Fatty acids and the control of adipocyte glycerolphosphate acyltransferase by noradrenaline

Lederer & Hers (1985) have recently demonstrated that long chain fatty acids influence the kinetics of GPAT in crude extracts from rat adipocytes. These authors reached three main conclusions. First, that fatty acids will alter GPAT kinetics with respect to the acyl-CoA substrate by displacing this from albumin present in the assay. This is as expected in view of the hydrophobic nature of both agents. Second, that GPAT is irreversibly inactivated on incubation of cell extracts for 30 min at 0 °C with oleate concentrations in excess of 1 mM (albumin apparently only being added to a concentration 1/200th of that of oleate). This also is as expected since these are most extreme conditions. Third, it was concluded that treatment of adipocytes with noradrenaline had no effect in its own right upon GPAT activity and that any hormone-induced changes in activity were secondary to elevation of fatty acid concentration and were attributable to one or other of the two effects described above. We have previously reported effects of adrenaline or noradrenaline to decrease GPAT *in vitro* (Sooranna & Saggerson, 1976a, 1978; Rider & Saggerson, 1983) and demonstrate here that these changes cannot be related to the effects described by Lederer & Hers (1985).

Before doing this we should firstly outline the procedures that have been used throughout all of our studies with catecholamines. Epididymal adipocytes are isolated from rats weighing 160–190 g. The average wet weight of a single fat pad is 424 mg. Cells are incubated with or without hormone at a cell density such that cells from 15–17% of a fat pad (40 mg average dry weight of cells) are in each 1 ml of incubation medium containing 490–600 μ M-albumin. At the appropriate time the cells are recovered by centrifugation, the incubation medium rapidly removed by aspiration and the cells freeze-stopped in liquid N₂. This whole procedure takes approx. 30 s. When crude homogenates are subsequently prepared from these cells the homogenate always contains adipocyte material from 0.5–0.7 fat pads/ml and GPAT assays are performed within 5 min of homogenization on 0.1 ml of extract in an assay final volume of 1.0 ml.

We have recently measured the NEFA concentration in such homogenates. When these are obtained from cells incubated for 40 min with 5 mM-glucose and 600 μ M-albumin this was found to be 0.36 ± 0.05 mM. In parallel incubations with 1 μ M-noradrenaline also present this homogenate concentration of fatty acid was increased to 0.58 ± 0.03 mM (means $\pm$ S.E.M. for four separate experiments in each case). It may appear surprising both that the homogenates from control cells contained so much nonesterified fatty acid and that the increase brought about by noradrenaline was so modest. However, this is not at variance with long-established literature. Adipose tissues contain sizeable amounts of NEFA and only a small fraction of this rapidly exchanges with the extracellular pool (Kerpel *et al.*, 1961; Borgstrom & Olivecrona, 1961; Winand *et al.*, 1969; Vaughan *et al.*, 1964). Furthermore, incubation of adipocytes for

45–60 min with 33 μ M-noradrenaline in the absence of glucose only expands the intracellular fatty acid pool by approx. 3-fold (Angel *et al.*, 1971). Our GPAT assays therefore contain fatty acids varying over a range of approx. 35–60 μ M and albumin at approx. 31 μ M (26 μ M added in the assay mixture and approx. 4.7 μ M carried over via the homogenate from the cell incubation medium – see below). It is clear from Fig. 3 of Lederer & Hers (1985) that such a change in fatty acid concentration at that albumin concentration could make very little contribution to a noradrenaline-induced decrease in GPAT activity when the enzyme is assayed at 100 μ M-palmitoyl-CoA. Furthermore the ‘fatty acid effect’ would also be expected to be negligible if the enzyme was assayed at 40 μ M-palmitoyl-CoA or might even cause a small increase in measured GPAT activity. This must be contrasted with the sizeable inactivations of GPAT with noradrenaline routinely observed in this laboratory. Indeed, Lederer & Hers (1985) do state “that this effect cannot simply be explained by the presence of an excess of fatty acids” but then suggest that our experimental conditions are likely to lead to irreversible, fatty-acid-dependent inactivation of GPAT in homogenates. This proposal is not in accord with several findings, some of which have been published for several years.

