Effect of phosphodiesterase inhibition with amrinone or theophylline on lipolysis and blood flow in human adipose tissue in vivo as measured with microdialysis

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Abstract Phosphodiesterase III (cyclic GMP-inhibited, particulate, low K_m) is believed to play a dominant role in the cyclic AMP breakdown and lipolysis regulation in fat cells. Its importance for lipolysis activity was investigated in situ in humans by comparing the effects of a selective (amrinone) and a nonselective (theophylline) inhibitor of the enzyme. Abdominal subcutaneous adipose tissue of healthy nonobese humans was microdialyzed with solvents containing one or both of these agents, and glycerol (lipolysis index) or the escape of ethanol from the dialysis solvent (blood flow index) was continuously monitored in the perfusate. Both agents caused a dose-dependent and sustained increase in the glycerol level in the perfusate for at least 2.5 h. Although amrinone was 5000 times more potent than theophylline on a molar basis, its maximum activity was only 35% as compared to the maximum activity of theophylline. Half-maximum lipolytic effect of the two drugs occurred at about 0.1 μmol/l and 1 mmol/l, respectively ($P < 0.001$). At maximum effective concentrations, amrinone stimulated lipolysis by about 63% and theophylline by about 200% ($P < 0.01$). At these concentrations amrinone increased the rate of disappearance of ethanol from the perfusate by about 20% and theophylline increased the rate by about 75%, the difference being statistically significant ($P < 0.01$). When the two drugs were added together, the level of lipolysis stimulation was not different from that with theophylline alone both at maximal and submaximal effective concentrations of drug combinations. In conclusion, amrinone stimulates lipolysis and blood flow in situ at much lower concentrations than theophylline does, but its maximal effect is only a fraction of that of theophylline. This suggests that PDE III is not the only phosphodiesterase that is involved in the regulation of lipolysis and blood flow in human adipose tissue in vivo.—**Arner, P., J. Hellmér, E. Hagström-Toft, and J. Bolinder.** Effect of phosphodiesterase inhibition with amrinone or theophylline on lipolysis and blood flow in human adipose tissue in vivo as measured with microdialysis. *J. Lipid Res.* 1993. **34:** 1737–1743.

Supplementary key words glycerol • fat cell • cyclic AMP

Phosphodiesterase (PDE) plays an important role in regulating cyclic AMP-mediated processes, such as lipolysis in fat cells. Multiple molecular forms of phosphodiesterase exist and many of these isoenzymes are selectively expressed in a limited number of cell types (1). The hormone-sensitive type III form (PDE III) is believed to be of particular importance for regulating lipolysis. As reviewed (2), this isoenzyme, which is located in the particulate fraction of fat cells, has a high affinity to cyclic AMP, can be inhibited by cyclic GMP, and is stimulated by the major lipolysis-regulating hormones, catecholamines and insulin. The hormone-sensitive properties of PDE III have been demonstrated in several tissues, as discussed (2, 3), including human adipose tissue (4). PDE III may also have indirect effects on lipolysis, as it is involved in the regulation of vascular tone (5–7) and changes in adipose tissue blood flow may alter the lipolytic rate (8).

A number of drugs that selectively inhibit PDE III have recently been developed (1, 9–12). One such agent is amrinone. As reviewed (9–12), it was the first to be used in clinical medicine as a cardiotonic agent taking advantage of the inotropic and vasodilating effects of the selective inhibition of PDE III. In addition to the PDE inhibitory effect of amrinone, this agent can stimulate the lipolysis rate in fat cells through competitive antagonism of antilipolytic adenosine receptors (13). The latter mechanism is shared with nonselective PDE inhibitors, such as theophylline (14).

Our knowledge of the role of phosphodiesterase in the regulation of lipolysis in adipose tissue has hitherto been based on in vitro studies. Recently, a method for in vivo lipolysis investigations was developed, based on continuous monitoring of glycerol (lipolysis index) in the extracellular water space of adipose tissue, with the aid of

Abbreviations: PDE, phosphodiesterase.

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microdialysis (15). When ethanol is included in the microdialysis solvent and its clearance from the perfusion medium is measured, it is possible to detect blood flow changes indirectly in the tissue regions surrounding the microdialysis probe (16). The alcohol readily diffuses through the probe membrane and is not metabolized by the peripheral tissues (17).

