Metabolic Regulation and Adenyl Cyclase Activity of Adrenocortical Carcinoma Cultured Cells

James S. Brush, Lynda S. Sutliff, and Rameshwar K. Sharma

Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tennessee 38163

SUMMARY

In order that the mechanisms involved in the altered control of steroidogenesis by adrenocorticotropic hormone (ACTH) in the adrenal tumor 494 might be elucidated, various metabolic properties of this tumor maintained in tissue culture have been studied. It has been shown in the cultured cells that: (a) neither ACTH nor adenosine cyclic 3',5'-monophosphate stimulates steroidogenesis; (b) the normal adrenal biosynthetic pathway from pregnenolone to corticosterone is intact; (c) the biosynthesis of deoxycorticosterone to corticosterone from pregnenolone is at a reduced level as compared to the normal adrenal cell, indicating a reduced 11β-hydroxylase activity; and (d) (20S)-20-hydroxycholesterol can be converted to corticosterone, thus indicating a defect in the tumor system normally stimulated by ACTH to convert cholesterol to (20S)-20-hydroxycholesterol.

The adenylate cyclase activity of the cultured cells has been evaluated with an improved methodology. In confirmation of previous studies by others, it is herein shown that high concentrations of ACTH (460 milliunits) and epinephrine (10^-8 M) stimulate the activity of the whole homogenate 2.9-fold and 4.1-fold, respectively, whereas 20 mM fluoride has no effect on its activity. In addition it has been demonstrated that a partially purified particulate fraction of the cultured tumor cells containing plasma membrane was stimulated only 32% by ACTH, whereas the stimulation by epinephrine was 108% suggesting more sensitivity of the membrane receptor to epinephrine than to ACTH. Similarly, epinephrine significantly stimulated the adenylate cyclase activity of isolated nuclei, but no stimulation by ACTH was obtained. It is, therefore, proposed that distinct tumor receptors are at least as sensitive to epinephrine as they are to ACTH.

INTRODUCTION

In contrast to normal isolated adrenal cells (6, 12, 23), which are markedly stimulated by microunit quantities of ACTH2 to form corticosterone, adrenocortical carcinoma 494 (24), tissue slices (10), or cells (22) do not respond to this hormone or to cAMP. It has been further demonstrated that the inability of the adrenal tumor to synthesize increased amounts of corticosterone from endogenous precursors in response to ACTH is not due to accelerated cAMP breakdown (15).

The studies described herein and in a previous report (1) have reexamined the above defective control mechanisms of the tumor maintained in tissue culture. The unique observation that the adenyl cyclase of this tumor has multiple specific hormone receptors (13, 14) has been reexamined in the tumor cultured cells. In order that adenyl cyclase activity might be localized within the tumor cell, methods have been developed for the isolation of various cellular particulate components and for the assay of the enzyme in response to ACTH and epinephrine.

MATERIALS AND METHODS

Chemicals. 3',5'-Cyclic nucleotides were purchased from Sigma Chemical Company, St. Louis, Mo., and Boehringer-Mannheim, New York, N. Y. All the other chemicals were reagent grade and were obtained commercially. (20S)-20-Hydroxycholesterol-7α-3H (specific activity, 25 Ci/m mole), pregnenolone-7α-3H (specific activity, 5 Ci/m mole), ATP-3H (20 to 40 Ci/m mole), and Aquasol scintillation solvent were purchased from New England Nuclear, Boston, Mass. Pregnenolone-4-14C (specific activity, 50 mCi/m mole) was obtained from Amersham Searle, Arlington Heights, Ill.

Chromatography. Precoated silica gel plates (Silica Gel F254, Brinkmann Instruments, Westbury, N. Y.) were used for thin-layer chromatography in the indicated solvent systems. Thin-layer plates were used for the final purification of deoxycorticosterone and corticosterone. Chromatographically homogeneous products were further checked for purity and identity by recrystallization to constant specific activity and constant 3H:14C ratio.

