EVIDENCE FOR THE STIMULATION BY ADRENOCORTICOTROPIC HORMONE OF THE CONVERSION OF CHOLESTEROL ESTERS TO CHOLESTEROL IN THE ADRENAL, IN VIVO

Warren W. Davis[o] and Leonard D. Garren[+]

National Heart Institute
and National Institute for Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland

Received August 11, 1966

Previous studies demonstrated that the inhibition of adrenal protein synthesis in vivo by cycloheximide blocked the ACTH* stimulation of corticosterone secretion (Garren, Ney, and Davis, 1965). It has been shown that cycloheximide acts by inhibiting the first step in the conversion of cholesterol to Δ5-pregnenolone in the adrenal (Davis, Ney, and Garren, 1966; Davis and Garren, 1966). The present study demonstrates that ACTH activates the conversion of adrenal cholesterol esters to free cholesterol even when adrenal protein synthesis and steroidogenesis are inhibited by cycloheximide.

METHODS

Adrenals were removed from recently hypophysectomized rats (Charles River Farms), trimmed, and weighed. Cholesterol and total lipids were extracted by the Folch technique (Folch, et al., 1957). Free cholesterol was isolated by thin layer chromatography on Silica gel using benzene: ethyl acetate 5:1 (Avigan, Goodman, and Steinberg, 1963). Total cholesterol was isolated following saponification and chromatography in the above system. Free and total cholesterol were also obtained

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[o] Clinical Associate, National Heart Institute

[+] Present address: Department of Medicine, Yale University School of Medicine, New Haven, Connecticut

* Abbreviating: ACTH, Adrenocorticotropic Hormone; TLC, Thin Layer Chromatography GLC, Gas Liquid Chromatography.

by the digitonin method according to Schoenheimer and Sperry (1934). The isolated cholesterol was determined by a colorimetric procedure (Abell, et al, 1952) and by gas liquid chromatography using a Glowall apparatus equipped with a Strontium 90 detector. Operating conditions consisted of a 1% SE-30 column maintained at 225°C with detector and flash temperatures at 275° C, using argon as carrier gas.

RESULTS

Hypophysectomized rats were injected with 10 mgm of cycloheximide, which was previously shown to almost completely inhibit adrenal protein synthesis, and the steroidogenesis which is stimulated by ACTH (Garren, Ney, and Davis, 1965). Twenty minutes later 0.1 unit of ACTH was injected intravenously. The animals were sacrificed 90 minutes after the injection of ACTH and adrenal cholesterol determined. These animals were compared with animals which were untreated or were injected with either ACTH or cycloheximide. To confirm the methodology used in obtaining these results, different techniques were used to determine cholesterol. These yielded similar findings (Table 1).

As illustrated in Table I and previously demonstrated, the depletion of adrenal cholesterol which follows ACTH treatment involves the cholesterol-ester fraction (Sayers, et al, 1944). This ACTH stimulated cholesterol depletion is blocked by the prior administration of cycloheximide (Davis, Ney, and Garren, 1966; Davis and Garren, 1966). The adrenal free cholesterol fraction which comprises approximately 10% of the total cholesterol does not change after ACTH treatment. However, as shown in Table I, after treatment with cycloheximide, the ACTH stimulated conversion of cholesterol-esters to free cholesterol is now manifested, because the antibiotic blocks further utilization of cholesterol in the pathway of corticosterone biosynthesis.

Although, it is obvious that after cycloheximide treatment ACTH stimulates

a fall in the concentration of cholesterol esters and an associated rise in the free cholesterol fraction, the level of esterified cholesterol was not decreased as much as when ACTH was injected without cycloheximide (Table I). The reason for this result is unknown; perhaps, the increased concentration of free cholesterol which accumulates when ACTH is administered after cycloheximide, in some way, (eg. mass action) prevents the further breakdown of cholesterol esters to free cholesterol.

Table I

Experiment	Cholesterol mg/50 Adrenal Wgt.	
	Free Cholesterol	Esterified Cholesterol
No. 1*		
Control (4)#	0.109 ± .002	0.93 ± .09
Cycloheximide (4)	0.144 ± .018	0.93 ± .12
ACTH (4)	0.104 ± .009	0.50 ± .09
ACTH cycloheximide (4)	0.409 ± .028	0.67 ± .07
No. 2**		
Control (4)	0.125 ± .016	
ACTH (4)	0.130 ± .025	
ACTH cycloheximide (4)	0.564 ± .090	
No. 3***		
Control	0.08	0.92
ACTH	0.06	0.44
ACTH cycloheximide	0.31	0.64

Rats were treated as described in text. All values are expressed as mean ± standard error of the mean. * = cholesterol determined by Schoenheimer and Sperry, 1934. ** = cholesterol determined by TLC and gas liquid chromatography. *** = cholesterol determined by TLC and GLC and colorimetric procedure using aliquots from 6 rats in each group. Duplicates agreed within 5%. # = number of rats in group.

