CONTROL OF PYRUVATE DEHYDROGENASE INTERCONVERSION IN ADIPOSE TISSUE BY INSULIN

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1. Introduction

As has been shown for heart muscle, kidney, liver and brain, pyruvate dehydrogenase (PDH) is an interconvertible enzyme, which exists in an active and inactive form, PDH_a and PDH_b respectively [1, 2, 3, 4]. Furthermore it has been demonstrated, that the dynamic equilibrium between PDH_a and PDH_b is under metabolic and hormonal control, which enables pyruvate in these tissues to be converted to a greater or lesser extent to acetyl-CoA [5, 6, 7]. Considering the essential role of insulin in stimulating fatty acid synthesis from carbohydrate in adipose tissue, it was of interest to study the influence of insulin on the PDH-system in this tissue.

2. Experimental

Male Wistar rats (150–180 g) fed ad libitum with a standardized chow diet (Labortierfutter 57Z, J. Zahn II, Hockenheim, Germany) were used. Incubation of pooled epididymal fat pads was performed in Krebs-Ringer-bicarbonate-buffer pH 7.4 containing 2.5% bovine serum albumin (Behring Werke, Marburg, Germany) dialyzed for 16 hr against Krebs-Ringer-bicarbonate-buffer. The initial glucose concentration in the incubation medium was 5.5 mM. At the end of the incubation period fat pads were blotted and rapidly frozen in liquid nitrogen. Preparation of adipose tissue extracts was performed by homogenisation of 1 g of the frozen tissue with 1 ml 10 mM phosphate buffer, pH 7.0 containing 0.25 M sucrose and 2.5% bovine serum albumin together with 6 ml silicone-oil (density 0.98 lot No. AK 20, Wacker, Burghausen, Germany). The fat pads were homogenized at 0 °C with a high speed tissue desintegrator (Ultra-Turrax TP 10, Firma Jahnke und Kunkel, Stauffen, Germany) 4 times 30 sec each with 15 sec intervals to avoid warming of the homogenate.

The homogenates were centrifuged 10 min at 39,000 *g* at 0 °C. PDH activity was measured spectrophotometrically at 405 nm in the clear infranatant aqueous phase using the arylamine acetyltransferase (EC 2.3.1.5) system as indicator reaction [6]. The substrates and coenzymes were products of Boehringer Mannheim GmbH (Germany). *p*-nitroaniline was from E. Merck AG, Darmstadt (Germany).

3. Results and discussion

By the silicone-oil procedure PDH activity is completely extracted from the mitochondria into the aqueous phase. No enzyme activity was detectable in the precipitate. Homogenisation in the presence of silicone-oil results in the removal from the infranatant of endogenous lipid which floats entirely to the top of the tube during centrifugation (fig. 1a). This was demonstrated by addition of Sudan-red to the extraction mixture. Phase (b) represents the silicone-oil separated from the clear fat-free aqueous phase (d) by a small interphase (c) of unknown constitution. This interphase showed no staining with Sudan-red.

Fig. 2 illustrates that PDH-activity decreases in a time dependent reaction upon incubation of adipose tissue extract with ATP (A), while in the presence of high concentrations of magnesium a rapid activation occurs (B). Thus adipose tissue PDH behaves in a manner identical to the enzyme from heart muscle,

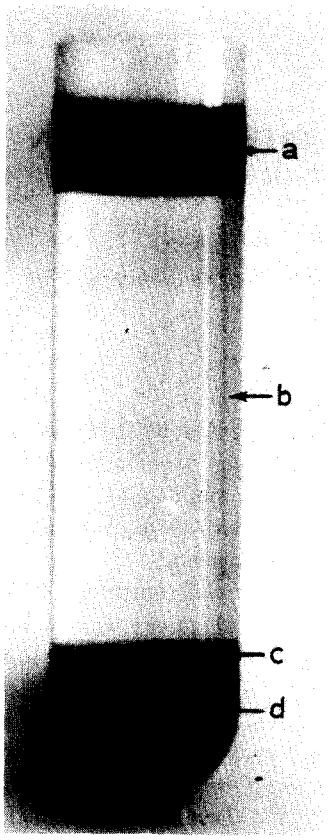

Fig. 1. Silicone oil technique for preparation of adipose tissue extracts. For experimental details see section 2. For explanation see text. The small insoluble sediment is not visible on the photograph.

Table 1
Effect of insulin *in vivo* on PDH_a-activity in rat adipose tissue.

Experimental conditions	*n*	PDH_a activity % ± S.E.M.
Controls	4	56.7 ± 11.1
i.p. injection of 250 mU insulin/kg 15 min before sacrifice	3	102.3 ± 7.2

PDH_a activity is given in percent of the total activity, the latter being obtained after 15 min preincubation of the homogenate at 25 °C in the presence of 10 mM Mg^{++}.

kidney and liver. Though incorporation of phosphate into adipose tissue PDH has not been demonstrated, the data shown in fig. 2 suggest that this enzyme is also regulated by phosphorylation–dephosphorylation reactions.

