Adenyl Cyclase of Rat Cerebral Cortex

ACTIVATION BY SODIUM FLUORIDE AND DETERGENTS*

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SUMMARY

Sodium fluoride activates adenyl cyclase at a rate that is dependent on temperature and the concentration of NaF. The activation is not readily reversed by dilution, extensive washing of the membrane-bound enzyme, or dialysis. Activation occurs in the absence of added Mg++, but the rate of activation is increased 2-fold by 5 mM Mg++. The NaF-activated enzyme is more stable at 4°C and during dialysis than the native enzyme. The enzyme also is activated by Triton X-100 and to a lesser extent by sodium dodecyl sulfate. The activity of the detergent-treated enzyme is not stimulated by NaF, but the activity of the NaF-activated enzyme can be increased by Triton X-100. Thus, NaF does not cause full expression of adenyl cyclase activity. Freezing and thawing of the cerebral cortex of young (1 to 20 days) rats prior to homogenization causes a 2-fold increase in enzyme activity. It was concluded that adenyl cyclase exists in its membranous environment in a state of inhibition. Treatment with detergents or freezing of the tissue could lead to nonspecific changes in membrane structure which cause reduction of the inhibition and increased catalytic activity.

It is now well established that the activity of adenyl cyclase is regulated by specific hormones. The enzyme from a particular tissue is stimulated only by those humoral substances which elicit a physiological response in that tissue. This selective stimulation of adenyl cyclase has been observed in both whole cell and broken cell preparations and has been used as one of the criteria (1) for the demonstration of the role of cyclic adenosine 3',5'-phosphate as a second messenger in a particular hormone-target tissue relationship.

In all mammalian tissues examined adenyl cyclase is associated with membrane fragments of homogenates and is assumed to be a component of the plasma membrane in most cell types (2). Robison, Butcher, and Sutherland (3) have suggested a model of adenyl cyclase that depicts the enzyme as an integral part of the plasma membrane with a receptor-regulatory component exposed on the outer surface and a catalytic component exposed on the inner surface. Hormone specificity is attributed to a selective interaction of a particular hormone with the receptor component.

Sodium fluoride also increases the activity of adenyl cyclase (4, 5). However, unlike the effects of hormones, the stimulatory effect of NaF is nonselective in that the enzyme activity from most tissues is increased. Although the mechanism of action of NaF is not known, certain characteristics of the effect have been established by recent studies. Unlike hormones, NaF does not stimulate adenyl cyclase activity of intact cells, having an effect only on broken cell preparations (6). Furthermore, agents that specifically block the effect of certain hormones on adenyl cyclase (e.g. β-adrenergic blocking agents) do not prevent stimulation by NaF (7). Also, certain nonspecific agents can destroy hormonal responsiveness without decreasing the effect of NaF (8, 9). This evidence indicates that NaF stimulates adenyl cyclase by a somewhat different mechanism than do the hormones. However, the effects of NaF and hormones cannot be completely independent since hormones usually have no additional stimulatory effect in the presence of optimal concentrations of NaF (5, 10; however, see 7).

In this paper further studies on the mechanism of action of NaF are presented. Also, we have examined the effects of detergents and freezing on adenyl cyclase activities of developing rat cerebral cortex. The results of these studies are discussed in relation to a general model for the regulation of adenyl cyclase activity.

MATERIALS AND METHODS

Materials—Cyclic adenosine 3',5'-phosphate, ATP (disodium salt), and pyruvate kinase type II (crystalline) were purchased from Sigma. Dowex 50W-X8, 200 to 400 mesh, H+ form, was obtained from Bio-Rad, Richmond, California, theophylline from Nutritional Biochemicals, and phosphoenolpyruvate-triclo-

hexylammonium salt from Boehringer Mannheim. 3H-ATP tetralithium salt was purchased from Schwarz BioResearch. Triton X-100 was purchased from Sigma and sodium dodecyl sulfate from Matheson, Coleman and Bell.

Preparation of Enzyme—Freshly prepared rat cerebral cortex was homogenized in 10 volumes of 50 mM Tris HCl, pH 7.5, with a Teflon-glass apparatus. The homogenate was centrifuged at 1000 X gmax in the SS-34 rotor of the Sorvall RC-2-B centrifuge for 10 min at 4°C. The precipitate was washed twice by resuspension and centrifugation in the same volume of Tris buffer. The precipitate was resuspended to about 5 mg per ml of protein in Tris buffer prior to assay and designated P1.

