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EFFECT OF ACTH ON DEHYDROGENASE ACTIVITY OF CELLS AND TISSUES

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KEY WORDS: ACTH; dehydrogenases; muscles; microbial cells.

One manifestation of the action of hormones of the pituitary-adrenocortical system in man or animals is modification of energy metabolism. Effects of glucocorticoids on intermediate metabolism of carbohydrates and lipids and on the level of high-energy compounds linked with it [1, 5], on activity of enzymes of the glycolytic [6] and respiratory chains [4, 5, 7] and on concentrations of metabolites and activity of enzymes of the Krebs' cycle [2, 3] are known. However, there is no information on the effect of ACTH on respiratory function of the tricarboxylic acid cycle.

In the investigation described below the effect of ACTH on dehydrogenation in the Krebs' cycle was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-200 g. Dehydrogenase activity was determined colorimetrically, by a method based on reduction of triphenyltetrazolium chloride to formazan.

After decapitation of the rats strips of muscle weighing 100 mg were cut from the diaphragm and placed in Krebs–Ringer medium with phosphate buffer, without glucose, pH 7.4, in a volume of 3 ml, containing 0.2 ml of a 0.5% solution of 2,3,5-triphenyltetrazolium chloride and 0.01 M of the corresponding dehydrogenation substrate. ACTH (Kaunas Endocrine Preparations Factory) or Synacten ($ACTH_{1-24}$; Ciba-Geigy) was added to the incubation medium up to a concentration of 0.005-0.5 U/ml. The samples were incubated at 37°C for 60 min, after which the tubes were placed in melting ice, and the incubation medium was replaced by 3 ml of a 2% solution of Trition X-100 in a mixture of ehter and alcohol (8:2) to extract the formazan produced. Complete extraction of formazan from the diaphragm was achieved by homogenization of the muscle in a glass homogenizer with the ether–alcohol mixture. Pooled extracts were centrifuged at 10,000g for 10 min. The supernatant was decanted into graduated tubes and the volume made up with the ether–alcohol mixture to 10 ml; colorimetry was carried out at 540 nm in a 10-mm cuvette. Dehydrogenase activity was calculated by a calibration curve plotted for diformazan and expressed in nanomoles diformazan per gram tissue. Similar tests were carried out with a suspension of *Escherichia coli* serotype O III B_4 (stoke W strain), grown on nutrient agar. Dehydrogenase activity of the *E. coli* cells was determined colorimetrically by estimation of reduction of 2,3,5-triphenyltetrazolium chloride and expressed in micrograms formazan formed during incubation of a suspension containing 10^{12} microbial cells for 2 h at 37°C [8]. ACTH was added to a concentration of 0.005-0.5 U/ml of incubation medium. The results were subjected to statistical analysis.

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TABLE 1. Effect of ACTH on Dehydrogenase Activity of Rat Diaphragm (nanomoles diformazan/g tissue; M ± m)

Substrate	Control	ACTH, U/ml	
		0,5	0,05
Pyruvate	1715,28±177,42 (10)	1912,62±143,32 (10)	2237,28±136,81* (10)
α-Ketoglutarate	2565,35±104,53 (11)	2532,46±120,68 (6)	3131,55±119,92† (10)
Malate	1498,44±153,86 (7)	2864,60±169,94‡ (7)	3002,52±195,44† (7)
Succinate	2738,39±218,24 (6)	3029,83±257,33 (6)	3002,51±299,68 (6)
β-Hydroxybutyrate	1778,54±64,36 (8)	1840,59±132,96 (8)	2512,87±182,26† (8)
D-aspartic acid	1070,16±99,23 (7)	1543,26±118,00* (7)	1280,14±142,14 (7)

Legend. Number of experiments given in parentheses. Here and in Table 2: *P < 0.05, †P < 0.01, ‡P < 0.001.

TABLE 2. Effect of ACTH on Dehydrogenase Activity of *E. coli* Cells (μg formazan/10^{12} microbial cells) (M ± m n = 7-8)

Substrate	Control	ACTH, U/ml		
		0,5	0,05	0,005
Oxaloacetate	22,47±0,68	43,03±1,98‡	50,26±0,82‡	22,82±1,40
Isocitrate	27,50±2,45	74,95±9,70‡	45,04±0,90‡	35,70±1,74*
α-Ketoglutarate	9,19±1,10	47,35±1,45‡	50,70±1,17‡	16,10±0,93‡
Succinate	5,74±0,82	28,73±3,68‡	24,87±2,04‡	7,20±1,30
Fumarate	1,93±0,03	25,00±2,48‡	7,20±1,32‡	—
Malate	26,53±3,78	54,34±4,43‡	47,06±2,45‡	30,43±2,19
Pyruvate	7,49±0,93	16,54±1,75‡	28,16±3,00‡	10,20±1,47
Lactate	22,93±0,98	37,43±2,19‡	37,93±1,51‡	27,85±0,80†
Glucose	21,66±1,16	38,16±2,64‡	20,59±1,75	18,41±1,33
β-Hydroxybutyrate	00,00±0,00	12,25±1,52	15,70±1,40	35,70±2,00
D-aspartic acid	00,00±0,00	33,66±5,51	5,26±0,91	00,00±0,00

