Guanylic Triphosphate-sensitive Adenylate Cyclase of Adrenocorticotrophic Hormone- and Prostaglandin-resistant Human Adrenocortical Tumors

Guy P. Tell, Anne-Marie Cathiard, and José M. Saez

Unité de Recherches sur le Contrôle Hormonal des Activités Cellulaires, INSERM, U. 162, Hôpital Debrousse, 29 Rue Soeur Bouvier, 69322 Lyon Cedex 1, France

ABSTRACT

The guanylic triphosphate (GTP)-sensitive adenylate cyclase was studied in the crude membranes of three adrenocorticotrophic hormone (ACTH)-resistant tumors and one prostaglandin E, (PGEi)-resistant human adrenocortical tumor. In the ACTH-resistant tumors, the steroidogenesis of isolated adrenocortical cells was not stimulated by ACTH, but was enhanced by PGEi, whereas in the PGEi-insensitive tumor, PGEi had no effect but ACTH stimulated steroidogenesis. The cortisol production of cells from all four tumors was stimulated by N6,O2-dibutyryl cyclic adenosine 3':5'-monophosphate. Binding studies with labeled ACTH, ACTH1-24, and PGEi revealed that the hormone resistance was associated with a selective alteration of the ACTH or the PGEi receptor. The adenylate cyclase activity of ACTH-resistant tumor membranes was not stimulated by ACTH but was normally stimulated by PGEi. Conversely, in the PGEi-insensitive tumor, the enzyme activity was not enhanced by PGEi, but was normally increased by ACTH1-24. In all four tumors, the adenylate cyclase activity was normal regarding basal levels and NaF stimulation. In addition, 5'-guanylylimidodiphosphate and, to a lesser extent, GTP and 5'-guanylylmethylenediphosphonate were effective in stimulating the basal adenylate cyclase activity of the four tumors and in enhancing the hormone effect on the intact hormone receptor complex (i.e., the PGEi receptor of the ACTH-resistant tumors and the ACTH receptor of the PGEi-resistant tumor). Our results are thus consistent with the fact that, in human adrenocortical tumors, the ACTH and PGEi receptors can be independently affected, the adenylate cyclase catalytic subunit and the GTP-sensitive subunit being unaltered by this selective tumoral process. This suggests that the GTP-sensitive subunit may be more closely related to the catalytic subunit of the enzyme than to the hormone-binding site.

INTRODUCTION

cAMP4 plays an important role in the control of normal cell growth, and alterations in many steps of its metabolism (synthesis, degradation, activation of protein kinases) have been associated with cancer (1, 13). In the case of cells in which cAMP is the second messenger of hormone action, cAMP synthesis is regulated by the activation of a membrane-bound hormone receptor-adenylate cyclase complex (14), the integrity of which is essential to the normal cell metabolism. In normal cells, the receptor complex is composed of at least 2 subunits, i.e., the hormone-binding site and the catalytic subunit of adenylate cyclase (15) and of a putative GTP-sensitive transmission system that would convey the information from hormone-binding site to catalytic subunit (9). The loss or the defect of hormonal receptors has been demonstrated in a number of malignant cultured cells of animal (12, 21) and human (6) origin and probable alterations of the adenylate cyclase catalytic site discussed for various malignant or transformed cell lines (1). Recently, more attention has been given to the guanylnucleotide GTP, which seems to have an important regulatory function in the transmission of the hormonal message (16), and studies with labeled GTP or GTP derivatives have shown that there is a specific GTP-binding site in plasma membranes from adipocytes and hepatocytes (20), solubilized myocardium (8), and bovine adrenal cortex (4). In our laboratory, the ACTH receptor complex of normal and tumoral human adrenal cortex has been under investigation for several years and has proven to be a good model for studying the possible architecture and alterations of the ACTH-binding site (17, 18). Since GTP plays a regulatory function in the normal mammalian ACTH receptor complex of the adrenal cortex (4, 5), we have examined the adenylate cyclase sensitivity to GTP and GTP derivatives in 4 human adrenocortical tumors that are characterized by a selective resistance to ACTH or to prostaglandin stimulation, the defects being related to anomalies of the specific hormone receptors. Our purpose was to determine: (a) whether the adenylate cyclase resistance to hormone stimulation was total or only relative, a minimal sensitivity being then revealed by the use of guanylnucleotides; and (b) whether the alteration of 1 hormone receptor would be accompanied by any anomaly of the GTP-sensitive adenylate cyclase of the cell membrane.
MATERIALS AND METHODS