Adenyl Cyclase Activity—Adenyl cyclase activity was meas-
ured by a modification of the method of Krishna, Weiss, and Brodie (11). The standard reaction mixture included Tris-HCl, pH 7.5, 40 μmoles; theophylline, 10 μmoles; cAMP, \(^{1}\) 0.8 μmole; phosphoenolpyruvate, 2.5 μmoles; pyruvate kinase, 40 μg (including 5 μmoles of (NH₄)₂SO₄); MgSO₄, 5 μmoles; ATP, 1.65 μCi, 0.4 μmole; enzyme sample, containing 0.5 to 1.0 mg of protein; and water in a final volume of 1.0 ml. The reaction mixture was incubated at 30° for 10 min and then placed in a boiling water bath for 5 min. After cooling in ice for 5 min, the samples were centrifuged to remove heat-denatured protein. The supernatant fluid was quantitatively transferred to Dowex 50W-X8 columns (0.4 x 4.0 cm) prepared in Pasteur pipettes. Elution of cAMP from the columns was as described by Krishna et al. (11). Carrier ATP was added to the 3.5 ml of cAMP fraction to give a final concentration of 0.63 mM. ZnSO₄ (5%) and Ba(OH)₂ (0.3 x) were then added (0.3 ml of each) to precipitate the remaining ²H-ATP. After centrifugation, a portion of the supernatant was analyzed for radioactivity and another used to determine the absorbance at 260 μm, which provided a measure of the recovery of cAMP throughout the entire procedure. The enzyme activity was linear with time for 30 min over the range of protein concentrations used in these studies. All values reported are corrected for recovery and are expressed as nanomoles X min⁻¹ X mg of protein⁻¹. Protein was determined by the biuret reaction with bovine serum albumin as standard.

**Fluoride Assay**—Fluoride was measured by the procedure of Yamamura, Wade, and Sikes (12) with Amadac-F reagent supplied by Burdick and Jackson Laboratories, Muskegon, Michigan.

**General Properties of Enzyme**—A detailed report of the properties of adenyl cyclase of rat cerebral cortex has been submitted for publication elsewhere. In general the enzyme of cerebral cortex displays properties quite similar to those reported recently for adenyl cyclase activity (in the absence of hormones) of rat adipose tissue (13) and rabbit heart (14). Activation by NaF did not lead to significant changes in the apparent K₅₀ value (0.18 mM) for the substrate, ATP-Mg. Both Mg⁺⁺ and Mn⁺⁺ caused stimulation of enzyme activity at concentrations above that required to complex all the ATP in a manner similar to that reported for this enzyme from other sources (13, 14).

**RESULTS**

**Preliminary Observations**—In previous reports (4, 5) it was demonstrated that the addition of NaF (usually 10 mM) to the assay mixture for adenyl cyclase resulted in an increase in enzyme activity. When enzyme activity was plotted against time in the presence of NaF, the relationship was linear and could be extrapolated back through the origin of the axes of the graph (15). Thus, it was not apparent that the stimulation might result from other than an instantaneous interaction of the enzyme with NaF.

Birnbaumer, Pohl, and Rodbell (13) observed that the relation of adenyl cyclase activity to NaF concentration was sigmoidal in nature and that the concentration of NaF causing a half-maximal effect was decreased by increasing the temperature of the assay from 30° to 37°. They interpreted these results as indicating the order of the reaction to be greater than unity and that the activation process might be cooperative in character. We have made similar observations as shown in Fig. 1. An equally probable explanation for the sigmoidal shape of the curves and the displacement of the curves to lower NaF concentrations at higher temperatures is as follows. If the stimulation by NaF is the result of a time-dependent reaction of such a rate that at low concentrations of NaF the reaction is not complete during the 10-min assay period, then activation curves such as those in Fig. 1 and those obtained by Birnbaumer et al. (13) would be generated. The shift to the left at higher temperature could be a reflection of an increased rate of activation at higher temperatures.

