

Metabolic Regulation and Relationship of Endogenous Protein Kinase Activity and Steroidogenesis in Isolated Adrenocortical Carcinoma Cells of the Rat¹

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SUMMARY

In the adrenocortical carcinoma cell, in contrast to normal isolated adrenal cells, 10 to 50 μ units of ACTH do not raise the level of adenosine cyclic 3':5'-monophosphate (cyclic AMP), protein kinase activity, and steroidogenesis. This indicates a lesion in the tumor adenylate cyclase system.

Two-tenths to 10 mM cyclic AMP and guanosine cyclic 3':5'-monophosphate (cyclic GMP), which stimulate steroidogenesis in a normal cell, activate protein kinase activity in a concentration-response manner without any detectable rise in steroidogenesis in the adrenocortical carcinoma cell. Cycloheximide and actinomycin D do not inhibit the stimulation of the phosphorylation. These results suggest that the tumor cyclic nucleotide-dependent protein kinase activity is unrelated to steroidogenesis and is also not under the transcriptional or translational control steps.

Curiously, μ M concentrations of cyclic AMP, in contrast to cyclic GMP, stimulate protein kinase activity. In a normal cell, both cyclic AMP and cyclic GMP, in this concentration range, stimulate protein kinase without an increase in steroidogenesis. It is therefore proposed that, in contrast to the normal cell, there is an additional defect in cyclic GMP-dependent protein kinase.

INTRODUCTION

In vitro studies, with isolated adrenocortical carcinoma cells (21) and cultured cells (1) have revealed various biochemical lesions both before and after the events leading to the cleavage of the cholesterol side chain; these metabolic lesions are responsible for the altered but unique ACTH³-controlled system in these cells. These abnormalities have been observed at the level of plasma membrane (14), adenylate cyclase system (1, 11), phosphodiesterase activity (12), and in the steroidogenic biosynthetic transformations of (20S)-20-hydroxycholesterol (19, 20), pregnenolone (5-preg-

nen-3 β -ol-20-one), progesterone (4-pregnen-3,20-dione), and deoxycorticosterone (21-hydroxy-4-pregnen-3,20-dione) (13) to corticosterone (11 β ,21-dihydroxy-4-pregnen-3,20-dione). On the basis of these studies, it was postulated that one of the reasons for the lack of ACTH-stimulated corticosterone synthesis is the defective cyclic AMP-dependent protein kinase system (19, 20). The present investigation was designed to investigate further this property in the intact tumor cells and to relate it to the abnormal ACTH control as mediated by cyclic AMP and cyclic GMP.

MATERIALS AND METHODS

The isolated adrenocortical carcinoma cells of rat were prepared by trypsin digestion (18). The method of incubation with ACTH, cyclic nucleotides, and other agents has been already described (13, 21). In general, for each isolated adrenocortical carcinoma cell preparation, 2.6 g of tumor tissues were used, and the cells, representing 30 to 35 mg of adrenal tumor (approximately 2×10^6 cells), were resuspended in 0.8 ml of Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose.

The incubation experiments, in which corticosterone and cyclic AMP were measured simultaneously, were conducted in sextuplicate; 2 of the samples were used for the determination of corticosterone (6), 2 for the measurement of cyclic AMP, and 2 for the assay of phosphorylation. The experiments in which corticosterone and protein kinase activity were measured, were done in quadruplicate; 2 of the samples were used for the estimation of corticosterone, and the other 2, for the assay of protein kinase activity.

Extraction of cyclic AMP and cyclic GMP was as described previously (18). The assay of cyclic AMP was then accomplished (18) by the method of Gilman (5), using the cyclic AMP-binding protein isolated from bovine kidney (2).

After 1 hr of incubation, the isolated adrenocortical carcinoma cell samples were processed for the estimation of protein kinase activity by the modified techniques (24) developed for measurement in Islets of Langerhans (8) and collagenase-treated adrenal cells (10). After incubation, 2 ml of ice-cold Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 0.2% glucose, were added to the cell suspensions and the tubes were centrifuged at $800 \times g$. To the cell pellet was added 0.45 ml of ice-cold protein kinase buffer (22) containing 50 mM sodium glycerate (pH 6.0), 10 mM magnesium acetate, 20 mM NaF, 0.3 mM ethylene glycol

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³ The abbreviations used are: ACTH, adrenocorticotrophic hormone; cyclic AMP, adenosine cyclic 3':5'-monophosphate; cyclic GMP, guanosine cyclic 3':5'-monophosphate.

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bis(β -aminoethyl ether)- N,N' -tetraacetic acid, and 0.5 mg of histone. The reaction mixture was treated sonically for 10 sec at 0°. [γ - 32 P]ATP (100 μ M; 1 μ Ci) in 0.05 ml protein kinase buffer was then added, and the assays were performed at 37° for 5 min. The reaction was terminated by the addition of 2 ml of 10% trichloroacetic acid, and the samples were processed as previously described (22). Background radioactivity of 32 P, which was measured in the presence of sonically disrupted cells but without other agents, was subtracted for each reaction tube.