Materials. [α-32P]ATP, [3H]cAMP, and 125I were purchased from the Radiochemical Centre, Amersham, England. Pyruvate kinase, phosphoenolpyruvate, theophylline, ATP, GTP, (-)-isoproterenol, glucagon, and dbcAMP were from Sigma Chemical Co., St. Louis, Mo. Synthetic ACTH, and ACTH were kindly given by Dr. W. Rittel from Sigma Chemical Co., St. Louis, Mo. Synthetic ATP, GTP, (-)-isoproterenol, glucagon, and dbcAMP were chased from the Radiochemical Centre, Amersham, Eng. beled ACTH, and ACTH, and PGE, and (c) basal, NaF-zoo, Mich. Human chorionic gonadotrophin was from Ser- and PGE, by was given Dr. J. E. Pike, Upjohn Co., Kalama- and Dr. P. A. Desaulles, Ciba-Geigy AG, Basel, Switzerland, land. Pyruvate kinase, phosphoenolpyruvate, theophylline, ACTH, and ACTH, were labeled, and their binding and degradation measured according to previously published methods (18). The binding of [3H]PGE, to the same mem- branes was performed as described (3).

Cortisol Production by Isolated Cells. Methods for isolating adrenal cells and for measuring cortisol production have been reported elsewhere (17).

Adenylate Cyclase Assay. Adenylate cyclase was as- sayed essentially as already described (23). In the present conditions, the incubation mixture (0.1 ml) contained 25 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 0.1 mM MnCl2, 8 mM theophylline, 0.1% (w/v) albumin, 2 to 3 x 10⁴ cpm [α- 32P]ATP (0.5 mM ATP), and 50 to 100 μg membrane proteins; 5 mM phosphoenolpyruvate and pyruvate kinase (60 μg/ml) were used to regenerate ATP. After 10 min at 30°, the reaction was stopped by boiling for 3 min. The enzyme activity was linear during the incubation time. After addition of 15 x 10⁶ cpm [3H]AMP (30 Ci/mmol) to each sample, cAMP was isolated on a column containing 1 g of alumina (neutral) that was eluted with 2.0 ml of 25 mM Tris-HCl (pH 7.5).

RESULTS

Among the adrenal tumors studied in our laboratory, 4 were selected for the investigation of the GTP regulatory role. Criteria for selection were based upon: (a) cortisol production by isolated cells under basal and stimulatory (ACTH, PGE, dbcAMP) conditions; (b) binding of la- belled ACTH, ACTH, and PGE; and (c) basal, NaF-stimulated, and hormone-stimulated adenylate cyclase activities of membrane preparations.

Cortisol Production by Isolated Cells

Maximal stimulation of cortisol production by isolated cells of the normal human adrenal cortex was obtained with about 10⁻⁶ M ACTH, 8 x 10⁻⁸ M PGE, and 10⁻³ M dbcAMP (Chart 1).

In the first 3 tumors (Table 1), the production of cortisol by isolated cells was not stimulated by concentrations of ACTH, up to 10⁻⁶ M, whereas significant responses were observed with 3 x 10⁻⁶ M PGE, and 10⁻³ M dbcAMP.

Binding of ACTH, and ACTH, to Adrenocortical Membranes

Results obtained with the first 3 tumors (Table 2) extend to a 20,000 x g pellet of homogenized adrenal cortex (3).

<table>
<thead>
<tr>
<th>Membranes</th>
<th>125I-ACTH</th>
<th>125I-ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adrenal</td>
<td>0.42 ± 0.08</td>
<td>4.1 ± 1.6</td>
</tr>
<tr>
<td>Tumor 1</td>
<td>3.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Tumor 2</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Tumor 3</td>
<td>5.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Tumor 4</td>
<td>0.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

a Mean ± S.D. of triplicate replications.