Culture of Cells. Tissue culture media and sera were purchased from Grand Island Biological Company, Grand Island, N. Y.; sterile plastic ware was manufactured by the Falcon Plastics Company, Oxnard, Calif.
Adrenocortical carcinoma 494 (24) maintained by i.p. and s.c. transplantation in Sprague-Dawley rats was removed aseptically. Each tumor nodule was ruptured and the cells were expressed from the nodules and suspended in Ham's F-10 medium (5) supplemented with 15% horse serum and 2.5% fetal calf serum. The cell suspension was drawn up into a syringe (without a needle) approximately 10 times to obtain suspension of cells. The cell suspension was then transferred to a sterile tube and allowed to stand for 5 min to remove the cell debris. The suspended cells were then drawn up into a syringe through a 20-gauge needle. The cells were resuspended in fresh medium containing penicillin and streptomycin, and aliquots (usually 1 x 10^6 cells) were transferred to tissue culture flasks containing 30 ml of the above medium and incubated in a CO₂:O₂ incubator at 37°. The medium was changed twice a week. Viable cells attached to the flask within 24 hr after incubation in tissue culture, and distinct colonies of dividing tumor cells could be seen within 48 hr (Fig. 1). The cells divide every 24 hr thereafter; the division rate decreases as the maximum cell density of approximately 10^6 cells/ml is approached.

The cultured cells were further propagated by the technique of alternate culture and animal passage (2).

For experimental work cell stocks were grown in 6- or 10-cm-diameter Petri dishes at 37° in a water-saturated atmosphere of 5% CO₂ in air.

**Chromosome Preparation of Adrenocortical Carcinoma 494 Cultured Cells.** The chromosome preparation of the cultured carcinoma cells was achieved using the method of Moorehead et al. (9). Aliquots of the pellets were then fixed on slides (9), and the chromosomes were examined under the microscope and photographed. From 70 to 80% of the metaphase spreads examined were found to have 58 chromosomes (Fig. 2). An investigation was not made, however, to pinpoint marker chromosomes.

**Incubations of Cell Cultures with ACTH, Cyclic Nucleotides, and Nonradioactive Steroids.** A series of cultures each of which contained 1 x 10^6 cells were incubated with the complete medium supplemented with ACTH, pregnenolone, or an appropriate cyclic nucleotide. A comparable series was incubated in medium alone to serve as controls. At the end of the incubation period the cultures were twice extracted with 5 ml of methylene chloride. Corticosterone was measured fluorometrically (4). The results are reported as µg of corticosterone produced per ml of incubation medium per 4 hr in excess of the amount present at zero time.

The experiments with radioactive precursors were conducted in a similar fashion, except that incubation was carried out with 30 x 10^6 cells. To observe the incorporation of (20S)-20-hydroxycholesterol into deoxycorticosterone and corticosterone, 1.30 µCi of (20S)-20-hydroxycholesterol-7α-3H were used. In the double-labeled experiment where a mixture of pregnenolone-4-14C and pregnenolone-7α-3H was used, the radioactive contents of the 2 isotopes were 0.20 and 6.0 µCi, respectively. The reaction was stopped by the addition of methylene chloride; deoxycorticosterone and corticosterone were purified by thin-layer chromatography and crystallized as previously described (16, 17, 19). The radioactive extracts of deoxycorticosterone and corticosterone were diluted with nonradioactive carrier compounds. Deoxycorticosterone was acetylated (21) and purified by thin-layer chromatography on silica gel (16, 17, 19). Deoxycorticosterone and corticosterone were then crystallized 3 times until the specific activities were constant.

**Adenyl Cyclase Activity.** In general adenyl cyclase was measured as described by Pohli et al. (11) with specific exceptions as follows. To 0.12 ml of incubation media was added 0.30 ml of tumor homogenate or other cyclase containing subcellular components suspended or dissolved in SMT buffer. The final concentrations in the assay solution were the following: 0.025 M Tris-HCl buffer, pH 7.6; 5 mm MgCl₂ or MgSO₄; 1 mm EDTA; 47.6 mm phosphocreatine; 0.476 µg creatine phosphokinase per ml; and 4.16 mm cAMP. The solution contained 3 µCi of ATP-3H (final volume, 0.42 ml). The reaction was started with the addition of an amount of enzyme to each tube that was the same in any 1 experiment. This amount varied between experiments but was obtained from 60 to 130 mg of wet tissue cells. The incubation was carried out for 10 min at 30°. It was terminated by the addition to 0.5 ml of 1 M HClO₄. After centrifugation at 4°, 0.5 ml of the supernatant was removed to a tube containing 0.18 ml of 1.5 x KOH from which KClO₄ precipitated. The mixture was adjusted with glacial acetic acid or concentrated NH₄OH to a pH of 8 to 9 with pH paper. The supernatant solution was transferred to 1.2 to 1.5 ml of QAE Sephadex A-25 in the acetate form contained in a glass column, 0.6 cm in diameter. The resin was washed with 5 ml of 0.02 M ammonium acetate and the eluate was discarded followed by 5 ml of 0.5 M ammonium acetate; this 2nd eluate was then collected and lyophilized to dryness.

