Adenylate Cyclase System of Bovine Adrenal Plasma Membranes*

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SUMMARY

The adenylate cyclase system present in a preparation enriched in plasma membranes derived from bovine adrenal cortex was investigated in considerable detail. This system is stimulated by adrenocorticotropic hormone (ACTH), by biologically active analogs of this hormone, and by fluoride ion. The preparation contains sodium-potassium- and magnesium-dependent ATPases that are markedly inhibited by 50 mM sodium fluoride. Incorporation of a pyruvate phosphokininase ATP generating system into the adenylate cyclase assay medium provided constant substrate levels. In the presence of the ATP generating system, the rate of cyclic AMP formation (basal, fluoride, and ACTH-activated) was proportional to enzyme concentration and was linear with time. Proportionality with respect to enzyme concentration as concerned the hormone-activated adenylate cyclase was achieved only when the ratio of hormone to enzyme protein was kept constant. The temperature optimum of the adenylate cyclase, basal or activated, was approximately 30°C. Michaelis-Menten kinetics were observed when the ratio of Mg2+ to ATP was approximately 6:1. Both calcium and ethylene glycol bis(β-aminoethyl ether)-N,N’-tetraacetic acid completely inhibited the adenylate cyclase system at concentrations of 5 and 0.5 mM, respectively. GTP was inhibitory at concentrations of 10−3 M but had little effect at lower concentrations.

Freezing in liquid nitrogen and storage at −60°C exerted little effect on the fluoride-stimulated enzyme but lowered hormone-stimulated activity. Preincubation in the presence of ACTH afforded a high degree of stabilization of the enzyme system while preincubation with a biologically inactive analog afforded no protection.

The earliest demonstration of the general nature of Sutherland’s discovery (1) of the involvement of cyclic AMP in peptide hormone action was provided by the finding of Haynes (2) that cyclic AMP accumulated in beef adrenal cortical slices in response to stimulation by natural ACTH. This observation was extended by Grahame-Smith et al. (3) who showed that formation of cyclic AMP preceded steroidogenesis in quartered rat adrenals and that cyclic AMP production could be enhanced by ACTH in rat adrenal homogenates. In the intervening years, a large body of evidence has accumulated in support of the view that cyclic AMP serves as the intracellular “second messenger” for many peptide hormones. In fact the first event between hormone binding to its receptor and physiological response is apparently activation of an adenylate cyclase system. Unfortunately, owing to the location of the mammalian enzyme in the plasma membrane and the problems encountered in attempts to solubilize adenylate cyclase, information regarding the relationship between hormone binding and adenylate cyclase activation is difficult to obtain. Investigations of the enzyme system located in the adrenal cortex have largely been confined to studies carried out with crude adrenal particulates (4, 5) and particulate fractions obtained from an ACTH-sensitive mouse adrenal tumor (6).

We have described (7) a subcellular fraction derived from bovine adrenal cortex shown by electron microscopy, electron microscopic cytochemistry, and marker enzyme assays to be enriched in plasma membranes. Structure-function studies have demonstrated that this preparation specifically binds ACTH and biologically active congeners and that these hormones activate a membrane-bound adenylate cyclase system. Furthermore, a number of ACTH analogs exhibiting low in vivo biological activity are capable of binding to the membrane preparation and acting as competitive inhibitors of the ACTH sensitive adenylate cyclase system (8).

In assessing the relative abilities of various hormone analogues to stimulate adenylate cyclase, it is essential to establish conditions that result in linear reaction rates. The assay is complicated by the presence in the membrane preparation of enzymes that metabolize ATP and cyclic AMP. Although adenylate cyclases derived from various sources share many properties (9), significant differences in levels of associated ATPases and phosphodiesterase activities exist to merit a thorough investigation of these enzymes.

The present communication examines certain parameters of the bovine adrenocortical plasma membrane adenylate cyclase system and the effect of other related phosphate-metabolizing
enzymes present in the preparation on the levels of substrate and product.

**EXPERIMENTAL PROCEDURE**

**Materials**—Pyruvate phosphokinase (rabbit skeletal muscle, type II), P-enolpyruvate (trisodium salt, hydrate), dithioerythritol, dithiothreitol, EGTA, and ouabain were purchased from Sigma. Cyclic AMP, ATP (disodium salt), GTP (disodium salt), and [3H]ATP (specific activity, 21 Ci per mmol) were obtained from Schwarz-Mann. Theophylline was purchased from Nutritional Biochemicals, cyclic [3H]AMP (20.7 Ci per mm mol) from Amersham, and AG 1 X2 (200 to 400 mesh) and AG 50W X4 (200 to 400 mesh) from Bio-Rad. Corticotropin-1-24 (ACTH) was a gift from Dr. W. Rittel of Ciba-Geigy Corporation, Basel, Switzerland. N' Acetyl[HisGlu'-N'-formyl]-Lys[24]"'-"'Corticotropin-124 amide (protected ACTH) was prepared in this laboratory (10).

