ANALYSIS OF THE ROLE OF GLYCOLYSIS IN THE RESPONSE OF ADRENAL CELLS TO ACTH

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The dynamics of steroid formation was found to coincide with that of lactic acid synthesis during perfusion of rat adrenal slices with ACTH. Inhibition of glycolysis by monoiodoacetate prevented the development of the specific effect of ACTH; the effect was restored on the addition of pyruvate to the medium. A decrease in the P/O ratio was found in the adrenal slices under the influence of ACTH, progesterone, and deoxycorticosterone (DOC). Steroid formation was inhibited by 2,4-dinitrophenol (2,4-DNP). The possibility of secondary activation of glycolysis in the adrenals under the influence of ACTH by a mechanism of self-regulation of the glycolytic chain through a decrease in the phosphate potential of the cell is discussed.

KEY WORDS: ACTH; adrenal cortex; lactic acid; oxidative phosphorylation.

The action of ACTH on metabolic processes in the adrenals [1, 4] (including glycolysis [5, 10]) has been demonstrated. However, the role of glycolysis and the concrete mechanism of its activation by ACTH has not yet been explained [5].

The object of this investigation was to study glycolysis and oxidative phosphorylation in the tissues of the adrenals and the role of these processes in the specific function of the glands.

EXPERIMENTAL METHOD

After decapitation of male Wistar rats (150-200 g) the adrenals were removed and slices prepared from them at 2°C. The slices were incubated in a Warburg's apparatus at 37°C in Krebs–Ringer–phosphate buffer with glucose (20 mM). The concentration of 11-hydroxycorticosteroids (11-HCS) in the incubated samples was measured fluorimetrically [3]. In the experiments were perfusion of the slices a modified apparatus of Flack and Ramwell [8] was used. The O_2 consumption of the slices, the decrease in inorganic phosphate in the incubation medium [6, 12], and the lactic acid production [11] were determined.

EXPERIMENTAL RESULTS AND DISCUSSION

The addition of ACTH to the perfusion medium led to marked activation of glycolysis and steroid formation (Fig. 1). Activation of the two processes coincided in time and developed during the first 20 min after the addition of ACTH. The coupling of the steroidogenic and glycolytic responses to ACTH suggests that the two processes have a relationship of cause and effect.

This hypothesis was confirmed by the fact that monoiodoacetate (MIA), an inhibitor of glycolysis, completely abolished the action of ACTH on steroid formation in the adrenal slices (Table 1). Since one of the functions of glycolysis is to provide substrates for the tricarboxylic acid cycle, it might be expected that the inhibitory effect of MIA would be abolished by the addition of pyruvate, the end product of glycoly-

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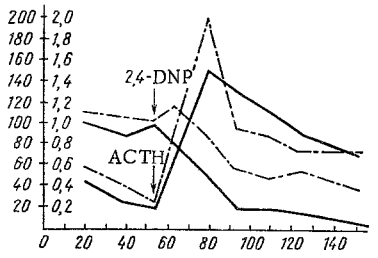

Fig. 1

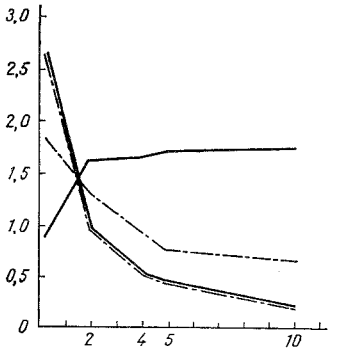

Fig. 2

Fig. 1. Dynamics of steroid formation and lactic acid synthesis during perfusion of adrenal slices with ACTH (1 unit/ml) and 2, 4-DNP (10^{-4} M). Continuous line represents 11-hydroxycorticosteroids, broken line – lactic acid. Abscissa, times of investigation after beginning of perfusion (in min); ordinate, left: lactic acid concentration in μg/100 mg/h, right: concentration of 11-HCS (in μg/100 mg/h).

