CONTROL OF PYRUVATE DEHYDROGENASE INTERCONVERSION BY PALMITOYL-COENZYME A AS RELATED TO ADENINE NUCLEOTIDE TRANSLOCATION IN ISOLATED FAT CELL MITOCHONDRIA

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1. Introduction

It is now well documented that insulin, in vitro, activates pyruvate dehydrogenase (EC 1.2.4.1) (PDH) in adipose tissue by promoting conversion of the inactive form of the enzyme (PDH_b) to the active form (PDH_a) [1–11]. This effect was suggested to result from an action of insulin on the energy metabolism of the fat cell rather than from a direct hormonal interaction with the mitochondrial PDH-system. According to our hypothesis [9,11] insulin, by lowering long chain acyl-CoA in adipose tissue [12,13] leads to deinhibition of the mitochondrial adenine nucleotide (AN) translocase system and thereby to a drop in the mitochondrial ATP/ADP ratio. The relative increase of ADP which inhibits the PDH kinase competitively with ATP is than thought to shift the phosphorylation equilibrium of the PDH system towards the active dephosphoenzyme. Experimental evidence for such an interrelationship between the mitochondrial AN and PDH activity so far stems mainly from work on liver mitochondria [14,15] or isolated liver cells [26]. In the present work isolated fat cell mitochondria were chosen as a model for studying the possible involvement of long chain acyl-coenzyme A in the activation of adipose tissue PDH by insulin. Some of the results have been reported briefly elsewhere [29].

2. Materials and methods

Isolated fat cells were prepared from epididymal fat pads of normally fed Sprague–Dawley rats weighing 120–150 g according to [16]. Preparation of mitochondria was essentially as described [17] with the following modifications: the isolated fat cells were suspended in sucrose medium (sucrose 0.25 mol/litre, glutatione-SH 7.5 mmol/litre, EDTA 2 mmol/litre, Tris-buffer pH 7.4 20 mmol/litre, bovine serum albumin 2%), and homogenised in a glass/teflon homogenizer (Potter–Elvehjem). After removing unbroken cells, fat, nuclei and cell debris by centrifuging at 3000 *g* for one minute, the mitochondria were spun down at 10 000 *g* for 4 min. The pellet was resuspended in KCl medium (KCl 130 mmol/litre, $MgCl_2$ 2 mmol/litre EDTA 2 mmol/litre, KH_2PO_4 2 mmol/litre, Tris–HCl buffer 5 mmol/litre, pH 7.4). After centrifugation at 10 000 *g* for 4 min mitochondria were suspended in KCl medium and used for the experiments. All steps were carried out at 4°C. The respiratory control quotient (substrate succinate) of mitochondria prepared by this procedure was 3.8 ± 0.2 (n = 72).

Incubation: 50 µl of the mitochondrial suspension (corresponding to about 350 mg fat cell dry weight) were incubated in 550 µl of KCl medium. Gas phase: air, temperature 25°C. For other additions see individual experiments.

For PDH determinations the cups were placed on ice at the end of incubations, and centrifuged for 1 min at 8000 *g* (Eppendorf centrifuge) in the cold room. After discarding the supernatant the mitochondrial pellet was suspended in 100 µl potassium phosphate buffer pH 7.0, 15 mmol/litre containing 2% bovine serum albumin, and frozen in liquid nitrogen. After thawing and another freeze and thaw step the suspension was rigorously shaken on a vortex mixer

for 10 sec. The resulting extract was used without centrifugation for the determination of PDH_a and total PDH activity in the optical assay described previously [11]. PDH activities are expressed as mU/U GDH (glutamate dehydrogenase, EC 1.4.1.2) the latter being determined according to [18] in the same extract. Mitochondrial AN were determined in parallel incubations. After centrifugation the mitochondrial pellet was mixed with 100 μl 6% $HClO_4$, and after centrifugation reextracted with the same amount of $HClO_4$. The combined supernatants were neutralized with solid $KHCO_3$. ATP and ADP were measured according to [19] and [20], respectively using a spectro-photofluorometer (Aminco–Bowman, USA). This required the following modifications: in the ATP assay triethanolamine buffer was exchanged against Tris–buffer, and the glucose concentration was 25 mmol/litre. In the ADP assay triethanolamine buffer was exchanged against phosphate buffer 50 mmol/litre, pH 7.5, and the NADH concentration was reduced to 1.25 μmol/litre. α-Glycerophosphate was determined according to [21]. Oxygen consumption was recorded polarographically with a Clark-type electrode (Radiometer, Copenhagen) fitted to a thermostated respiration chamber.

