THE EFFECT OF ACTH UPON ADRENAL GLUCOSE-6-PHOSPHATE METABOLIZING ENZYMES*

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Haynes, Sutherland and Rall (1960) have proposed a mechanism of action of ACTH upon the adrenal gland in which G-6-P** plays a most important role. ACTH was shown to activate adrenal phosphorylase, causing an increased production of G-1-P, and the subsequent conversion of G-1-P by phosphoglucomutase to G-6-P, results in an increased metabolism of G-6-P by oxidation to 6-phosphogluconic acid. This oxidation via the hexose monophosphate shunt produces TPNH and TPNH serves as a source of energy for various steps in the production of steroids (Haynes and Berthet, 1957). However, G-6-P may also be metabolized by dephosphorylation, affected by the enzyme G-6-Ptase.

Since we have been studying the effect of ACTH treatment upon certain adrenal enzymes (Hilf et al., 1961), we felt that an investigation of the effects of ACTH upon G-6-Ptase might provide further insight into the metabolism of G-6-P. The activities of G-6-P dehydrogenase and 6-phosphogluconic dehydrogenase were also measured in ACTH stimulated adrenals. In this communication data are presented indicating that ACTH treatment caused a rapid decrease in G-6-Ptase activity.

Method: Various doses of ACTH (corticotrophin, Armour, 25 I.U./mg) were injected subcutaneously into male Fischer rats. Animals were sacrificed by decapitation, blood was collected, and adrenals were removed immediately. Plasma corticosterone determinations were performed by fluorometric methods (Hilf et al., 1960).

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** Abbreviations used are: G-6-P, glucose-6-phosphate; G-1-P, glucose-1-phosphate; G-6-Ptase, glucose-6-phosphatase; TPNH, reduced triphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; ACTH, adrenocorticotrophic hormone.

Adrenals from two or three animals of the same dose groups were pooled and homogenized in conical ground glass homogenizers, using deionized water as diluent. G-6-Ptase was assayed on aliquots of the whole homogenate by the method of Swanson (1955), final incubation mixture contained 30 millimoles maleate buffer, pH 6.5, 10 millimoles G-6-P (disodium salt) and tissue in a final volume of 0.7 ml. The reaction was terminated after one hour at 38°C by the addition of 0.3 ml of 50% trichloroacetic acid and the inorganic phosphate (P_i) released in the supernatant was determined (Fiske and Subbarow, 1925). G-6-P dehydrogenase and 6-phosphogluconic dehydrogenase activities were assayed on aliquots of the 21,000 x g supernatant of the same adrenal homogenate by the method of Glock and McLean (1954). Nitrogen determinations were performed using a modification of the Pregl micro-Kjeldahl method (Pregl and Roth, 1935).

Results and Discussion: In Table I are summarized the data obtained on the enzyme activities, plasma corticosterone and adrenal nitrogen following stimulation by different doses of ACTH. No change in nitrogen content occurred with the doses of ACTH employed. Plasma corticosterone levels clearly indicate the responsiveness of the adrenals to exogenous ACTH, the levels returning to control values at 24 hours after a single dose of 10 I.U.

G-6-Ptase activity decreased rapidly following the injection of ACTH. One half hour after stimulation with 10 I.U. of ACTH, the G-6-Ptase activity was reduced by approximately 50%. A slight further reduction was obtained at one hour following this same dose of ACTH. Injection of 2 I.U. of ACTH also reduced G-6-Ptase, but to a lesser extent than the 10 I.U. dose, suggesting the possibility of a dose-response of G-6-Ptase activity to ACTH. By 24 hours after the injection of 10 I.U. of ACTH, adrenal G-6-Ptase activity was approximately equal to the control levels. Preliminary investigations have indicated that ACTH treatment caused a reduction in the G-6-Ptase activity of the microsomal fraction (Hilf et al., 1962).

If one assumes that a change in G-6-Ptase activity is a reflection of some alteration in the metabolism of G-6-P, then the data presented here certainly suggest that ACTH treatment causes a rapid change in the metabolism of this

TABLE I

THE EFFECT OF ACTH UPON ADRENAL GLUCOSE-6-PHOSPHATE METABOLIZING ENZYMES, ADRENAL NITROGEN AND PLASMA CORTICOSTERONE LEVELS

Group	# Animals	ACTH Dose (I.U.)	Time After Dose (hrs.)	Glucose-6-Phosphatase[1]	Glucose-6-Phosphate Dehydrogenase[2]	6-Phosphogluconic Dehydrogenase[2]	Corticosterone (μg/ml)	Nitrogen (mg/100 mgs)
Control	24	-	1	5.27 ± 0.50[3]	1.047 ± 0.021	0.156 ± 0.012	0.348 ± 0.018	2.41 ± 0.09
ACTH	24	2	1	3.46 ± 0.35	0.972 ± 0.078	-	0.469 ± 0.029	2.06 ± 0.07
ACTH	24	10	1/2	2.47 ± 0.07	1.005 ± 0.087	0.156 ± 0.012	0.526 ± 0.016	2.38 ± 0.03
ACTH	39	10	1	2.25 ± 0.10	1.020 ± 0.018	0.168 ± 0.006	0.594 ± 0.011	2.27 ± 0.06
ACTH	18	10	24	4.52 ± 0.14	0.963 ± 0.003	-	0.414 ± 0.034	2.25 ± 0.16
ACTH	33	100	3	3.37 ± 0.38	0.996 ± 0.024	0.174 ± 0.012	0.534 ± 0.013	2.29 ± 0.10

1 Activity expressed in terms of micrograms P_i/10 mg/hr.

2 Activity expressed in terms of micromoles TPNH/minute/100 mg.

3 Standard error of the mean.

compound. However, the exact relationship of these enzyme changes to the production of adrenocortical steroids is not known. One possible explanation is that the decrease in total G-6-Ptase activity might shunt the metabolism of G-6-P partly towards the oxidative pathway, with the resultant increase of production of TPNH. It has been reported that there is an increase in adrenal TPNH levels following stimulation by ACTH (Haynes et al., 1960). Furthermore, it has been shown that an increase in G-6-Ptase activity favors the production of TPN (Glock et al., 1956), and one might speculate that a decrease in G-6-Ptase activity may favor the formation of TPNH.

It is of interest that neither G-6-P dehydrogenase nor 6-phosphogluconic dehydrogenase levels were altered at the doses of ACTH employed here. Although this might appear to be in conflict with the histochemical results of Greenberg and Glick (1960), it should be noted that these investigators employed much larger doses of ACTH (7.5-10.0 mg). Thus, it would appear that the relatively high levels of these dehydrogenases in the adrenal are sufficient to handle the increased G-6-P metabolism induced by the small doses of ACTH employed in these studies.

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