**Activation of Adenyl Cyclase by NaF**—Adenyl cyclase preparations were incubated with or without NaF (5 mM) for 20 min at 30°, and the enzyme activity was measured in a subsequent 10-min assay in the presence and absence of NaF (Table I). For the assay the incubation mixture was diluted into the other-
Fig. 2. Effects of dialysis on NaF-activated adenyl cyclase. One-half (C) of a washed P1 fraction (5 mg of protein per ml) was incubated at 30° for 30 min in the presence of 5 mM MgSO4. The other one-half (A) was incubated in the presence of 5 mM MgSO4 plus 5 mM NaF. Samples were then assayed for adenyl cyclase activity in the presence (dark bars) or absence (open bars) of 5 mM NaF following the standard procedure. The samples were then dialyzed for 5 hours against 500 volumes of Tris-HCl-MgSO4 in the presence or absence of 5 mM NaF. The dialysis of NaF had reached equilibrium in 5 hours as determined by direct measurement of fluoride (see section under “Materials and Methods”).

wise complete reaction mixture such that the NaF concentration was diluted to 0.50 mM or 0.25 mM. It is apparent from the results that the activation of the enzyme by NaF was not reversed upon dilution of the NaF to concentrations that were insufficient to cause activation during the 10-min assay (see Fig. 1). Furthermore, adenyl cyclase activity was not decreased by extensive washing (NaF diluted 1000-fold) of the particulate enzyme by repeated centrifugation (Table I). Incubation of the enzyme preparation at 30° for 20 min in the absence of NaF caused no loss of basal activity and did not alter the sensitivity of the preparation to NaF as was indicated by a normal response to different concentrations of NaF in the subsequent assay (not illustrated). When NaF was included in the assay mixture no further activation of samples incubated with NaF was observed.

The effects of dialysis on the NaF-activated enzyme also have been examined (Fig. 2). Enzyme preparations were incubated with and without NaF, then dialyzed (500 volumes) at 4° for 5 hours in the presence or absence of NaF. Dialysis of nonactivated enzyme in the absence of NaF caused a marked loss of activity. However, the activity remaining was still increased by the inclusion of NaF in the assay mixture. Dialysis in the absence of NaF of samples incubated with NaF resulted in a small loss of total activity, but the activity remaining was not increased by NaF in the assay mixture. More extensive dialysis (16 hours, 2 x 500 volumes) of the NaF-activated enzyme resulted in greater loss of total activity, but again the remaining activity was not increased by NaF in the assay mixture. Complete retention of activity was observed when the NaF-activated enzyme was dialyzed for up to 16 hours in the presence of NaF (Fig. 2). We have concluded from these observations that during dialysis of the NaF-treated enzyme, activation is reversed slowly. The basal activity state produced by the reversal is then rapidly denatured during the dialysis. The result of a slow reversal of activation followed by a rapid loss of the basal activity is that at any point in time only enzyme in the activated state exists in significant amounts.

We have carried out a number of experiments in an attempt to find conditions for the rapid reversal of the NaF activation.
Studies on stability of NaF-activated adenyl cyclase

One portion of a washed P1 fraction was incubated at 30° for 30 min in the presence of 5 mM MgSO4 and another in the presence of 5 mM MgSO4-5 mM NaF. A portion of each sample was then (a) stored at 4° for 16 hours, (b) dialyzed twice against the buffer (500 volumes) used in the activation mixtures for 16 hours at 4°, (c) incubated at 40° for 60 min, and (d) incubated in the presence of 50 μg per ml or 12 μg per ml of trypsin for 30 min and 60 min. Aliquots (0.1 ml) of each sample were then assayed for adenyl cyclase activity. Soybean trypsin inhibitor (12 μg per ml) was included in those assay mixtures to which trypsin-treated enzyme was added.

Properties of NaF-Activated Adenyl Cyclase—We have examined some of the properties of the enzyme activated by NaF after removal of the supernatant fraction by centrifugation. No reversal was observed. Incubation of the activated enzyme with ATP and Mg++ had no effect on the activated state. As yet no conditions have been found that will cause the rapid reversal of NaF stimulation.

Kinetic Studies of NaF Activation—The time course of the activation of adenyl cyclase at different concentrations of NaF is shown in Fig. 3. The activity at various concentrations of NaF did not always increase with time to the same plateau. The absolute rate of activation varied from one preparation to another, and in some cases the activity would increase to a maximum and then slowly decrease with time. This may reflect variations in enzyme stability of different preparations.

The effect of changes in temperature on the rate of activation of the enzyme by NaF is shown in Fig. 4. A preparation of adenyl cyclase was incubated with or without 5 mM NaF at various temperatures for 10 min. After the incubation aliquots of each sample were diluted (1:10) into the assay mixture and the enzyme activity measured in the presence and absence of NaF. The crosshatched bars of Fig. 4 indicate that the rate of activation of the enzyme is a function of the temperature of the incubation reaction. With this preparation the rate of activation was somewhat lower at 37°. Since the basal activity measured at 37° also was lower than at 30°, the decrease in activation rate may have been due to enzyme instability.

