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Concerning the Relationship Between Protein Synthesis and Adenosine-3′, 5′-Cyclic Phosphate-Stimulated Steroidogenesis in Isolated Rat Adrenal Cells[1] (38897)

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(Introduced by I. L. Schwartz)

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The possibility that the action of the adrenocorticotropic hormone (ACTH) on the adrenal cortex may involve the stimulation of the synthesis of proteins which regulate steroidogenesis has prompted a number of studies. Using rat adrenal slices, amino acid analogs were found to have no effect on the steroidogenic response to ACTH despite a 20–25% inhibition of protein synthesis (1). Hechter and Halkerston (2) found that puromycin, under proper conditions of concentration and incubation, inhibited protein synthesis with little effect on the stimulation of steroidogenesis by ACTH or adenosine 3′,5′-cyclic phosphate (cAMP). Similar results have been obtained by Kowal using adrenal tumor cells (3). On the other hand, it has been found by Ferguson (4) that puromycin prevented the steroidogenic response to both ACTH and cAMP, and Garren *et al.* (5) showed that cycloheximide given after ACTH stimulation of steroidogenesis in the perfused rat adrenal results in a rapid decrease of the rate of steroidogenesis to basal levels. The conclusion was drawn that a protein, or proteins, with a rapid turnover rate ($t_{½}$ of about 8 min) was involved. It has also been found that the inhibition of steroidogenesis brought about by inhibitors of protein synthesis is located in the conversion of cholesterol to pregnenolone (6), the same site where ACTH and cAMP stimulate steroidogenesis (7, 8).

In this report we have presented the results of an examination of the relationship between cAMP-stimulated steroidogenesis and protein synthesis using isolated adrenal cortical cells. Cyclic AMP rather than ACTH has been used to obviate any possible effects of the inhibitors on adenyl cyclase. The data obtained suggest that the presence of a rapidly turning-over protein, but not the stimulation of its synthesis, is required if cAMP is to effect steroidogenesis.

Methods. Adrenal cells were prepared by the method of Sayers *et al.* (9) and were suspended in Krebs-Ringer bicarbonate medium which contained 0.2% glucose, 0.5% bovine serum albumin, and 0.1% lima bean trypsin inhibitor. The incubation medium contained the indicated additions and about 3×10^5 cells per ml. Unless otherwise noted, in those experiments in which protein synthesis was measured 0.5 μCi of U-^{14}C-L-leucine with a specific activity of 342 mCi per mmole was added per ml of incubation medium.

The experiments were carried out at 37° in silicone-coated beakers in a Dubnoff incubator in an atmosphere of 95% O_2 and 5% CO_2. At the end of the incubation aliquots were extracted with dichloromethane and corticosterone determined as described previously (10). When protein synthesis was determined, the cells were collected at the end of the incubation by centrifugation at 3000*g* for 3 min and washed twice with 0.15 *M* NaCl which contained 2 mg of L-leucine per ml. The cells were resuspended in 0.25 *M* sucrose and an equal volume of 20% trichloroacetic acid added. The incorporation of the ^{14}C-leucine into proteins was determined by the procedures described by Beattie *et al.* (11). Protein was determined by the method of Lowry *et al.* (12).

Results. The data in Table I are representative of experiments which show the effects of cycloheximide and puromycin on both steroidogenesis and protein synthesis in the isolated rat adrenal cells used in this study. At the lower concentrations of the inhibitors

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TABLE I. THE EFFECT OF CYCLOHEXIMIDE AND PUROMYCIN ON PROTEIN SYNTHESIS AND STEROIDOGENESIS IN RAT ADRENAL CELLS.[a]

Inhibitor $M \times 10^6$	Corticosterone nmoles/ml	Corticosterone % decrease	Protein synthesis cpm/μg protein	Protein synthesis % decrease
Cycloheximide				
0	9.9		63	
0.18	8.6	13	42	33
0.36	6.5	34	30	52
0.71	5.6	43	23	64
Puromycin				
0	12.0		31	
2.5	12.0	0	20	35
5.0	9.8	18	15	52
10.0	5.9	51	11	64

[a] The incubation medium contained 5 μmoles of cAMP per ml. The incubation time was 60 min. The data for the two inhibitors were obtained in separate experiments.

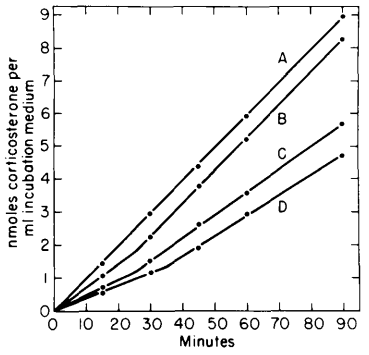

FIG. 1. The time-course of steroidogenesis in the presence of cycloheximide. The incubation medium contained 5 μmoles of cAMP per ml. Curve A, no cycloheximide; curve B, 0.36×10^{-6} *M* cycloheximide; curve C, 1.07×10^{-6} *M* cycloheximide; curve D, 1.78×10^{-6} *M* cycloheximide.

it is seen that the inhibition of protein synthesis is greater than that of steroidogenesis.