DISCUSSION

The presented data indicate that the injection of ACTH activates the conversion of cholesterol-esters to free cholesterol despite the inhibition of adrenal protein synthesis and steroidogenesis by cycloheximide. It has been known that ACTH stimulates the breakdown of tryglycerides to fatty acids in the fat cell (Engel, 1962; Vaughan, 1961; Jeanrenaud, 1961) and more recently a similar

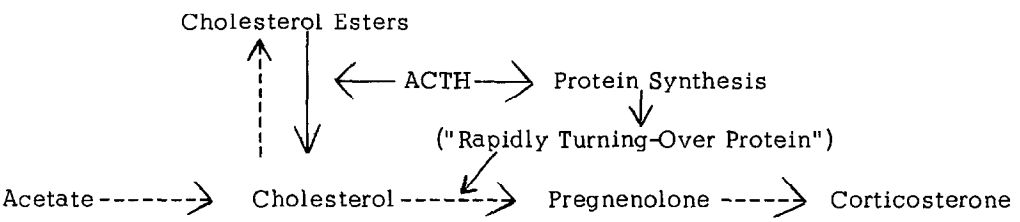
phenomenon was observed in the adrenal (Rudman and Garcia, 1966; Palkovic, Macho, and Mosinger, 1965). Presumably, ACTH activates the hydrolysis of cholesterol esters by a similar mechanism. Indeed, recent experiments have shown that when ACTH is added to rat adrenal slices, an increased activity of cholesterol esterase results (Vaughan, Ursardi, and Steinberg, 1966).

Because it had been known that following ACTH treatment the cholesterol ester fraction was markedly depleted, the possibility was considered that cholesterol esters represented the direct precursors of the steroid hormones. However, this was shown to be unlikely and therefore it was suggested that the cholesterol esters serve as the storage depot for the active substrate of steroidogenesis, the free chol esterol (Goodman, 1965).

Since ACTH or a metabolite which appears in response to the administration of ACTH activates the transformation of cholesterol esters to free cholesterol, the possibility should at least be considered that the mechanism of ACTH action involves the formation of free cholesterol, the concentration of which, in turn, regulates the rate of steroidogenesis. It is unlikely, however, that this explanation can entirely account for the stimulation of steroidogenesis by ACTH for the following reasons: 1. The cholesterol of the blood which is in equilibrium with the adrenal free cholesterol provides a sufficiently large extra-adrenal source of substrate, which could potentially be utilized in steroidogenesis and thus by-pass this proposed site of ACTH action. 2. The findings of Stone and Hechter (1954), and Koritz (1965) that ACTH stimulates the conversion of radioactive cholesterol into corticosterone and that pregnenolone and progesterone are almost maximally converted to corticosterone without the addition of ACTH, strongly indicated that the site of ACTH action was the transformation of cholesterol to pregnenolone. If ACTH acted solely by converting cholesterol esters to cholesterol, the incor-

poration from radioactive cholesterol into the steroid hormone would have been diluted, rather than increased.

Previous studies demonstrated that when adrenal protein synthesis is inhibited *in vitro* (Ferguson, 1963; Farese, 1964) and *in vivo* (Garren, Ney, and Davis, 1965), ACTH action is blocked. Furthermore, the site of the inhibition was shown to occur at the first step in the conversion of cholesterol to pregnenolone, the suggested rate limiting step in the pathway of steroidogenesis (Stone and Hechter, 1954). These studies and the data from the present experiments suggest the following hypothesis to explain the stimulation of steroid hormone biosynthesis by ACTH:



As illustrated above and previously postulated (Garren, *et al.*, 1965), ACTH activates the synthesis of a rapidly turning over protein which acts by stimulating the rate limiting step in steroidogenesis. ACTH also by a mechanism not dependent on protein synthesis regulates the availability of free cholesterol from the cholesterol ester depot. Thus, an ample supply of the active substrate is maintained when steroidgenesis is stimulated.

The presented model in which the mechanism of ACTH action is postulated to involve both enzyme activation and protein synthesis has certain similarities with that proposed for the regulation of numerous biosynthetic pathways in bacteria. Here, it has been shown that a single substance can regulate the biosynthetic pathway by both, directly inhibiting the first enzyme of the pathway (i.e., feedback inhibition), and in conjunction with other cellular factors, repressing protein synthesis (Moyed and Umbarger, 1963).

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