Legend. In the case of malate incubation was for 48 h, in all other cases 2 h.

EXPERIMENTAL RESULTS

The data in Table 1 show that ACTH changed the dehydrogenase activity of the rat diaphragm. For instance, in a concentration of 0.05 U/ml it enhanced dehydrogenase activity with pyruvate and α-ketoglutarate by 30.43% (P < 0.05) and by 22.07% (P < 0.01), respectively. In medium with succinate ACTH did not change the dehydrogenase activity of rat diaphragm. Succinate dehydrogenase activity was unchanged in the gastrocnemius muscles and other organs of rabbits and rats after intraperitoneal injection of ACTH into these animals in a dose of 1 U/100 g body weight [5].

In medium with malate ACTH increased the dehydrogenase activity of the diaphragm in concentrations of 0.5 and 0.05 U/ml by 97.17% (P < 0.001) and by 100.37% (P < 0.001), respectively.

It was decided to study the effect of ACTH on dehydrogenase activity of the muscle in medium with β-hydroxybutyrate, becuase ketone bodies can be utilized to support the respiratory function of the Krebs' cycle [13], especially in emergency, in carbohydrate deficiency [6]. The experiments showed that ACTH in a concentration of 0.05 U/ml increased the dehydrogenase activity of the diaphragm with β-hydroxybutyrate by 41.28% (P < 0.05). A link between protein and energy metabolism was demonstrated by the transamination reaction. Bearing this in mind, it was decided to undertake experiments using D-aspartic acid as the dehydrogenation substrate. It was found that ACTH, in a concentration of 0.5 U/ml, increased the dehydrogenase activity of the diaphragm in medium with aspartic acid by 44.20% (P < 0.05).

In other words, ACTH enhances the dehydrogenase activity of the isolated rat diaphragm muscle with pyruvate, α-ketoglutarate, malate, and -hydroxybutyrate, i.e., with substrates whose redoxe potential is very high, but does not change dehydrogenase activity with succinate.

Meanwhile analysis of the data encounters certain difficulties because it is impossible to rule out the participation of endogenous substrates in dehydrogenation processes; muscle

tissue, moreover, contains active endopeptidases which are responsible for ACTH degradation [14].

It was accordingly decided to carry out experiments with a suspension of *E. coli* cells in which the whole Krebs cycle functions during aerobic growth in the absence of sugars, when it performs a respiratory function (similar to that of mammalian cells), and whose dehydrogenases are located on the cytoplasmic side of the membrane [10]. This model is also perfectly adequate because skeletal muscle is also a facultative anaerobic system [15].

It will be clear from Table 2 that ACTH enhances dehydrogenase activity of the microbial cells with all substrates used. For instance, in medium with oxaloactate ACTH increased the dehydrogenase activity of the microbial cells by 91.50% ($P < 0.001$) and 132.57% ($P < 0.001$), respectively, in concentrations of 0.5 and 0.05 U/ml. In medium with isocitrate, the dehydrogenase activity of the microbial cells under the influence of ACTH was increased by 172.54% ($P < 0.001$), 67.78% ($P < 0.001$), and 29.82% ($P < 0.05$), respectively, in concentrations of 0.5, 0.05, and 0.005 U/ml, respectively. In medium with α-ketoglutarate, ACTH increased dehydrogenase activity of the microbial cells by 415.23% ($P < 0.001$), 451.68% ($P < 0.001$), and 75.08% ($P < 0.001$) with a decrease in its concentration. ACTH sharply increased the dehydrogenase activity of the microbial cells (unlike rat diaphragm) in medium with succinate also: by 400.52% ($P < 0.001$) and 333.27% ($P < 0.001$) in concentrations of 0.5 and 0.05 U/ml, respectively.

