A kinetic study of glycerophosphate acyltransferase of rat adipocytes in relation to its control by noradrenaline

B. LEDERER and H.-G. HERS

Laboratoire de Chimie Physiologique, Université de Louvain and International Institute of Cellular and Molecular Pathology, UCL 75.39, Avenue Hippocrate 75, B-1200 Brussels, Belgium

(Received 10 September 1984/Accepted 23 October 1984)

Glycerophosphate acyltransferase present in an extract of rat adipocytes is strongly inhibited by excess palmitoyl-CoA. This inhibition is released by serum albumin but an excess of serum albumin is inhibitory, particularly at low palmitoyl-CoA concentrations. An optimal activity is reached when the ratio palmitoyl-CoA/albumin is in the range of 3–6. In the absence of albumin, oleic acid inhibits the activity at all palmitoyl-CoA concentrations. This inhibition is released by albumin and, inversely, oleic acid releases the inhibition by high concentrations of albumin. Another effect of fatty acids is to favour the inactivation of the glycerophosphate acyltransferase in extracts of adipocytes kept at 0°C. This inactivation is time-dependent and cannot be reversed by the addition of albumin to the assay mixture. Treatment of adipocytes with noradrenaline had no effect on the activity of the enzyme as long as the cells had been separated from fatty acids and albumin. With extracts of unwashed cells, the effect of noradrenaline on both the activity and stability of glycerophosphate acyltransferase could be explained by the presence of fatty acids in the extract.

Several effects of noradrenaline on isolated adipocytes have been reported in recent years. These include an activation of phosphofructokinase (Sooranna & Saggerson, 1982), an inhibition of glycerophosphate acyltransferase (Sooranna & Saggerson, 1976*a,b*, 1978; Rider & Saggerson, 1983) and also an inactivation of phosphatidate phosphohydrolase (Cheng & Saggerson, 1980). Whereas all these effects were reported to be cancelled by an excess of serum albumin, they apparently could not be mimicked by the addition of free fatty acids to the cell suspension or to the cell-free extract. More recently, however, we were able to show that the activity of phosphofructokinase present in an extract of adipocytes could be greatly increased by oleic acid, at least if added with a minimal amount of albumin (Lederer & Hers, 1984). Consequently, the effect of noradrenaline to activate phosphofructokinase could be adequately explained by its well-known lipolytic action.

The purpose of the present paper was to investigate if the effect of noradrenaline to

decrease the activity of glycerophosphate acyltransferase of rat adipocytes could also be explained by the presence of fatty acids, as long as the concentration of serum albumin was closely controlled. We observed a complex interaction between the concentration of a substrate, palmitoyl-CoA, and that of albumin and of fatty acids. We reached the conclusions that the effect of noradrenaline is mediated by fatty acids but that this effect can be a stimulation as well as an inhibition, according to the concentration of palmitoyl-CoA and albumin present in the assay.

Materials and methods

Male fed Wistar rats weighing 150–160 g were used throughout. L-Glycerol 3-phosphate was obtained from Boehringer (Mannheim, Germany). L-[U-¹⁴C]Glycerol 3-phosphate (ammonium salt) was purchased from Amersham International (U.K.). Palmitoyl-CoA, fatty acid-free bovine serum albumin and oleic acid were from Sigma Chemical Co. (St Louis, MO, U.S.A.). A suspension of oleic acid which was stable at 25°C was obtained by vigorous shaking in the presence of bovine serum albumin (10 mM oleic acid in 50 μ M-

Abbreviations used: GPAT, glycerophosphate acyltransferase (EC 2.3.1.15).

albumin). L-Noradrenaline bitartrate was from Winthrop Laboratories (Brussels, Belgium).