RESULTS AND DISCUSSION

It has been proposed (15, 16, 18) that ACTH regulation of steroidogenesis in a normal adrenal cell is mediated by both cyclic AMP and cyclic GMP. It has been further suggested that cyclic AMP-regulated hormonal response is mediated by the holoenzyme, protein kinase (3). This enzyme has been purified and has been shown to be activated by exogenous cyclic AMP (4), according to the mechanism originally proposed for skeletal muscle cyclic AMP-dependent protein kinase (24). According to this scheme, the association of cyclic AMP with the regulatory subunit of the protein kinase dissociates the catalytic subunit of the enzyme which modulates a large number of phosphorylation processes. Walton and Gill (25) have reaffirmed that cyclic AMP-dependent protein kinase phosphorylation leads to ribosomal phosphorylation, and that this is the obligatory step in adrenal steroidogenesis.

The present studies conducted with isolated adrenocortical carcinoma cells show that, in contrast to the stimulatory effect of 10 to 50 μ units of ACTH, in the synthesis of cyclic

AMP and steroidogenesis in the normal cell (18), the hormone does not activate the synthesis of cyclic AMP, protein kinase activity, or corticosterone synthesis in the tumor cell (data not shown). This appears to suggest that the tumor adenylate cyclase, unlike normal cell cyclase, is unresponsive to these concentrations of the hormone. This supports the previous conclusion (1) that the adrenocortical carcinoma cell adenylate cyclase, although stimulated by very high concentrations of ACTH, is not activated by the physiological concentrations of the hormone. This fact alone, however, does not explain the lack of stimulation of corticosterone by ACTH, since exogenous cyclic AMP and cyclic GMP also are unable to stimulate steroidogenesis (21), despite the undetectable levels of phosphodiesterase activity in these cells (16).

Concentrations of cyclic AMP and cyclic GMP, which stimulate corticosterone synthesis in a normal adrenal cell (7), do not activate the onset of steroidogenesis, but they stimulate the protein kinase activity in a typical sigmoid concentration-response manner in a tumor cell (Chart 1, A and B). This signifies the lack of association of the process of phosphorylation with steroidogenesis in the tumor cell. Curiously, concentrations of the nucleotides higher than 5 mM reduced the rate of phosphorylation. This could possibly be due to accelerated phosphatase activity or product inhibition of the kinase enzyme. Cyclic AMP- and cyclic GMP-activated protein kinase is inhibited neither by cycloheximide nor by actinomycin D (Table 1), indicating that the cyclic nucleotide-dependent activation of the kinase is not under transcriptional or translational control.

Of additional significance is the finding that cyclic GMP (1 to 10 μ M) does not stimulate the protein kinase activity,

