



Hormone Receptor Modulates the Regulatory Component of Adenylyl Cyclase by Reducing Its Requirement for Mg^{2+} and Enhancing Its Extent of Activation by Guanine Nucleotides

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Hormone receptor modulates the regulatory component of adenylyl cyclase by reducing its requirement for Mg^{2+} and enhancing its extent of activation by guanine nucleotides

(guanosine 5'-[γ -thio]triphosphate/reconstitution analysis/ cyc^- S49 cell membrane adenylyl cyclase/glucagon)

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ABSTRACT *N*-Ethylmaleimide treatment of rat liver plasma membranes results in an adenylyl cyclase (EC 4.6.1.1) system that shows no measurable cyclizing activity but retains both an active glucagon receptor and a receptor-sensitive regulatory component N as assessed by reconstitution into cyclase-negative (cyc^-) membranes from S49 murine lymphoma. Treatment of such *N*-ethylmaleimide-treated membranes, termed C^- liver membranes, with guanosine 5'-[γ -thio]triphosphate (GTP[γ S]) and Mg^{2+} , followed by the removal of GTP[γ S] by washing, yields an activated N which upon mixing with cyc^- S49 membranes reconstitutes the cyc^- S49 membrane adenylyl cyclase in the absence of added GTP[γ S]. It was found that GTP[γ S] activation of the N at saturating concentrations of GTP[γ S] is slow at low Mg^{2+} concentration and accelerated by increasing Mg^{2+} concentrations. Addition of glucagon during the activation results in a lowering of the Mg^{2+} requirement for full activation from 25 mM to around 10 μ M and in concomitant increases in both the rate and the extent of N activation. In contrast to its dramatic effect on Mg^{2+} requirement, glucagon has little (less than 2-fold) effect on the GTP[γ S] requirement of N activation. These experiments indicate that the glucagon receptor facilitates activation of N by: (i) decreasing the apparent K_m of N for Mg^{2+} , and (ii) increasing the extent of activation that can be elicited by saturating concentrations of guanine nucleotide. It is postulated that the mechanism by which Mg^{2+} and receptors facilitate N activation involves dissociation of n_α activated ADP-ribosylatable subunits (with guanine nucleotide bound to them) from n_β non-ADP-ribosylatable subunits (with receptor and Mg^{2+} bound to them).

Adenylyl cyclases [adenylate cyclase; ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] are signal transduction systems for hormones and neurotransmitters that affect cellular function via alterations in the concentrations of cAMP, their "second messenger." To initiate their actions hormones bind to distinct entities on the plasma membranes called receptors, which then interact with adenylyl cyclase to increase reaction velocity (1). Early studies showed that the enzyme activity is dependent not only on the presence of substrate, MgATP, but also on excess Mg^{2+} , which modulates activity by interacting with an allosteric site. Hormone stimulation led both to a decrease in the requirement for Mg^{2+} at the allosteric site and to an increase in the maximal velocity of the enzyme (2).

Since those studies were performed, much information has been gained about the molecular nature of adenylyl cyclase. It is now known that guanine nucleotides play key regulatory roles (3, 4) and that their effect is mediated through a component distinct from the catalyst (5, 6). This component, termed guanine nucleotide-binding regulatory component of adenylyl cy-

clase, abbreviated here as N, but also called G/F (7) or G (8), has been shown to interact not only with guanine nucleotides but also with Mg^{2+} (9) and has been purified (7). It has been shown that stimulation of cAMP production by the catalytic component C is a consequence of activation of N (9, 10), which is measurable in reconstitution assays (10–12). It has also been shown that activation of N is the rate-limiting step in the stimulation of adenylyl cyclase and that Mg^{2+} is an important modulator of the rate at which N is activated by saturating concentrations of guanine nucleotides (9).

Studies by Citri and Schramm (13) on the mode of action of agonist-occupied β -adrenergic receptors from turkey erythrocyte membranes showed that these receptors, like guanine nucleotides and Mg^{2+} , also act on N and that receptor-mediated activation of N can occur regardless of whether a functionally active catalyst is present or not. However, these studies did not address the mechanism by which hormone receptors modulate the activation of N. We have now tested whether hormone-occupied receptors act by altering the Mg^{2+} requirement in the activation of N. These studies are reported here, and the results obtained are discussed in light of the recently reported two-subunit structure of N (7).