Isolated fat cells were prepared by the method of Rodbell (1964) and incubated for 1 h at 37°C according to Rider & Saggerson (1983) except that the fatty acid-free albumin was at 280 μM . The cells were then recovered by centrifugation (20s at 100g) and, unless otherwise stated, washed twice in 10 ml of a Krebs-Henseleit (1932) buffer without albumin and finally frozen in a mixture of solid CO_2 and acetone. The frozen cells (0.2–0.3 g of adipose tissue), stored at -80°C for up to 3 weeks, were homogenized with a glass tissue-grinder (Kontes, Vineland, NJ, U.S.A.) in 1.8 ml of a solution containing 0.25 M-sucrose, 10 mM-Tris/HCl buffer, pH 7.4, 1 mM-EDTA and 1 mM-dithiothreitol (Rider & Saggerson, 1983). The homogenate was centrifuged for 1 min at 7000g in an Eppendorf centrifuge and the resulting extract (infranatant) was used immediately for analytical purposes.

GPAT was assayed at 30°C in a final volume of 0.25 ml containing 0.1 M-Tris/HCl buffer, pH 7.4, 0.25 mM-L-[U- ^{14}C]glycerol 3-phosphate (0.1 $\mu\text{Ci}/\text{assay}$), palmitoyl-CoA and fatty acid free-albumin at the concentrations indicated, and 0.7 mM-dithiothreitol. The reaction was initiated with 25 μl of tissue extract. Assays were performed for 10 min and were linear with time over this time interval. The reaction was stopped with 2 ml of 0.1 M-HCl-saturated butan-1-ol and incorporation of radioactivity into butanol-soluble products determined as described by Dae & Bremer (1970). *N*-Acetyl- β -D-glucosaminidase was measured at 37°C by the hydrolysis of methylumbelliferyl *N*-acetyl- β -glucosamine as described by Leaback & Walker (1961). One unit of enzyme is the amount that converts 1 μmol of substrate/min in the conditions of the assay. The average activity of the *N*-acetyl- β -D-glucosaminidase was 2.15 m-units/ml of extract. In order to correct for incomplete cell recovery or extraction, GPAT activity is expressed as μmol of L-glycerol 3-phosphate incorporated into lipid/min per unit of *N*-acetyl- β -D-glucosaminidase (activity ratio). Fatty acids were measured by the method of Duncombe (1963) after extraction of 0.25 ml of the cellular extract with 3 ml of chloroform.

Experiments shown are representative of several similar experiments that gave essentially the same results.

Results

Kinetic properties of GPAT in an extract of control adipocytes

Saturation curves of GPAT for its substrate palmitoyl-CoA obtained in the absence and in the

presence of various concentrations of albumin are shown in Fig. 1. The interplay between palmitoyl-CoA and albumin is also illustrated by another experiment shown in Fig. 2, the data being now

Fig. 1. Effect of albumin and of oleic acid on the GPAT activity measured in an adipocyte extract at different concentrations of palmitoyl-CoA

Fig. 2. Effect of the concentration of palmitoyl-CoA on the GPAT activity measured in an adipocyte extract in the presence of increasing concentrations of albumin

plotted as a function of albumin concentration. When no albumin was added to the assay, the activity did not exceed 10% of the value reached under optimal conditions and there was a strong inhibition by excess substrate with a nearly complete loss of activity at $40\text{ }\mu\text{M}$ -palmitoyl-CoA. When a small amount of albumin ($2.3\text{ }\mu\text{M}$) was added to the assay, the activity was increased about 3-fold at low substrate concentrations, and much more in the presence of inhibitory concentrations of palmitoyl-CoA, at least up to a certain concentration of this substrate. Indeed, the inhibition by $100\text{ }\mu\text{M}$ -palmitoyl-CoA was not released by low, but only by high, albumin concentrations. Higher concentrations of albumin decreased the activity measured at low palmitoyl-CoA concentrations, but increased the activity at high substrate concentrations; consequently, the substrate-saturation-inhibition curves (Fig. 1) were shifted to the right. Albumin had a biphasic effect at all palmitoyl-CoA concentrations, being first stimulatory but becoming inhibitory at higher concentrations, and the albumin-saturation-inhibition curves were shifted to the right in the presence of increasing concentrations of palmitoyl-CoA (Fig. 2). As a rule, an optimal activity was reached when the ratio palmitoyl-CoA/albumin was in the range 3–6.