The residue from the lyophilization procedure was dissolved in 0.05 ml of distilled water and spotted along a line parallel to the short dimension and 8.3 cm from the end of the paper on Whatman No. 1 filter paper (46 x 57 cm) that had been washed with distilled water, then ethanol, and dried prior to being impregnated with 10% saturated (NH₄)₂SO₄ (3). The Rₚ values of the various pertinent nucleotides in this system were: ATP, 0.07; ADP, 0.19; AMP, 0.38; cAMP, 0.58. The spots were developed in the long dimension with 80% ethanol:water for 17 hr. A strip from the chromatogram containing the row of spots of cAMP was sewn with nylon thread to a sheet of filter paper treated identically to that described above. Overlapping paper was cut away leaving only a 0.5-cm strip near each line of the stitching. The paper was developed with saturated (NH₄)₂SO₄:isopropyl alcohol (80:2) for 18 hr. The Rₚ values of the various relevant nucleotides were: ATP, 0.48; ADP, 0.46; AMP, 0.34; and cAMP, 0.11. After drying, the strips containing the rows of spots of cAMP were cut from the chromatograms and sewn to a 3rd sheet washed as before but not impregnated with (NH₄)₂SO₄ and developed for 18 hr with 80% ethanol. This 3rd system desalted the purified cAMP. After drying, each individual spot of cAMP was eluted with about 5 ml of 0.01 M NaCl into tared scintillation vials and filtered to remove any particulate matter. The absorbance at 260 nm was measured and the
volume of solution was determined from the weight of the liquid to determine the final recovery of cAMP. After lyophilization to dryness, 1 ml of distilled water and 10 ml of Aquasol scintillation fluid were added to the vials, and the radioactivity present was determined in a Nuclear Chicago Mark II scintillation counter. The absolute dpm were determined from a standard curve relating efficiency of counting to the external standard ratio.

Membrane, Mitochondria, and Nuclei Preparation. Homogenates of tumor cells were centrifuged at 700 x g for 10 min. The pellet was designated the 700 x g particles. The supernatant suspension was then centrifuged at 17,000 x g for 10 min to obtain the 17,000 x g particles (mitochondria), and the supernatant suspension was then centrifuged at 100,000 x g for 90 min to sediment the 100,000 x g particles (microsomes) above which was the 100,000 x g supernatant solution used in 1 experiment. The 17,000 x g particles in 1 experiment were washed 4 times in SMT buffer.

The 700 x g particulate fraction was resuspended in 3 to 4 ml of SMT buffer and carefully layered on top of a sucrose density gradient as prepared as follows. On the bottom of a centrifuge tube were placed 5.0 ml of 2.3 M sucrose. On top of this was placed a linear sucrose gradient varying from 2.0 M at the bottom to 1.0 M at the top.

The gradient was centrifuged at 25,000 rpm at 4\(^\circ\)C in an SW 27 rotor for 90 min. A band of particulate material was observed at 1.7 M sucrose in the gradient and is referred to as the purified 700 x g particles. After this layer was pumped from the gradient, it was washed twice by centrifugation at 12,000 x g for 10 min in SMT buffer. This preparation, after staining with Wright-Giemsa stain, was examined under the light microscope and was found to contain primarily membrane-like material with only a slight contamination by trace amount of nuclei and unbroken cells.

Nuclei were found at the bottom of the 2.3 M sucrose layer. The remaining sucrose solution was decanted and the nuclei were washed twice with SMT buffer. Smears of the preparation that were stained (Wright-Giemsa) showed only nuclei by light microscopy.

The preparation of all tissue components was carried out at 0-4\(^\circ\)C.

RESULTS

Ultrastructural Characteristics of the Cultured Adrenocortical Carcinoma Cells. The histological features of these cells vary as previously described (22). The most pronounced difference between the isolated adrenocortical carcinoma cells and normal adrenal cells was in the morphology of the mitochondria. Mitochondrial cristae were thin and tubular and no vesicular cristae characteristic of isolated adrenal cells were observed (Fig. 3).

