Studies on Adrenocortical Carcinoma of Rat Cyclic Nucleotide Phosphodiesterase Activities

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SUMMARY

Cyclic 3',5'-nucleotide phosphodiesterase activities of the homogenates of normal adrenal and adrenocortical carcinoma tissues of the rat have been measured. The rates of hydrolysis of cyclic adenosine 3',5'-monophosphate (cAMP), cyclic guanosine 3',5'-monophosphate, cyclic inosine 3',5'-monophosphate, cyclic uridine 3',5'-monophosphate, cyclic cytidine 3',5'-monophosphate, and cyclic thymidine 3',5'-monophosphate by the homogenates of normal rat adrenal were considerably higher than those shown by the homogenates of the adrenocortical carcinoma of the rat. A multiplicity of Km's for cAMP phosphodiesterase enzymes was not observed in either the normal rat adrenal or in the adrenocortical tumor. The Km values for cAMP phosphodiesterases of normal adrenal and adrenocortical carcinoma 494 were 23.9 and 17.8 μM, respectively. These results suggest that lack of corticosteroidogenic response to adrenocorticotropic in the tumor may not be explained on the basis of its phosphodiesterase activity but may be at a site beyond the formation of cAMP.

INTRODUCTION

We have recently reported an isolated adrenocortical carcinoma 494 cell preparation that is useful for study of the abnormalities in control mechanisms of steroidogenesis in isolated target tissue (14). In contrast to the isolated cells prepared from the adrenal gland (6, 12), it was demonstrated that the tumor cells are not stimulated for corticosteroidogenesis by ACTH.2 cAMP, N6-2'O-dibutyryladenosine 3',5'-monophosphate, cyclic inosine 3',5'-monophosphate, or cGMP (14). This is in spite of the fact that the adenyl cyclase activity in response to ACTH is higher in the tumor than in the normal adrenal (13). For a better understanding of the control mechanisms of ACTH or cAMP for the lack of corticosteroidogenesis, the phosphodiesterase activities of the adrenals of normal rat and adrenocortical carcinoma of the rat were determined. These studies suggest that the lack of steroidogenesis to ACTH or cAMP in the tumor cells cannot be explained on the basis of phosphodiesterase activities of the tissue.

MATERIALS AND METHODS

Animals. Adrenocortical carcinoma 494, a spontaneously occurring tumor discovered in Osborne-Mendel rats by Snell and Stewart (15), has been maintained in our laboratories by i.p. and s.c. transplantation in Sprague-Dawley rats at 3 to 4 weeks of age. Of the rats receiving implantation, 60 to 70% successfully developed the tumor.

Chemicals. cAMP-3H was purchased from New England Nuclear, Boston, Mass. Nonradioactive cyclic nucleotides and Crotalus atrox snake venom were purchased from the Sigma Chemical Company, St. Louis, Mo., and Boehringer-Mannheim, New York, N.Y. All other chemicals were purchased commercially and were of analytical reagent grade.

Cyclic 3',5'-Nucleotide Phosphodiesterase. Cyclic 3',5'-nucleotide phosphodiesterase was obtained from the rat adrenal tumor tissue and normal rat adrenal by the method of Cheung (3). The tissues were homogenized in 5 volumes of ice-cold, glass-distilled water, sonically dispersed, and then centrifuged at 30,000 X g for 30 min. The supernatant solution was then dialyzed against 20 mM Tris-HCl, pH 7.5, for 48 hr at 4°. This enzymic preparation was stored at −20° in 1-ml aliquots. Once the aliquots were thawed for assay, the remainder of the enzyme preparation was rejected.

Assay of Cyclic 3',5'-Nucleotide Phosphodiesterase Activities. Rate of hydrolysis of various cyclic nucleotides was measured by the method of Butcher and Sutherland (2) as modified by Cheung (3), and the Km values of the phosphodiesterase preparations for cAMP were evaluated by the modified radioactively labeled method of Loten and Sneyd (8), substituting QAE Sephadex (Cl−) for Bio-Rad ACl-X2 Cl− column in the procedure for the isolation of adenosine.

The assay mixture for phosphodiesterase activities consisted of 40 mM Tris-HCl (pH 7.5), 1.8 mM MgSO4, 2 mM cNMP, and crude cyclic phosphodiesterase in a total volume of 0.5 ml.

The reaction was started by the addition of cNMP. Incubation was carried out at 30° for 60 min. After 50 min, 0.5 mg of Crotalus atrox snake venom in 0.05 ml of 10 mM Tris-HCl (pH 7.5) was added to convert the 5'-nucleotide produced to nucleoside, and the reaction mixture was incubated further for 10 min. For every nucleotide, a control tube without phosphodiesterase was included to correct for

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1 This work was supported by Veterans Administration Institutional grants.
2 The abbreviations used are: ACTH, adrenocorticotropic; cAMP, cyclic adenosine 3',5'-monophosphate; cGMP, cyclic guanosine 3',5'-monophosphate; cNMP, cyclic mononucleotide.