In the absence of albumin, oleic acid was inhibitory at all concentrations of palmitoyl-CoA. In the presence of albumin, it increased the affinity of the enzyme for palmitoyl-CoA, shifting the saturation-inhibition curve to the left (Fig. 1). Symmetrically, it also shifted the albumin-saturation-inhibition curve to the right (Fig. 3) having then the same effect as an excess of palmitoyl-CoA (Fig. 2); in other words, more albumin was required both for its positive and for its negative effects in the presence of fatty acids.

GPAT activity of an adipocyte extract was also measured in the presence of $27\text{ }\mu\text{M}$ -albumin and of 10 mM -*N*-ethylmaleimide, a known inhibitor of the microsomal, although not of the mitochondrial, enzyme (Saggerson *et al.*, 1980). The inhibition by *N*-ethylmaleimide was 96% at $100\text{ }\mu\text{M}$ -palmitoyl-CoA and at least 88% at lower substrate concentrations, indicating that the participation of mitochondrial GPAT in our observation was minimal.

Effect of noradrenaline treatment on the kinetics of GPAT

Incubation of adipocytes in the presence of noradrenaline was without effect on the activity of GPAT present in extracts of cells that had been freed of albumin and fatty acids by two washings, as they also had been in the experiments shown in Figs. 1–3. However, if the washing of the cells was

Fig. 3. *Effect of albumin and of oleic acid on GPAT activity measured in an adipocyte extract*

The activity was measured at $100\text{ }\mu\text{M}$ -palmitoyl-CoA. Superimposable results were obtained with palmitic acid added instead of oleic acid.

omitted, the extracts of noradrenaline-treated cells contained an excess of fatty acids and, as expected, the albumin-saturation-inhibition curve was shifted to the right (Fig. 4; to be compared with Fig. 3).

Effect of fatty acids and of noradrenaline treatment on the inactivation of GPAT

Rider & Saggerson (1983) described that treatment of adipocytes with noradrenaline decreased the activity of GPAT over a large range (40 – $140\text{ }\mu\text{M}$) of palmitoyl-CoA concentration. Since this effect, as indicated by Fig. 1, cannot be simply explained by the presence of an excess of fatty acids, we have considered the possibility that fatty acids could cause an inactivation of the enzyme. We show in Fig. 5 that when the cellular extracts were kept for 30 min at 0°C in the presence of various concentrations of fatty acids, the enzyme was inactivated to various degrees, at least when the concentration of fatty acids in the extracts exceeded a threshold value of approx. 1 mM . The results were the same if this concentration was reached by adding exogenous fatty acids to the extract or, at least in part, by treating the cells with noradrenaline. This inactivation was time-dependent (Fig. 6) and could not be reversed by the addition of albumin to the assay mixture. The loss of activity was observed at all concentrations of palmitoyl-CoA present in the assay mixture (results not shown).

Fig. 4. Effect of noradrenaline treatment on the GPAT activity measured in an adipocyte extract in the presence of increasing concentrations of albumin

The cells were incubated with (---) or without (—) $1 \mu\text{M}$ -noradrenaline, isolated by centrifugation and immediately frozen. The two usual washings of the cells were, therefore, omitted. The concentration of fatty acids carried over with the cellular extract was $5 \mu\text{M}$ in the control enzymic assay and $45 \mu\text{M}$ in the assay made with extracts of noradrenaline-treated cells. The assay were performed at $100 \mu\text{M}$ -palmitoyl-CoA.