A linear relationship was established between the number of cells and corticosterone synthesized from exogenously added pregnenolone to study the formation of corticosterone from pregnenolone. The latter substrate was used in concentrations of 0.0032 to 0.316 mM. Although not shown, the results obtained were very similar to those previously reported for the isolated tumor cell (22). The concentration of pregnenolone required to achieve one-half the maximal synthesis of corticosterone was 6 \mu M.

Since previous studies (16, 19) have shown that the isolated adrenal tumor cells produce relatively low concentrations of corticosterone from pregnenolone as compared to the formation of deoxycorticosterone, this property in the cultured cells was examined. The cultured cells were therefore incubated with pregnenolone-4,11\(^\alpha\)-C, and deoxycorticosterone and corticosterone were isolated and crystallized to constant specific activity (16, 17, 19). These cells, like the isolated adrenocortical carcinoma cells (16, 19), can transform pregnenolone into the measured steroid products. Of the total added labeled precursor, 10.7 and 0.62% of the total radioactivity were incorporated into deoxycorticosterone and corticosterone, respectively. This again corroborates the earlier finding that there is a deficiency of 11\(\beta\)-hydroxylase activity in the tumor (19). Although not shown, the conversion of 0.063 mM pregnenolone to corticosterone was measured using the fluorometric assay (4). Like the isolated tumor cell preparation (16) this conversion was also linear with time. To determine whether these cultured cells have the same property as the isolated adrenocortical carcinoma cells (19), the cells were incubated with (20S)-20-hydroxycholesterol-7\alpha-3H, and deoxycorticosterone and corticosterone were crystallized to constant specific activity. The results obtained indicated that 6.28 and 0.86% of the total radioactivity were incorporated into deoxycorticosterone and corticosterone, respectively. This appears to support the previous proposal (19, 20) that the cAMP-dependent step responsible for the conversion of cholesterol to (20S)-20-hydroxycholesterol may be defective.

Time Course of Steroidogenesis. The measurement of corticosterone production in the presence of pregnenolone (0.0632 mM) was carried out with cultured adrenal tumor cells over a period from 0 to 240 min. As demonstrated previously, the formation of corticosterone was found to be linear with time. No lag period was noted.

Effect of ACTH and Cyclic Nucleotides. The studies with adrenal tumor slices (10) and with isolated adrenocortical carcinoma cells (16, 22) have shown that ACTH and cyclic nucleotides have no effect in isolated adrenocortical carcinoma cells (19). The cells were incubated with (20S)-20-hydroxycholesterol-7\alpha-3H, and deoxycorticosterone and corticosterone were crystallized to constant specific activity (16, 19). These cells, like the isolated tumor cell (22). The concentration of pregnenolone required to achieve one-half the maximal synthesis of corticosterone was 6 \mu M.

In order that the effects of ACTH and various cyclic nucleotides upon the synthesis of deoxycorticosterone and corticosterone might be studied in the cultured tumor cells, the following cyclic nucleotides were tested at the concentrations noted: cAMP, 10 mM; N\(\alpha\)-2'-O-dibutyryl-cAMP, 1 mM; cyclic 3',5'-GMP, 10.0 mM; cyclic 3',5'-UMP, 10.0 mM; and cyclic 3',5'-CMP, 10.0 mM. None of these compounds had activity in stimulating corticosterone synthesis.

Adenyl Cyclase Assay. The method for the purification of the cAMP produced by adenyl cyclase action was developed to eliminate nonphosphorylated nucleosides by adsorption and elution from QAE Sephadex followed by subsequent...
purification by paper partition chromatography in 3 systems. The determination of recoveries of added cAMP included in the original assay mixture was checked in 1 experiment by also including cAMP-8-14C. Recoveries by the 2 methods were identical within a standard deviation of 2.2%. Since all ATP-3H preparations contained significant amounts of cAMP-3H (0.01 to 0.03%), controls were run to correct for this value. No cAMP was formed by the methods used for extraction. This was indicated by paper chromatographic analysis of ATP-3H prior to incubation or treatment with HClO4 and KOH.

Although not shown, it was ascertained that the assay of activity was linear over the 10 min incubation time utilized.

Tissue Particulates Preparation. The method for the simultaneous preparation of purified 700 × g particles and nuclei was adapted and modified from the method of McKeel and Jarret (8) for the preparation of fat cell membrane and from the method of Whittle et al. (26) for the preparation of nuclei.

