INTERACTING ACTIONS OF INSULIN AND UNESTERIFIED FATTY ACIDS ON LIPOGENIC ENZYMES IN RAT ADIPOCYTES

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1. Introduction

It has recently been shown that glycerol phosphate acyltransferase activity in fat cell extracts is modified by prior exposure of fat cells to insulin or adrenalin in vitro [1]. This investigation examines the effect of long chain fatty acids upon glycerolphosphate acyltransferase activity and the manner in which fatty acids modify insulin actions. In addition, interactions of fatty acids and insulin in modification of acetyl CoA carboxylase and pyruvate dehydrogenase$_a$ activities were examined since these enzymes are believed to be important in the hormonal regulation of fatty acid synthesis, a process which shows interacting responses to long chain fatty acids and insulin [2].

2. Materials and methods

Sources and treatments of animals and chemicals were as described previously [1]. Fat cells were prepared by the method of Rodbell [3]. Previous techniques for cell incubation and extraction were miniaturised as follows: Cells from eight epididymal fat pads were suspended in a final volume of 12 ml in Krebs–Ringer bicarbonate containing 10 mg of albumin per ml. One ml portions of this stock cell suspension were then dispensed into siliconised 25 ml flasks and incubated with shaking at 37°C in a total volume of 4 ml under $O_2 + CO_2$ (95:5%). After 60 min the cells were separated from incubation media and frozen in liquid N_2 [1]. Tissue extracts were made from the cells of each incubation by homogenisation at 4°C for four 15 sec periods in an Ultra Turrax homogeniser fitted with a small probe. Fat-free homogenates were produced by centrifugation for 30 s in an Eppendorf 3200 centrifuge. Extracts for estimation of Acyl CoA: L-glycerol 3-phosphate-O-acyl transferase (EC 2.3.1.15) and pyruvate dehydrogenase$_a$ (EC 1.2.4.1.) were prepared with 1.0 ml of ice-cold 0.25 M sucrose containing 1 mM EDTA, 1 mM dithiothreitol and 10 mM Tris–chloride buffer (pH 7.4). These enzymes, together with glutamate dehydrogenase (EC 1.4.1.2) and lactate dehydrogenase (EC 1.1.1.27), were assayed as described previously [1] with the exception that glycerolphosphate acyltransferase activity was only assayed at 0.5 mM glycerol phosphate. Extracts for estimation of acetyl-CoA carboxylase (EC 6.4.1.2) were prepared with 1.0 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.3) containing 2 mM EDTA and 4 mM GSH [4]. Acetyl-CoA carboxylase in fat-free homogenates was assayed in triplicate by following [^{14}C]bicarbonate fixation over 3 min at 30°C [4]. Suitable blanks were employed.

Glycerol phosphate acyltransferase, pyruvate dehydrogenase$_a$ and acetyl-CoA carboxylase activities are expressed as nmol of L-[U-^{14}C]glycerol phosphate incorporated/min per unit of lactate dehydrogenase, μmol of acetyl-CoA formed/min per unit of glutamate dehydrogenase and nmol of [^{14}C]bicarbonate incorporated/min per unit of lactate dehydrogenase respectively.

Unesterified fatty acids and glycerol in incubation media were assayed as described previously [1].

Where appropriate, statistical differences between experimental values were *t*-tested on the basis of paired differences.

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3. Results and discussion

It was shown recently that incubation without substrates in the presence of adrenalin considerably decreased fat cell glycerol phosphate acyltransferase activity [1]. This correlated inversely with increased lipolysis as measured by extracellular unesterified fatty acid accumulation. Pyruvate dehydrogenase$_a$ activity was decreased under the same conditions. We have considered the possibility that these effects may be secondary to accumulation of lipolysis products. Figure 1 demonstrates that under incubation conditions similar to those used previously (fig.3 of [1]), with the exception that extracellular fatty acid was increased by palmitate addition rather than by adrenalin-stimulated lipolysis, extracellular fatty acids had no effect on glycerol phosphate acyltransferase activity. Palmitate may however mimic the effects of accumulation of lipolysis products on pyruvate dehydrogenase$_a$ activity. Under these incubation conditions (i.e. no carbohydrate substrate