In the present study we have for the first time studied the role of PDE III in the regulation of lipolysis and blood flow in human adipose tissue in vivo. This was done by using microdialysis of subcutaneous adipose tissue in nonobese healthy volunteers. The in situ effects of amrinone (selective inhibitor of PDE III plus adenosine antagonist) and theophylline (nonselective PDE inhibitor plus adenosine antagonist) on the glycerol and ethanol levels in the microdialysate were investigated.

MATERIALS AND METHODS

Subjects

The study group comprised 22 healthy and drug-free nonobese volunteers (9 men, 13 women), aged 18–51 years (mean 29 years). One woman (51 years old) was postmenopausal. Her results did not differ from those of the other women. Body mass index (kg/m^2) ranged from 19.4 to 26.2 (mean 22.7). The study was approved by the Ethics Committee of the Karolinska Institute. The subjects were given a detailed description of the experiments and their consent was obtained.

Microdialysis probe

The microdialysis probe has been described in detail previously (18). Dialysis tubing (10 × 0.5 mm; 20,000 mol wt cut-off) is glued to the end of a double-lumen steel cannula. The perfusion solvent enters the probe through the inner cannula, streams upwards in the space between the inner cannula and the outer dialysis membrane, and leaves the probe through the outer cannula. Glycerol or ethanol is determined in the perfusate and used as an index of lipolysis and blood flow, respectively.

Experiment protocol

The subjects were investigated in the supine position at 8 AM after an overnight fast. The dialysis probe was inserted percutaneously without anesthesia into the abdominal subcutaneous adipose tissue immediately to the right or left of the umbilicus. Two or three dialysis probes were used simultaneously in each experiment. The distance between adjoining probes was always 30 mm. The probe was connected to a microinjection pump and was continuously perfused (2.5 μl/min in the glycerol experiments and 1.5 μl/min in the ethanol experiments) with 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). The following agents in a sterile solution were added to the basal dialysis solvent: theophylline (Draco, Lund, Sweden), amrinone (Sterling-Winthrop, New York, NY), and ethanol (Vin-och Spritcentralen, Stockholm, Sweden).

In each experiment 15-min fractions of the outgoing dialysate were collected. The first two 15-min fractions were excluded because methodological experiments (19) have shown that there is a transient rise in the ATP concentration in the outgoing dialysate during the first 30 min of dialysis, which probably reflects the initial trauma when the dialysis probe is inserted into the adipose tissue. At the perfusion velocity (2.5 μl/min) used in this study, the in vitro recovery of glycerol, determined as described previously (20), is 15%. Venous blood samples were drawn simultaneously with the dialysis experiments at 30 min from an indwelling polyethylene catheter in the cubital vein simultaneously with the dialysis experiments. Plasma glycerol was also determined in these samples. The pulse rate was taken at 30-min intervals.

Analysis of glycerol

Twenty microliters of plasma or perfusate was used for the analysis of glycerol. An automatic ultrasensitive kinetic bioluminescent assay of glycerol (20) and a bioluminescent analyzer were used for the assay.

Analysis of ethanol

Ten microliters of dialysis solvent or perfusate was used to measure of ethanol, using an enzymatic fluorometric method (21). The ratio of ethanol concentration in the outgoing and ingoing dialysis solvents was determined when blood flow was evaluated. Changes in this ratio indirectly reflect changes in the blood flow of the tissue surrounding the microdialysis probe, as discussed recently in detail (16).

Statistical analysis

Values are means ± SE. The Student's paired *t*-test or analysis of variance for repeated measures (ANOVA) was used for the statistical comparisons. When drug concentrations causing a half-maximum effect (ED_{50}) were compared, the values were converted into their logarithmic form before the statistical analysis.

RESULTS

The adipose tissue blood flow method was evaluated in methodological experiments (**Fig. 1**). These experiments were performed previously (21) but calculated in a different way presently. They showed that when the perfusion of 5.6 mol/l of ethanol was started, the concentration of the alcohol in the microdialysate gradually increased during the first 20 min; thereafter it stabilized at about 70% of the concentration in the ingoing perfusion medium. An