A number of substances have been assayed for their effect on the rate of activation of the enzyme by NaF. Of those examined only ATP in the absence of Mg++ and Ca++ caused appreciable inhibition, while Mg++ and ATP-Mg caused about a 2-fold increase in the rate of activation.

Properties of NaF Activated Adenyl Cyclase—We have examined some of the properties of the enzyme activated by NaF after removal of the NaF by washing. In most regards the properties are similar to those determined by adding NaF (10 mM) to the assay mixture.2 The Km value for ATP-Mg (0.16 mM) is slightly increased or unchanged; Mg++ and Mn++ stimulate activity (apparently by interaction at a metal ion-binding site), Ca++ inhibits activity, and ATP in excess of Mg++ causes inhibition.

As mentioned above, the NaF-activated enzyme was more stable than the native enzyme during dialysis at 4°. Further studies (Table II) demonstrated that the NaF-activated enzyme was more stable when incubated at 40° for 1 hour or stored at 4° for 16 hours. Experiments also were carried out to compare the basal and activated enzymes with regard to inactivation by trypsin. No significant difference in the rate or extent of inactivation was observed when the enzyme preparations were incubated at 30° with 12 or 50 μg per ml of trypsin.

Effects of NaF on Adenyl Cyclase Activity of Other Tissues—NaF (5 mM) causes activation (not reversed by 1:10 dilution) during a 20-min incubation with adenyl cyclase preparations from rabbit heart, turkey erythrocyte ghosts, human tumor astrocyte cell lines 113, 118, and rabbit iris.

Effects of Detergents—The effect of Triton X-100 on the activity of adenyl cyclase is shown in Fig. 5. The enzyme preparation was incubated for 10 min at 30° in the presence of various concentrations of Triton indicated in the figure. The open bars indicate the adenyl cyclase activity in a subsequent assay that contained no added Triton. The dark bars indicate the activity in a subsequent assay that contained a concentration of Triton equal to that in the first incubation mixture. The carry over of Triton into the adenyl cyclase assay mixture was 10% in each case.

### Table II

| Sample               | Percentage of change in enzyme activity |
|----------------------|----------------------------------------|
| Storage, 4°, 16 hours|                                       |
| Dialysis, 4°, 16 hours|                                       |
| Incubation, 40°, 60 min|                                       |
| Trypsin (12 μg per ml)|                                       |
| Trypsin (50 μg per ml)|                                       |
| Enzyme               | -50 -75 -55 -8 -40 -72 -88             |
| NaF + enzyme         | 0 0 -10 -20 -40 -67 -88                |

Fig. 5. The effect of Triton X-100 on adenyl cyclase activity. Portions of a washed P1 fraction, 5 mg of protein per ml, were incubated for 10 min at 30° in the presence of the concentrations of Triton indicated in the figure. The open bars indicate the adenyl cyclase activity in a subsequent assay that contained no added Triton. The dark bars indicate the activity in a subsequent assay that contained a concentration of Triton equal to that in the first incubation mixture. The carry over of Triton into the adenyl cyclase assay mixture was 10% in each case.
The cerebral cortex of an 8-day-old rat was divided in half. One half was frozen and the other kept at 0°C. After 30 min the frozen tissue was thawed, and both tissues were homogenized in 50 mM Tris-HCl, pH 7.5 (1 g per 10 ml). The adenosyl cyclase activity of each preparation was determined in a standard assay with and without 10 mM NaF. The cerebellum of an adult rat was treated in an identical manner.

| Tissue sample | Fresh | Frozen |
|---------------|-------|--------|
|               | Control | NaF | Control | NaF |
| Cerebellum    | 0.31   | 1.44  | 1.14 | 1.32 |
| Cerebrum      | 0.23   | 0.41  | 0.41 | 0.52 |

![Graph showing effects of freezing and thawing on enzymatic activity](http://www.jbc.org/)

**Table III**

**Effect of freezing on adenosyl cyclase of rat cerebral cortex and cerebellum**

The cerebral cortex of an 8-day-old rat was divided in half. One half was frozen and the other kept at 0°C. After 30 min the frozen tissue was thawed, and both tissues were homogenized in 50 mM Tris-HCl, pH 7.5 (1 g per 10 ml). The adenosyl cyclase activity of each preparation was determined in a standard assay with and without 10 mM NaF. The cerebellum of an adult rat was treated in an identical manner.

reversal of activation. It is more probable that 0.02% Triton does not cause activation of the enzyme at the high concentration of protein in the first incubation but is sufficient to activate the enzyme when the protein concentration is diluted 10-fold. Further studies (not illustrated) have supported the contention that activation of the enzyme by Triton is not reversed upon dilution.

