EFFECT OF HYPOXIA, ISCHEMIA AND BARBITURATE ANESTHESIA ON INTERCONVERSION OF PYRUVATE DEHYDROGENASE IN GUINEA PIG BRAIN

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Received 12 January 1976

1. Introduction

Experiments of Reed [1] and Wieland [2] revealed that pyruvate dehydrogenase (PDH) activity may be regulated by reversible interconversion from the active (dephosphorylated) to the inactive (phosphorylated) form of the enzyme. In the experiments performed in vivo it was found that PDH in various tissues including the brain occurs in the active as well as in the inactive state [2,3]. The ratio of the two forms depends on nutritional and hormonal conditions in kidney, heart and liver [3,4] but not in the brain [5].

From the studies of Wieland [6], Martin [7] and Wałajtys [8] it results that PDH activity in isolated rat liver mitochondria depends on their energy state. Under conditions of controlled respiration the percentage of PDH in the active form varied directly with ADP content and inversely with ATP/ADP ratio. This mechanism has been shown recently to operate also in isolated brain mitochondria [9].

In the present study we investigate the possibility that PDH activity may be regulated in vivo by interconversion of the active to inactive form depending on the energy state of the brain. Experimental conditions have been sought which either increase (barbiturate anesthesia) or decrease (hypoxia, postdecapitative ischemia) the energy state of the brain.

2. Materials and methods

Experiments were carried on 200–250 g guinea pigs fed a standard diet. Five experimental models were used:

(1) Pentobarbital anesthesia was applied by introducing Nembutal i.p. in doses of 35 mg/kg body weight. Anesthetized animals were decapitated after 30 min of sleep.

(2) The animals were subjected to 3 min hypoxia in a chamber with continuous flow of 2–3% O_2 in N_2.

(3) In the experiments on postdecapitative ischemia heads of animals were kept in a thermostat for 3 min at 37°C.

(4) and (5) Hypoxia or ischemia as described in points (2) and (3) was preceded by 30 min of pentobarbital anesthesia.

Immediately after decapitation heads of the animals were immersed in liquid nitrogen to prevent post-mortem changes in the active/inactive PDH forms. Frozen brain tissue (forebrain) was homogenized with 5 vol. of a medium containing: 20 mM Tris–HCl pH 7, 1 mM dithiothreitol and 50% glycerol v/v at about −10°C [4].

Active PDH form was assayed after sonication of the homogenate for 3 × 10 sec at −10°C, 20 kHz in MSE Ultrasonic Disintegrator (150 W), micro-probe, amplitude 14 μm. Total PDH activity was determined after enzymatic activation consisting in 5 min incubation of the homogenate with PDH-phosphatase in the presence of 10 mM $MgCl_2$ at 25°C (fig.1). PDH-phosphatase was purified from pig heart muscle up to step 6 of the procedure described by Siess and Wieland [10].

PDH activity was determined radiochemically after

Land and Clark [11]. The assay mixture contained: 100 mM Tris–HCl, pH 8, 0.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 6.7 mM NAD, 2.7 mM thiamine pyrophosphate, 0.5 mM CoA, 1 mM L-malate, LDH 50 U/ml, 8.3 mM pyruvate ([1-^{14}C]pyruvate, 0.1 μCi/ml) and about 1 mg of protein in a total vol of 1.5 ml. The addition of L-malate was shown by Patel and Tilghman [12] to inhibit carboxylation of pyruvate by pyruvate carboxylase. The reaction was run in the main chamber of the Warburg's flask with a center well containing Hyamine with continuous shaking at 25°C. The reaction was started by adding the homogenate and stopped after 30 min by adding 0.5 ml of 4 M HCl to the main chamber and the contents of the flask were shaken for a further 60 min to facilitate complete absorption of the released $^{14}CO_2$ by Hyamine. Hyamine was transferred to the counting vials with 7 ml of Bray's scintillator and counted for radioactivity in Isocap 300 (Nuclear Chicago liquid scintillation counter). All assays were run in duplicate in parallel with control determinations where homogenate or CoA were omitted. Protein was estimated by the method of Lowry et al. [13]. Statistical significance was evaluated by Student's *t*-test.