Discussion

The kinetics of GPAT

The dominant feature in the control of GPAT activity is the inhibition by excess acyl-CoA. Abou-Issa & Cleland (1969) have interpreted this inhibition by the formation of micelles when the concentration of palmitoyl-CoA exceeds $4 \mu\text{M}$. Furthermore, mixed micelles are formed in the presence of microsomes and/or albumin ($170 \mu\text{M}$ -albumin was used in their experiments), lowering then the concentration of monomeric palmitoyl-CoA in solution. These mixed micelles were reported not to cause substrate inhibition as do pure palmitoyl-CoA micelles, at least up to a certain proportion of palmitoyl-CoA in them.

According to this view, the stimulatory effect of albumin at concentrations lower than approximately one-fourth that of palmitoyl-CoA [a value which, according to Spector *et al.* (1971), corresponds to the binding capacity of albumin for fatty acids] could be understood by its ability to form non-inhibitory micelles, in equilibrium with the free substrate. Larger concentrations of albumin would be inhibitory because of binding too much of the substrate and decreasing its availability for the enzyme. Increasing palmitoyl-CoA concentration would of course increase the concentration of albumin required for both its positive and negative effects. Free fatty acids would also bind part of the

Fig. 5. Effect of fatty acids on the inactivation of GPAT in adipocyte extracts incubated at 0°C
(a) The cells were incubated with (closed symbols and broken line) and without (open symbols and full line) $1 \mu\text{M}$ -noradrenaline; the two usual washings of the cells were omitted. The cellular extracts were maintained for 30 min at 0°C in the presence of the indicated concentrations of oleic acid. GPAT was assayed at $100 \mu\text{M}$ -palmitoyl-CoA and in the presence of $27 \mu\text{M}$ -albumin before and after this incubation. Different symbols correspond to different experiments all performed under identical conditions. The concentration of fatty acids carried over with the cellular extract were $60 \mu\text{M}$ in the controls and 330 ($\blacksquare$), 360 ($\bullet$), 440 ($\blacktriangle$) and 600 ($\blacktriangledown$) μM in the extracts of noradrenaline-treated cells. The mean initial activity ratio was 1.75 for the control and 1.53 for the extracts of noradrenaline-treated cells. In (b), the same data were plotted as a function of the total fatty acid concentration in the cellular extracts.

Fig. 6. Time-course of the inactivation of GPAT in an adipocyte extract

Same general procedure as in Fig. 5 was used. Closed symbols in this Figure correspond to the presence of 1 mM-oleic acid in the cellular extracts during the incubation at 0°C.

albumin competing with palmitoyl-CoA and therefore a larger concentration of albumin would be necessary to reach the same positive or negative effects (see Fig. 3).

The noradrenaline effect

No stable effect of noradrenaline, i.e. an effect that would persist after removal of the incubation medium and washing of the cells, on GPAT activity was observed. This negative result is against the proposal made by Nimmo & Houston (1978) that rat adipose tissue GPAT can be inactivated by cyclic AMP-dependent protein kinase. The results of Nimmo & Houston (1978) could not be confirmed by Rider & Saggerson (1983); they might be explained by an activation of the hormone-sensitive lipase which presumably contaminated the unwashed preparation of microsomes used as a source of GPAT.

The incubation of adipocytes in the presence of noradrenaline is well known to cause an activation of lipase and liberation of free fatty acids. Rider & Saggerson (1983) have reported up to 7 mM-fatty acids in their incubation medium in the presence of noradrenaline, corresponding to approx. 0.1 mM

in the assay mixture. We observed a lower degree of lipolysis, and the concentration of free fatty acids in our incubation medium rarely exceeded 2 mM, i.e. 40 μ M in the assay performed with extracts of unwashed cell. This concentration is sufficient to explain the effects of noradrenaline shown in Fig. 4 and in Figs. 5 and 6. The effect shown in Fig. 4 was indeed to shift the albumin-saturation-inhibition curve to the right, exactly like fatty acids do (see Fig. 3). The effect shown in Figs. 5 and 6 was to favour the irreversible inactivation of GPAT in the presence of intermediary concentrations of oleic acid, insufficient to cause by themselves the complete inactivation of GPAT within 30 min at 0°C.

