CYTOPLASMIC DEHYDROGENASE ACTIVITY IN THE ADRENAL GLANDS DURING PROLONGED ACTH ADMINISTRATION

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Activity of cytoplasmic dehydrogenases of adrenal cells was investigated during prolonged administration of ACTH (5 units/100 g body weight) to Wistar rats. The indices of the specific steroid-synthesizing function of the glands remained relatively high throughout the experiment. Changes in the rate of corticosterone synthesis and in dehydrogenase activity were phasic in character and included an initial synchronized activation (1-2 days) followed by a decrease of activity (7 days of ACTH administration). In the final phase of the experiment (13 days) predominance of activity of NADP-dependent dehydrogenases was combined with reactivation of steroidogenesis. The possibility of an adaptive role of selected activation of NADP-dependent enzymes in the maintenance of a high level of hormone synthesis during prolonged stimulation of steroidogenesis by ACTH is discussed.

KEY WORDS: adrenal cortex; ACTH; dehydrogenases; steroidogenesis.

The writers showed previously that stimulation of steroid synthesis by ACTH is accompanied by lowering of the phosphate potential and stimulation of glycolysis in the adrenal cells [5]. The ATP deficiency developing under these circumstances [7] may be the cause of exhaustion of the adrenocortical cells. However, during prolonged administration of ACTH the specific function of the adrenals is preserved, despite morphological signs of cortical damage appearing at a certain stage of the course of stimulation [1].

Considering that cytoplasmic enzymes play an important role in the maintenance of the structure and

TABLE 1. Indices of Steroidogenesis during Prolonged Administration of ACTH (M ± m)

Parameter	Control	Time of administration of ACTH (in days)				
		1	3	5	7	13
11-Hydroxycorticosteroids of blood plasma (in μg %; n = 8-12)	8,4±0,7	17,8±0,7	15,35±2,03*	14,25±1,04*	13,1±0,4*	23,3±4,3*
Corticosterone production by adrenal slices, second hour of incubation (in μg/100 mg/h; n = 4)	1,60±0,13	4,43±0,50*	3,96±0,64*	2,95±0,10*	2,495±0,101*	5,70±0,43*
The same + ACTH (2 units/ml, n = 4)	3,908±0,23	13,35±2,06*	10,59±0,76*	6,605±0,765	4,26±0,14	12,95±1,57*
The same + progesterone (10 μg/ml, n = 4)	3,25±0,26	14,16±0,32*	13,58±0,33*	6,61±0,17*	5,00±0,14*	17,625±1,248*
The same + DOC (10 μg/ml, n = 4)	5,85±0,61	28,16±2,05*	20,06±0,94*	9,87±0,48	8,46±0,85	29,02±0,38*

* $P < 0.05$.

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TABLE 2. Activity of Cytoplasmic Dehydrogenases (in μmoles NADPH [mg protein/min] of adrenal) Cells during Prolonged Stimulation by ACTH (M ± m)

Parameter	Control	Time of administration of ACTH (in days)					
		1	2	3	5	7	13
Protein (in mg/100 mg tissue)	5,86± 0,19	6,12± 0,14				8,80± 0,49*	6,58± 0,91
LDH	342,8± 14,12	1069,4± 33,5*	847,96± 12,91*	651,1± 32,8	315,3± 18,1	224,4± 11,67*	312,4± 14,7
n	20	5	11	4	4	17	16
MDH	117,03± 5,65	189,24± 5,35*	—	—	—	59,73± 3,53*	94,1± 16,9
n	17	10				12	13
Glucose-6-phosphate dehydrogenase+ 6-phosphogluconate dehydrogenase	95,37± 3,77	162,13± 3,16*	119,52± 7,76	86,88± 2,55	51,12± 2,61*	55,65± 3,69*	362,18± 14,00*
n	20	9	4	4	4	16	16
MDH	3,63± 0,30	21,66± 1,94*	16,42± 0,90*	15,1± 0,49*	10,22± 0,39*	3,11± 0,24	9,91± 1,04*
n	21	4	9	4	4	16	18

*P < 0.05.

function of organs [9, 13], an attempt was made to compare the character of changes in the indices of steroid secretion and activity of certain NADP- and NAD-dependent dehydrogenases with time during prolonged administration of ACTH.

