Adrenal Protein Synthesis and Corticosteroid Production in Man[1] (34901)

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An intimate association between adrenal protein synthesis and the ACTH stimulation of corticosteroid production has been suggested on the basis of several careful studies (1, 2). The exact relationship between protein synthesis and hormone action has not been clearly defined, and most of the attempts to study this relationship have been done *in vitro* utilizing adrenals from animal sources. A previous attempt to study the association between protein synthesis and adrenal function in man was done *in vivo* with patients receiving fluorinated pyrimidines as cancer chemotherapy (3). Under the conditions of the study, it was impossible to demonstrate that the fluorinated pyrimidines had an inhibitory effect on adrenal function. Since species differences exist with regard to adrenal function, it was of interest to study the relationship in man between adrenal protein synthesis and corticosteroid production *in vitro*.

Material and Methods. Adrenals were obtained at the time of operation from three patients undergoing bilateral adrenalectomy for Cushing's Disease. The glands were hyperplastic with a combined weight of 15 g in two cases, and 23 g in the other. Histological examination revealed generalized hyperplasia in two instances and cortical nodular hyperplasia in one. The glands were kept in ice immediately after removal and sectioned in a Stadie-Riggs microtome within 30 min.

Approximately 125 mg of adrenal tissue was preincubated in Krebs–Ringer–bicarbonate buffer (KRB) at 37° for 15–30 min. The buffer was changed, 1.0 μCi of ^{14}C-leucine (0.1 m*M* final concentration) was added, and the incubation was carried out for 120 min. Various flasks also contained ACTH 1 U/ml, cyclic AMP 2.5 m*M*, cycloheximide 1.0 m*M*, or testosterone 100 μg/ml. In some instances 0.5-ml aliquots of incubation medium were removed at 30-min intervals. At the end of the incubation, the adrenal tissue was homogenized in 10% TCA with 0.5% ^{12}C-leucine, and the medium along with a subsequent wash was saved for steroid determination.

The adrenal homogenate was successively heated at 90° for 15 min in 5% TCA, solubilized in 1.5 ml of 1.0 *N* NaOH, reprecipitated with 5% TCA, washed with ethyl acetate and ether, resolubilized in 0.5 ml of 1.0 *N* NaOH, spotted on paper discs, and counted in a liquid scintillation counter. The counts were expressed as cpm/mg of adrenal protein. Adrenal protein was determined by the Folin–Lowry method using serum protein as a standard (4).

The steroids were extracted from the incubation medium with methylene chloride. They were then washed with 0.05 *M* NaOH, 0.1 *M* acetic acid, and water, and total 11-hydroxysteroids were determined by the acid fluorescence method, using corticosterone as a standard (5).

For the separate determinations of cortisol and corticosterone, ^{14}C-corticosterone and ^{14}C-cortisol were added to the extraction tubes to measure losses. The steroids were chromatographed in a Bush C system (6) for 3 hr, and eluted in absolute alcohol. The amount of steroid was determined by the acid fluorescence method using cortisol and corticosterone as appropriate standards. Recovery losses were corrected as determined by ^{14}C-cortisol.

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TABLE I. Effect of ACTH, Cycloheximide, and Testosterone on Adrenal Protein Synthesis and Corticosteroid Production.[a]

Group	Amino acid incorporation (cpm/mg of adrenal protein)	Corticosterone (μg/100 mg of adrenal/120-min incubation)	Cortisol (μg/100 mg of adrenal/120-min incubation)
Control	721 ± 89[b]	1.9 ± 0.2	10.4 ± 2.1
ACTH	340 ± 54	3.3 ± 0.6	20.1 ± 1.0
ACTH + cycloheximide	13 ± 1	0.9 ± 0.2	4.5 ± 0.3
Testosterone	121 ± 31	1.1 ± 0.2	2.5 ± 0.8
ACTH + testosterone	81 ± 36	1.1 ± 0.3	2.4 ± 0.6

[a] Slices of human adrenal weighing about 125 mg were preincubated for 15–30 min in KRB at 37° with gaseous phase of 95% O_2/5% CO_2. The adrenal slices were then placed in fresh buffer containing 1.0 μCi of ^{14}C-leucine, with ACTH, 1.0 U/ml; cycloheximide, 1.0 m*M*; and testosterone, 100 μg/ml; where indicated, and the incubation was continued for 120 min. Cortisol and corticosterone production were determined by the acid fluorescence method. The values were expressed as mg of cortisol or corticosterone/100 mg of adrenal/120 min of incubation. The incorporation of ^{14}C-leucine into adrenal protein was expressed as cpm/mg of adrenal protein. The values represent the mean ± SEM of two experiments done in duplicate.

[b] Mean ± SEM.

Results. When ACTH (1 U/ml) was added to incubation medium containing the adrenal slices, there was approximately a 100% increase in cortisol (F) and corticosterone (B) production and about a 50% inhibition of the incorporation of ^{14}C-leucine into adrenal protein (Table I). The addition of 1.0 m*M* cycloheximide not only blocked the ACTH stimulation of cortisol and corticosterone production, but also lowered the amount of corticosteroid produced to below control values. Testosterone (100 μg/ml) inhibited adrenal protein synthesis and blocked the basal production of cortisol and corticosterone, as well as the ACTH stimulation of corticosteroid production.