Sodium dodecyl sulfate also causes an increase (2-fold) in adenosyl cyclase activity over the range of 0.02 to 0.04% sodium dodecyl sulfate in the assay mixture. The range of stimulation with this substance is quite limited. No effect is observed at 0.01% and complete inhibition is observed at 0.06%.

**Effect of Freezing and Thawing on Enzyme Activity**—Early in the study we had observed that when intact cerebella from adult rats were frozen and later thawed and homogenized, adenylyl cyclase activity was higher than in homogenates of nonfrozen cerebella (Table III). The addition of NaF to the assay mixture had little or no effect on the previously frozen samples but activated the fresh sample. The stimulatory effect of freezing was not so apparent with the enzyme from the cerebrum of adult animals. We now have examined the effect of freezing on the activity of the enzyme using cerebral cortex from young (1–20 days) rats. The results presented in Table III were obtained with an 8-day-old rat. In general, freezing caused a 2-fold increase in enzyme activity of young rats. Freezing of homogenates (rather than intact tissue) of fresh rat cerebral cortex or cerebellum does not change enzyme activity or the degree of the activation by NaF.

The stimulatory effect of NaF on adenylyl cyclase of nonfrozen cerebral cortex of young rats was seldom greater than 50%, and it was apparent that NaF was not causing full expression of enzyme activity (see Table III). However, the effects of NaF shown in Table III were obtained by adding the halide to the assay mixture with no prior incubation time. In Fig. 6 is shown a comparison of the effects of NaF and Triton X-100 on adenylyl cyclase activity in homogenates of adult and young (5-day) rat cerebral cortex. When NaF (10 mM) is included in the assay mixture without prior incubation only the typical small degree (35%) of activation is observed. A 20-min incubation with NaF (5 mM) preceding assay in the presence of NaF (5 mM) results in a 3-fold activation. However, even in this case NaF does not cause complete activation since the enzyme can be stimulated 5-fold by Triton.

**DISCUSSION**

The details of the mechanism whereby adenylyl cyclase is regulated are yet to be elucidated. Two models (3, 16) have been proposed which have in common the basic concept that adenylyl cyclase is composed of a receptor-regulatory component(s) and a catalytic component(s). A multicomponent enzyme was suggested by the observations that different hormones stimulate the same catalytic activity in different tissues and that sensitivity to hormonal stimulation is a labile property. Further support for such a model has come from the recent observations that hormonal stimulation of adenylyl cyclase of thyroid, adrenal cortex, liver (8), and fat cells (9) can be blocked selectively without reduction in basal enzyme activity or response to NaF. In brain slices, cAMP levels can be increased by neurohumoral substances such as norepinephrine and histamine (17), but the response of adenylyl cyclase to these agents is not demonstrable in broken cell preparations. Presumably, the process of cell disruption is adequate to dissociate the functional relationship of the receptor and catalytic components of brain adenylyl cyclase. Similar loss of hormone sensitivity occurs upon homogenization of liver (18) and turkey erythrocytes (6).

The results presented here demonstrate that NaF activates brain adenylyl cyclase at a rate that is dependent on temperature and the concentration of NaF. The activation process is not easily reversed since little or no activity was lost upon dilution, extensive washing, or dialysis. It is not clear from these studies whether the effect of NaF is a result of chemical or enzymatic activation. It is clear, however, that activation by NaF can occur in the absence of exogenously added substances, although the reaction rate is increased about 2-fold by Mg++, Mn++, or ATP-Mg. The reaction rate is not appreciably decreased by extensive washing of the particulate enzyme, suggesting that soluble protein components are not involved in the reaction.
Schrampa and Naim (19) recently demonstrated that Mg$^{++}$ was required for the activation by NaF of adenylyl cyclase from rat parotid gland. These investigators did not carry out rate studies but made their observations after a 5-min incubation of the enzyme at 25° in the presence of NaF (10 mM) and other additives. The difference in the brain and parotid gland enzymes with respect to the requirement for Mg$^{++}$ in the activation process probably does not represent a qualitative difference but rather reflects the use of different experimental material and conditions. We have recently measured the rate of activation of rabbit heart adenylyl cyclase by NaF (5 mM) in the presence of 5 mM MgSO$_4$. The degree of activation was a function of the length of the incubation. After 30-min incubation at 30° the enzyme specific activity had increased 8-fold to a plateau, and addition of NaF (10 mM) during the subsequent assay did not increase activity further.

