INTERCONVERSION OF PYRUVATE DEHYDROGENASE IN RAT HEART MUSCLE UPON PERFUSION WITH FATTY ACIDS OR KETONE BODIES

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1. Introduction

Pyruvate dehydrogenase (PDH) is an interconvertible enzyme which exists in active or inactive forms, PDH_a and PDH_b, respectively [1–3]. In heart muscle and in kidney from normal fed rats, PDH_a amounts to about 2/3 of total PDH, whereas in starvation or in acute diabetes it is about 1/6 [4]. Under the latter conditions, the plasma levels of free fatty acids (FFA) and of ketone bodies are increased, and it was suggested that these metabolites favor the transition $PDH_a \rightarrow PDH_b$ [4]. In the present investigation, the role of FFA and of ketone bodies in the control of the PDH system was studied isolated perfused rat hearts.

2. Experimental

Male Sprague Dawley rats (150 to 200 g; Fa. Wiga, Munich, Ottobrunn) fed ad libitum with a standardized chow diet (Labortierfutter 57 Z, J. Zahn II, Hockenheim, Germany) were used. Hearts were prepared in nembutal anaesthesia (5 mg/100 g body weight i.p.) according to the Langendorff procedure [5] modified by Morgan et al. [6]. After pre-perfusion (open system) with 8 ml of substrate-free Krebs-Henseleit bicarbonate buffer [7] saturated with 95% O_2–5% CO_2, the hearts were perfused with 15 ml of recirculating Krebs-Henseleit buffer, pH 7.4, containing 2% bovine albumin (Cohn Fr. V., Serva Entwicklungslabor, Heidelberg) and 5 mM glucose. Palmitate (Fa. Schuchardt, Munich) was added as an albumin complex prepared according to Bode and Klingenberg [8]. The hearts were perfused for 20 min at 37° with a constant pressure of 60 mm Hg maintained by an excenter pump (Fa. Deuster, Munich). Flow rates (12–15 ml/min) and frequency of heart contractions (240–280/min) remained constant during the perfusion period. Glucose, lactate, pyruvate, acetoacetate and β-hydroxybutyrate were determined in $HClO_4$-deproteinized and neutralized perfusate samples by standard enzymatic procedures [9]. Acetate assays were carried out according to Holz and Bergmeyer [10], FFA determinations by a modified [11] Duncombe procedure [12]. L-β-Hydroxybutyrate was measured by titration with NaOH. Protein was determined by the Biuret Method using Labtrol serum (DADE Div. Miami USA) as a standard. After freeze clamping the beating hearts according to Wollenberger et al. [13] tissue homogenates were prepared by pulverizing the frozen hearts and homogenisation with a high speed tissue desintegrator for small samples (Ultra Turrax TP 10 N, Fa. Jahnke and Kunkel, Staufen, Germany). PDH activities were measured by the dismutation assay as described previously [4]. Enzymes, coenzymes and D,L-β-hydroxybutyrate were products of Boehringer Mannheim GmbH. Lithium-acetoacetate was prepared according to Hall [14]. Quinine salt of L-β-hydroxybutyrate kindly donated by Prof. Lynen, Munich, was converted to the sodium salt [15]. All other reagents were from E. Merck, Darmstadt. The significance of differences between means was calculated by the *t* test method.

3. Results and discussion

Addition of palmitate, acetoacetate, β-hydroxybutyrate, or acetate decreased glucose uptake of the

Table 1

Group	Additions to 5 mM glucose	N	Metabolite changes of perfusate (Δ μmole per g w.w./20 min)							
			Glucose	Palmitate	Acetoacetate	D,L-β-Hydroxy-butyrate	Acetate	Lactate	Pyruvate	L/P
A	none	6	−28.0 ±0.9	–	–	–	–	+4.27 ±0.64	+1.24 ±0.13	3.5 +0.2
B	1 mM palmitate	5	−18.7 ±2.9	−6.93 ±0.77	–	–	–	+14.3 ±2.37	+0.77 ±0.18	19.7 ±1.6
C	4 mM acetoacetate	6	−17.8 +2.8	–	−22.1 +1.3	+7.50 ±2.4	–	+6.77 ±0.75	±0.30	27.2 ±6.6
D	10 mM D,L-β-hydroxybutyrate**	6	−17.8 ±0.8	–	+5.43 ±0.5	−23.8 ±2.6	–	+11.4 ±2.70	+1.20 ±0.33	11.3 ±2.1
E	8 mM acetate	6	−15.3 ±1.2	–	–	–	−52.3 +3.0	+5.98 ±0.89	+0.54 ±0.06	11.5 ±1.6
F	8 mM acetate + 4 mM acetoacetate	5	−15.8 ±2.6	–	−21.1 ±2.6	+6.67 ±1.0	−39.5 ±4.4	+9.04 ±3.19	+0.74 ±0.25	13.4 ±2.0
G	4 mM L-β-hydroxy-butyrate	6	−26.3 ±4.4	–	–	–	–	+7.77 ±1.34	+1.36 ±0.14	6.0 ±0.8

Effect of fatty acids and ketone bodies on glucose metabolism of isolated perfused heart from fed rats. The reduction of glucose uptake in groups B−F vs. A is statistically significant at $0.001 < p < 0.01$. Group G vs. A = n.s.