In Table 1 it is seen that as previously reported significant adenylyl cyclase activity can be found in a number of fractions isolated by differential centrifugation. The activity present in the microsomal and 100,000 × g supernatant fractions was, however, much less than that in the crude 700 × g and the 17,000 × g particulate fractions.

Table 2 shows that both ACTH and epinephrine stimulated adenylyl cyclase activity in the whole homogenate, whereas 20 mM fluoride had no effect upon the activity as previously reported (13, 14). Although not shown, and in accord with observations (13), there was no stimulation of the activity at a concentration of ACTH (0.1 milliunit/ml) that markedly affects pregnenolone metabolism. Also, in Table 2 it is seen that epinephrine and ACTH both stimulated adenylyl cyclase in the 700 × g particulate fraction, whereas in the 17,000 × g particles neither epinephrine nor ACTH had a significant effect upon enzymatic activity. The results obtained for the control activity in the 17,000 × g particles were much lower relative to the heavier particles. The activity in the 17,000 × g particles in the experiment of Table 1 indicated that activity was relatively much higher in this tissue component. The diminution of activity in the latter fraction seems to have been produced by the washing procedure.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylyl cyclase activity of cellular components obtained from adrenocortical carcinoma cultured cells</td>
</tr>
<tr>
<td>Each fraction was incubated for 10 min with ATP-3H at 30°, and the cAMP was isolated by ion-exchange and paper chromatography as described in &quot;Materials and Methods.&quot; The activity in each fraction was obtained from the same amount of cells.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction</th>
<th>dpm incorporated into cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>700 × g particles</td>
<td>10,400 ± 2400* (5)</td>
</tr>
<tr>
<td>17,000 × g particles (unwashed)</td>
<td>13,100 ± 470 (6)</td>
</tr>
<tr>
<td>100,000 × g particles</td>
<td>4,210 ± 600 (6)</td>
</tr>
<tr>
<td>100,000 × g supernatant solution</td>
<td>4,560 ± 700 (6)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Numbers in parentheses, number of determinations.

Table 3 shows that there was significant cyclase activity in the nuclear fraction. The nuclear activity was stimulated significantly by epinephrine. Purified 700 × g particles contained the greatest amount of activity which again was stimulated 108% by epinephrine but only 32% by ACTH. Both of the latter results are highly significant, which is in contrast to the lack of significance in the ACTH stimulation of the crude fraction (Table 2) from which the purified particles were prepared. This may be due to the greater inherent experimental error involved in aliquot measurements of the much more heterogeneous unfractoned 700 × g particles.

DISCUSSION

In order to evaluate properly the effects of the various hormones upon the adrenal tumor it is far more advantageous to use as pure a cell preparation of this tissue as is achievable. Previous studies on this aspect have been conducted in adrenal tumor slices (10), tumor tissue homogenates (10, 13, 15), and isolated adrenocortical carcinoma cells (16, 18, 22).

Reexamination of some of the metabolic properties of the adrenal tumor cells grown in culture indicates that, in confirmation of previous findings obtained with tumor slices (10) and isolated adrenocortical carcinoma cells (16, 19, 22), (a) the cultured cells are not stimulated by ACTH and its presumed intermediary messenger substance, cAMP, in the formation of corticosterone; (b) the lack of stimulatory effects by these agents cannot be explained on the basis of their cAMP phosphodiesterase activity (15); and (c) the tumor has the enzymic system responsible for the conversion of pregnenolone to corticosterone. Furthermore, the present studies with (20S)-20-hydroxycholesterol support the previous hypothesis that one of the defects responsible for the nonstimulatory effect of ACTH may be in the protein kinase system (19, 20).

The methodology described for determining adenylyl cyclase was developed to permit the measurement of relatively low amounts of activity, which is not permitted by most of the common methods. In earlier experiments it was determined that there was very significant conversion of ATP to a component tentatively identified by its absorption spectrum and Rf as being inosine. Because the cAMP product is extensively purified of this and other contaminants by ion-exchange and paper chromatography, it allows the use of ATP-3H, the specific radioactivity of which is much more reproducible from experiment to experiment than the more commonly used ATP-α-32P. The paper chromatographic systems utilized are those that were described previously for the separation and purification of noncyclic mononucleotides (3).