It is not clear how our results allow to explain the effect of noradrenaline to decrease the activity of GPAT, as described by Rider & Saggerson (1983). Since, according to these authors, the noradrenaline effect can be prevented by inclusion of albumin in the homogenization buffer, it is likely to be related to the excess of fatty acids resulting from the hormonal treatment. The previous failure of Sooranna & Saggerson (1976b) to mimic the noradrenaline effect by addition of fatty acids to the incubation medium can presumably be explained by the excess of albumin that was added simultaneously. Since the noradrenaline effect was observed at all palmitoyl-CoA concentrations, we are tempted to believe that it corresponds to an inactivation of the enzyme in the presence of the large excess of fatty acids, which, in the work by Rider & Saggerson (1983), characterizes the hormonal effect. This inactivation would, however, occur only if the assay was not performed immediately after the preparation of the extract, which is not specified by the authors. We also have no interpretation for the observation reported by Rider & Saggerson (1983) that the response to adrenaline could be reversed by insulin under conditions in which lipolysis was not arrested.

The physiological implication of the fatty acid-mediated effect of noradrenaline treatment on GPAT activity is difficult to evaluate. As shown by Figs. 1 and 3, fatty acid can cause a stimulation or an inhibition of GPAT activity according to the concentration of albumin and of palmitoyl-CoA. Assuming that these concentrations are low inside the cells, it is more likely that the general effect of fatty acid, and therefore of the noradrenaline treatment, is a decrease in the activity of GPAT.

Note added in proof (received 5 December 1984)

When this paper was in press, Nimmo & Nimmo (1984) have reported that, in contrast with Sooranna & Saggerson (1976a), they were unable to observe a significant and reproducible inactivation

of GPAT in adipocytes treated with adrenaline. Their assays were performed at 28 μ M-albumin and at either 20 or 125 μ M-palmitoyl-CoA. This finding is in agreement with our data (Fig. 1) which indicate that fatty acids, expected to be formed in the presence of adrenaline, cause a slight stimulation of GPAT in the first condition and a slight inhibition in the second, the overall effect being most likely not significant.

This work was supported by the Belgian Fonds de la Recherche Scientifique Médicale and by the U.S. Public Health Service (Grant AM 9235).

References

- Abou-Issa, H. M. & Cleland, W. W. (1969) *Biochim. Biophys. Acta* **176**, 692–698
- Cheng, C.H.K. & Saggerson, E. D. (1980) *Biochem. J.* **190**, 659–662
- Daae, L. N. W. & Bremer, J. (1970) *Biochim. Biophys. Acta* **210**, 92–104
- Duncombe, W. G. (1963) *Biochem. J.* **88**, 7–10
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Leaback, D. H. & Walker, P. G. (1961) *Biochem. J.* **78**, 151–156
- Lederer, B. & Hers, H. G. (1984) *Biochem. J.* **217**, 709–714
- Nimmo, G. A. & Nimmo, H. G. (1984) *Biochem. J.* **224**, 101–108
- Nimmo, H. G. & Houston, B. (1978) *Biochem. J.* **176**, 607–610
- Rider, M. H. & Saggerson, E. D. (1983) *Biochem. J.* **214**, 235–246
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Saggerson, E. D., Carpenter, C. A., Cheng, C. H. K. & Sooranna, S. R. (1980) *Biochem. J.* **190**, 183–189
- Sooranna, S. R. & Saggerson, E. D. (1976a) *FEBS Lett.* **64**, 36–39
- Sooranna, S. R. & Saggerson, E. D. (1976b) *FEBS Lett.* **69**, 144–148
- Sooranna, S. R. & Saggerson, E. D. (1978) *FEBS Lett.* **90**, 141–144
- Sooranna, S. R. & Saggerson, E. D. (1982) *Biochem. J.* **202**, 753–758
- Spector, A. A., Fletcher, J. E. & Ashbrook, J. D. (1971) *Biochemistry* **10**, 3229–3232