EXPERIMENTAL PROCEDURES

Materials and Methods. Sources of all materials used in the preparation of membranes from adenylyl cyclase-negative (cyc^-) S49 murine lymphoma cells and rat liver cells and assay conditions for determination of adenylyl cyclase in liver membranes have been described recently (14–18).

***N*-Ethylmaleimide (MalNEt) Treatment of Liver Membranes.** Liver membranes (8–10 mg of protein per ml) were exposed to 2 mM MalNEt for 20 min at 0°C in the presence of 25 mM Tris·HCl (pH 7.5) in a final volume of 5 ml. This membrane suspension was then diluted to 25 ml with ice-cold 25 mM Tris·HCl (pH 7.5) and centrifuged at $14,000 \times g$ for 15 min. This washing procedure was repeated once, followed by a second MalNEt treatment as described above. After the second treatment, the membranes were subjected to four 25-ml washes with 25 mM Tris·HCl (pH 7.5). The final pellet was resuspended in 2.5 ml of 25 mM Tris·HCl (pH 7.5), divided into aliquots, rapidly frozen in a dry ice/acetone bath, and stored at -70°C until use. Such MalNEt-treated liver membranes are termed C^- liver membranes because they display no measurable adenylyl cyclase activity.

Treatment of C^- Liver Membranes for Activation of the Regulatory Component. C^- liver membranes (2–2.5 mg of pro-

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Abbreviations: N and C, nucleotide-binding regulatory subunit and catalytic subunit, respectively, of adenylyl cyclase; MalNEt, *N*-ethylmaleimide; GTP[γ S], guanosine 5'-[γ -thio]triphosphate.

tein per ml) were treated in the presence of 12.5 mM Tris·HCl (pH 7.5), 0.2 mM dithiothreitol, 0.5 mM ATP, 16 mM creatine phosphate, creatine kinase (≈ 100 units/mg) at 0.16 mg/ml, myokinase ($\approx 1,000$ units/mg) at 0.016 mg/ml, and 0.02% bovine serum albumin in a final volume of 100 μ l at 32.5°C for the indicated time periods. Unless stated otherwise, the treatment mixtures also contained 30 nM glucagon, 2.5 mM EDTA, and specified concentrations of guanosine 5'-[γ -thio]triphosphate (GTP[γ S]) and MgCl₂. These effectors were added individually or in appropriate combinations. After treatment, the membranes were diluted to 4.0 ml with ice-cold 25 mM Tris·HCl, pH 7.5/20 mM MgCl₂ and washed by centrifugation at 100,000 \times g for 20 min. This washing procedure was repeated once more. The final pellet was suspended in 50 μ l of ice-cold 1% Tris cholate (pH 8.0) and mixed thoroughly for 1 min. The cholate suspension was then diluted to 250 μ l with 25 mM NaHepes (pH 8.0) and 10- μ l aliquots were added to the assay system for measurement of N activity.

Assay for the Active Regulatory Component (*cyc*⁻ Reconstituting Activity). This was measured by its capacity to enhance [³²P]cAMP production by *cyc*⁻ S49 membrane adenylyl cyclase in the absence of added GTP analog or hormone (glucagon) with MgATP as substrate. The assay was performed at 32.5°C; the mixture contained, in a final volume of 50 μ l, 0.1 mM [α -³²P]ATP (2,000–3,000 cpm/pmol), 25 mM MgCl₂, 1 mM EDTA, 1 mM [³H]cAMP ($\approx 10,000$ cpm), 20 mM creatine phosphate, creatine kinase at 0.2 mg/ml, myokinase at 0.02 mg/ml, and 25 mM NaHepes (pH 8.0). Unless stated otherwise, *cyc*⁻ S49 membrane protein was 25–35 μ g per assay tube and protein from liver membranes was 5–10 μ g per assay tube. After incubation for 15 min, the reaction was stopped and the [³²P]cAMP formed was quantified.