Reagents: Chemical and biochemical reagents were products of E. Merck AG (Darmstadt, West Germany) and of Boehringer GmbH (Mannheim, West Germany), respectively. Bovine serum albumin (BSA) (pure) was from Serva (Heidelberg, West Germany); crude collagenase from Worthington Biochemical Corp. (Freehold, New Jersey, USA). Arylamine-acetyltransferase (EC 2.3.1.5), and pyruvate dehydrogenase phosphatase were prepared from pigeon liver [22,23] and from pig heart [24], respectively.

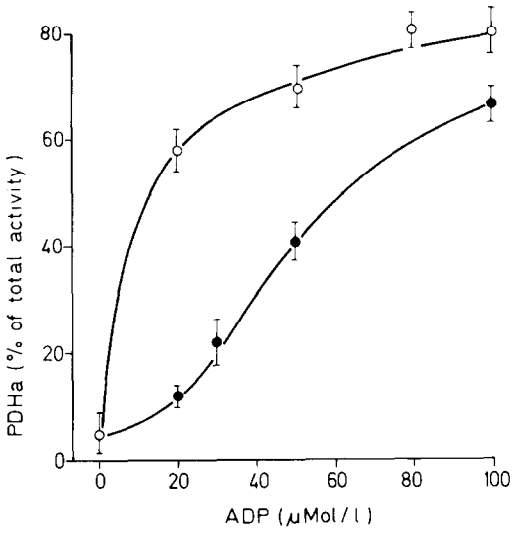

Fig.1. Effect of ADP on PDH_b to PDH_a conversion in fat cell mitochondria. Mitochondria were incubated 7 min at 25°C at increasing concentrations of ADP either in the absence (•) or the presence (○) of glycerol, 10 mmol/litre, and glycerokinase (EC 2.7.1.30), 250 μg/ml. For further experimental details see Materials and methods. Mean values from 6 experiments ± SEM.

3. Results and discussion

In fat cell mitochondria only a small portion (less than 10%) of the PDH complex exists in the active form. As illustrated in fig.1, addition of ADP to

Table 1
Effect of atractyloside on PDH_b to PDH_a conversion in fat cell mitochondria

Atractyloside (μmol/litre)	PDH_a (% of total activity)	Glyc-3-P-formation (μmol × U GDH^{-1} × 7 min^{-1})
–	81.8 ± 2.4	1.5 ± 0.12
0.1	52.9 ± 3.4	1.2 ± 0.1
0.25	36.0 ± 2.8	1.0 ± 0.09
0.5	28.0 ± 3.3	0.9 ± 0.06
1.0	12.7 ± 1.3	0.6 ± 0.08
2.0	13.4 ± 1.3	0.5 ± 0.05
10.0	11.2 ± 1.8	0.5 ± 0.09

Fat cell mitochondria were incubated for 7 min at 25°C in 1 ml KCl medium containing 0.05% BSA, 10 mmol/litre glycerol, 250 μg/ml glycerokinase. Carboxyatractyloside was added as indicated. Figures represent mean values ± SEM from 6 experiments.

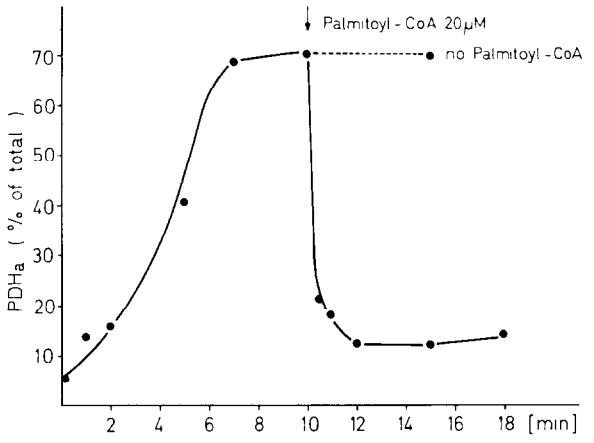

Fig.2. Time course of PDH activation by ATP trapping and its reversal by palmitoyl-CoA. Fat cell mitochondria were incubated for the time indicated in 1 ml KCL-medium containing 0.05% BSA, 10 mmol/litre glycerol and 250 µg/ml glycerokinase. t = 25°C; gas phase air. Each point represents the mean of two determinations.