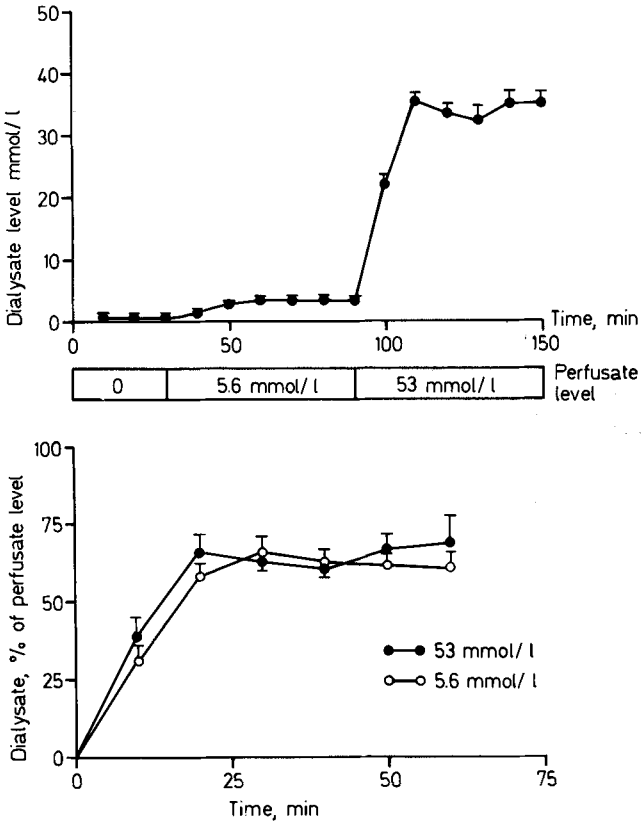

Fig. 1. Microdialysis of subcutaneous adipose tissue with ethanol. Adipose tissue was microdialyzed with Ringer's solution for 60 min. Ethanol (5.6 mmol/l) was added to the solution for 60 min, and this was followed by the addition of ethanol (53 mmol/l) for another 60 min. Microdialysate was collected every 15 min and analyzed for ethanol. The upper graph shows absolute values for ethanol. The lower graph shows the ratio of the ethanol concentration in the dialysate divided by the concentrations in the perfusion medium during the 2 h of ethanol perfusion. Values are mean ± SE of four experiments.

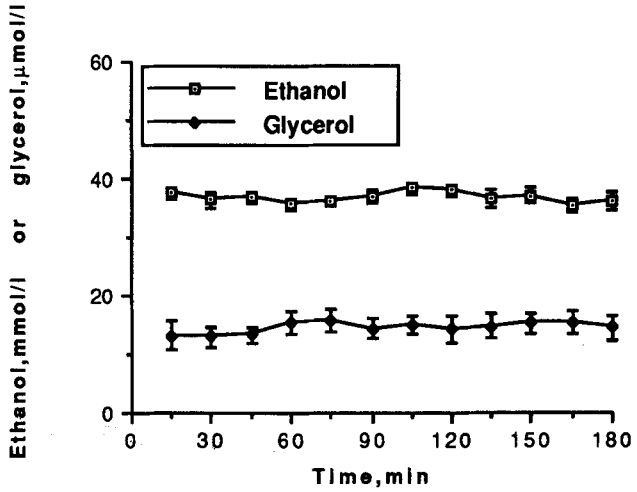

Fig. 2. Changes in the levels of glycerol or ethanol in microdialysates of adipose tissue at rest. Two probes were implanted in the subcutaneous adipose tissue and perfused with Ringer's solution alone or supplemented with 50 mmol/l of ethanol. Microdialysate fractions were collected every 15 min and analyzed for either glycerol or ethanol. Values are mean ± SE of seven experiments. The actual concentration of ethanol in the ingoing perfusion solvent was 48.4 ± 0.9 mmol/l giving an average outflow/inflow ratio of ethanol of 75 ± 2%.

almost identical pattern of increase was seen when the concentration of ethanol in the ingoing dialysis solvent was increased to 53 mol/l. The finding of consistent outflow/inflow ethanol levels over a 10-fold range of alcohol concentration indicates that the rate of escape of ethanol from the dialysate solvent in the probe to the surrounding tissue does not depend on the actual concentration of ethanol in the perfusion medium. This is important from a methodological standpoint, as discussed in detail (16). In addition, the methodological data suggest that ethanol at these levels does not directly influence local blood flow.

The levels of glycerol and ethanol in the microdialysate were investigated for 3 h with the subject resting (**Fig. 2**). Both levels remained almost constant throughout the experimental period. The coefficients of variation for glycerol and ethanol were only 6% and 2%, respectively. These data suggest, first, that the resting rates of lipolysis and blood flow are markedly constant and, second, that the current microdialysis method can detect small variations in lipolysis and blood flow.