3. Results

Table 1 illustrates the contribution of the active form of PDH to the total enzyme activity in the forebrains of guinea pigs subjected to various experimental conditions. The total PDH activity remained unchanged in all the conditions examined. In the control conditions the active form of PDH made 62% of the total enzyme activity. The active form of the enzyme was observed to increase by about 40% in hypoxia and ischemia, showing a 40% drop in anesthesia. This means that in hypoxia or ischemia the active form of PDH makes as much as 90% and in anesthesia only 38% of the total enzyme activity.

The conclusion that in anesthesia the equilibrium

Table 1
Active form and total activity of pyruvate dehydrogenase in guinea pig brain. Effect of hypoxia, ischemia and barbiturate anesthesia

Conditions	Pyruvate dehydrogenase activity, nmoles/mg of protein/h				
	Active form	% of control	Total	% of control	Fraction of total activity
Control	248 ± 35 (4)	100	398 ± 22 (4)	100	0.62
Hypoxia	339 ± 18 (4)	137	400 ± 30 (4)	101	0.85
Ischemia	359 ± 36 (4)	145	384 ± 20 (4)	96	0.93
Anesthesia	145 ± 29 (3)	58	379 ± 17 (4)	95	0.38
Anesthesia + Hypoxia	233 ± 50 (4)	94	358 ± 9 (3)	90	0.65
Anesthesia + Ischemia	368 ± 86 (3)	148	363 ± 36 (3)	91	1.01

Values represent means ± SD for number of experiments given in brackets.

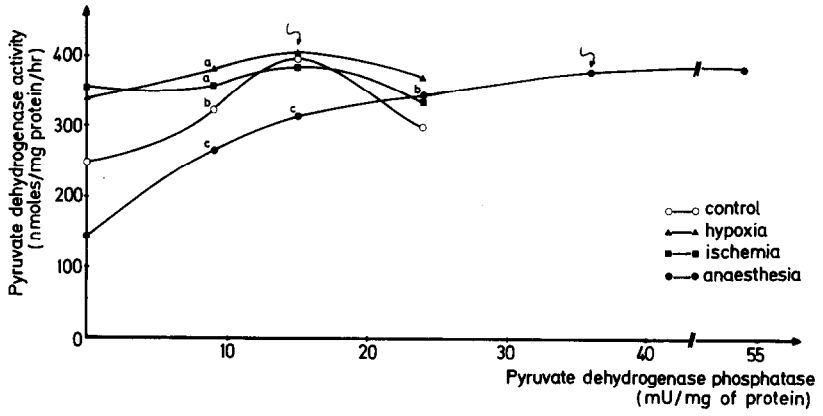

Fig.1. Effect of hypoxia, ischemia and barbiturate anesthesia on the activation of pyruvate dehydrogenase in guinea-pig brain homogenate. The homogenate corresponding to 10 mg of protein was incubated for 5 min at 25°C with 9–54 mU PDH phosphatase/mg of protein in the presence of 10 mM $MgCl_2$. PDH phosphatase activity was 3 IU/ml as assayed according to [10]. Each point represents the mean for 3-4 experiments. ^{a} = NS, ^{b} = $P < 0.05$, ^{c} = $P < 0.01$ with respect to maximum values in each group indicated by arrows.

between the active and inactive form of PDH is shifted towards the latter phosphorylated one finds support in the results of experiments on the effect of the PDH phosphatase on the PDH activation (fig.1). PDH obtained from the brains of anesthetized animals requires 2.5 times more PDH phosphatase for its full activation as compared to the enzyme derived from the control animals. In contrast, PDH from hypoxic or ischemic animals achieved near maximum activation with less PDH-phosphatase than PDH from control animals.

When ischemia or hypoxia were applied to previously anesthesized animals, these two models of oxygen deficiency appeared to affect differently the proportion of the active and inactive form of the enzyme. In animals in which anesthesia was followed by hypoxia, the active form of PDH remained on the control level, suggesting that the effects of anesthesia and hypoxia were additive. No such additive effect was observed when anesthesia was followed by ischemia. In these conditions all the PDH is in the active form.