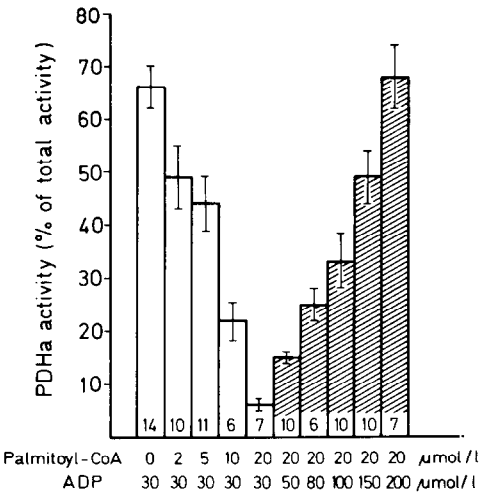

Fig.3. Dose dependency of the palmitoyl-CoA effect on PDH_b to PDH_a conversion and reversal by ADP. Fat cell mitochondria were incubated in KCl medium containing 0.05% BSA, 30 µmol/litre ADP, 10 mmol/litre glycerol, 250 µg/ml glycerokinase for 6 minutes with increasing amounts of palmitoyl-CoA (☐). After 6 min ADP was added to give the final concentrations as indicated, and incubation was continued for 6 min (▨). Values are given as means ± SEM. Figures in parentheses represent the number of experiments. Maximal PDH_a activities at 250 µmol/litre ADP and in the absence of palmitoyl-CoA were 287 ± 8 mU/U GDH (n = 4).

Table 2
Effect of palmitoyl-CoA on PDH_a activity and adenine nucleotide levels of isolated fat cell mitochondria

Exp.	Additions	PDH_a (% of total)	ADP (nmol/U GDH)	ATP	$\frac{ATP}{ADP}$	α-Glycerophosphate (µmol/U GDH)
A	ADP 20 µM (n = 12)	11.9 ± 1.7	4.1 ± 0.4	12.8 ± 1.0	3.2 ± 0.1	
B	ADP 20 µM Glycerol 10 mM Glycerokinase 250 µg/ml (n = 18)	63.9 ± 2.4	6.4 ± 0.6	8.1 ± 0.5	1.3 ± 0.1	1.2 ± 0.06
C	ADP 20 µM Glycerol 10 mM Glycerokinase 250 µg/ml Palmitoyl-CoA 5 µM (n = 6)	42.5 ± 2.8	5.7 ± 0.8	11.2 ± 1.3	2.1 ± 0.3	1.1 ± 0.08
D	ADP 20 µM Glycerol 10 mM Glycerokinase 250 µg/ml Palmitoyl-CoA 20 µM (n = 9)	10.0 ± 1.0	3.4 ± 0.4	10.3 ± 0.9	3.2 ± 0.3	0.3 ± 0.04

Fat cell mitochondria were incubated in 1 ml KCl medium containing 0.05% BSA and the further additions as indicated. t = 25°C, incubation time 7 min, gas phase air. Figures represent mean values ± SEM, number of experiments as indicated. Total PDH activity = 348 ± 67 mU/U GDH ($\bar{X}$ ± SEM, n = 25).