* Corresponding to 4 mM D-β-hydroxybutyrate by enzymatic determination.

hearts (table 1). Lactate production and lactate/pyruvate concentration ratios were increased which agrees with earlier observations [for review, see 16]. So far, diminished rates of glucose uptake have been reported only under conditions where insulin was available either endogenously bound to the heart muscle or by addition to the medium. After a perfusion period of about half an hour most of endogenous insulin bound to rat heart muscle is removed [17, 18]. Since in our studies the pre-perfusion period was only 2−3 min, it is assumed that sufficient amounts of endogenous insulin were still present to allow the fatty acid induced inhibition of glucose uptake. The rates of acetoacetate and β-hydroxybutyrate utilization were similar to those reported by Williamson and Krebs [19]. Acetate was also rapidly used. However, acetate uptake was diminished when added together with acetoacetate indicating preferential utilization of acetoacetate as substrate. Similarly, acetate has

has been shown to be ineffective in competing for the oxidation of palmitate in rat hearts [20].

PDH_a activity in perfused hearts accounts for about 60% of total PDH activity (fig. 1) corresponding well with the values found in heart muscle rapidly removed from normally fed rats by freeze-clamping [4]. Furthermore, 20 min of perfusion with 5 mM glucose did not change either total activity or the active portion of the enzyme. When palmitate, acetoacetate, D,L-β-hydroxybutyrate or acetate was added to the perfusate PDH_a activity decreased to about half of the control value ($p < 0.001$). The total PDH activity was unchanged exept in the experiments with acetate, where both total PDH and PDH_a were decreased. However, the decrase in PDH_a relative to total activity was also highly significant in the presence of acetate. As also shown in fig. 1 perfusion with the unphysiological L-form of β-hydroxybutyrate did not result in a lowering of PDH_a. Perfusions with 4 mM
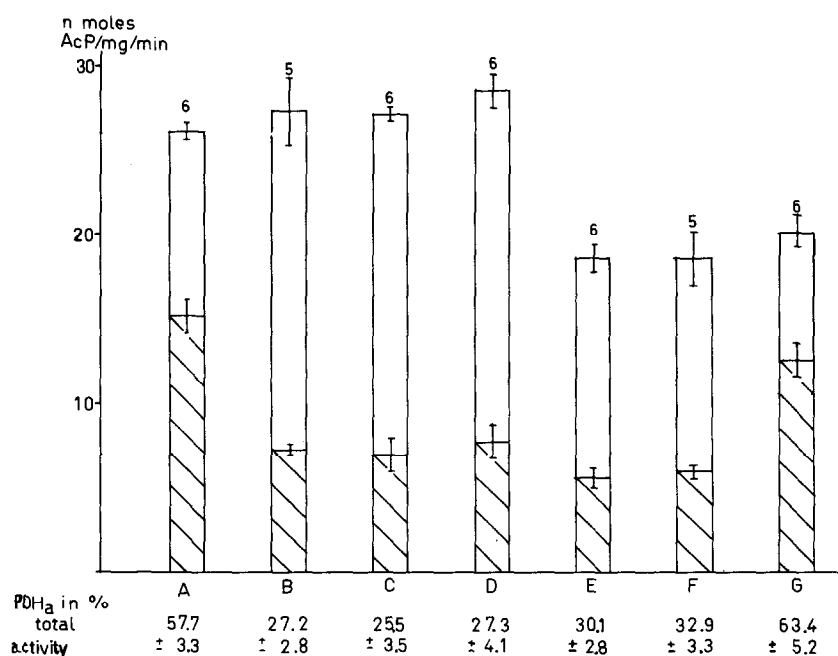
Fig. 1. Effect of FFA and ketone bodies on PDH activities in isolated perfused heart from fed rats. Total height of bars represents total PDH activity (after activation) and the height of hatched areas the PDH_a activity. Mean values $\pm$ S.E.M. are given. Results are expressed as nmoles acetylphosphate formed mg protein/min. Groups A–G are the same as in table 1.

lithium chloride instead of lithium acetoacetate showed no differences as compared to controls.

The present experiments with isolated perfused rat hearts support our hypothesis that an increased supply of long chain FFA is responsible for the conversion of active to inactive PDH in heart muscle [4] . Moreover, ketone bodies and acetate have been shown to exert the same effect. The mechanism for this interconversion is not yet clear, but it is obviously related to metabolic alterations induced by the oxidation of fatty acids and ketone bodies, substrates which are readily utilized by heart muscle. So far, the diminished pyruvate oxidation observed upon perfusion with fatty acids or ketone bodies has been ascribed to feedback inhibition of PDH by acetyl-CoA and/or NADH [21–23] . However, interconversion from active to inactive PDH may also be considered as a mechanism for the control of pyruvate oxidation.

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