The effects of phosphodiesterase inhibitors on the glycerol levels in adipose tissue were investigated in the experiments depicted in **Fig. 3.** Two dialysis probes in the same subject were first perfused for 60 min with the basal solution (e.g., Ringer's solvent). Thereafter theophylline

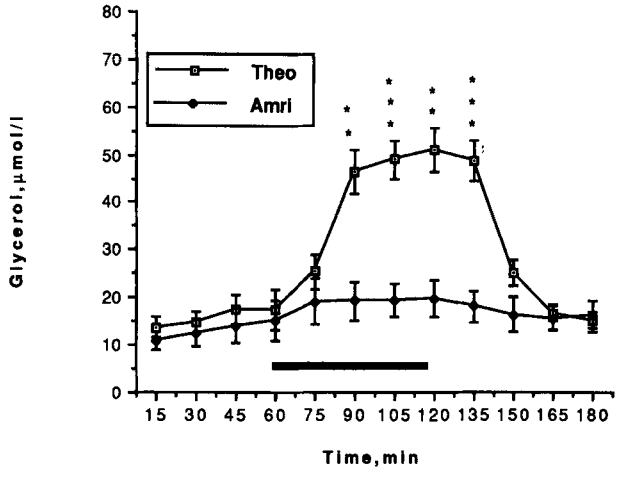

Fig. 3. Effect of theophylline (10^{-2} mol/l) or amrinone (10^{-4} mol/l) on glycerol in microdialysate of subcutaneous adipose tissue. Two probes were implanted and microdialyzed for 180 min with Ringer's solution. Either theophylline or amrinone was added to the perfusion solvent during the second 60-min period (as indicated in the graph). Thereafter the drug was removed from the perfusion solvent. Microdialysate was collected every 15 min and analyzed for glycerol. Values are mean ± SE of eight experiments. They were statistically compared using Student's paired *t*-test. $^{*}P < 0.01$; $^{**}P < 0.01$; $^{**}P < 0.001$.

(10^{-2} mol/l) or amrinone (10^{-4} mol/l) were added to the dialysis solvent for 60 min. This caused a rapid increase in the dialysate glycerol concentration; the latter level reached a maximum within 30 min after the addition of the agents. With both drugs glycerol rose significantly in the microdialysate from 15 to 75 min after the addition of the drugs (P from 0.001 to 0.042 with the paired t-test as compared to the dialysis fractions before addition of drug). However, theophylline stimulated lipolysis much more effectively than amrinone did. In the presence of theophylline, glycerol increased 3-fold, whereas it increased only 50% in the presence of amrinone. When the phosphodiesterase inhibitors were removed from the perfusion solvent, the glycerol level in the microdialysate gradually decreased towards baseline after a period of 15 min (P from 0.005 to 0.02 during the 150- to 180-min dialysis period as compared with the period 90–135 min). The plasma glycerol levels and the pulse rate did not change throughout these experiments, despite the increase in adipose tissue glycerol levels (data not shown). In uncharted experiments theophylline (10^{-2} mol/l) or amrinone (10^{-4} mol/l) caused a sustained increase in the microdialysate glycerol level for at least 2.5 h. The experiments in Fig. 3 were also analyzed using ANOVA. The changes in glycerol after addition of either theophylline or amrinone were statistically significant (P = 0.001; F=70 and 5, respectively). The glycerol curves for the theophylline and amrinone experiments also differed from each other (F=4.84, P = 0.048).

In further studies, the lipolytic actions of amrinone and theophylline were compared in dose-response experiments. The drugs were added to the dialysis solvent in increasing concentrations; each concentration was used for 45 min. The mean glycerol value (from the three 15-min fractions) was calculated at each concentration. Values are expressed as a percent of the basal value (e.g., perfusion with Ringer's solution alone). Concentrations of amrinone and theophylline below 10^{-8} and 10^{-5} mmol/l, respectively, were without significant effect of the glycerol level (data not shown).