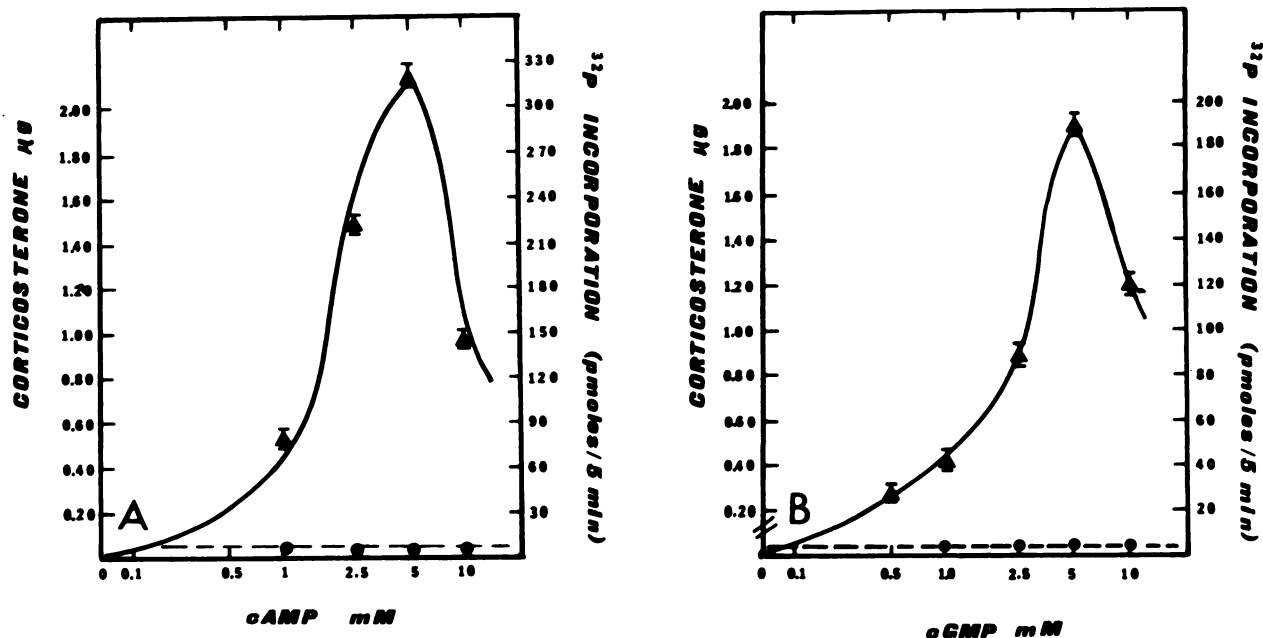


Chart 1. A, corticosterone production (●) and phosphorylation (▲) in isolated adrenocortical carcinoma cells in response to cyclic AMP (mM concentrations). Incubation system: adrenal cell suspension, 0.8 ml; reagents were dissolved in 0.2 ml of Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose, or protein kinase buffer. Total volume of 1st incubation, 1 ml, and of 2nd incubation, 0.5 ml. Results are the average of 6 observations. Basal values have been subtracted from the experimental results. (Average basal value for corticosterone, 0.050 μ g, and for phosphorylation, 395 pmoles). B, corticosterone production (●) and phosphorylation (▲) in isolated adrenocortical carcinoma cells in response to cyclic GMP (mM concentrations). The conditions of the experiment are identical to those described in A.

Table 1

Effect of cycloheximide and actinomycin D on cyclic AMP- and cyclic GMP-induced phosphorylation in adrenocortical carcinoma cells

Incubation system: adrenal cell suspension 0.8 ml; reagents dissolved in 0.2 ml of Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose, or protein kinase buffer. Total volume of 1st incubation, 1 ml, and of 2nd incubation, 0.5 ml. Results are the average of 6 observations. Basal values have been subtracted from the experimental results. No stimulation of steroidogenesis was observed in any of the experiments. The average basal value of corticosterone was 0.05 μg /incubation tube and, for phosphorylation, 395 pmoles.

Cyclic nucleotides (mM)	^{32}P incorporation (pmoles/5 min)					
	Cyclic AMP			Cyclic GMP		
	Control	+ cycloheximide	+ actinomycin D	Control	+ cycloheximide	+ actinomycin C
0.5				27	23	24
1.0	80	69	72	42	37	50
2.5	222	216	218	88	104	94
5.0	320	339	337	189	182	194
10	145	135	138	121	127	115

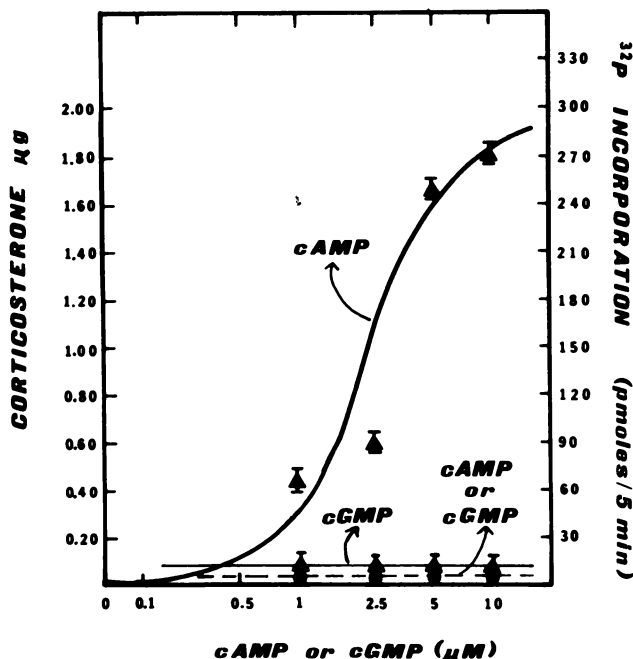


Chart 2. Corticosterone production and phosphorylation in isolated adrenocortical carcinoma cells in response to cyclic AMP and cyclic GMP (μM concentrations). The conditions of the experiment are identical to those described for Chart 1A.

whereas, cyclic AMP is quite effective (Chart 2) in doing so. This again is in contrast to the normal adrenal cell, where both the cyclic nucleotides in this concentration range stimulate the phosphorylation but do not stimulate steroidogenesis (17). This indicates an additional defect in cyclic GMP-dependent tumor protein kinase. It is noteworthy that, in these concentrations of the cyclic nucleotides, the process of phosphorylation does not result in steroidogenesis in a normal adrenal cell.

The results shown herein and elsewhere (1, 13, 14, 19-21) further reaffirm the fact that adrenocortical carcinoma 494 (23) is not unresponsive to cyclic AMP and ACTH as originally proposed (9). Rather, the tumor possesses the cyclic nucleotide-sensitive control steps which are uniquely differ-

ent from that of normal tissue. The techniques described demonstrate a means of elucidating these systems.

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