Received February 8, 1972; accepted May 1, 1972.
Table 1
Hydrolysis of cyclic nucleotides by adrenal homogenates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Normal rat adrenal</th>
<th>Rat adrenal tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>146 ± 3</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>cGMP</td>
<td>160 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>cCMP</td>
<td>145 ± 7</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>cUMP</td>
<td>15 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>cIMP</td>
<td>25 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>cTMP</td>
<td>33 ± 1</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

b cIMP, cyclic inosine 3',5'-monophosphate; cUMP, cyclic uridine 3',5'-monophosphate; cCMP, cyclic cytidine 3',5'-monophosphate; cTMP, cyclic thymidine 3',5'-monophosphate.

trace hydrolysis of cNMP by snake venom. The reaction was terminated by the addition of 0.05 ml of 55% chilled trichloroacetic acid. The reaction mixtures were centrifuged, and the supernatants were assayed for inorganic phosphate by the method of Fiske and SubbaRow (5) as modified by Butcher and Sutherland (2). Protein was determined by the method of Lowry and Lopez (9), with bovine serum albumin as standard. All assays were performed in quadruplicate. The cyclic phosphodiesterase values (Table 1) are expressed as nmoles of Pl released per mg protein in 60 min at 30°.

The enzyme kinetic constants, K_m and V_max, for cAMP hydrolysis by various cyclic phosphodiesterase preparations were determined by use of an incubation mixture that consisted of 40 mM Tris-HCl buffer, pH 7.5, 1.8 mM MgSO_4, cAMP-3H (approximately 20,000 dpm), and varying concentrations of nonradioactive cAMP. The reaction was started by the addition of the enzyme preparation, and the incubation was carried out as described above. The reaction was stopped by the addition of 50 μl of 10 mM adenosine in 50 mM EDTA. Adenosine formed during the reaction was removed from the unreacted cAMP by passing the reaction mixture through QAE Sephadex (Cl-) column and eluting the column (5 x 0.5 cm) with 4 ml of distilled water.

Radioactivity in aqueous solutions was determined by counting a sample in 15 ml Bray's solution in the Nuclear Chicago Mark II automatic liquid scintillation counter. Although not shown, cAMP hydrolysis was directly proportional to enzyme concentration. Enzyme concentration used in each tube was calculated to give less than 10% substrate decomposition. cAMP concentrations used to determine K_m values ranged from 0.00625 to 2 mM.

A computer program "hyper" for determining enzyme kinetics constants was that of Cleland (4). It is based on the nonlinear regression methods described by Wilkinson (18).

RESULTS AND DISCUSSION

The K_m values of cAMP phosphodiesterases are 23.9 and 17.8 μM, respectively, for normal adrenal and adrenocortical carcinoma of the rat (Table 2). The results show that there is no significant difference in the K_m values for cAMP between normal and cancer tissue in the rat. The cyclic phosphodiesterase activity of rat adrenal tumor was shown to be inhibited in a noncompetitive fashion by cGMP (R. K. Sharma, unpublished observations). As shown in Table 1, the rates of hydrolysis of 6 of the cyclic nucleotides at a relatively high substrate concentration (2 mM) have been determined in the different tissues, confirming the earlier findings from our laboratories (7). Normal rat adrenals showed significant cyclic phosphodiesterase activity for 6 of the cyclic nucleotides.
cAMP, cGMP, and cyclic inosine 3',5'-monophosphate were degraded at the highest rates, followed by cyclic thymidine 3',5'-monophosphate and cyclic uridine 3',5'-monophosphate. The nucleotide degraded at the lowest rate was cyclic cytidine 3',5'-monophosphate. The rat adrenal tumor, however, showed considerably lower phosphodiesterase activities for all the cyclic nucleotides, as compared to the normal adrenal.

The radioactive methods for assay of cyclic phosphodiesterase activity (1, 8, 16) have demonstrated the presence of multiple cyclic phosphodiesterase enzyme system in most of the tissues analyzed. The multiplicity of this enzyme system was substantiated when the supernatants of the homogenates of various tissues of the rabbit and rat were examined by starch-gel electrophoresis (10). However, as shown in Chart 1, A and B, the normal adrenal and adrenocortical carcinoma tissues of the rat show that there is no break in the slope of the curve, thus suggesting 1 $K_m$ for cAMP phosphodiesterase enzyme. These results are of interest, since the concept of high and low $K_m$ enzymes for cAMP has been proposed (16) to explain the regulation of intracellular concentrations of cAMP. The low $K_m$ cyclic phosphodiesterase enzyme, which would be membrane bound in such a case, in conjunction with adenyl cyclase, could contribute to control of the availability of cAMP necessary for the regulation of various metabolic functions of the hormones. Of particular interest are the very low phosphodiesterase activities toward all 6 of the cyclic nucleotides tested in rat adrenal tumor as compared to the normal rat. Since the $K_m$ values of the enzymes in both tissues are the same, the implication is that there is considerably lowered enzymic activity in the adrenal tumor, possibly because of a lower amount of enzyme produced. Nevertheless, that the rat adrenocortical carcinoma 494 has developed an independent method for maintaining high cellular cAMP levels since cAMP levels of the rat adrenal tumor are reported to be relatively high and are unaffected by further stimulation of the adenyl cyclase by ACTH (11).

These studies further support our previously proposed hypothesis that the steps of steroidogenesis in the adrenal tumor are impaired after the adenyl cyclase step (14). From the present studies, the above hypothesis may be extended to suggest that the lack of corticosteroidogenic response to ACTH in the tumor may be at a site beyond the formation of cAMP.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. James Brush and Dr. Abbas Kitabchi for useful and stimulating discussions. The excellent technical assistance of Mrs. Margaret Cirtain is also appreciated.

REFERENCES

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