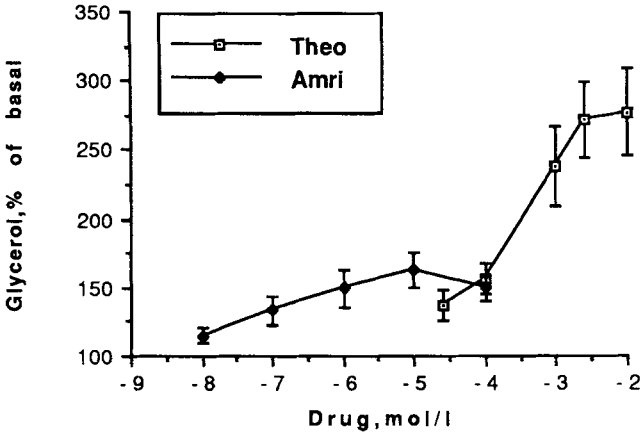

Fig. 4. Dose-response relationship for the effects of amrinone or theophylline on glycerol in a microdialysate of subcutaneous adipose tissue. Two probes were implanted and microdialyzed with Ringer's solution. Either amrinone or theophylline was added to the perfusion solvents in increasing concentrations, starting with no addition. Each concentration was used for 45 min, starting every 15 min, and analyzed for glycerol. The mean glycerol concentration of the three fractions was calculated at each drug concentration and expressed as a percent of the basal value (no drug added). Values are mean ± SE of 11 experiments.

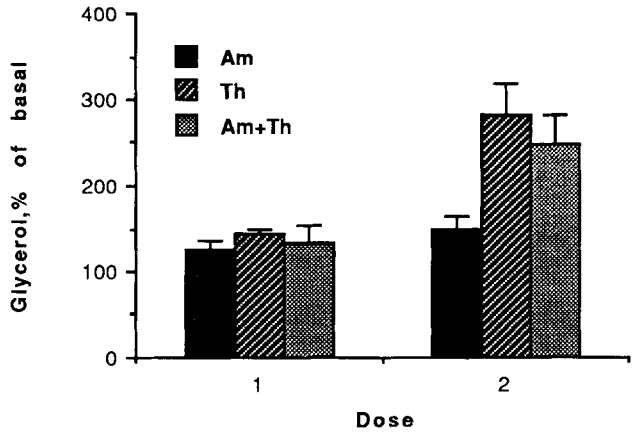

Fig. 5. Effect of amrinone and theophylline alone or in combination on glycerol in microdialysate of subcutaneous adipose tissue. Three probes were implanted and microdialyzed, as described in Fig. 4. In one probe amrinone was added at 0, 10^{-7}, and 10^{-5} mol/l. In a second probe theophylline was added at 0, 10^{-4}, and 10^{-2} mol/l. In the third probe, amrinone plus theophylline were added at 0, 10^{-7} plus 10^{-4}, and 10^{-5} plus 10^{-2} mol/l. The effects of the drugs were calculated as in Fig. 4. 1, Effect of the low drug concentration; 2, effect of the high drug concentration. Values are mean ± SE of five experiments.

Fig. 4 depicts the lipolytic dose-response relationship in situ for amrinone and theophylline when determined simultaneously in the same subjects, using two different probes. Both drugs caused a dose-dependent stimulation of the glycerol level. On a molar basis, amrinone was much more potent than theophylline. The concentration of drug causing a half-maximum effect (ED_{50}) was calculated from each individual dose-response curve (n = 11). The mean ED_{50} values (log mol/l) for amrinone and theophylline were 6.8 ± 0.3 and −3.3 ± 0.2, respectively ($P < 0.001$), which indicates a 5000-times difference in potency. However, at the maximum effective concentration, theophylline was a much more powerful lipolytic agent than amrinone. In the individual experiments theophylline maximally stimulated the basal glycerol level by 199 ± 27%. The corresponding value for amrinone was 73 ± 11%. This difference was significant ($P < 0.01$). Thus, the intrinsic activity of amrinone as compared to the maximum lipolytic effect of theophylline was about 35%.

The in situ lipolytic action of a combination of amrinone and theophylline is shown in **Fig. 5.** In these experiments, adipose tissue in each subject was microdialyzed with three different probes containing increasing doses of

amrinone and theophylline either alone or in combination. As expected, a much higher degree of lipolytic stimulation was obtained with theophylline than with amrinone alone. However, neither at submaximum or maximum effective concentrations was lipolysis significantly more stimulated with amrinone plus theophylline than with theophylline alone. Thus, there were no additive effects of amrinone on theophylline-induced lipolysis.

It should be noted that the final concentration of the lipolytic agent in the extracellular space is unknown in the present experiments. The concentration of the drug entering the extracellular space is probably the same as that in the dialysis solvent, because the molecules can move freely across the dialysis membrane. However, there may be a further dilution before the drug reaches the target tissue level.