The studies with the cultured adrenal tumor homogenates support the findings from another laboratory (13) that various subcellular fractions of this tumor have significant adenylyl cyclase activity. The activity in the 100,000 × g particulate and supernatant fractions was, however, much less than that in the 700 × g or 17,000 × g particulate...
Table 2

Adenylyl cyclase activity of whole homogenate and fractionated particulates obtained from adrenocortical carcinoma cells

Conditions are the same as in Table 1. The 17,000 x g particles were washed 4 times with SMT buffer.

<table>
<thead>
<tr>
<th>Cell fraction</th>
<th>Addition</th>
<th>dpm incorporated into cAMP</th>
<th>Fold stimulation</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>10 μM epinephrine</td>
<td>12,700 ± 580 (5)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>460 milliunits ACTH</td>
<td>53,000 ± 9,500 (5)</td>
<td>4.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>20 mM NaF</td>
<td>37,200 ± 5,300 (4)</td>
<td>2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>700 x g particles</td>
<td>10 μM epinephrine</td>
<td>11,200 ± 2,700 (4)</td>
<td>0.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>700 x g particles</td>
<td>460 milliunits ACTH</td>
<td>15,300 ± 1,300 (3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>17,000 x g particles</td>
<td>10 μM epinephrine</td>
<td>30,600 ± 2,300 (3)</td>
<td>2.0</td>
<td>0.003</td>
</tr>
<tr>
<td>17,000 x g particles</td>
<td>460 milliunits ACTH</td>
<td>28,600 ± 7,800 (3)</td>
<td>1.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>17,000 x g particles</td>
<td>10 μM epinephrine</td>
<td>6,390 ± 250 (3)</td>
<td>1.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>17,000 x g particles</td>
<td>460 milliunits ACTH</td>
<td>6,560 ± 230 (4)</td>
<td>1.0</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Probability that there is no significant difference between the listed mean for each cellular component (whole homogenate, 700 x g particles, and 17,000 x g particles) and the mean for the same component obtained with no additions.

N.S., no significant difference between the compared means.

Table 3

Adenylyl cyclase activity of isolated tumor nuclei and membrane stimulated by epinephrine and ACTH

See "Materials and Methods" for the technique used for the preparation of the cellular particulate fractions. Reaction conditions are the same as in Tables 1 and 2.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Addition</th>
<th>dpm incorporated into cAMP</th>
<th>Fold stimulation</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>10 μM epinephrine</td>
<td>4,140 ± 250 (5)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Nuclei</td>
<td>460 milliunits ACTH</td>
<td>5,580 ± 520 (4)</td>
<td>1.35</td>
<td>0.03</td>
</tr>
<tr>
<td>Nuclei</td>
<td>12,710 ± 530 (4)</td>
<td>1.00</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Purified 700 x g particles</td>
<td>10 μM epinephrine</td>
<td>26,400 ± 1,130 (5)</td>
<td>2.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Purified 700 x g particles</td>
<td>460 milliunits ACTH</td>
<td>16,800 ± 610 (4)</td>
<td>1.32</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Probability that there is no significant difference between the listed mean for each purified subcellular fraction and the mean for the same fraction obtained with no additions.

The adenyl cyclase activity in the purified 700 x g particles was stimulated by ACTH and epinephrine but no such stimulation was obtained in the 17,000 x g particles. Since most of the hormone-sensitive adenyl cyclase from the homogenate is in the purified 700 x g particles, that fraction must contain the plasma membrane. Of particular importance may be the results obtained with whole homogenate and the latter membrane-containing fraction with regard to the higher stimulation of adenyl cyclase activity by epinephrine as compared to ACTH. The diminished stimulation by the hormones in the purified membrane fraction when compared to the whole homogenate suggests the possible involvement of a factor that has been removed in the purification procedure. The ACTH receptor system seems more sensitive to the removal of the factor than does the epinephrine system. From the work of Pohl et al. (11), one possibility is that this factor is GTP.

The finding of significant adenyl cyclase activity in the purified nuclei is of interest as confirmatory of earlier reports (7, 25) demonstrating activity in this component from other tissues. The stimulation of the activity by epinephrine was significant but requires further study.

A possible role for cAMP in plasma membrane transport mechanisms in this tissue has been reported recently (18). The finding of nuclear adenyl cyclase may suggest a role for its nucleotide product in controlling transport mechanisms in that component as well.

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