**Methods**—The bovine adrenocortical plasma membrane preparation was obtained as described (7). Protein was determined according to the method of Lowry et al. (11) using bovine serum albumin as standard. Plasma membranes were solubilized in 0.1 N sodium hydroxide containing sodium lauryl sulfate (0.75 mg per ml).

Cyclic AMP was determined by a modification (7) of the method of Krishna et al. (12). Cyclic AMP and AMP are simultaneously eluted with 0.1 N H3PO4 from the AG 1 X2 column. Further development of the columns with 0.01 N KCI-0.01 M NaCl (50 ml) allows recovery of ADP and finally ATP is obtained by elution with 0.1 N KCl-0.05 M NaCl (50 ml).

Sodium-potassium- and magnesium-dependent ATPases were determined by the method of Usugi et al. (13). Total ATPase activity was measured in a medium containing NaCl (142 mM), KCl (20 mM), MgCl2 (4 mM), ATP disodium salt (4 mM), and pH 7.5 imidazole-HCl buffer (30 mM). Magnesium-dependent ATPase activity was measured by including ouabain (0.1 mM) in the incubation medium. Reagents were preincubated at room temperatures for 15 min and plasma membrane suspensions containing 0.1 to 0.4 mg of protein were added to initiate the reaction. Total reaction volume was 1.2 ml. Following incubation for 15 min at 37°C, the reaction was terminated by addition of ice cold 5% trichloroacetic acid in chloroform-methanol (1:1, v/v). Following centrifugation, an aliquot of upper phase was withdrawn for determination of inorganic phosphate by the method of Bartlett (14). Sodium-potassium ATPase activity is defined as the difference between total ATPase and magnesium-dependent ATPase activity. Results are expressed in micromoles of Pi released per mg of protein per hour. Enzyme activity was linear with respect to protein concentration and incubation time.

Adenylyl cyclase was determined as described (7) except that 0.01 ml of dithiothreitol or dithioerythritol (0.8 M) was added to the membrane suspensions. ATP reaction mixture (0.6 ml) consisted of [3H]ATP (0.5 to 0.6 mM), 0.04 mM Tris-HCl buffer (pH 7.3), 0.01 M theophylline, and 4.4 mM MgSO4 (4.4 mM MgCl2 was substituted in experiments dealing with the effects of Ca2+ or EGTA). Sodium fluoride, when used, was present at a concentration of 10-5 M. ATP generating system contained pyruvate phosphokinase (0.01 ml) and aqueous solution (90 mg per ml) of P-enolpyruvate (0.1 ml). Final volume of the assay solution was 0.82 ml.

The Ca2+ content of the membrane preparation was determined by the method of Diehl and Ellingboe (15) using a Corning model 940 calcium analyzer.

**RESULTS**

The high ATPase activity of plasma membranes is well documented (16). Indeed, it was in an effort to inhibit these enzymes that the effect of fluoride ion on hormone-sensitive adenylate cyclases was actually discovered (17). Bovine adrenal plasma membranes contain both sodium potassium and magnesium-activated ATPases. The specific activities of the two enzymes measured in a series of experiments were 3.15 ± 0.27 µmol of P1 per mg of protein per hour for the sodium potassium and 7.52 ± 1.14 µmol of P1 per mg of protein per hour for the magnesium-dependent enzyme. The decline in enzyme activity with time is illustrated in Fig. 1.

As might be expected (18, 19) both types of ATPase activity are inhibited by fluoride ion (Table I). It should be noted that the fluoride ion concentration necessary for full activation of the adenylate cyclase system (10 mM) (8) does not completely inhibit either ouabain-sensitive or ouabain-insensitive activity. The degree of inhibition observed even with 50 mM fluoride ranged from 81 to 94% in various membrane preparations examined.

The efficiency of the pyruvate phosphokinase ATP generating system in maintaining ATP concentration is illustrated in Fig. 2. The generating system preserves substrate both in the presence and absence of sodium fluoride throughout the course of the assay.