Fig. 2. Effect of ACTH on oxidative phosphorylation of adrenal cells. Abscissa, dose of ACTH (in units/100 mg tissue); ordinate, O_2 consumption (continuous line), decrease in inorganic phosphate (broken line) (in μA/100 mg/h) and P/O ratio (double line).

TABLE 1. Effect of ACTH, Corticosterone Precursors, MIA, and Pyruvate on Steroid Formation, Glycolysis, and Oxidative Phosphorylation in Rat Adrenal Slices (M ± m)

Parameter studied	Control	Progesterone	DOC	ACTH	ACTH + MIA	ACTH + MIA + pyruvate
11-hydroxycorticosteroids (μg/100 mg/h)	2,81 ± 0,45 n = 8	5,30 ± 0,24 n = 4 P < 0,01	9,90 ± 0,12 n = 4 P < 0,001	7,32 ± 0,71 n = 7 P < 0,002	1,12 ± 0,09 n = 5 P < 0,002	6,62 ± 1,23 n = 5 P < 0,05
P/O ratio	18,00 ± 2,23 n = 8	34,20 ± 3,26 n = 4 P < 0,05	37,60 ± 2,52 n = 4 P < 0,01	35,18 ± 1,86 n = 7 P < 0,002	2,80 ± 0,32 n = 5 P < 0,01	31,40 ± 2,06 n = 5 P < 0,025
Lactic acid (μg/100 mg/h)	2,43 ± 0,078 n = 10	0,390 ± 0,081 n = 4 P < 0,001	0,16 ± 0,01 n = 4 P < 0,001	—	—	—

Legend: n) number of investigations; P) significance of difference compared with control.

sis, to the medium. The results fully confirmed this hypothesis. Similar data have recently been published [9].

Most workers consider that ACTH directly activates individual enzymes of the glycolytic chain, especially phosphofructokinase [5, 10]. However, a change in the phosphate potential of the cell – [ATP]/[ADP][P_i] – is known to be a physiological regulator of the activity of glycolysis [2].

The state of oxidative phosphorylation in the adrenal cells on the addition of ACTH was accordingly investigated (Fig. 2). A decrease in the P/O ratio dependent on the dose of ACTH (mainly on account of a decrease in the utilization of inorganic phosphate) was found. However, this effect could hardly reflect the uncoupling of oxidative phosphorylation, for the typical uncoupling of oxidative phosphorylation, for the typical uncoupler 2, 4-DNP, unlike ACTH, inhibits steroid formation (Fig. 1).

In experiments in which progesterone and deoxycorticosterone (DOC), precursors of corticosterone, were added to the incubation medium (in substrate concentrations), the same effects of stimulation of glycolysis and lowering of the P/O ratio were observed as during the action of ACTH (Table 1). This suggests that stimulation of the enzymes of glycolysis under the influence of ACTH may be a secondary effect, through an increase in the concentration of precursors of the steroid hormones.

The possibility of competition for the primary high-energy intermediate between the electron transport chain and the hydroxylating system in adrenal mitochondria in response to an increase in the concentration of unhydroxylated steroid substrate has been shown in the literature [7]. It is assumed that the energy of the primary high-energy compound in this case is utilized for maintaining the transhydrogenase reaction in the presence of an increased requirement of mitochondrial NADP · H_2 by the system for hydroxylation to the steroid ring. The results of the present experiments confirm this hypothesis. It is therefore logical to suppose that the primary cause of the activation of glycolysis under the influence of ACTH is a decrease in the phosphate potential of the cell.

Stimulation of glycolysis under these conditions makes good the deficiency of intracellular ATP and also increases the synthesis of pyruvate, an essential substrate in the Krebs cycle. Activation of glycolysis can therefore be regarded as an essential component in the reorganization of the energy balance of the adrenal cells during acute mobilization of the specific function of the gland.

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