4. Discussion

The existence of the active and inactive form of PDH in rat brain was demonstrated by Wieland et al. [2,5]. They found that in brains from adult, normal fed rats the active form made up some 70% of the total PDH activity. In our experiments the percentage of PDH in the active form amounted to 62% in guinea pig brain. The present results indicate that the amount of the active form of PDH in the brain strongly depends upon its functional state.

A number of studies have shown that barbiturate anesthesia and hypoxia essentially differ in their effects on both the functional and energy state of the brain. Anesthesia and in particular barbiturate anesthesia is considered as a high energy state of the brain, characterized by inhibition of the energy-consuming processes [14]. This finds manifestation in slowing down of cellular syntheses, rise in the high energy compounds' level (e.g. phosphocreatine), increase of the ATP/ADP ratio and decrease of the NADH/NAD or lactate/pyruvate ratios [15,16]. In contrast to anesthesia, hypoxia and ischemia are accompanied by a decreased supply of the high energy compounds and well-known disturbances of the above mentioned metabolic indices [15–19].

Studies on isolated liver mitochondria [6,7,20] have demonstrated that operation of the PDH activity control mechanism consisting in the active–inactive form interconversion, mainly depends upon the level of ADP which protects PDH against the phosphorylating and inactivating action of the PDH kinase, but

Table 2
Comparison of various data concerning the level of several substrates in the brain under various conditions (% of control value)

Substrates	Hypoxia			postdecap. Ischemia			Anesthesia		Anesthesia + ischemia
	100% N_2 1.5 min	5% O_2 3 min	5% O_2 15 min	3 min			Pheno-barbital	Pento-barbital	3 min
ATP		55	96	18	11		106		53
ADP		244	116	190	136		79		155
ATP/ADP		23	83	10	8		135		34
Pyruvate			460	167			30		695
AcCoA	124					66		98	
Animal	rat	rat	mouse	10-days-old mouse	adult mouse	rat	mouse	rat	10-days-old mouse
References	[17]	[18]	[19]	[16]	[16]	[22]	[15]	[17]	[16]

also upon the pyruvate level, which plays a protective role too.

To analyze the possible correlation between the changes in the level of the active form of PDH and the known changes in the concentration of metabolites affecting interconversion, literature data concerning the ADP, pyruvate and AcCoA levels and the ATP/ADP ratio in the brains of animals subjected to similar experimental conditions were gathered and are presented in table 2. This presentation is limited to the data obtained on rats and mice, since those for guinea pigs are incomplete in the available literature.

A strong correlation between those parameters may be concluded. The proportion of the active form of PDH was found to increase in the conditions known to lead to elevation of the ADP and pyruvate levels and decrease of the ATP/ADP ratio in the brain, the conditions including hypoxia, ischemia and ischemia after anesthesia. The lack of data on these compounds' level in hypoxia after anesthesia excludes the possibility to discuss this model.

A similar correlation, though in the opposite direction, characterizes the effect of barbiturate anesthesia.

The above analysis of the present results and of the literature data allows to suggest the existence in brain of the interconversion mechanism of PDH activity regulation, with ADP and pyruvate serving as factors protecting the enzyme against inactivating action of PDH kinase; the same mechanism has been demonstrated in purified enzyme [5,21] and in isolated liver [6,7,20] and recently brain [9] mitochondria.

The question arises as to the physiological significance of the observed changes in the active form of PDH and as to the relation of the amount of this form to the actual enzyme activity in vivo.

There is an evident lack of correlation between the AcCoA level (table 2 after Schuberth [17,22], and PDH activity in a number of energy states of the brain (table 1). This is not a surprising finding, since the AcCoA level depends upon a number of enzyme activities and this is not an appropriate index of the rate of PDH-mediated pyruvate metabolism.

In addition, regulation of PDH activity underlies some other mechanism, such as inhibition by the products: AcCoA [5] or NADH [23], of which the level is related to the functional state of mitochondria and the rate of aerobic and anaerobic glycolysis. More detailed, dynamic studies, taking into account factors like concentration of Ca^{2+} and Mg^{2+} ions known to activate PDH phosphatase [1] and the cellular- and mitochondrial membrane permeability for pyruvate [24], are needed for further interpretations.

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