Table 2

Apparent dissociation constants of ACTH, and ACTH, to adrenocortical membranes

Values for tumors represent the mean of 3 determinations.
our previous observation (17) that in some adrenocortical tumors the binding affinity of ACTH\(_{1-24}\) for its receptor is about 10 times lower than that of the normal gland and very similar to the binding affinity of the ACTH\(_{1-24}\) fragment, this modification reflecting an alteration of the ACTH-binding site (17). Alternatively, binding studies with the fourth tumor (Table 2) revealed that, in this last case, the binding affinity of ACTH\(_{1-24}\) was similar to that of the normal adrenal membranes.

The differences observed for the dissociation constants of the first 3 tumors and the normal adrenal are possibly not related to an increased degradation of ACTH\(_{1-24}\) by tumor membranes, since degradation of unbound \(^{125}\)I-ACTH\(_{1-24}\) was less marked in Tumors 1 and 2 than in normal adrenals and in Tumor 4 (Table 3). In all tumors, bound \(^{125}\)I-ACTH was protected from degradation (data not shown), confirming our previous observations with normal and tumoral adrenals (17, 18).

**Binding of \(^{[3]H}\)PGE, to Adrenocortical Membranes**

The dissociation constants of PGE, for the first 3 tumors ranged from 2 to 6 \(\times 10^{-8}\) M; these values being similar to those obtained with the normal adrenal (3). On the contrary, no specific binding of PGE, was observed with crude membranes prepared from Tumor 4.

Binding studies with ACTH fragments and PGE, thus confirm that Tumors 1, 2, and 3 have an abnormal ACTH receptor but a normal PGE, receptor whereas Tumor 4 has an altered PGE, receptor, the ACTH receptor having normal characteristics.

Adenylate cyclase sensitivity to NaF, hormones, and GTP and its derivatives was then studied in the same membrane material from all 4 tumors.

**Basal, NaF-stimulated, and Hormone-stimulated Adenylate Cyclase Activities**

Basal and 10 mM NaF-stimulated adenylate cyclase activities were different in normal and tumoral membranes (Table 4). Results presented in Table 3 also confirm the predictable adenylate cyclase insensitivity to ACTH of Tumors 1, 2, and 3 and the PGE, resistance of Tumor 4.

**ACTH-insensitive Tumor Adenylate Cyclase**

**Guanylnucleotides and ACTH Effects.** The effects of GTP and of its 2 derivatives Gpp(NH)p and Gpp(CH)\(_2\)p, which are potent stimulants of adenylate cyclase activity (2, 7, 10), were measured in the absence and in the presence of 10\(^{-8}\) to 10\(^{-5}\) M ACTH\(_{1-24}\) in membranes of normal adrenal (Chart 2, left) and of Tumors 1, 2, and 3 (Chart 2, right). The normal human adrenocortical tissue is sensitive to guanylnucleotides, and Gpp(NH)p is the most potent in enhancing both basal and ACTH-stimulated adenylate cyclase (Chart 2). Similar results have been obtained with bovine and rat adrenals (5). In contrast, adenylate cyclase of the first 3 adrenocortical tumors is not stimulated by ACTH\(_{1-24}\); however, GTP and its derivatives are effective in stimulating the basal activity of the enzyme but no further stimulation is observed in the presence of ACTH\(_{1-24}\) (Chart 2, right). These results indicate that no effect of ACTH can be revealed by guanylnucleotides and that, although the ACTH receptor is impaired, the adenylate cyclase activity (basal) remains sensitive to GTP and its derivatives. The latter observation has also been extended to the prostaglandin receptor-adenylate cyclase.