Dehydrogenation of fumarate by the *E. coli* cells was weak, but ACTH in a concentration of 0.5 U/ml increased it by 1195.33% ($P < 0.001$), and in a concentration of 0.05 U/ml, by 272.54% ($P < 0.001$). Dehydrogenation of pyruvate was stimulated by ACTH by 120.83% ($P < 0.001$) and by 257.96% ($P < 0.001$) in concentrations of 0.5 and 0.05 U/ml, respectively (Table 2). Increased dehydrogenase activity also was observed when glucose and lithium lactate were used as dehydrogenation substrates (Table 2). Incidentally, in *E. coli* cells glucose is metabolized by the Embden–Meyerhof–Parnas pathway, i.e., the same as in mammalian cells [10].

Investigation of malate dehydrogenase activity showed that intact microbial cells utilize malate only weakly. The cell suspension was therefore incubated with this substrate for 48 h. Under these circumstances dehydrogenase activity was enchanged by ACTH by 104.80% ($P < 0.001$) and 77.40% ($P < 0.001$) in concentrations of 0.5 and 0.05 U/ml, respectively (Table 2).

The study of the effect of the synthetic preparation Synacten (ACTH 1-24), which contains not 39, but 24 amino-acid residues, showed that in a concentration of 0.05 U/ml ($5 \cdot 10^{-7}$ g/ml) it increased the dehydrogenase activity of *E. coli* cells from 23.05 ± 0.31 to 51.65 ± 3.00 μg formazan per 10^{12} microbial cells, i.e., by 124.08% ($P < 0.001$). In a concentration of 0.005 U/ml ($5 \cdot 10^{-8}$ g/ml) Synacten had no effect. Clearly ACTH 1-24, like the native ACTH preparation, has similar (in magnitude as well as character) effects on dehydrogenase activity of *E. coli* cells (Table 2). However, Synacten differed from the native ACTH preparation in not changing the dehydrogenase activity of rat diaphragm in medium with α-ketoglutarate. The reason evidently was that ACTH 1-24 and shorter ACTH fragments are degraded rapidly and intensively into amino acids under the influence of muscle endopeptidases [14].

The effect of ACTH on dehydrogenase activity of *E. coli* cells also was studied in medium with β-hydroxybutyrate, since in these cells, just as in skeletal muscle, β-hydroxybutyrate is converted into acetyl-CoA by the same pathway [10]. The results showed that intact *E. coli* cells take up exogenous β-hydroxybutyrate only weakly, but ACTH sharply increased its dehydrogenation (Table 2), i.e., dehydrogenases of the Krebs' cycle in striated muscle cells and in *E. coli* cells react to ACTH in the same direction: ACTH stimulates them.

It will be noted that these effects of ACTH are not mediated through the adrenals, for they are manifested on isolated muscles and microorganisms. It is also unlikely that they are realized through the action of ACTH on specific receptor structures, for no such structures have been demonstrated on *E. coli* cells. The effects of ACTH found in this investigation are perhaps realized through its influence on adenylate cyclase, in the absence of receptors interacting with it directly or indirectly, as has been shown on plasma membranes of fat cells [11]. ACTH evidently modifies phospholipids of the cell membrane and thus influences the activity of membrane enzymes. ACTH is known to increase the flowability of the synaptic plasma membrane isolated from the rat forebrain [12].

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EFFECT OF ANTIBRAIN ANTIBODIES ON LIPID PEROXIDATION IN THE BRAIN

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Disturbance of the functional and structural integrity of the neuroglial cell membrane in multiple sclerosis and experimental allergic encephalomyelitis (EAE) has been demonstrated in many investigations [2, 4]. There are also insolated publications reporting intensification of lipid peroxidation (LPO) in patients with multiple sclerosis and schizophrenia, and this may be a factor involved in modification of the membranes and various disturbances of brain functions [5, 6].

The blood sera of patients with multiple sclerosis and schizophrenia, and also of animals with EAE are known to have an injurious membranotropic action which, according to some authorities, is due to a factor (factors) contained in the γ-globulin fraction of serum [7, 9, 10]. One such factor may be the antibrain antibodies that are found in the serum of patients with schizophrenia and multiple sclerosis [3, 4].

The effect of blood sera of normal dogs and of dogs immunized with brain, of IgG isolated from these sera, and of blood sera from patients with neuropsychic diseases, with certain somatic diseases, and of normal subjects, on LPO in brain tissue *in vitro*, and also in effect of the IgG fraction isolated from blood serum of dogs immunized with brain tissue on LPO processes after intracisternal injection, were studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-180 g. The relative antioxidant properties of the blood sera were judged by their ability to inhibit LPO in homogenates of surviving rat brain and liver tissue. The incubation medium contained 0.5 ml of a 3.3% homogenate made up in 0.05 M Tris-HCl buffer, pH 7.4, 0.3 ml of the test blood serum,

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