The effects of cyclic AMP on adrenal steroid production and protein synthesis are shown in Table II. CAMP did not enhance steroid production as did ACTH, but there was an inhibition of adrenal protein synthesis.

Corticosteroid production in adrenal slices during 90 min of incubation is shown in Fig. 1. The values represent both cortisol and corticosterone measured together by acid fluorescence with corticosterone as a standard. However, corticosterone fluoresces more strongly than cortisol, and this obviously affects the interpretation of total 11-hydroxysteroid production (7). ACTH (1 U/ml) stimulated corticosteroid production, and this was blocked by the addition of 1.0 m*M* cycloheximide.

Discussion. Inhibition of human adrenal protein synthesis by cycloheximide resulted in a decrease in the basal level of adrenal steroid production as well as a block in ACTH-stimulated corticosteroid production. Time course studies suggested that cycloheximide was having its effect within 30 min. This inhibitor of protein synthesis also blocked corticosteroid production in the presence of cyclic AMP. It was of interest that cyclic AMP under the conditions of this study did not stimulate corticosteroid production to the same extent as did ACTH.

Ferguson (1) demonstrated that puromycin could inhibit adrenal protein synthesis and block ACTH and cyclic AMP stimulation of corticosterone production in rat adrenals *in vitro*. On the basis of these findings, he suggested that adrenal protein synthesis might be necessary for ACTH responsiveness. Garren *et al.* (2) have presented data to suggest that adrenal steroidogenesis is regulated by a protein with a rapid rate of turnover. Subsequent studies have shown that cyclic AMP appears to play a role in ACTH action as a "second messen-

TABLE II. Effect of Cyclic AMP, Cycloheximide, and Testosterone on Adrenal Protein Synthesis and Corticosteroid Production.[a]

Group	Amino acid incorporation (cpm/mg of adrenal protein)	Corticosterone (μg/100 mg of adrenal/120-min incubation)	Cortisol (μg/100 mg of adrenal/120-min incubation)
Control	836	1.8	7.8
	684	2.1	14.6
CAMP	203	1.9	12.8
	409	2.7	12.1
Cycloheximide	12	0.3	2.8
	11	0.5	5.9
CAMP + cycloheximide	7	0.5	2.7
	7	0.5	2.5
CAMP + testosterone	39	1.4	1.8
	48	1.4	2.5

[a] The experimental conditions were the same as those described in Table I. 3′,5-AMP was added in a final concentration of 2.5 m*M*. The values were expressed as μg of cortisol or corticosterone/100 mg of adrenal/120 min of incubation. The incorporation of ^{14}C-leucine into adrenal protein was expressed as cpm/mg of adrenal protein. The values represent one experiment done in duplicate.

ger" (8) and have suggested the possibility of a specific protein associated with cyclic AMP (9). Whatever the relationship of protein synthesis to ACTH action, the human adrenal appears to be very responsive to inhibitory effects on adrenal protein synthesis. For in the human adrenal, unlike the rat (1), basal corticosteroid production was blocked by inhibitors of protein synthesis. Another difference between rat and human adrenal responsiveness was the lack of corticosteroid stimulation with cyclic AMP. This might reflect differences in membrane permeability in the human adrenal; unfortunately dibutyryl cyclic AMP was not used to test this possibility (10).

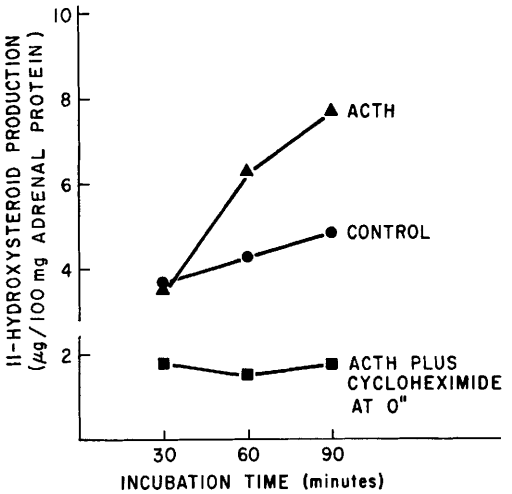

FIG. 1. The experimental conditions were the same as those described in Table I: 0.5-ml aliquots of incubation medium were taken at 30-min intervals during the incubation. Total 11-hydroxysteroid production was measured by the acid fluorescence method, with corticosterone as standard. Each point on the graph represents one experiment done in duplicate.

In addition to stimulating corticosteroid production, the addition of ACTH also inhibited ^{14}C-leucine incorporation into adrenal protein by 53%. Previous studies from this laboratory have suggested that inhibition of adrenal protein synthesis is due to the increased steroid concentration in response to ACTH (11, 12). When testosterone (100 μg/ml) was added in the present study as a model steroid to test this hypothesis, since it was neither a precursor for the corticosteroids nor interfered in their determination, there was a marked inhibition of both adrenal protein synthesis and corticosteroid production. In a previous study with rat adrenals this

inhibition was shown to be due to an effect on both the respiratory chain and on the steroid hydroxylation chain of adrenal mitochondria (13).

There appeared to be no difference in the responsiveness of the adrenal tissue considered to be cortical nodular hyperplasia compared with that interpreted as generalized hyperplasia. Although it should be kept in mind that all the human adrenal tissue used was hyperplastic and may not be representative of normal human adrenals, an *in vitro* association between protein synthesis and ACTH responsiveness could be demonstrated in the human adrenal *in vitro*.

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