METABOLIC REGULATION OF STEROIDOGENESIS IN ADRENOCORTICAL CARCINOMA CELLS OF RAT. EFFECT OF ADRENOCORTICOTROPIN AND ADENOSINE CYCLIC 3',5'-MONOPHOSPHATE ON THE INCORPORATION OF (20S)-20-HYDROXY[7α-^{3}H]CHOLESTEROL INTO DEOXYCORTICOSTERONE AND CORTICOSTERONE.

Rameshwar K. Sharma and James S. Brush
With the Technical Assistance of Lynda Sutliff
Department of Biochemistry
University of Tennessee
Medical Units
Memphis, Tennessee 38163

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Summary: ACTH in the isolated adrenocortical carcinoma cell inhibits the incorporation of (20S)-20-hydroxycholesterol into deoxycorticosterone and corticosterone. These results are in direct contrast to those obtained with the normal isolated adrenal cell where it has been shown that ACTH stimulates the synthesis of deoxycorticosterone and corticosterone from the above precursor. N^{6}-$O^{2'}$-dibutyryl adenosine 3',5'-monophosphate, the nucleotide, which does not stimulate steroidogenesis from (20S)-20-hydroxycholesterol in a normal adrenal cell, inhibits its incorporation into deoxycorticosterone and corticosterone. The results presented further demonstrate the uniqueness of the ACTH-sensitive control system in the tumor.

Introduction

Studies with isolated adrenocortical carcinoma 494 cells have investigated various aspects of the markedly altered control of steroidogenesis by ACTH[1] and cAMP (1-5). These biochemical abnormalities have been localized in the reactions both before and after the cleavage of the cholesterol side chain.

The chemical methods have demonstrated in the adrenal

Trivial Names and Abbreviations
ACTH, adrenocorticotropic hormone; cAMP, adenosine cyclic 3',5'-monophosphate; Bt_2cAMP, N^{6}-2'O-dibutyryl adenosine 3',5'-monophosphate; pregnenolone, 5-pregnen-3β-ol-20-one; corticosterone, 11β,21-dihydroxy-4-pregnen-3,20-dione; deoxycorticosterone, 21-hydroxy-4-pregnen-3,20-dione.

tumor an ACTH- and cAMP-insensitive conversion of endogenous precursor (presumably cholesterol) to corticosterone (1). More precise radioactive labeling methods have indicated that the hormone and cAMP insensitivity are probably due to the loss of the cycloheximide-inhibited part of the system which converts cholesterol to (20S)-20-hydroxycholesterol in the normal adrenal cell (6). It has been further demonstrated, that although the tumor cells have the capacity to synthesize corticosterone from exogenous pregnenolone, progesterone and deoxycorticosterone, the incorporation of pregnenolone and progesterone is inhibited by ACTH (3), whereas the incorporation of exogenous deoxycorticosterone into corticosterone is stimulated. This latter observation has been related to an effect of the hormone upon transport mechanisms carrying deoxycorticosterone into the cell (5). The pattern of inhibition by cAMP of the incorporation exogenous pregnenolone and progesterone into corticosterone was different from that of ACTH (3). Furthermore, in direct contrast to the ACTH effect, Bt_2cAMP was devoid of any stimulatory effect on the incorporation of deoxycorticosterone into corticosterone in adrenal tumor cells (5).

In continuation of the above studies upon the alteration in control processes in the tumor, the effect of ACTH and Bt_2cAMP on the incorporation of (20S)-20-hydroxycholesterol into deoxycorticosterone and corticosterone has been studied and is reported herein.

Materials and Methods

The isolated adrenocortical carcinoma 494 cells were prepared by trypsin digestion (1). The method of incuba-

tion of ACTH or Bt_2cAMP with (20S)-20-hyroxy[7α-^{3}H]cholesterol was as previously described (6): Incubation was carried out in Teflon flasks. Each flask contained 20 ml of suspended isolated adrenal tumor cells prepared from 1.5 g adrenal tumor tissue. In addition to the appropriate amount of suspended tumor cells, each flask contained 1.60 μCi of (20S)-20-hydroxy[7α-^{3}H]cholesterol and ACTH (200 microunits per ml) or Bt_2cAMP (1 mM). The incubation was conducted for 150 min and the reaction was stopped by the addition of 15 ml of distilled water and 75 ml of methylene chloride to each flask. To the reaction mixture in each flask 15 mg of deoxycorticosterone and 15 mg of corticosterone were added and the products processed identically.

Deoxycorticosterone and corticosterone were purified by thin-layer chromatograghy (3,6). The isolated deoxycorticosterone was acetylated (3,7), further purified by thin-layer chromatography (3,6) and crystallized from acetone-n-hexane to constant specific activity. The purified corticosterone was crystallized from acetone-ligroin until the specific activity was constant.

ACTH, a United States Pharmacopeia standard, was purchased from United States Pharmacopeia. (20S)-20-Hydroxy[7α-^{3}H]cholesterol (specific activity, 25 Ci per mmole) was purchased from New England Nuclear, Boston, Mass.