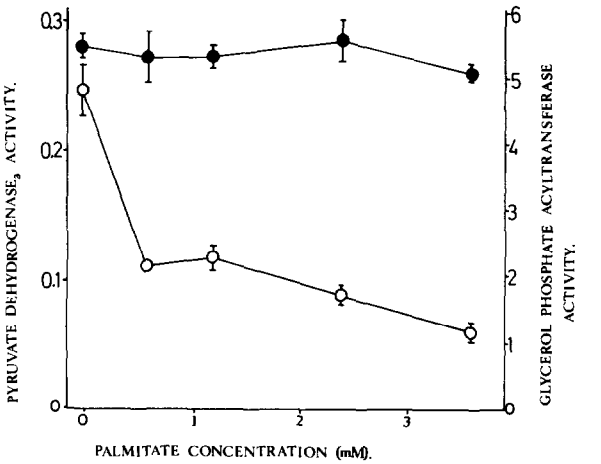

Fig.1. Cells were incubated without added carbohydrate substrates for 1 h in Krebs–Ringer bicarbonate containing albumin (47.5 mg/ml) and initial palmitate concentrations as indicated. The results are means and S.E.M. of 4 experiments using 4 cell preparations. The mean fat cell DNA was 8.7 μg/ml of incubation medium. (○) pyruvate dehydrogenase$_a$ activity; (●) glycerol phosphate acyltransferase activity.

Table 1
Effects of Palmitate and Insulin on glycerol phosphate acyltransferase activity

Initial palmitate concn. (mM)	Insulin (20 munits/ml)	Glycerol phosphate acyltransferase		Final fatty acid concn. (mM)
		Activity	% Effect of palmitate	
0	–	7.64 ± 0.60	–	0.153 ± 0.020
0.25	–	8.33 ± 0.90	+8 ± 5	0.214 ± 0.008
0.5	–	8.09 ± 0.86	+6 ± 6	0.417 ± 0.020
1.0	–	7.63 ± 0.69	+1 ± 10	0.889 ± 0.036
0	+	6.85 ± 0.91 (−11 ± 10)	–	0.114 ± 0.014*
0.25	+	6.41 ± 0.75* (−23 ± 5)**	−5 ± 3	0.121 ± 0.012****
0.5	+	7.58 ± 0.87 (−5 ± 10)	+13 ± 6 +19 ± 7$^{\neq}$	0.245 ± 0.024**‡
1.0	+	9.01 ± 1.13$^{\neq,\ddagger\ddagger}$ (+17 ± 8)	+33 ± 7$^{\neq\neq}$ +40 ± 6‡‡	0.685 ± 0.022**

Cells were incubated for 1 h in Krebs–Ringer bicarbonate with albumin (21 mg/ml), fructose (5 mM) and other additions, as indicated. The results are means and S.E.M. of 5 experiments using 5 cell preparations. Values in parentheses indicate percentage effects of insulin. The mean cell DNA was 7.1 μg/ml of incubation medium. Significance tests for glycerol phosphate acyltransferase are applied both to absolute values and to values normalised by expression as percentages of controls.

*, **, ***, **** Indicate $P < 0.05, < 0.02, < 0.01, < 0.001$ respectively for insulin-treated cells versus controls.
$^{\neq},^{\neq\neq}$ Indicate $P < 0.02, < 0.01$ versus appropriate zero-palmitate values.
‡,‡‡ Indicate $P < 0.05, < 0.01$ versus appropriate 0.25 mM palmitate values.

present) the addition of fatty acid did not significantly influence the effect of insulin [1] on glycerol phosphate acyl transferase (results not shown).

