ENZYMIC BASIS OF THE STIMULANT EFFECT OF ACTH ON THE ADRENOCORTICAL CELL

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Intensive incorporation of uracil-C^{14} and methionine-S^{35} into the protein fraction of the adrenal cortex under the influence of ACTH is combined with high activity of the enzymes steroid-3β-ol dehydrogenase and NADP · H_2 diaphorase. The stimulant action of ACTH on steroidogenesis is based on intensification of the biosynthesis of enzyme proteins through a mechanism of increased RNA metabolism.

Information in the literature on the action of ACTH on ribonucleoprotein and protein metabolism is extremely contradictory (see the survey by Bransome [3]).

A histochemical study has been made of enzymes participating in steroid biosynthesis and in the general metabolism of the adrenocortical cells.

* Deceased.

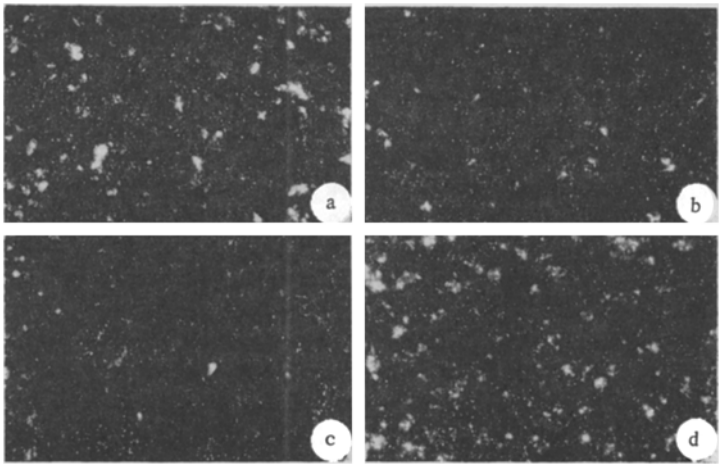

Fig. 1. Cholesterol in zona fasciculata of the adrenal cortex: a) control; b) reduction of crystals 3 h after a single injection of ACTH; c) low content of cholesterol 10 days after beginning of ACTH injections; d) high concentration of birefringent material after 5 days of ACTH injections. Polarization microscopy, 72×.

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TABLE 1. Enzyme Activity of Cells of Zona Fasciculata (logarithmic scale of microphotometer)

Group of animals	Duration of injection of hormone	Steroid-3β-ol dehydrogenase		NADP · H_2 diaphorase		Succinate dehydrogenase	
		$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
Control	—	47,6±0,70	—	89,2±1,05	—	85,3±1,06	—
Receiving ACTH injections	Single	85,6±1,38	<0,001	125,2±1,81	<0,001	87,5±1,42	<0,05
	5 days	78,2±0,93	<0,001	108,0±2,12	<0,01	94,3±1,39	<0,02
	10 days	73,8±1,49	<0,001	102,6±1,78	<0,01	104,6±1,15	<0,001

TABLE 2. Radioactivity (pulses/min/mg) of Native Tissue and Total Protein of Adrenal Cortex (M±m)

Group of animals	Duration of injection of hormone	Uracil-C^{14}		Methionine-S^{35}	
		native tissue	protein	native tissue	protein
Control	—	30,3±2,2	11,3±2,6	2 166±217	1 160±88
Receiving ACTH injections	Single	110,3±12,3	20,0±4,8	1 944±166	1 661±126
	6 days	114,6±8,3	23,1±3,2	3 047±196	1 986±152
	10 days	68,2±8,2	15,6±1,7	2 950±104	1 661±130

EXPERIMENTAL METHOD

Wistar rats received a single injection of ACTH (3 units/100 g body weight) or repeated injections for 5 and 10 days. To determine the rate of biosynthesis of RNA and sulfur-containing proteins, the animals were injected with uracil-C^{14} (0.5 μCi/g 3 h before sacrifice) or methionine-S^{35} (1 μCi/g 6 h before sacrifice), respectively. The cortical substance of the adrenals, freed from medullary tissue, was examined radiometrically and treated biochemically to obtain the total protein fraction. Weighed samples of the dried protein were also examined radiometrically with a T-25-B FL end-window-type counter. Glands fixed in Carnoy's fluid and embedded in paraffin wax were used for the histochemical detection of ribonucleoproteins and sulfur-containing proteins. Phospholipids, ketosteroids, and cholesterol were detected in adrenals fixed in calcium-formol (by polarization microscopy). Some unfixed frozen glands were used for the histochemical determination of enzyme activity. Reactions for steroid-3β-ol dehydrogenase [4], for NADP · H_2 diaphorase [2], and for succinate dehydrogenase, by Nachlas' method, were carried out on frozen sections. The intensity of the histochemical reactions for ribonucleoproteins, proteins, phospholipids, and ketosteroids was estimated by means of an automatic extinction recorder with ERJ-10 integrator. Activity of the enzymes was determined in the sections from readings on the logarithmic scale of the MF-3 microphotometer.

EXPERIMENTAL RESULTS

The content of sudanophilic material in all zones of the cortex fell sharply after a single injection of ACTH. The decrease was particularly marked in the zona glomerulosa, which lost its distinctive character. Cells of the zona fasciculata retained a very small number of tiny lipid droplets. The content of ketosteroids in both zones fell appreciably. There was also a reduction in the number of large cholesterol crystals, whereas the small accumulations of birefringent material remained unchanged (Figs. 1a, b, c).

A single injection of ACTH caused a sharp increase in the intensity of the reactions for steroid-3β-ol dehydrogenase and NADP · H_2 diaphorase (Table 1). Succinate dehydrogenase activity, on the other hand, was substantially unchanged. Activity of steroid-3β-ol dehydrogenase and NADP · H_2 diaphorase remained high compared with the control throughout the experiment, although after 10 days it showed a distinct tendency

to diminish. The intensity of the reaction for succinate dehydrogenase was increased 5 and, in particular, 10 days after the beginning of the ACTH injections.

Radiobiochemical analysis of the material showed that ACTH stimulates the incorporation of uracil-C^{14} and methionine-S^{35} into the protein fraction and native tissue of the adrenal cortex (Table 2). The maximal effect of ACTH in stimulating biosynthesis of ribonucleoproteins and sulfur-containing proteins was exhibited after repeated injections [1]. The intensity of the histochemical reactions for RNA and SH-SS groups varied in accordance with the dynamics of incorporation of uracil-C^{14} and methionine-S^{35}. These reactions can thus be regarded as additional criteria of the level of biosynthesis of RNA and proteins [1].

The results of these experiments thus show that the initial effect of ACTH on adrenocortical cells is manifested not only by the mobilization of hormonal material, but also by simultaneous stimulation of biosynthesis of enzyme proteins catalyzing steroid formation. The stimulant effect of pituitary corticotropin on steroidogenesis is based on increased biosynthesis of enzyme proteins through a mechanism of increased RNA metabolism.

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