The concentration of glycerol in the dialysate in the experiments depicted in Figs. 1–3 is lower than the actual level in the extracellular space because of incomplete recovery. It is possible to determine the true adipose tissue level of glycerol indirectly by performing equilibrium microdialysis experiments (22). For methodological reasons discussed in detail previously (23), it is not, however, possible to combine the present kinetic experiments with equilibrium experiments.

The effects of amrinone and theophylline on the ethanol outflow/inflow ratio were compared in the same subjects (**Fig. 6**). Both agents were added at the concentrations which in the previous lipolysis experiments induced maximum effects (10^{-4} and 10^{-2} mol/l of amrinone and theophylline, respectively). The addition of theophylline caused a marked and significant decrease in the ethanol outflow/inflow ratio of about 45% (P from 0.008 to 0.019 during the 30- to 75-min period after the addition of theophylline as compared to the period before addition of drug, using the paired t-test). The outflow/inflow ratio returned towards baseline when the drug was removed from the dialysate solvent. A similarly significant, but smaller (about 20%), effect was observed with amrinone (P from 0.09 to 0.027 during the 45- to 75-min period after the addition of amrinone as compared with the period before addition of drug, using the paired t-test). The ratio gradually returned to baseline when amrinone was removed from the dialysis solvent. The difference in effect on the outflow/inflow ratio of ethanol between theophylline and amrinone was statistically significant (P from 0.004 to 0.026 during the dialysis time points of 105–135 min by paired t-test). The results with ethanol were also statistically evaluated using ANOVA. The effect of theophylline on ethanol ratio was significant when the whole experimental period was analyzed ($F=6.8$; $P = 0.01$). However, the effect of amrinone was statistically significant only when the results from the first 120 min were analyzed ($F=2.44$; $P = 0.03$). The curves for amrinone and theophylline differed significantly when the 75- to 135-min time period was compared ($F=6.55$; $P = 0.023$).

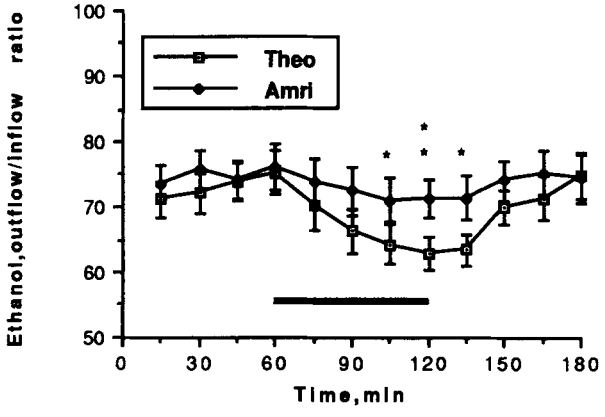

Fig. 6. Effect of theophylline (10^{-2} mol/l) or amrinone (10^{-4} mol/l on blood flow in subcutaneous adipose tissue. Eight experiments were performed and analyzed as in Fig. 3 but the perfusion solvent was supplemented with 50 mmol/l of ethanol throughout the experimental period. Ethanol was determined in the ingoing and outgoing solvents. The ratio (in percentage) of these two values was calculated.

DISCUSSION

PDE III is believed to play an important role in controlling the cyclic AMP level and thereby lipolysis in fat cells (2, 3). In this study the role of this enzyme in regulating lipolysis has been investigated in vivo for the first time. Using microdialysis, it is demonstrated that the selective PDE III inhibitor amrinone causes a small but dose-dependent stimulation of lipolysis in situ in human adipose tissue. However, a much larger (and dose-dependent) lipolytic effect was observed in situ with the nonselective PDE inhibitor theophylline. The two agents are reported to have similar properties as regards adenosine antagonism (13, 14). The only major difference in action seems to be in PDE selectivity. Thus, it appears that PDE III is not the only isoenzyme of importance for in vivo lipolysis regulation, at least not in human fat cells. This agrees with in vitro data on rat fat cells showing that at least two other PDEs besides PDE III can regulate lipolysis (24).

