# INHIBITION OF ADRENAL FUNCTION BY AN ACETYLATED DERIVATIVE OF ACTH

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An acetylated derivative of ACTH inhibits adrenal function and lowers the blood hydrocortisone level in guinea pigs and the corticosterone level in rats.

In connection with the important role of increased corticotropic function in the development of several pathophysiological processes and diseases (obesity, Cushing's disease, diabetes mellitus in the elderly, tumors, etc.), the search for pharmacological agents to inhibit ACTH secretion or adrenal function is an important field for research. The use of glucocorticoids for this purpose is unsuitable in many cases because of their own hormonal action.

Substances which block the synthesis of some adrenal hormones, notably metapyron, lead to a compensatory increase in the adrenocorticotropic function of the pituitary and to increased synthesis of hormonally active metabolites in the adrenals [6]. DDD derivatives,* causing lasting damage to the adrenals, possess marked toxicity and also stimulate pituitary function [1]. Some antiepileptic drugs, notably aminoglutethimide, block the conversion of cholesterol into pregnenolone, and because of the complete inhibition of synthesis of adrenal hormone, they produce a particularly marked increase in ACTH production [4]. All these facts determine the urgent need for the discovery of drugs to inhibit either the synthesis or secretion of ACTH in the pituitary.

The object of this investigation was to study an acetylated derivative of ACTH, on the basis of the concept of anahormones [2], and also of data indicating that native ACTH can directly inhibit the adrenocorticotropic function of the pituitary in adrenalectomized animals.

## EXPERIMENTAL METHOD

Adrenocorticotropic hormone (ACTH) with an activity of 40 units/mg, obtained from the Medical Preparations Factory, S. M. Kirov Leningrad Meat Combine, was used in the experiments. The acetylated ACTH was obtained by treatment of the hormone with acetic anhydride by the Fraenkel-Conrat method [5]. A difference from the original method was that detergents were added to the hormone solution before the chemical reaction: sodium dodecylsulfate and nonylphenol with 10 moles ethylene oxide up to a final concentration of 0.1%.

To study the properties of the derivative, in vitro determinations were made of its steroidogenic [9], lipolytic [8], and melanophore activity [10], and of its free $NH_2$ groups [8].

*1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane.

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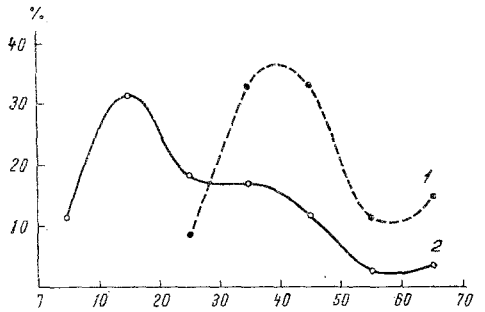


Fig. 1. Distribution of animals by hydrocortisone level in blood plasma: 1) before injection of acetylated ACTH; 2) 4 h after injection of acetylated ACTH. Abscissa, hydrocortisone concentration (in μg%); ordinate, number of animals (in %).

The state of function of the pituitary – adrenal system was estimated from the blood concentration of hydrocortisone in guinea pigs. Acetylated ACTH was injected intramuscularly once or twice a day in a dose equivalent to 20 units of active hormone. In some experiments a control injection of active ACTH or acetylated albumin was given. Blood was taken from the heart at the same time in order to exclude diurnal fluctuations, and the hydrocortisone concentration in the circulating blood was determined [3] before the experiment began and 4 h after injection of the derivative. For injection, the preparation was dissolved in 0.1 N NaOH solution and neutralized in 0.1 N or 1 N HCl to pH 8.0.

The effect of acetylated ACTH on the corticosterone level was also studied during prolonged administration to rats. In this case, acetylated ACTH in zinc phosphate solution was injected subcutaneously, daily for 7 days, in a dose equivalent to 5 units of active hormone. Control animals received injections of zinc phosphate solution only. The corticosterone level was determined by the method of Pankov and Usvatova in blood taken from the heart under nembutal anesthesia (5 mg/100 g body weight).

TABLE 1. Changes in Blood Hydrocortisone Level in Guinea Pigs 4 h after Injection of Acetylated ACTH

| Preparation | Number of animals | Blood hydrocortisone concentration | |
|---|---|---|---|
| | | μg% | percent of initial |
| Before injection of preparations | 63 | 43±1.52 | – |
| Acetylated ACTH | 36 | 27±2.83 | 63* |
| Acetylated albumin | 9 | 43±2.54 | – |
| Active ACTH | 5 | 217±12.16 | 504* |

*P < 0.001.

TABLE 2. Changes in Blood Corticosterone Level of Rats after Administration of Acetylated ACTH for 7 Days

| Preparation | Number of animals | Blood corticosterone concentration | |
|---|---|---|---|
| | | μg% | percent of control |
| Zinc phosphate solution (control) | 23 | 9.0±1.22 | – |
| Acetylated ACTH | 12 | 6.4±0.8 | 71* |

*P < 0.05.

## EXPERIMENTAL RESULTS

A study of the properties of acetylated ACTH in vitro showed that it possesses marked steroidogenic (30% of the initial) and lipolytic (40% of the initial) activity, and retains the whole of the melanophore action of active ACTH. Determination of free amino groups showed that they are completely blocked by acetylation. Dissolving the derivative in alkali excludes the presence of acetylated phenolic hydroxyl groups. At the same time, in experiments in vivo, the acetylated ACTH had virtually no steroidogenic activity, so that it could be used to study possible inhibition of adrenal function.

The results in Table 1 show that acetylated ACTH, when injected into guinea pigs, considerably lowered the blood hydrocortisone level (on the average by 37%). In one-third of cases, this decrease was greater still (about 70%), and the hydrocortisone concentration fell to 8-12 μg%. This can be seen by analysis of the distribution curves of the animals by their blood hydrocortisone level (Fig. 1). Before injection of the derivative, a normal Gaussian distribution was found, with a maximum at a concentration of 40 μg%, while after administration of acetylated ACTH the maximum was shifted considerably toward lower values, and lay in the region of a hydrocortisone concentration of 10-20 μg%. On the other hand, after injection of acetylated albumin, no change in the blood hydrocortisone level was observed. In experiments in which acetylated ACTH was injected for 4 days, the blood hydrocortisone level fell by an amount which corresponded approximately to its decrease following a single injection of the compound.

The study of the effects of prolonged administration of acetylated ACTH was continued in rats. Injection of the preparation for 7 days lowered the blood corticosterone level by 29% (Table 2).

The results show that administration of acetylated ACTH caused a marked decrease in the blood hydrocortisone or corticosterone levels in guinea pigs and rats, respectively. The absence of any such action of acetylated albumin indicates the specificity of the observed effect. It is proposed to study next whether, under these circumstances, the adrenocorticotropic function of the pituitary is inhibited or whether the derivative acts directly at the adrenal level, competing with endogenous ACTH.

From the theoretical standpoint, it is also extremely interesting to determine whether the inhibitory action of the acetylated derivative is due to the preservation of its melanophore activity or of some other "extra-adrenal" activity.

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