A STABLE DECREASE IN LONG CHAIN FATTY ACYL CoA SYNTHETASE ACTIVITY AFTER TREATMENT OF RAT ADIPOCYTES WITH ADRENALINE

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1. Introduction

ATP-dependent fatty acyl CoA synthetase activity initiates the intracellular metabolism of fatty acids and is, therefore, a potential site of regulation of adipose tissue triacylglycerol synthesis. The specific activity of this enzyme in microsomes prepared from adipocytes [1] is considerably increased by a brief exposure of these cells to insulin. This suggested that this enzyme might be subject to acute control by hormones. We have investigated changes in fatty acyl CoA synthetase activity that result from treatment of adipocytes with adrenaline. Similar experiments have suggested acute hormonal regulation of glycerol phosphate acyl transferase and Mg^{2+}-dependent phosphatidate phosphohydrolase activities in this tissue preparation [2,3].

2. Materials and methods

Sources and treatments of animals and chemicals were as in [2]. [G-^{3}H]coenzyme A was obrained from NEN Chemicals GmbH, Frankfürt/Main. Adipocytes were prepared as in [4] and incubated and extracts prepared from freeze-stopped cells as in [5].

ATP-dependent long chain fatty acyl CoA synthetase (EC 6.2.1.3) was assayed at 30°C in final vol. 0.2 ml containing 0.1 M Tris–Cl buffer, pH 7.4, 0.2–0.5 μCi [^{3}H]coenzyme A, 8 mM $MgCl_2$, 10 mM ATP, 0.5 mM dithiothreitol, 50 μM coenzyme A, 30 μM palmitic acid and 0.2 mg Triton X-100 [6]. The reaction was initiated with 0.01 ml tissue extract. Assays were performed in duplicate for 1 min, but were usually linear for at least 2 min. The reaction was terminated with 2 ml ice-cold 0.3 M trichloroacetic acid and the acid-precipitable palmitoyl [^{3}H]CoA was separated from [^{3}H]coenzyme A by Millipore filtration [6]. Filters were counted in 10 ml 2,5-bis-(5-*t*-butylbenzoxazol-2-yl) thiophen in toluene (4 g/litre) with a counting efficiency of 40%. Appropriate blanks were conducted in parallel with all experiments. Fatty acyl CoA synthetase activity was expressed as nmol palmitoyl CoA formed/min/unit lactate dehydrogenase in order to correct for any incompleteness in recovery of cells after incubation or for incomplete cell breakage during homogenisation. Lactate dehydrogenase (EC 1.1.1.27) was assayed as in [2].

Non-esterified fatty acids in incubation media and DNA contents of adipocyte preparations were measured as in [2].

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

3. Results and discussion

Previous studies [2,3] have shown that after incubation of adipocytes with catecholamines in the absence of carbohydrate substrates, glycerolphosphate acyltransferase and phosphatidate phosphohydrolase activities are decreased. Under similar conditions (fig.1), 0.63 μM adrenaline decreased fatty acyl CoA synthetase activity, the effect being significant at all tested times ($P<0.01$ in every case). The activity observed in the absence of adrenaline also decreased

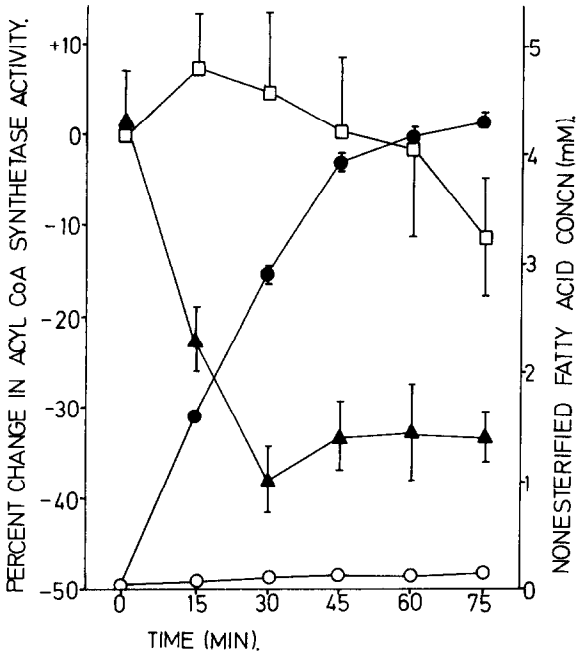

Fig.1. Time course of adrenaline effect on fatty acyl CoA synthetase activity. Adipocytes were incubated without added substrates for the indicated times in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) in the presence and absence of 0.63 μM adrenaline. The results are the means of 6 expts. and the bars represent SEM. The mean fat-cell DNA was 5.2 μg/ml flask contents. The fatty acyl CoA synthetase activity at zero-time was 11.74 ± 3.82 nmol palmitoyl CoA formed/min/unit lactate dehydrogenase (or 6.66 ± 1.79 nmol palmitoyl CoA formed/min/10 μg DNA). (▲) Effect of adrenaline on fatty acyl CoA synthetase activity (compared against control at the same time). (□) Change in control fatty acyl CoA synthetase activity compared to zero-time. (●, ○) Extracellular concentration of nonesterified fatty acids with and without adrenaline, respectively.