ACTH has no detectable effect on the ATPase activity of the adrenocortical plasma membrane preparation. Neither total ATPase nor magnesium-dependent ATPase activity is affected when tested at a hormone to membrane-protein ratio that elicits full adenylate cyclase activity (Table II). The relation between enzyme concentration (expressed as milligrams of membrane protein) and the rate of cyclic AMP formation was investigated with the unstimulated, as well as the ACTH- and fluoride ion-stimulated enzyme (Fig. 3). All three rates were proportional to enzyme concentration. In the case of the ACTH-sensitive enzyme, a constant ratio between hormone and membrane protein had to be maintained in order to achieve linearity. The rate of product formation was also linear throughout the time course investigated (25 min) both as concerns ACTH- and fluoride ion-stimulated enzyme (Fig. 4).
Fig. 2. Influence of ATP generating system on substrate levels of adenylate cyclase assays. ○, incubation in the absence of generating system; □, incubation in the presence of generating system; ●, incubation in the presence of sodium fluoride (10^{-2} M) and generating system. For details see text.

TABLE II

<table>
<thead>
<tr>
<th>Additions</th>
<th>Total ATPase</th>
<th>Magnesium ATPase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.97</td>
<td>4.67</td>
</tr>
<tr>
<td>ACTH (3.98 μM)</td>
<td>7.93</td>
<td>4.75</td>
</tr>
</tbody>
</table>

* Magnesium-dependent ATPase is the enzyme activity remaining after addition of ouabain (see text for details). In each assay, 0.3 mg of membrane protein was used.

Fig. 3. Cyclic AMP production as a function of plasma membrane protein concentration. ○, fluoride-stimulated adenylate cyclase; incubation solution contained sodium fluoride (10^{-2} M). ●, ACTH-stimulated adenylate cyclase; a constant ratio of hormone to membrane protein (8.13 nmol of ACTH per mg of membrane protein) was employed. This concentration of ACTH is sufficient to activate the adenylate cyclase system fully under the conditions of the assay. ×, unstimulated adenylate cyclase.

Routinely a 15-min incubation time was employed. The ratio of fluoride ion to ACTH stimulation varied considerably from one membrane preparation to another; however, in most instances fluoride stimulation markedly exceeded that observed with the hormone.

The temperature profiles for basal, ACTH, and fluoride ion-stimulated adenylate cyclase coincide and results from several experiments indicate that the temperature optimum is about 30° (Fig. 5).

Fig. 4. Formation of cyclic AMP as a function of time. ○, incubation solution contained sodium fluoride (10^{-2} M); ●, incubation solution contained ACTH (3.98 μM). For details of assay see text.

Fig. 5. Adenylate cyclase activity as a function of temperature. ×, fluoride-stimulated (10^{-2} M); ●, ACTH-stimulated (3.98 μM); ○, basal activity.

The relationship between ATP concentration and adenylate cyclase activity at constant and varying concentrations of added MgSO_4 is shown in Fig. 6.

The effect of added CaCl_2 on membrane adenylate cyclase is shown in Fig. 7. A significant decrease in the activity of ACTH-stimulated cyclase occurred in the presence of 50 μM Ca^{2+} and complete inhibition was observed when the calcium concentration reached 5 mM. Basal activity also was abolished at this concentration.

Addition of the calcium complexing agent, EGTA, had a similar inhibitory effect on the activity of the hormone sensitive cyclase (Fig. 8). However, basal activity was somewhat elevated by the chelating agent. The two activity curves converged at an EGTA concentration of approximately 500 μM with the greatest changes in both activities occurring when the EGTA concentration was between 10 and 100 μM.

The calcium content of several membrane preparations ranged from 0.02 to 0.06 μmol per mg of protein. Thus, using 0.4 mg of membrane protein for adenylate cyclase assay, the total calcium added with the membranes did not exceed 10 to 29 μM.
Effect of substrate and Mg\(^{2+}\) concentration on adenylate cyclase activity. Curve a, ACTH-stimulated, ratio of Mg\(^{2+}\):ATP was 6:1. Curve b, ACTH-stimulated, Mg\(^{2+}\) concentration was 4.4 mM. Curve c, unstimulated, Mg\(^{2+}\) concentration was 4.4 mM. ACTH when present was 3.98 pM.

Effect of added Ca\(^{2+}\) on adenylate cyclase activity. O, ACTH stimulated; incubation solutions contained ACTH (3.98 pM). 0, unstimulated. The points on the ordinate represent the adenylate cyclase activity in the absence of added Ca\(^{2+}\).