Previously, when cells were incubated in the presence of 5 mM fructose without added fatty acid, insulin decreased glycerol phosphate acyltransferase activity [1]. Table 1 shows that palmitate tended to reverse this effect of the hormone and, in particular, insulin appeared to facilitate a significant effect of palmitate upon glycerolphosphate acyltransferase activity which was not seen in the absence of the hormone. Although this is a model system it is possible that it pertains to a more physiological situation. Insulin stimulation of lipoprotein lipase should increase the availability of unesterified fatty acids for intracellular esterification by hydrolysis of plasma lipoprotein in the post-absorbtive state. Table 1 also demonstrates that extracellular fatty acid concentrations were lower at the end of incubations when insulin was present. This was due both to a significant antilipolytic effect of the hormone as measured by glycerol release (results not shown) and to promotion of esterification [2]. Net uptake of fatty acids was observed in incubations containing added palmitate.

Figure 2 shows that after 1 h of incubation in the presence of 5 mM fructose, which is a convenient model substrate [5], pyruvate dehydrogenase$_a$ activity was steeply related to the extracellular fatty acid concentration at the time of freeze-stopping. This is similar to the relationship between hepatic pyruvate dehydrogenase activity and serum unesterified fatty acids observed by Wieland et al. [6]. The steep curves shown in fig.2 suggested that effects of insulin on adipose tissue pyruvate dehydrogenase activity that are apparent in similar closed incubation systems [5,7–9] should be considered as two separate processes: (1) modulation of the extracellular fatty acid concentration. At low fatty acid concentrations this effect may be quite large. We suggest that an appreciable proportion of the insulin effects previously demonstrated on this enzyme in incubated fat pads [5,7–9] may therefore be due simply to manipulation of fatty acid concentrations in the interstitial spaces of the tissue pieces. (2) An effect which is quite small in percentage terms and is represented by the difference between the two curves in fig.2. This effect increases as the fatty acid concentration is increased i.e. fatty acids may be said to 'permit' this action of insulin. The data of fig.2 can be satisfactorily linearised by reciprocal plots of (pyruvate dehydrogenase$_a$ activity)$^{-1}$ (y) versus (fatty acid concentration)$^{-1}$ (x). The regression lines describing these are $y = 8.08 - 0.843x$ in the absence of insulin ($r = -0.983$) and $y = 5.05 - 0.402x$ in the presence of insulin ($r = -0.995$). These intercept at a value corresponding to 147 μM unesterified fatty acid, i.e. the effect is only seen above this concentration. At a hypothetical fatty acid concentration of infinity this effect would amount to a 60% increase in pyruvate dehydrogenase$_a$ activity.

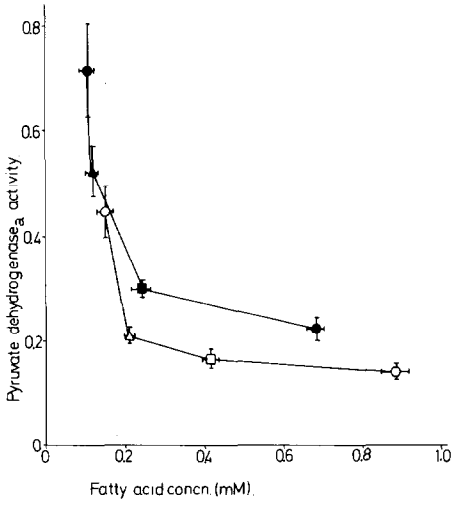

Fig.2. The data were obtained in the experiment described in table 1. The fatty acid measurements refer to the final concentrations in incubation media. Open symbols, without insulin; closed symbols, with insulin. Initial palmitate concentrations: zero (●,○); 0.25 mM, (▲,△); 0.5 mM (■,□); 1.0 mM (◀,◁).

This interacting pattern of fatty acids and insulin influencing pyruvate dehydrogenase$_a$ activity is somewhat different from the interaction of these agents with fatty acid synthesis when [^{14}C]fructose is substrate (fig.5 of [2]).

