisms for the inotropic effect of the cardiotonic drug milrinone. *J Cardiovasc Pharmacol* 8:864–872, 1986
41. Londos C, Cooper DMF, Schlegel W, Rodbell M: Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process and methylxanthines on cyclic AMP production and lipolysis. *Proc Natl Acad Sci USA* 7:5362–5366, 1978
42. Simonsen L, Bülow J, Madsen J: Adipose tissue metabolism in humans determined by vein catheterization and microdialysis techniques. *Am J Physiol* 266:E357–E365, 1994