It should be pointed out that NaF does not necessarily cause full expression of adenylyl cyclase activity (see Table III, Fig. 6). Furthermore, the maximal effect may not be expressed if NaF is simply added to assay mixtures (Fig. 6), especially when short assay periods and low NaF concentrations are used.

The observation (15, 19) that the formation of cAMP during the assay is linear with time in the presence of NaF does not appear to be consistent with the demonstration of time-dependent activation process. A possible explanation is that in the presence of 10 mM NaF (the concentration commonly employed in assay mixtures), activation is essentially complete by the earliest time cAMP is measured (usually 2 to 4 min). The activation (Fig. 5) is about 60% of maximum after 4 min in the presence of 5 mM NaF. If the reaction rate doubles by increasing the NaF concentration to 10 mM, the activation process would be essentially complete in 4 min. The presence of the other assay constituents also may affect the activation rate. This is consistent with the observation that activation by 10 mM NaF in a 10-min assay seldom exceeds 2.5-fold, while 5 mM NaF causes a 4- to 5-fold activation during a 10-min incubation preceding the assay. Further experiments to clarify these anomalies are currently underway.

The mechanism of activation of adenylyl cyclase by NaF remains unclear. However, the activated states brought about by treatment of adenylyl cyclase with NaF, hormones, or non-specific treatments such as with detergents or by freezing are either the same or mutually exclusive since the optimal effects of the various agents are not additive (7, 10). The observation that NaF stimulates the enzyme from all mammalian sources studied, suggests that it acts at a site common to all species of adenylyl cyclase. In the two-component model proposed by Robison et al. (3) the most probable common feature of adenylyl cyclase from different sources would be the catalytic component. To a degree this is borne out by the observations that the adenylyl cyclases of fat cells (13, 15), adrenal tumor (20), rabbit heart (14), and rat cerebral cortex (4) demonstrate similar kinetic properties in the absence of added hormones.

In the broadest sense hormones could increase adenylyl cyclase activity by two mechanisms. The hormone could supply a missing component, necessary for the expression of complete enzymic activity. Alternatively, the hormone could reduce or prevent the effect of an inhibitor of enzymic activity. In the former case the stimulation would be effected, in all probability, only by close structural analogues of the hormone. In the latter case, however, stimulation could be brought about by any treatment which would selectively inactivate an inhibitor substance or dissociate the inhibitor-catalytic component complex.

If the inhibition was due to the interaction of macromolecular substances with the catalytic component of the enzyme, then even nonselective alterations of macromolecular structure (denaturation of proteins or protein-lipid complexes) could lead to an increased expression of enzyme activity. The stimulation of brain adenylyl cyclase by Triton X-100 supports the hypothesis that this enzyme exists in its membranous environment in a partially inhibited state. It is probable that Triton does not act at a specific, physiologically significant receptor sites but activates the enzyme as a result of nonselective alterations in membrane structure. The suggestion that adenylyl cyclase can be activated by a nonselective mechanism is supported by the observation that sodium dodecyl sulfate, an anionic detergent, also stimulated enzyme activity. Øye and Sutherland (6) reported that adenylyl cyclase of turkey erythrocyte ghosts was stimulated by deoxycholate, a neutral detergent. Furthermore, freezing and thawing of tissue from rat brain produced marked increases in enzyme activity, presumably in a nonspecific manner.

At present we are extending our study of the regulation of adenylyl cyclase within the guidelines of the following working hypothesis. Adenylyl cyclases are multicomponent macromolecular systems existing as integral parts of mammalian plasma membranes. The catalytic activity of the enzyme is normally inhibited due to interaction of these components. Specific hormones interact with the receptor component of the system. This interaction results in increased activity due to a reduction in the normal inhibition imposed on the catalytic component. In some instances agents that disrupt the structure of macromolecules (e.g., detergents) also can activate the enzyme by dissociation of the inhibitor-catalytic component complex. NaF activates the enzyme by interaction with some component of the system common to all species of adenylyl cyclase to bring about a similar dissociation. In all three instances activation is the result of the dissociation of an inhibitory complex.

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