Results and Discussion

It has been well documented, from the studies of various laboratories, that ACTH in the adrenal cell, stimulates the rate limiting step which consists of the

conversion of cholesterol to pregnenolone (8-10). That this stimulation is mediated by cAMP has been shown (8,11, 12) and supported by this laboratory (3,13,14). Recent studies (6,15) have led to the modification of the hypothesis of Garren, et al. (16), regarding the mode of ACTH action. According to this scheme, ACTH stimulation in the normal isolated adrenal cell is both dependent and independent of cAMP. It has been proposed that ACTH stimulates two biosynthetic steps in the conversion of cholesterol to pregnenolone. The first step consists in the conversion of cholesterol to (20S)-20-hydroxycholesterol and is cycloheximide sensitive; the second step stimulates the synthesis of pregnenolone from (20S)-20-hydroxycholesterol which is cycloheximide insensitive. The latter effect of ACTH is not mediated by cAMP.

The present studies conducted with the isolated adrenocortical carcinoma cells show that in contrast to the stimulatory effect of ACTH in the transformation of (20S)-20-hydroxycholesterol into deoxycorticosterone and corticosterone in the normal adrenal cell (6), the hormone inhibits the incorporation of (20S)-20-hydroxycholesterol into deoxycorticosterone and corticosterone (Table I) in the tumor cell. Bt_2cAMP, which has been shown to be devoid of any stimulatory activity in the transformation of (20S)-20-hydroxycholesterol into steroid hormones in normal adrenal cell, also inhibits the incorporation of the above precursor into deoxycorticosterone and corticosterone in adrenocortical carcinoma cells (Table II). These results appear to suggest that this is an additional biochemical alteration in the control by ACTH and cAMP in the adrenal tumor.

Table I

Effect of ACTH on transformation of (20S)-20-hydroxy[7α-^{3}H]-cholesterol into deoxycorticosterone and corticosterone in isolated adrenocortical carcinoma cell preparation.

The total ^{3}H-disintegrations per min of the products (and their derivatives) obtained after the incubation of (20S)-20-hydroxy[7α-^{3}H]cholesterol with isolated adrenal cells. Incubation was carried out in Teflon flasks containing 20 ml of isolated adrenocortical carcinoma cell preparation as mentioned under "Experimental Procedure". Each flask contained (20S)-20-hydroxy[7α-^{3}H]cholesterol (1·60 μCi) and ACTH (200 microunits per ml) or Bt_2cAMP(1 mM). The incubation was for 2.5 hours and the reaction was stopped by the addition of 75 ml of methylene chloride to each flask. Deoxycorticosterone and corticosterone were isolated as described under "Experimental Procedure".

Compound	Crystallization	^{3}H dpm of compound from Control	+ACTH
Deoxycorticosterone acetate	Crude Product	794,000	373,000
	1st	786,000	362,000
Corticosterone	1st	200,000	98,000
	2nd	183,000	87,000
	3rd	176,000	77,000

In view of these observations, coupled with earlier ones (2,3,4), it is evident that control by ACTH and cAMP of cholesterol side chain hydroxylation and subsequent conversion to pregnenolone is highly modified in

Table II

Effect of Bt_2cAMP on transformation of (20S)-20-hydroxy[7α-^{3}H]-cholesterol into deoxycorticosterone and corticosterone in isolated adrenocortical carcinoma cell preparation.

The total ^{3}H-disintegrations per min of the products (and their derivatives) obtained after the incubation of (20S)-20-hydroxy[7α-^{3}H]cholesterol with isolated adrenal cells. Conditions of the experiment were similar to the experiment in Table I.

Compound	Crystallization	^{3}H dpm of compound from Control	^{3}H dpm of compound from +Bt_2cAMP
Deoxycorticosterone acetate	Crude Product	674,000	133,000
	1st	668,000	132,000
Corticosterone	1st	170,000	31,000
	2nd	155,000	29,000
	3rd	141,000	27,000

the tumor. By elimination this suggests that the rate limiting enzyme(s) which converts cholesterol to (20S)-20-hydroxycholesterol (20-hydroxylase) may be, (1) missing, (2) present but not stimulated because of a defective activating protein for the process, (3) present but not stimulated because of the lack of synthesis of the activating protein. Of these possibilities it is thought the first one is unlikely for the reason that the (20S)-20-hydroxycholesterol side chain cleavage enzyme(s) and the 11β-hydroxylase are present and it is probable, therefore, that the 20-hydroxylase is also present. Based on the

premises that a cAMP-dependent protein kinase is involved in hormonal action, these studies suggest that the modification in the tumor may be in one of the following factors: a) cAMP binds to the regulatory subunit of the protein kinase but does not dissociate the catalytic subunit or b) cAMP dissociates the catalytic subunit of the protein kinase but the latter does not stimulate the ATP-dependent phosphorylation of ribosomes which in turn results in the lack of the translation of mRNA.

The results shown here and elsewhere (1-5) demonstrate that adrenocortical carcinoma is not unresponsive to cAMP and ACTH as originally proposed (17). Rather the tumor possesses an ACTH-sensitive control system which is uniquely different from that of normal tissue. The techniques described demonstrate a means of elucidating these systems.

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