It is most likely that amrinone acts on the same steps on the lipolytic process as theophylline does. Amrinone was 5000 times more potent than theophylline in terms of apparent drug sensitivity (ED_{50}), which points to the selectivity of amrinone. However, when the drugs were added simultaneously at submaximal or maximal effective concentrations there was no additive effect of amrinone to that of theophylline. This suggests that the selective lipolytic action of amrinone is part of the nonselective action of theophylline.

One drawback of the microdialysis technique is that it measures net changes in the metabolite concentrations in

the extracellular water space of adipose tissue. These changes are influenced by production/uptake as well as by changes in the removal of the metabolite from the compartment via the blood flow. Because glycerol—unlike free fatty acids—is not metabolized by adipose tissue to a significant extent (25), the contribution of fat cells to the glycerol level in the extracellular compartment is influenced only by the rate of production. Local blood flow may be of importance for lipid mobilization in adipose tissue because an increase in flow can stimulate lipid mobilization and vice versa (8), so that changes in blood flow may alter the glycerol level in the extracellular water space independently of lipolysis. Thus, a decrease in blood flow may slow down the removal of glycerol from the extracellular water space, so that the glycerol level increases. This may be erroneously interpreted as a lipolytic effect if glycerol and tissue flow are not considered together in the microdialysis experiment. The opposite phenomenon may occur with glycerol if blood flow in increased.

It is not possible at present to determine blood flow in the tissue surrounding the microdialysis probe directly. However, an ethanol technique has recently been developed by Hickner et al. (16) to estimate local blood flow indirectly in muscle. Present methodological data suggest that this technique can also be used in microdialysis experiments with human adipose tissue. Our data show that the escape of ethanol from the dialysate into the extracellular space is stimulated by phosphodiesterase inhibition. The effect of theophylline was much more marked than that of amrinone. These findings suggest that PDE III regulates the vascular tone in human adipose tissue only to a minor extent. Other PDEs also seem to be important with regard to this as well as to lipolysis. This is in line with recent in vitro data that show that other PDEs besides PDE III are involved in the regulation of vasorelaxation in human arteries (26). As regards flow, we have tested only the maximum effective doses of the PDE inhibitors, which were obtained from the lipolysis experiments. Unfortunately, the changes in the escape of ethanol induced by the phosphodiesterase inhibitors were too small for detailed dose-response experiments to be performed.

When glycerol and ethanol data are considered together, the differences in the effects of amrinone and theophylline on lipolysis may be even more striking. Referring to the discussion above, a vasodilating effect of theophylline (and amrinone) may lower the glycerol concentration in the interstitial fluid and thereby also in the dialysate. This probably underestimates the true lipolysis effect of theophylline.

The present finding concerning lipolysis stimulation in situ differs from that of previous microdialysis experiments with catecholamines. In this study a constant marked stimulation (at least for 2.5 h) of lipolysis was observed with theophylline. On the other hand, the in situ lipolytic action of noradrenaline was observed to be transient and disappeared within 1 h, in spite of the presence of catecholamines (27); the tachyphylaxia appears to be due to an acute desensitization primarily of $beta_1$-adrenoceptors (28). When the data are considered together, it appears that the sites for in vivo tachyphylaxia of the lipolytic system are localized at the hormone receptor–adenylate cyclase complex rather than at the phosphodiesterase level.

The experiments in this study were performed in the basal resting state. Because PDE III is hormone-sensitive, it is possible that the activity of this enzyme varies according to the physiological state. In vitro data suggest that PDE III in human adipose tissue can be altered by diabetes and thyroid disorders with consequences for lipolytic activity (29, 30). Furthermore, microdialysis studies performed under different conditions, such as during exercise, hormone infusion, or carbohydrate ingestion, are necessary for a full understanding of the in vivo regulation of PDE III. It is also possible that there may be sex-related or regional variations in the effects of phosphodiesterase inhibitors, such differences in blood flow and lipolysis are known to exist in humans as previously reviewed (31). Only abdominal tissue was used in the present study. Although subjects of either sex were induced, the number of women and men in each specific type of experiment was too small to allow a statistical comparison.

In summary, the present study points to a role of PDE III in the regulation of lipolysis and blood flow in vivo. However, other phosphodiesterases are very probably also involved in controlling these processes in human adipose tissue.

This study was supported by Grant 19X-01034 from the Swedish Medical Research Council, the Swedish Diabetes Association, the Swedish Athletics Council, the Karolinska Institute, and the Foundations of Osterman, Nordic Insulin, and Krefting.

Manuscript received 22 December 1992 and in revised form 7 May 1993.

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