By following the time-course of cAMP-stimulated steroidogenesis in the presence of varying concentrations of cycloheximide, it was found (Fig. 1) that the percentage of inhibition of steroidogenesis by cycloheximide decreases with time, and at the lowest concentrations the rate of steroidogenesis, with time, can approximate, or become equal to, that obtained in the absence of the inhibitor. Protein synthesis inhibition, however, continues in a linear manner (Fig. 2). With greater concentrations of the inhibitor, there is an increase in the time required to obtain a decrease in the inhibition of the rate of steroidogenesis. There is also a decrease in the extent of recovery from the effects of the inhibitor and at the highest concentration little or no recovery is observed. These experiments were carried out at saturation levels of cAMP, 5 μmoles per ml. If the amount of cAMP is decreased to

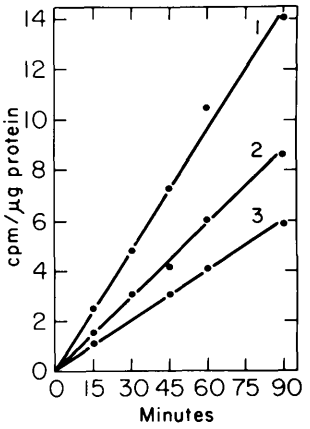

FIG. 2. The effect of cycloheximide on the time-course of protein synthesis. The incubation medium contained 5 μmoles of cAMP per ml. Curve 1, no cycloheximide; curve 2, 0.18×10^{-6} *M* cycloheximide; curve 3, 0.71×10^{-6} *M* cycloheximide. In these experiments 40 μg of leucine per ml of incubation medium was added in addition to 1.0 μCi of ^{14}C-leucine per ml to obtain linearity of protein synthesis for 90 min.

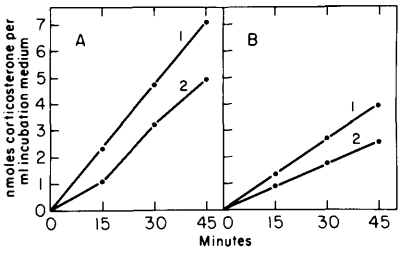

FIG. 3. The effect of puromycin on the time-course of steroidogenesis in the presence of different concentrations of cAMP. A, 5 μmoles cAMP per ml. B, 2 μmoles cAMP per ml. Curve 1, no puromycin; curve 2, 6×10^{-6} *M* puromycin.

a subsaturation level, then the recovery from the effects of a given concentration of puromycin is not observed (Fig. 3).

Discussion. Cells obtained from trypsin-dissociated rat adrenals are derived predominantly from the reticular zone, with no cells from the medullary zone (13). Such a preparation will thus minimize contributions to protein synthesis measurements by nonsteroidogenic cells, an important consideration in the present study.

At lower concentrations of cycloheximide and puromycin the inhibition of protein synthesis is greater than that of steroidogenesis (Table I). These results do not support the hypothesis that cAMP acts by increasing the rate of synthesis of a rapidly turning-over protein (Rt-protein) (14). One would expect that the concentration of a protein with such a short half-life would be rapidly and acutely lowered by inhibitors of protein synthesis and that effects on steroidogenesis would be observed at lower concentrations of inhibitors of protein synthesis, before inhibition of amino acid incorporation into the bulk of proteins is seen. Equally difficult to explain is the recovery of the rate of steroidogenesis with time (Figs. 1 and 3) while the inhibition of protein synthesis persists in a linear manner (Fig. 2).

The recovery in the rate of steroidogenesis with time suggests the accumulation of a substance essential for the stimulation of steroidogenesis by cAMP. The rate of accumulation of this substance, as expressed in the recovery of steroidogenesis, depends both on the concentration of the inhibitor of protein synthesis (Fig. 1) as well as the concentration of cAMP (Fig. 3). A possible explanation consistent with the above data assumes that cAMP affects the transformation of the RT-protein to an active form, perhaps by phosphorylation, which in turn, stimulates the transformation of cholesterol to pregnenolone by any of a number of mechanisms. The half-life of the activated RT-protein must be assumed to be somewhat longer than that of the RT-protein so that it will accumulate under conditions of low concentrations of inhibitors of protein synthesis and high concentrations of cAMP.

Summary. Inhibitors of protein synthesis decrease protein synthesis in isolated rat adrenal cells to a greater extent than they decrease adenosine-3′,5′-cyclic phosphate (cyclic AMP)-stimulated steroidogenesis. At low concentrations of the inhibitors, varying degrees of recovery of the rate of steroidogenesis occurs with increasing time of incubation. As the concentration is increased, the time required to achieve any recovery of the rate of steroidogenesis increases and the extent of recovery decreases. The recovery also depends on the amount of cyclic AMP present. During the process of recovery the inhibition of protein synthesis continues in a linear manner. An explanation consistent with these data involves a rapidly turning-over protein, the concentration of which is independent of the level of cyclic AMP present. However, the extent of transformation of this protein to an active form depends on the concentration of cyclic AMP.

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