THE MECHANISM OF ACTION OF ACTH ON THE ADRENAL CORTEX

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The mechanism of the acute stimulant action of ACTH on the adrenal cortex during exposure to the toxic effects of inhibitors of RNA synthesis (actinomycin D) and protein synthesis (chloramphenicol) was studied in experiments on hypophysectomized rats by the use of histochemical and histo-enzymological methods. Actinomycin D did not prevent the stimulant action of ACTH on the adrenal cortex as manifested by a selective increase in the activity of glucose-6-phosphate dehydrogenase, NADP·H_2-diaphorase, succinate dehydrogenase, acid phosphatase, and nonspecific esterase in the zona fasciculata and zona reticularis and by the disappearance of lipids, cholesterol, and steroids from these zones. Chloramphenicol blocked this effect of corticotropic hormone, indicating that a system for the consecutive transmission of generic information (DNA–RNA–protein) participates in the development of the steroidogenic response of the RNA–protein stage to ACTH.

Some hormones are known to regulate the secretory function of a target organ by their effect on the activity of the genetic apparatus of its cells; this can be manifested as an increase in the content of nucleic acids and protein and, ultimately, an increase in the synthesis of the specific product of that tissue [4].

However, an interesting feature is observed in the action of ACTH on the adrenal cortex: an acute effect, possibly not connected with the involvement of the genetic apparatus of the cells in the steroidogenic response at the DNA–RNA stage [6, 11, 14, 15, 17], whereas during the prolonged action of ACTH an increase in RNA synthesis and protein synthesis is observed [1-3, 7-9]. This naturally points to the participation both of the DNA–RNA stage and of the RNA–protein stage in the steroidogenic response. Consequently, it is very important to study the precise role, if any, of genetic mechanisms in the response of the adrenals to the acute action of ACTH. In addition, it is important to clarify at what stage of transmission of genetic information in the DNA–RNA–protein system the corticotropic hormone acts.

This paper describes an attempt to study the mechanism of action of ACTH on the adrenal cortex in vivo, using histochemical methods, during acute stimulation coupled with administration of inhibitors of RNA and protein synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 40 sexually mature male rats weighing 120-150 g from which the pituitary was removed by a transauricular approach 48 h before the experiment began. The animals were divided into four groups with ten in each group. Group 1, the control, consisted of hypophysectomized animals. The animals of group 2 received a single intramuscular injection of 10 units ACTH. The rats of group 3 received an intraperitoneal injection of a solution of actinomycin D in aqueous alcohol in a dose of 25 mg/kg, and an intramuscular injection of 10 units ACTH 3 h later. The animals of group 4 received an injection of a solution of chloramphenicol (500 mg/kg) in aqueous alcohol and, like the animals of group 3, an intramuscular injection of 10 units ACTH 3 h later.

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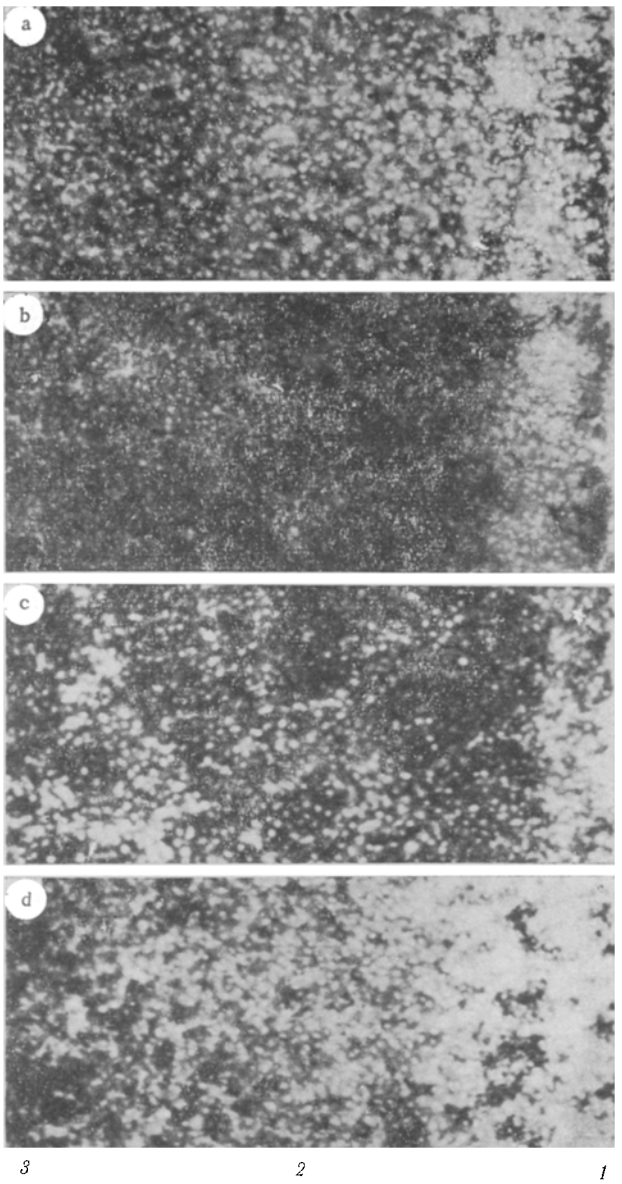