**Guanylnucleotides and PGE, Effects.** The adenylate cyclase activities of membranes of the normal adrenal and of Tumors 1, 2, and 3 were measured in the absence and in the presence of PGE, (1 and 10 \(\mu\)g/ml), with or without 10\(^{-5}\) M GTP, Gpp(CH)\(_2\)p, and Gpp(NH)p. In the normal gland all
3 nucleotides enhanced basal and PGE-stimulated enzyme activities (Chart 3, left). Essentially the same results were obtained with membranes of Tumors 1, 2, and 3 (Chart 3, right), Gpp(NH)p being the most potent of the 3 guanylnucleotides. These results together with the data that PGE binding was normal in these tumors (see above) indicate that the PGE receptor complex and its related GTP-sensitive adenylate cyclase are not affected by the tumoral process.

PGE-insensitive Tumor Adenylate Cyclase: Guanylnucleotides, ACTH, and PGE, Effects

The main effects of GTP and its derivatives, ACTH and PGE, on the adenylate cyclase activity of membranes of Tumor 4 are shown in Table 5. The results are opposite to those observed with Tumors 1, 2, and 3; i.e., no stimulation is observed with PGE, even when the enzyme activity has been enhanced by guanylnucleotides, whereas the responses to ACTH and/or GTP and its derivatives are in the normal range. Since basal (Tables 4 and 5) and NaF-stimulated (Table 4) adenylate cyclase activities of Tumor 4 were normal, it can be concluded that, in this PGE-insensitive tumor, only the PGE receptor complex is altered, the ACTH receptor-adenylate cyclase being essentially unaffected.

Tumor Adenylate Cyclase and Other Hormones

Because it is known that some adrenocortical tumors have ectopic hormone receptors that are not present in the normal gland (22, 24), we have tested the effects of various hormones on the adenylate cyclase activity of the 4 tumors. No effects were obtained with $10^{-6}$ M glucagon, $10^{-4}$ M (−)-isoproterenol, $10^{-7}$ M human chorionic gonadotrophin, and insulin (100 to 1000 microunits/ml) whether or not GTP or its derivatives were present in the incubation media (data not shown).

DISCUSSION

Our results essentially indicate that, in human adrenocortical tumors characterized by a selective resistance to ACTH or to PGE, stimulation: (a) the insensitivity of adenylate cyclase to ACTH or to PGE, is total and cannot be reversed by GTP and its derivatives (Chart 2, right; Table 5); (b) the alteration of the ACTH or the PGE, receptor is not accompanied by any evident modifications of the GTP-sensitive adenylate cyclase. This conclusion can be drawn from the following arguments: (a) in the absence of hormones, the adenylate cyclase activity of the tumor membranes was stimulated by GTP, Gpp(CH)₂p, and Gpp(NH)p (Charts 2 and 3; Table 5); each of the guanylnucleotides had been tested at concentrations ranging from $10^{-8}$ to $10^{-5}$ M, and no difference in their effects could be noted between normal tissue and tumors (data not shown); (b) in the ACTH-resistant tumors, the PGE, receptor and its related GTP-sensitive adenylate cyclase were normal (Chart 3); (c) the reverse was true for the PGE, resistant tumor (Tables 4 and 5). All these data are consistent with the fact that ACTH and PGE, receptors of the adrenal gland can be independently affected, and they confirm our previous observation that these receptors are discrete components of the adrenal cell membrane (17).

In addition, since the basal adenylate cyclase activity and the responses to NaF (Table 3) and to GTP and its derivatives (Charts 2 and 3; Table 5) were not lower than in the normal gland it is tempting to speculate that in these tumors, the GTP-sensitive adenylate cyclase may be more closely associated with the enzyme catalytic subunit than to the hormone-binding site. This also would be in agreement with the finding that, in normal myocardium membranes, the GTP-binding site is found in the solubilized fraction that contains the catalytic subunit of adenylate cyclase and not in the one that contains the hormone-binding sites (8).

Alternatively, this study does not exclude the possibility that, in addition to the observed alterations of the hormone receptors of the adrenal tumors, there may also be modifications of the intracellular steroidogenic pathway such as those described for the enzymatic pattern of some human adrenocortical tumors (19). This could explain our finding that the cortisol production was stimulated by dbcAMP and PGE, to a lesser extent in Tumors 1, 2, and 3 than in the normal gland (Table 1).

REFERENCES


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