Effect of EGTA on adenylate cyclase activity. O, ACTH stimulated; incubation solutions contained ACTH (3.98 pM). 0, unstimulated. The points on the ordinate represent the adenylate cyclase activity in the absence of EGTA.

<table>
<thead>
<tr>
<th>GTP molarity</th>
<th>Cyclic AMP added</th>
<th>Theophylline</th>
<th>Cyclic AMP remaining</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.17</td>
<td>+</td>
<td>0.14</td>
</tr>
<tr>
<td>0</td>
<td>0.17</td>
<td>–</td>
<td>0.05</td>
</tr>
<tr>
<td>10(^{-8})</td>
<td>1.70</td>
<td>+</td>
<td>1.63</td>
</tr>
<tr>
<td>10(^{-4})</td>
<td>1.70</td>
<td>–</td>
<td>1.20</td>
</tr>
<tr>
<td>0</td>
<td>17.00</td>
<td>+</td>
<td>10.90</td>
</tr>
<tr>
<td>0</td>
<td>17.00</td>
<td>–</td>
<td>12.90</td>
</tr>
</tbody>
</table>

ACTH concentration, 3.98 μM.

Phosphodiesterase activity of plasma membranes

Table III

Phosphodiesterase, an enzyme originally considered to be located exclusively (20) in the cytosol, recently has been shown to exist in at least two kinetically distinguishable forms (21), one of them associated with plasma membranes (22). The phosphodiesterase activity of the bovine adrenal plasma membrane preparation was examined at several cyclic AMP levels (Table III). At concentrations in the range normally produced in our adenylate cyclase assay (0.17 nmol) a significant proportion of the cyclic AMP was destroyed when theophylline was omitted from the incubation medium. Even in the presence of the inhibitor, complete recovery of added cyclic [\(^{3}H\)]AMP, and cyclic AMP as shown. Total volume of reaction mixture was 0.82 ml. Recovery of cyclic AMP (usually 90%) was determined using boiled plasma membranes.

Additions of various amounts of GTP to the bovine adrenocortical plasma membrane preparation failed to alter basal or hormone-stimulated activity significantly until the guanine triphosphate concentration was 10- to 30-fold in excess of the ATP concentration (Table IV). At GTP concentrations of 10\(^{-8}\) M complete inhibition occurred.

The stability of the adenylate cyclase system was investigated under a variety of conditions. Gradual inactivation resulting in total loss of activity occurred in every instance when the membranes were stored at 4°. Storage at -15° produced immediate (upon freezing) inactivation. Optimal stability resulted when the membranes were frozen rapidly in liquid nitrogen and stored at -60° (Table V). Under these conditions fluoride-stimulated activity was practically quantitatively retained while basal and, to a larger extent, ACTH-stimulated activities decreased.

Experiments designed to determine whether proteolysis plays a major role in terminating the interaction between ACTH and bovine adrenocortical plasma membranes led to unsuspected findings. The results presented in Table VI indicate that a marked deactivation of adenylate cyclase occurred when the membrane preparation was preincubated in the presence of ATP generating system, dithiothreitol, and bovine serum albumin for 45 min at 30°. Inclusion of ACTH in the preincubation medium afforded significant protection of the enzyme system. Protected ACTH, a biologically inert peptide that does not bind to the membranes (23), failed to stabilize the enzyme.

DISCUSSION

The ACTH-sensitive adenylate cyclase system of the adrenal cortex has been studied in crude systems by many investigators (4-6). Scant information is available pertaining to the validity...
of adenylate cyclase assays that are performed with such preparations. The present investigation defines apparently optimal conditions for assay of ACTH- and fluoride ion-sensitive adenylate cyclase in a bovine adrenocortical preparation enriched in plasma membranes. Information pertaining to substrate levels, proportionality of enzyme concentration and rate, as well as linearity of the reaction with time is critical for the development of an adenylate cyclase assay, particularly since the adrenal membranes contain ATPases and phosphodiesterase that influence the levels of substrate and product.