PDH_a-activity of adipose tissue from normal fed rats was in the range between 50–90 mμmoles acetyl-CoA formed per g wet weight per min at 25 °C. This corresponds to about 50% of the maximal activity obtained after incubation with magnesium. Intraperitonial injection of 250 mU insulin per kg rat 15 min before sacrifice results in a complete conversion of PDH_b to PDH_a (table 1). This mechanism would allow maximal rates of pyruvate oxidation and acetyl-CoA formation in adipose tissue. It therefore offers a reasonable explanation for the stimulation of pyruvate incorporation into fatty acids by insulin which has been demonstrated by several groups [8, 9, 10].

The stimulatory effect of insulin on the transition of PDH_b to PDH_a is demonstrated even more clearly after incubation of adipose tissue, *in vitro*. As may be seen from table 2 incubation of epididymal fat pads with insulin, 1 mU/ml, for 30 min leads to an increase of PDH_a from 31.6 ± 5.2 to 74.1 ± 14.3 mU per g wet weight. In preliminary studies 10^{-6} M ACTH or 10^{-3} M theophylline did not alter the level of PDH_a.

Table 2
Effect of insulin, *in vitro* on PDH activities in rat adipose tissue.

Experimental conditions	*n*	PDH_a	PDH total
Control	5	31.6 ± 5.2[a]	107.8 ± 14.6
Insulin 1 mU/ml	5	74.1 ± 14.3[a]	98.7 ± 16.9

Values are given in mU ± S.E.M. per g wet weight. 1 mU corresponds to the formation of 1 mμM acetyl-CoA per min at 25 °C. For composition of incubation medium see section 2. Incubation was carried out for 30 min at 37 °C in plastic vials under O_2/CO_2 (95 v %/5 v %) atmosphere in a shaker.

[a] At $p = 0.05$ the statistically significant difference is 7.4 mU considering the experimentally obtained mean-difference of 42.5 mU.

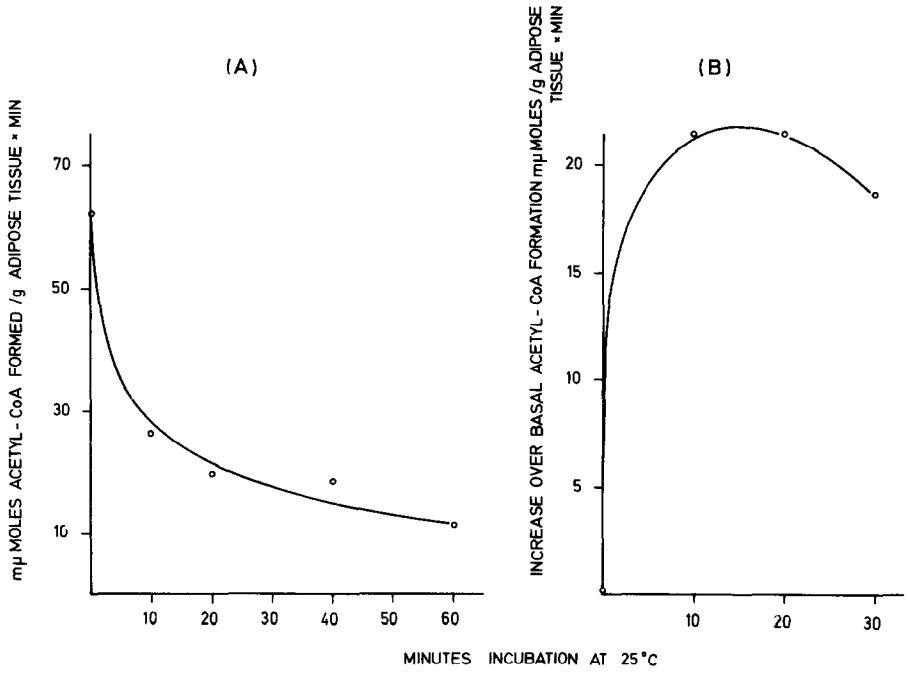

Fig. 2. Pyruvate dehydrogenase activities in extracts of rat adipose tissue in the presence of ATP or magnesium. (A) Adipose tissue extracts (see section 2) were incubated at 25 °C with an ATP regenerating system consisting of creatine phosphate 30 mM, ATP: creatine phosphotransferase (EC 2.7.3.2) 5 I.U., and ADP 1 mM, which maintained a level of 0.1 mM ATP. (B) Adipose tissue extracts were incubated at 25 °C in the presence of $MgCl_2$ 10 mM. At the times indicated samples were taken for the enzyme assay.

R.L. Jungas [8] recently reported data on the PDH activity in adipose tissue. In his studies PDH activity was measured by the formation of $^{14}CO_2$ from 1-^{14}C-pyruvate. On incubation with insulin he also observed an increase in PDH activity. The much smaller response to insulin and the lower basal values as compared to the data presented here may be due to the different experimental techniques applied.

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