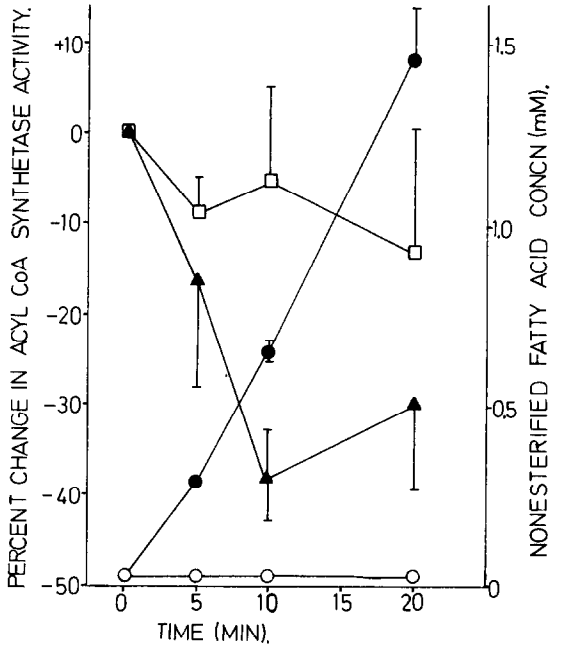

Fig.2. Time course of adrenaline effect on fatty acyl CoA synthetase in the presence of glucose. Adipocytes were incubated with 5 mM glucose for the indicated times in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) in the presence and absence of 0.63 μM adrenaline. The results are the means of 5 expts. and the bars represent SEM. The mean fat-cell DNA was 4.7 μg/ml flask contents. The fatty acyl CoA synthetase activity at zero-time was 3.60 ± 0.50 nmol palmitoyl CoA formed/min/unit lactate dehydrogenase (or 1.99 ± 0.36 nmol palmitoyl CoA formed/min/10 μg DNA) in the absence of adrenaline. Symbols as for fig. 1.

slightly during incubation of the cells. This latter finding is possibly at variance with [1] where it was found that adipocyte microsomal fatty acyl CoA synthetase activity increased during a 60 min incubation period.

When adipocytes were incubated in the presence of 5 mM glucose a decrease in fatty acyl CoA synthetase activity was apparent after 5 min with adrenaline (fig.2). This effect of adrenaline was significant at 10 min and 20 min ($P<0.01$ and $P<0.05$, respectively). Fatty acyl CoA synthetase activity was again observed to decrease slightly during control incubations.

The decreases seen in fatty acyl CoA synthetase activity with adrenaline both in the presence and absence of glucose were accompanied by a concomitant stimulation of lipolysis as seen by accumulation of nonesterified fatty acids in the incubation medium (fig.1,2). It is possible that the effect of adrenaline upon the enzyme may be secondary to accumulation of lipolysis products within the cells. Alternatively, the observed effect may be a more direct consequence of adrenaline stimulation of the adipocyte.

Table 1 shows that the decrease in fatty acyl CoA

Table 1
Effect of adrenaline, insulin and propranolol on adipocyte fatty acyl CoA synthetase activity

Additions to incubation medium	Extracellular unesterified fatty acid conc. (mM)	Fatty acyl CoA synthetase activity
None	0.12 ± 0.01	4.24 ± 0.22
Insulin (20 mU/ml)	0.11 ± 0.02	4.16 ± 0.63
Adrenaline (0.63 μM)	3.55 ± 0.36[a]	3.23 ± 0.36[a]
Adrenaline (0.63 μM) + insulin (20 mU/ml)	0.66 ± 0.13[a, c]	4.48 ± 0.68[b]
Propranolol (10 μM)	0.10 ± 0.01	4.35 ± 0.40
Propranolol (10 μM) + adrenaline (0.63 μM)	0.09 ± 0.01	4.21 ± 0.11

[a] Indicates $P < 0.01$ for adrenaline-treated cells versus appropriate controls
[b, c] Indicate $P < 0.05$, $P < 0.01$, respectively, for insulin-treated cells versus appropriate controls

Adipocytes were incubated without added carbohydrate substrates for 1 h in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) and other additions as indicated. The results are the means and SEM of 6 exps. The mean fat-cell DNA was 7.0 μg/ml flask contents

synthetase activity resulting from 1 h incubation with adrenaline in the absence of carbohydrate substrate was abolished by 10 μM propranolol (a β-blocker) which also inhibited the lipolytic action of the hormone. This suggests the involvement of a β-adrenergic receptor in the action of adrenaline upon the enzyme. Insulin was also able to abolish the decrease in the enzyme activity caused by adrenaline although no effect of insulin on basal activity was observed. This latter finding is in agreement with [7] but at variance with [1]. However, in [1] a freeze-stop procedure was not used and microsomes were isolated from insulin-treated adipocytes by a lengthy procedure prior to enzyme assay.

We conclude that brief exposure of adipocytes to adrenaline results in a relatively stable decrease in fatty acyl CoA synthetase activity in that the change persists through freeze-stopping and tissue extraction. The nature of this change is not understood at present. The possibility that palmitoyl CoA synthetase from rat liver microsomes might be controlled by covalent modification has been discussed [8], but this has not been clearly established. The decrease in activity reported here is consistently about 30% of the total fatty acyl CoA synthetase activity which is found in mitochondrial, microsomal and high speed supernatant fractions from adipose tissue and adipocytes [9,10]. At present the subcellular localisation of the adrenaline-sensitive activity is unknown.

Acknowledgements

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