The specific activities of sodium-potassium- and magnesium-dependent ATPases in adrenocortical plasma membranes are somewhat lower than is observed in plasma membranes from other sources (24-26). This may be attributed to inactivation during the isolation procedure employed for obtaining purified membranes. When the membrane preparation is stored at 4° in 1 mM bicarbonate buffer, pH 7.5, the decrease in enzymic activity follows first order kinetics with the rate of inactivation of the ouabain-sensitive enzyme being higher (Fig. 1). It is reasonable to assume therefore, that both the specific activities of the enzymes and the per cent contribution to activity of the sodium-potassium ATPase are lower than would be observed with fresh plasma membranes. The point designated as zero time in Fig. 1 corresponds to the time when purified plasma membranes are harvested, the residual activity is sufficient to warrant inclusion of an ATP generating system in the adenylate cyclase assay medium to maintain approximately constant substrate levels (Fig. 2). In the absence of a generating system, less than 40% of the substrate is available to the cyclase after 15 min of incubation using 0.4 mg of membrane protein. The pyruvate phosphokinase ATP generating system effectively maintains a constant level of substrate both in the presence and absence of fluoride ion. Over 95% of added ATP is preserved throughout the incubation period using standard conditions (see text) for adenylate cyclase assay. The proportion of substrate converted to cyclic AMP is approximately 0.5; of the total ATP added.

Hadden et al. (27) have reported that both norepinephrine and insulin stimulate human lymphocyte plasma membrane sodium-potassium ATPase activity. Stimulation of plasma membrane ATPases by ACTH was not observed in the present study (Table II).

The relationship between enzyme activity and ATP concentration is a complex one depending, as well, on magnesium concentration. Taunton et al. (6) working with a particulate preparation derived from an ACTH-sensitive mouse adrenal tumor, reported that no adenylate cyclase activity could be detected in the absence of Mg²⁺; however, these authors observed a decrease in ACTH and sodium fluoride-activated enzyme activity when Mg²⁺ was present in concentrations exceeding that of ATP and concluded that free ATP was the true substrate. In other tissues (9, 28) where the relationship between Mg²⁺ and ATP has been studied, quite the opposite has been found, namely that enzyme activity is inhibited when ATP concentration exceeds that of Mg²⁺. Consequently, a Mg²⁺:ATP complex is now considered to be the substrate for most of the adenylate cyclases investigated. The data obtained with ACTH-stimulated adrenocortical adenylate cyclase (Fig. 6) are in agreement with this postulate. At a fixed level of Mg²⁺ inhibition occurs even before ATP concentration exceeds that of Mg²⁺ whereas, when a constant molar ratio of Mg²⁺:ATP (6:1) is maintained, a typical Michaelis-Menten relationship between reaction velocity and substrate concentration is obtained. The apparent Kₘ for ATP measured with a constant Mg²⁺:ATP ratio (Fig. 6, Curve a) is

### Table V

<table>
<thead>
<tr>
<th>Additions</th>
<th>Cyclic AMP*</th>
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<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>None</td>
<td>137</td>
</tr>
<tr>
<td>ACTII</td>
<td>318</td>
</tr>
<tr>
<td>NaN</td>
<td>773</td>
</tr>
<tr>
<td>Frozen</td>
<td>114 (83%)</td>
</tr>
<tr>
<td>Control</td>
<td>385 (88%)</td>
</tr>
<tr>
<td>Frozen</td>
<td>1435</td>
</tr>
<tr>
<td>Experiment</td>
<td>755 (98%)</td>
</tr>
<tr>
<td>Frozen</td>
<td>95%</td>
</tr>
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</table>

* Measured in picomoles of cyclic AMP per mg of protein per 15 min.

### Table VI

<table>
<thead>
<tr>
<th>Additions to preincubation medium</th>
<th>Additions to assay medium</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td>231</td>
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<tr>
<td>Control</td>
<td>1578</td>
</tr>
<tr>
<td>None</td>
<td>192</td>
</tr>
<tr>
<td>ACTH</td>
<td>1536</td>
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<tr>
<td>Protected ACTH</td>
<td>205</td>
</tr>
<tr>
<td>NaN</td>
<td>1268</td>
</tr>
</tbody>
</table>

* No preincubation.
* An additional portion of NaF (8.2 nmol) was added.
* An additional portion of ACTH (3.25 nmol) was added.
approximately 0.1 mm for the adrenal system. The apparent
K_m for ATP of other adenylate cyclases is reported to be in the
range of 0.08 to 0.5 mM (9).

The stimulatory effect of calcium on steroidogenesis in adrenal
tissue preparations is well recognized (29, 30). More recently
Sayers et al. (31) have shown with rat adrenal cells that both
steroidogenesis and cyclic AMP formation are enhanced when the
cells are incubated in the presence of 7.65 mM calcium. The
cation produces quite a different effect on the adenylate cyclase
activity since the same
cells are incubated in the presence of 7.65 mM calcium.