Fig. 1. Histochemical reaction to glucose-6-phosphate dehydrogenase in the adrenal cortex under different experimental conditions: a) high enzyme activity in zona glomerulosa and reduced activity in zona fasciculata and zona reticularis in adrenals of the control group; b) sharp increase in enzyme activity in cells of the zona fasciculata and zona reticularis after injection of ACTH; c) increased enzyme activity after injection of ACTH coupled with the action of actinomycin D; d) inhibition of stimulant action of ACTH in animals poisoned with chloramphenicol; 1) zona glomerulosa; 2) zona fasciculata; 3) zona reticularis. Nachlas's method, MBI-6, objective 9, ocular 12.5.

The animals of all groups were decapitated 3 h after the injection of ACTH or, in the case of the control group, of physiological saline. The adrenals were quickly removed and frozen sections were cut to a thickness of 10 μ in a cryostat at between −8 and −12°C. Some sections, mounted on slides, were fixed in 10% formalin at 5°C for 1 h and then used for determination of lipids by staining with Sudan III after Jackson, cholesterol after Schultz, steroids after Lewis and Lobban, RNA after Brachet, and acid phosphatase and nonspecific esterase after Burstone, using phosphate-acetate-AS-LC-naphthol and a diazonium salt (PP blue). Glucose-6-phosphate dehydrogenase and succinate dehydrogenase were determined after Nachlas and NADP · H_2-diaphorase after Farber in unfixed sections.

EXPERIMENTAL RESULTS

The use of hypophysectomized animals in this investigation made it possible to distinguish the stimulant effect of ACTH on the adrenal cortex clearly: 48 h after removal of the pituitary gland the highest activity of the enzymes studied was found in the zona glomerulosa of the adrenals of the control group and less activity in the zona fasciculata and zona reticularis (Fig. 1a), whereas in the intact animals the activity of these enzymes was usually greatest in the zona fasciculata and zona reticularis in which the content of lipids, cholesterol, and steroids was relatively high. Injection of ACTH into hypophysectomized animals led to an increase in enzyme activity in the zona fasciculata and zona reticularis and the differences in activity between the cells of the zona glomerulosa on the one hand and the zona fasciculata and zona reticularis on the other hand (Fig. 1b) became indistinguishable and the lipids, cholesterol, and steroids disappeared from these zones.

In the writer's opinion the histochemical tests used reflect the functional state of the adrenal cortex adequately completely, an important matter when the action of corticotropic hormone is to be evaluated during exposure to the inhibitory effect of actinomycin D and chloramphenicol. Under these conditions hypophysectomy excluded the effect of endogenous ACTH, the secretion of which may have been increased as a result of the general toxic action of the inhibitors.

Injection of actinomycin D into the animals showed that inhibition of RNA synthesis does not prevent the stimulant effect of ACTH on the adrenal cortex as manifested by a decrease in the lipid content and an increase in the enzyme activity in the zona fasciculata and zona reticularis (Fig. 1c). The results agree with those of other investigations [11, 14, 15, 17] carried out in vitro and in vivo with the use of biochemical methods and they indicate that the acute stimulant action of ACTH on the adrenals is not effected through the DNA−RNA stage.

It must be pointed out that it was impossible to detect a decrease in the RNA content by the histochemical method in the adrenals of the hypophysectomized animals and also of the animals receiving actinomycin D. This does not mean, however, that, in the doses used, the inhibitor did not exhibit its effect. The stability of the ribosomal RNA of the adrenals is known to be high: its half-life period, according to Boyadjiev [5], is about 5 days. The expected effect naturally could not therefore have been manifested only 48 h after hypophysectomy, and also under the conditions of relatively short exposure to the action of the inhibitor. The RNA already present must therefore have enabled the adrenals to respond to the stimulant action of ACTH despite inhibition of its subsequent synthesis by actinomycin D.

The importance of the RNA−protein stage for the steroidogenic response of the adrenals was confirmed by the experiments using chloramphenicol − an inhibitor of protein synthesis: administration of ACTH, coupled with the action of chloramphenicol, prevented the stimulant action of corticotropic hormone usually observed on the zona fasciculata and zona reticularis of the cortex in which lipids, cholesterol, and steroids still remained, while the enzyme activity was not increased (Fig. 1d). These results agree with those of biochemical investigations carried out with this antibiotic in vitro [10, 13, 16].

The results indicate that the stimulant effect of ACTH on the adrenal cortex is connected with its action on the processes of protein synthesis, while the increase in the RNA content observed in some investigations [1, 2, 7, 8] is evidently a secondary process arising as a result of subsequent involvement of the DNA−RNA stage in the steroidogenic response; this involvement, in turn, is bound up with the need for protein synthesis in order that steroidogenesis may take place [18].

The dependence of steroidogenesis on protein synthesis revealed by these experiments confirms the view that special regulator proteins exist in the adrenal cortex [12, 13, 17]; the presence of these regulators depends on the presence of ACTH and, in turn, it determines the rate of synthesis of the steroid hormones.

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