(a) Our homogenate fatty acid concentrations are too low (see above) to cause the effects shown in Fig. 5 of Lederer & Hers (1985). Even if they were sufficiently high, our rapid assay procedures preclude the time-dependent inactivation seen in Fig. 6 of Lederer & Hers (1985). In support of this, Table 1 of Sooranna & Saggerson (1978) shows clearly that GPAT in homogenates from control cells or those pretreated for 75 min with noradrenaline is utterly stable when incubated for up to 70 min at 0 or 30 °C. To clarify this point still further we have measured the carry-over of [³H]sucrose in incubation media into homogenates. When cells from 0.67 of a fat pad were recovered after incubation the sucrose space carried over varied between 58 and 93 μ l (mean $\pm$ S.E.M. for six measurements from two separate cell preparations = 79 ± 6 μ l). This would represent a mean carry-over into homogenates of 47 μ M of albumin when cells are incubated with 600 μ M-albumin (see above). The highest concentration of fatty acid that we have ever obtained in incubation media on treatment with noradrenaline is 6.96 mM (Rider & Saggerson, 1983, Table 1). This would contribute a carry-over from the extracellular pool of 550 μ M into homogenates. Again, this is too low to cause the effects seen in Figs. 5 and 6 of Lederer & Hers (1985).

(b) Propranolol or insulin both cause significant restoration of GPAT activity after prior decrease due to noradrenaline (Rider & Saggerson, 1983, Fig. 5 and Table 1). These increases are seen in the presence of high concentrations (5–6 mM) of fatty acids that remain in the incubation medium. Therefore, the reversibility of the noradrenaline effect by either propranolol or insulin would be difficult to explain in terms of the ‘fatty acid effect’ of Lederer & Hers (1985) – see above.

(c) Lederer & Hers (1985) state “the previous failure of Sooranna & Saggerson (1976b) to mimic the noradrenaline

Abbreviations used: GPAT, glycerolphosphate acyltransferase; NEFA, non-esterified fatty acids.

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Fig. 1. Effect of fatty acid/albumin ratio on GPAT (○, ●) and pyruvate dehydrogenase (□, ■) activities

The data are derived from experiments of Sooranna & Saggerson (1976a,b) as explained in the text. Fatty acid/albumin molar ratios represent those present in adipocyte incubation media after 1 h of incubation. These different ratios were either brought about by addition of various concentrations of palmitate (○, □) or adrenaline (●, ■) at the start of the incubations. Carbohydrate substrates were absent from the incubations so that utilization of fatty acids by the cells was negligible. Enzyme activities in the two experiments are normalized relative to activities in the absence of added palmitate or adrenaline.

effect by addition of fatty acids to the incubation medium can presumably be explained by the excess of albumin that was added simultaneously". The experiment in question (Fig. 1 of Sooranna & Saggerson, 1976b) was specifically designed to test whether addition of fatty acid (at concentrations equivalent to those released by catecholamine-stimulated lipolysis) in the presence of normal albumin concentrations could decrease GPAT

activity. To make the point utterly clear we have constructed Fig. 1 which is a composite of data derived from Fig. 3 of Sooranna & Saggerson (1976a) and Fig. 1 of Sooranna & Saggerson (1976b). This makes a direct comparison showing that control levels of GPAT activity are maintained in the face of high NEFA/albumin ratios equivalent to those caused by adrenaline. Hence, the effect of adrenaline is not attributable to fatty acid accumulation. As proof that externally applied fatty acids enter the cell and influence metabolic events we have the parallel observation that pyruvate dehydrogenase activity is decreased by this treatment.

In conclusion, although the effect of noradrenaline to inactivate microsomal (but not mitochondrial) GPAT is abolished by inclusion of albumin in homogenization buffers (Rider & Saggerson, 1983) implying a role for some presumed hydrophobic inhibitory factor(s) in this system, we maintain that there is no evidence for a direct effect of fatty acids in the inactivating mechanism.

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