INTERCONVERSION OF INACTIVE TO ACTIVE PYRUVATE DEHYDROGENASE IN RAT LIVER AFTER FRUCTOSE APPLICATION IN VIVO

H.D.SÖLING and G.BERNHARD
with technical assistance of G.JANSON
Abt. f. Klin. Biochemie, Medizinische Universitätsklinik Göttingen, Germany

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1. Introduction

In a previous paper [1] we described the ketogenic effect of high concentrations of fructose in isolated perfused rat livers. The enhanced formation of ketone bodies resulted from an increased formation of acetyl-*S*-CoA from pyruvate. We concluded that the drop in the intracellular concentration of ATP which followed the application of fructose had led to a shift from the phosphorylated inactive form [2, see also 3] of pyruvate dehydrogenase (EC 1.2.4.1) to the dephosphorylated active form, and that this shift had resulted in an enhanced rate of pyruvate oxidation.

The present studies were undertaken in order to prove this hypothesis. According to the results obtained the intravenous injection of fructose leads to a significant increase in active pyruvate dehydrogenase within a few minutes without affecting total pyruvate dehydrogenase activity.

2. Experimental

Chow fed male Wistar rats of an inbred strain of the Zentralinstitut für Versuchstierzucht, Hannover–Linden/Germany (weight 200–240 g) were used. The animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and received then fructose (1.8 mmole/kg, i.v.) (femoral vein) within 3 min. Control animals received the same volume (5 ml/kg) of saline. Eight min after the beginning of the intravenous injection liver tissue was obtained by freeze-clamping [4] and pulverized carefully in a mortar under liquid nitrogen. The powder was transferred into a polypropylene centrifuge tube precooled in liquid nitrogen. After weighing the powder was brought to –45° by immersion of the centrifuge tube into precooled methanol. To 1 g of tissue powder 3 parts (w/v) of –23° C cold glycerol buffer mixture (glycerol 8.22 M, triethanolamine 40 mM, dithioerythritol 2 mM, EDTA 1 mM) were added. The mixture was homogenized 3 times for 15 sec with an Ultraturrax homogenizer. During the homogenization the tube was immersed into –45° cold methanol.

Immediately after homogenization aliquots were taken for measurement of pyruvate dehydrogenase activity. Other aliquots were brought to 15 mM Mg^{2+} by adding $MgCl_2$, incubated for various time periods at 37° and then used for the determination of pyruvate dehydrogenase activity.

2.1. *Determination of pyruvate dehydrogenase activity*

Pyruvate dehydrogenase activity was determined by coupling the reaction with the arylamine acetyltransferase (EC 2.3.1.5) reaction as described similarly by Schweet and Cheslock [5].

The assay mixture contained (final concentration given): potassium phosphate 50 mM pH 4.0, $MgCl_2$ 0.5 mM, NAD^+ 1.5 mM, nicotinamide 15 mM, CoA-SH 0.125 mM, thiaminepyrophosphate 0.27 mM, pyruvate 10 mM, GSH 2.5 mM, *p*-nitroaniline 0.15 mM, bovine serum albumin 1 mg/ml, and arylamine acetyltransferase 75 μg protein/ml.

The reaction was started by addition of 25 or 50 μl of homogenate and run at 30° for 30 min. The reaction mixture was rapidly cooled to +2° and im-

mediately spun at 20,000 *g* for 10 min. The supernatant was decanted into a cuvette and the decrease in absorption at 380 nm was measured against a blank reaction mixture which had been incubated in the absence of CoA-SH and thiaminepyrophosphate. All assays were run in triplicates.

2.2. *Preparation of arylamine acetyltransferase*

This enzyme was prepared from pigeon liver according to Tabor et al. [6] as described by Wieland [7] with the only variation that 20 mM phosphate instead of 10 mM phosphate was used for the elution of the enzyme from the $Al(OH)_3$ gel (C_γ). Arylamine acetyltransferase activity was measured in the combined assay with acetylphosphate and phosphotransacetylase as described by Tabor et al. [6]. The specific activity of the preparation used was 0.24 μmole *p*-nitroaniline acetylated/min/mg protein at 25° corresponding to a specific activity of 795 in terms of Tabor's [8] definition.

2.3. *Determination of protein and metabolites*

For determination of the protein concentration aliquots of the homogenates were first treated with desoxycholate (6% final concentration) and then measured by the biuret method. ATP [9], ADP [10] and AMP [10] were determined enzymic optically.

3. Results and discussion

When pyruvate dehydrogenase activity was tested immediately after homogenization the activity was rather low (table 1). During the incubation in the presence of 15 mM Mg^{2+} the activity increased gradually and reached a plateau after 90 to 120 min. When ATP (5 mM) was added after 60 min and the incubation was continued for another 60 min, pyruvate dehydrogenase activity declined instead of increasing (fig. 1).