Figure 3 shows an experiment in which cells were incubated without a carbohydrate substrate. There was then little esterification and insulin therefore caused little alteration in the terminal fatty acid concentration which showed a small net increase. Under these conditions insulin action not explicable by modulation of extracellular fatty acid concentration was only found at low fatty acid concentrations

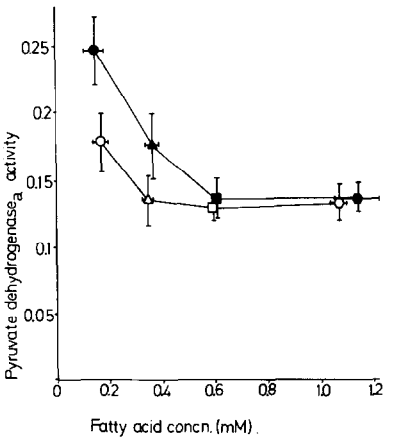

Fig.3. Cells were incubated without carbohydrate substrates for 1h in Krebs–Ringer bicarbonate containing albumin (21 mg/ml) and palmitate or insulin (20 munits/ml) as indicated. The results are means and S.E.M. of 4 experiments using 4 cell preparations. The mean fat cell DNA was 6.1 μg/ml of incubation medium. Symbols as for fig.2. The fatty acid measurements refer to the final concentrations in incubation media.

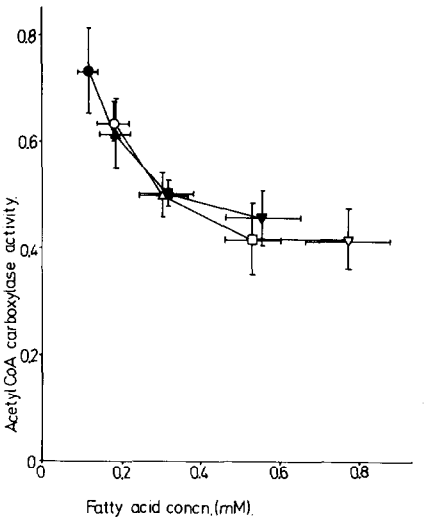

Fig.4. Cells were incubated for 1h in Krebs–Ringer bicarbonate containing albumin (21 mg/ml), fructose (5 mM) and palmitate or insulin (20 munits/ml) as indicated. The results are means and S.E.M. of 6 experiments using 6 cell preparations. The mean fat cell DNA was 5.3 μg/ml of incubation medium. The fatty acid measurements refer to the final concentrations in incubation media. Open symbols, without insulin; closed symbols, with insulin. Initial palmitate concentrations: zero (●,○); 0.25 mM (▲,△); 0.5 mM (■,□); 0.75 mM (▼,▽).

i.e. – the pattern was the reverse of that found with fructose present. Very similar interactions of insulin and unesterified fatty acids are seen on fatty acid synthesis measured using $[^3H]H_2O$ in fat-cells incubated without substrates (table 6 of [2]).

Figure 4 shows decreases in fat cell acetyl-CoA carboxylase activity on incubation with palmitate under conditions identical to fig.2 and table 1. The relationship between activity and terminal fatty acid concentration was not as steep as that found for pyruvate dehydrogenase and any apparent effects of the hormone under a given incubation condition could be explained in terms of modulation of the extracellular fatty acid concentration. Previous reports of insulin stimulation of fat pad acetyl-CoA carboxylase in vitro [4] could therefore be explained by alteration of extracellular fatty acid concentrations in the interstitial spaces. These are in a rather poorly defined environment, particularly when albumin is absent from incubations [4].

We conclude that insulin and unesterified fatty acids have interacting effects upon pyruvate dehydrogenase$_a$ activity in fat cells and that these actions are affected by the model substrate fructose which permits fatty acid esterification. In addition, glycerol phosphate acylation processes may also be subject to interacting effects of these agents. At present it is unknown whether these actions on the two systems are interrelated and the nature of the effects themselves is unresolved.

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