EXPERIMENTAL METHOD

A suspension of ACTH–zinc phosphate in a dose of 5 units/100 g body weight was injected intramuscularly daily into Wistar rats weighing 150-200 g. The animals were decapitated and the adrenal tissue was homogenized in a medium of 150 mM KCl containing 10 mM tris-HCl, pH 7.4; the cytoplasmic fraction was obtained by centrifugation at 20,000g for 20 min. Enzyme activity was determined fluorimetrically from changes in the concentration of reduced pyridine nucleotides: NADH for malate dehydrogenase (MDH) [6] and lactate dehydrogenase (LDH) [3], and NADPH for glucose-6-phosphate and 6-phosphogluconate dehydrogenases [14] and isocitrate dehydrogenase (IDH) [6]. The protein concentration in the samples was determined by the microbiuret method [2]. The corticosterone concentration in the blood plasma and the rate of its production by adrenal slices during incubation in a Warburg apparatus in Krebs–Ringer–phosphate buffer in an atmosphere of O_2 at 37°C were determined fluorimetrically. The reaction of the slices to ACTH and the biosynthesis of corticosterone in the presence of its precursors, progesterone and deoxycorticosterone (DOC) (10 μg/ml) also were studied.

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, administration of ACTH caused a significant increase in the secretion of hormones throughout the experiment, but the degree of activation of function depended on the time of stimulation. It was high on the first and second days of injection of the corticotropin, decreased relatively on the 7th day, and increased again on the 13th day of the experiment.

In a parallel study of the cytoplasmic dehydrogenases, the dynamics of their activity also showed three phases (Table 2). The high enzyme activity in the first phase (first to third days) is probably attributable both to the ability of ACTH to stimulate the initial stages of carbohydrate metabolism [8, 11] and to increased utilization of glycolysis products in the Krebs' cycle [10]. The writers showed previously that inhibition of glycolysis prevents the stimulant effect of ACTH whereas addition of pyruvate restores it [5].

Intensification of metabolic processes in the adrenal cells at this period led to an increase in the protein concentration per 100 mg wet weight and to an increase in the relative weight of the glands (Table 2).

The seventh day was a critical period for adaptation of the adrenals to the prolonged action of ACTH. decrease in enzyme activity coupled with morphological evidence of cortical injury described for this stage of ACTH administration [1] is evidence of a disturbance of plastic processes in the gland cells. However, the level of steroid synthesis at this period remained relatively high. This points to some independence of the reactions of hormone synthesis of the level of activity of the cytoplasmic dehydrogenases. Considering that five of the six steroid hydroxylation reactions are located on the inner membrane of the mitochondria, which is impermeable to cytoplasmic NADPH, in this phase of the experiment the main supplier of coenzyme for steroid biogenesis must be considered to be intramitochondrial oxidoreduction processes.

The phase of reactivation of specific adrenal function on the 13th day of ACTH administration deserves special attention. Analysis of the mechanisms for maintenance of a high level of steroid biosynthesis at this period emphasizes the autonomous activation of cytoplasmic NADP-dependent IDH and of enzymes of the pentose phosphate shunt, superposed upon low (close to the control level) activity of NAD-dependent LDH and MDH. During prolonged administration of ACTH appreciable activation of NADP-dependent mitochondrial IDH of adrenal cells is known to take place [12]. Comparison of these observations with the great effectiveness of isocitrate in the maintenance of steroid hydroxylation reactions points to the increasing role of the shuttle mechanism for the isocitrate–α-ketoglutarate pair [4]. Its active functioning makes the NADPH produced intensively in this phase in the course of the reactions of the pentose phosphate shunt in the cytoplasm accessible for utilization in the mitochondria.

Adaptation of the rat adrenals to prolonged maintenance of a high level of function is thus based on a mechanism which renders extramitochondrial reduced equivalents accessible for intramitochondrial hydroxylation reactions.

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