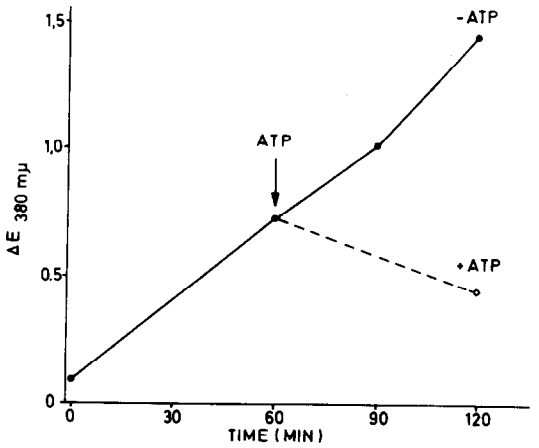

Fig. 1. Inhibition by ATP of the increase in pyruvate dehydrogenase activity during incubation at 37° in the presence of 15 mM Mg^{2+}. Liver tissue was obtained by freeze clamping and homogenized (for experimental details see text). Aliquots were used for determinations of pyruvate dehydrogenase activity before and after various periods of incubation in the presense of Mg^{2+}. After 60 min of incubation ATP (5 mM final concentration) was added to an aliquot of the incubated homogenate and the incubation with and without ATP was continued for another 60 min.

When the homogenate was incubated in the absence of exogenous Mg^{2+} there was likewise an increase in pyruvate dehydrogenase activity, but this increase was only about 1/3 of that seen in presence of exogenous Mg^{2+}.

Table 1

Activity of pyruvate dehydrogenase in livers from saline or fructose treated rats before and after incubation in the presence of Mg^{2+} (15 mM) for 120 min. The initial activity is also expressed as percentage of total activity of pyruvate dehydrogenase. The activities are expressed as μmoles *p*-nitroaniline acetylated/min/g wet liver. The values represent the means ± S.E.M. Each single experimental value was calculated as the mean value of triplicate determinations of enzyme activity.

Treatment	Activity before incubation (μmole/min/g liver)	Activity after incubation (μmole/min/g liver)	Initial activity as percentage of total activity (%)	Number of experiments (*n*)
Saline	0.0662±0.0071	1.070±0.053	6.20±0.67	11
Fructose	0.1340±0.0122*	1.020±0.099	13.64±1.38*	12

* Values differ significantly from the corresponding control value ($p < 0.001$).

Table 2
Concentrations of ATP, AMP and ATP + ADP + AMP, and ATP/ADP ratios in livers from saline or fructose treated rats (for experimental details see text). The values were obtained 7 min after the beginning of an intravenous injection of saline or 1.8 mmole/kg of fructose. The values represent the means ± S.E.M.

Treatment	ATP	ADP	AMP	ATP + ADP + AMP	ATP/ADP (ratio)	Number of experiments (*n*)
	(μmoles/g wet liver)					
Saline	2.568 ±0.032	0.770±0.120	0.321±0.080	3.677 ±0.216	3.543 ±0.611	5
Fructose	1.050*±0.080	0.564±0.081	0.400±0.062	2.034*±0.181	1.914**±0.261	5

*, ** Values differ significantly from the corresponding control values ($p < 0.001$ and $p < 0.025$, resp.).

The activity of pyruvate dehydrogenase immediately after homogenization was significantly higher in livers from fructose injected rats compared with control rats (table 1). When the activity of pyruvate dehydrogenase immediately after homogenization was expressed as percentage of total pyruvate dehydrogenase activity, again the percentage of active pyruvate dehydrogenase was significantly higher in livers from fructose treated rats (table 1). There was no significant difference in total pyruvate dehydrogenase activity between fructose treated and saline treated rats (table 1). The increase in active pyruvate dehydrogenase in livers from fructose treated rats was accompanied by a dramatic decrease in the concentration of ATP and of ATP + ADP + AMP and of the ATP/ADP ratio (table 2) which is in accordance with the findings of Mäenpää et al. [11, 12] and our previous results obtained in isolated perfused rat livers [1].

The low percentage of active pyruvate dehydrogenase in normal rat livers corresponds well with similar results reported recently by Wieland [13]. Though Wieland [13] could not detect a change in the percentage of active pyruvate dehydrogenase during starvation or diabetes, the present result demonstrates that an interconversion from inactive to active pyruvate dehydrogenase can take place also in liver under in vivo conditions. The rapid increase in the percentage of active pyruvate dehydrogenase after fructose explains well the rapid enhancement of pyruvate oxidation in the isolated perfused rat liver after the addition of fructose to the medium [1].

It remains to be clarified by further experiments whether smaller metabolic perturbations than those following the application of fructose will also affect metabolic flux rates in liver by an effect on the interconversion reaction of pyruvate dehydrogenase.

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