Table 3
PDH interconversion of fat cell mitochondria after prolonged incubation with palmitoyl-CoA

1st incubation	2nd incubation (5 min)							
20 μM palmitoyl-CoA	with ATP-trap				without ATP-trap			
no ATP-trap	Without palmitoyl-CoA		With palmitoyl-CoA		Without palmitoyl-CoA		With palmitoyl-CoA	
(min)	PDH_a % of total	α-GP μmol/U GDH	PDH_a % of total	α-GP μmol/U GDH	PDH_a % of total	α-GP μmol/U GDH	PDH_a % of total	α-GP μmol/U GDH
7.5	53.4 ± 7.2	1.4 ± 0.08	8.1 ± 1.0	0.6 ± 0.1	–	–	–	–
15	46.6 ± 7.2	1.4 ± 0.13	8.3 ± 1.4	0.5 ± 0.05	–	–	–	–
30	49.9 ± 4.4	1.5 ± 0.16	10.1 ± 1.7	0.6 ± 0.1	10.5	0	11.9	0
60	47.7 ± 3.8	1.4 ± 0.11	14.6 ± 3.9	0.4 ± 0.03	13.2	0	14.8	0

In the first incubation fat cell mitochondria were incubated in 1 ml KCl medium (see Methods) containing 0.05% BSA and 20 μmol/litre palmitoyl-CoA for the times indicated. After centrifugation mitochondria were washed 3 times with KCl medium containing 1.5, 2 and 0.5% BSA respectively. Control experiments with [^{14}C]palmitoyl-CoA indicated that at least 90% of palmitoyl-CoA were removed by this procedure. In the second incubation the mitochondria were resuspended in 0.5 ml KCl medium containing 0.05% BSA, 10 mmol/litre glycerol, 250 μg/ml glycerokinase, and 20 μmol/litre ADP either in the presence or absence of 20 μmol/litre palmitoyl-CoA. t = 25°C, gas phase air. Figures represent mean values ± SEM from 6 experiments. In the experiments where the 2nd incubation was carried out without the ATP-trap, glycerol and glycerokinase were omitted. Figures represent mean values from 3 experiments.

endogenously respiring mitochondria leads to conversion of PDH_b to PDH_a. This is potentiated upon addition of glycerol and glycerokinase as an ATP-trapping system, which leads to marked PDH_a formation at ADP concentrations which by themselves are hardly effective. At the same time there is a marked drop of the mitochondrial ATP/ADP ratio (table 2B). In the experiments illustrated in table 1, carboxyatractyloside was used for elevating the phosphorylation state of the mitochondrial AN [9,25,26]. As may be seen there is gradual PDH_a to PDH_b conversion at increasing concentrations of the inhibitor. The inhibition of AN translocation is indicated by the decreased formation of α-glycerophosphate.

Of greater physiological interest seems the observation that palmitoyl-CoA – a potent AN translocase inhibitor very similar to atractyloside [27,28] – also leads to PDH-inactivation due to conversion of PDH_a to PDH_b (fig.2). In these experiments the mitochondria were first incubated in presence of the ATP-trap, and after reaching a high and constant level of PDH_a, palmitoyl-CoA was added. No effect of palmitoyl-CoA could be observed after hydrolysis of the thioester for 10 min at 56°C and pH 12.5.

The dose–response dependency of the inactivation by palmitoyl-CoA is shown in fig.3. As may be furthermore seen here PDH_a to PDH_b conversion which is almost complete at 20 μmol/litre palmitoyl-CoA is fully reversible by adding increasing concentrations of ADP. The effect of palmitoyl-CoA on both the state of the PDH-system and the AN-system is shown in table 2. As may be seen palmitoyl-CoA by inhibiting AN translocation prevents the drop in the ratio of ATP:ADP (Exp. C,D) which is induced by the trapping system (Exp. B) and at the same time PDH_a is kept at a low level. Conversely, the low phosphorylation state of the adenine nucleotides in the absence of palmitoyl-CoA (Exp. B) is reflected by a low phosphorylation state of the PDH-system, i.e. a high level of PDH_a.

In mitochondria preincubated with palmitoyl-CoA up to 60 min, and then washed with BSA, the state of the PDH system and its response to the ATP-trap and to palmitoyl-CoA during a second incubation was much the same as in unpretreated preparations (table 3). This indicates that palmitoyl-CoA, even after prolonged contact with the particles, does not lead to irreversible damage, and thus cannot be regarded as an unspecific detergent only. Rather, in support of our view, it would appear from the present data that, in adipose tissue, long chain acyl-CoA, by reversible inhibition of the AN translocator, might be of physiological significance in the control of the PDH system by insulin.

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