Sayers et al. (31) have shown with rat adrenal cells that both
steroidogenesis and cyclic AMP formation are enhanced when the

cells are incubated in the presence of 7.65 mM calcium.

In every case the activity of the enzyme was inhibited by the
presence of GTP to the adrenal
membranes (Table IV) had little effect on either basal or ACTH-
stimulated adenylate cyclase until a GTP concentration of 10^{-2}
m was reached. The reason for the marked inhibition in the
presence of large amounts of GTP is unknown but does not ap-
tear to have physiological significance. Rodbell et al. (33) have
postulated that GTP binds at a regulatory site independent of
the glucagon binding site. Such a regulatory action for GTP is
not apparent from the results presented in Table IV.

Attempts to solubilize and isolate membrane-bound adenylate
cyclase thus far have resulted in only modest successes (9).

Inability of the enzyme, as reflected in large losses in specific
activity during storage, has been a major drawback. The

problem is particularly acute with the enzyme derived from adrenal
plasma membranes. Thus far activity has been preserved only
by rapid freezing in liquid nitrogen at -60°. Under these
conditions fluoride ion-sensitive activity can be virtually
quantitatively retained. In general fluoride-stimulated activity
is less sensitive to conditions that modify membranes, i.e. deter-
gents and phospholipase, than is hormone-stimulated activity
(38). Fluoride ion is considered to interact with the enzyme at a
site different from that influenced by hormones (28, 39).

The possibility that plasma membranes contain proteolytic
enzymes capable of destroying the peptide hormones that

specifically activate adenylate cyclases has been suggested by the
results of Pohl et al. (40). Glucagon (41) is rapidly inactivated
when incubated with liver membranes. Nearly 50% of the
hormone is destroyed within the first 2 min of incubation.
Furthermore, the presence of high concentrations of several
other peptide hormones did not protect glucagon from inactiva-
tion. When experiments designed to determine whether pro-
teolysis plays a major role in terminating the interaction be-

tween ACTH and its plasma membrane receptor were carried
out, very little evidence for ACTH destruction was found (Table
VI). The levels of both basal and ACTH-sensitive activity
were markedly decreased when the membranes were kept at 30°
for 45 min prior to assay. Surprisingly the presence of ACTH
in the preincubation medium afforded a high degree of stabiliza-
tion for the system. This result suggests that the membrane
preparation does not contain significant proteolytic activity.

That binding of the hormone to the membranes is necessary for
stabilization to occur is evident from results obtained when pro-
tected ACTH previously shown (23) to be incapable of binding
to the membranes was added to the preincubation medium.

Virtually the same degree of enzyme inactivation occurred
whether this peptide was present or absent. Fluoride activation
is not as sensitive to preincubation as is hormone stimulation.

Reactivation of preincubated membrane adenylate cyclase by
fluoride ion is approximately 80%.

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It appears unlikely that these results can be explained in terms
of stabilization of the receptor. The ability of the receptor to
bind ACTH is less sensitive to inactivation than is the complete
adenylate cyclase system; for example, repeated freezing and
thawing of the membrane preparation does not affect the capac-
itvity of the receptor to bind hormone (7) while the same treatment
totally inactivates the cyclase. At the present state of knowl-
dge of hormone-sensitive adenylate cyclase systems it is difficult
to interpret these observations. Since both hormone-receptor
interaction and adenylate cyclase activation by fluoride ion are
more stable than hormone activation of adenylate cyclase, it may
be tentatively concluded that inactivation during preincubation
results from disruption, in some manner, of the communication
between receptor and cyclase.
The adenylate cyclase system of bovine adrenal cortical plasma membranes exhibits many of the same characteristics of cyclases derived from other tissues. Because the enzymes are located in the plasma membrane they are associated with ATPase and phosphodiesterase activities. Provided that proper precautions are taken, however, linear reaction rates can be obtained. In agreement with previous reports the substrate appears to be an ATP-magnesium complex.

Cyclase hormonal control is the most sensitive component of the adrenocorticotroic plasma membrane adenylate cyclase system. In contrast to other reports the hormone effect is not associated with stimulation of ATPase activity, nor does GTP appear to modulate the cyclase system.

Acknowledgments—The authors wish to express their appreciation to Mr. I. Rosen of Western Packers for his cooperation in supplying the bovine adrenal glands. They also wish to thank Dr. Warren Diven for determining the Ca²⁺ content of the membrane preparation.

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