Except for one experiment (see *Results*), the Mg²⁺ concentrations stated represent values of MgCl₂ added in excess over ATP and EDTA and hence should be taken only as approximate concentrations of "free" Mg²⁺. When a more precise estimate of free Mg²⁺ was required, the total MgCl₂ that was added to achieve the desired concentrations was calculated by using the formula

$$Mg_{\text{total}} = Mg_{\text{free}} \left[1 + \frac{A_{\text{total}}}{(Mg_{\text{free}} + K_A)} + \frac{B_{\text{total}}}{(Mg_{\text{free}} + K_B)} \right],$$

in which A_{total} and B_{total} are the concentrations of added ATP and EDTA, and K_A and K_B are the stability constants of MgATP (60 μ M) and MgEDTA (0.4 μ M) at pH 7.5. All experiments were repeated at least twice and all values reported are the means \pm SD of triplicates.

RESULTS

We have shown recently that the characteristic lag with which GTP analogs stimulate adenylyl cyclase can be decreased by increasing the concentration of Mg²⁺ in the incubation medium, and that such lags are observed also with GTP at low Mg²⁺ concentration (12). Fig. 1 shows that, at limiting Mg²⁺ concentrations, glucagon addition to liver membranes results in an increase in the rate of adenylyl cyclase activation by guanine nucleotide. This is reminiscent of the effect observed upon increasing Mg²⁺ concentration (12).

In view of the recent findings that the rate of guanine nucleotide activation of solubilized N can be regulated by Mg²⁺ (9), we searched for conditions that would allow us to test the hypothesis that the hormone-receptor complex may be acting on N by reducing its apparent K_m for Mg²⁺, thereby allowing guanine nucleotides to activate N rapidly and independently of the presence of a catalytic component. Treatment of liver mem-

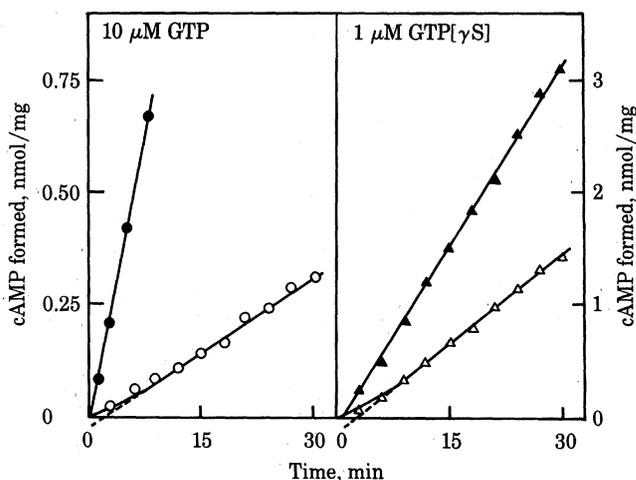


FIG. 1. Effect of glucagon on the rate of activation of liver membrane adenylyl cyclase. In addition to GTP or its analog GTP[γ S], the assay mixture contained 0.1 mM ATP, 1 mM EDTA, 0.5 mM MgCl₂, and 30 nM glucagon (\bullet , \blacktriangle) or no glucagon (\circ , \triangle).

branes with MalNET led to a system devoid of measurable catalytic activity in which receptor regulation of N could be studied.

Table 1 shows the effect of MalNET treatment on liver adenylyl cyclase activity and on *cyc*⁻ reconstituting activity in these membranes. It can be seen that, after treatment of the membranes as described under *Experimental Procedures*, they have lost all measurable adenylyl cyclase activity but retain a substantial amount of *cyc*⁻ reconstituting activity, which in several different experiments varied between 60% and 70%. Such MalNET-treated liver membranes are termed C⁻ liver membranes. The reconstituted activity measured in the experiment shown in Table 1 and in all subsequent experiments was always proportional to the amount of C⁻ liver membrane protein and hence to added N.

Table 1. Effect of MalNET treatment of liver membranes on intrinsic adenylyl cyclase and S49 *cyc*⁻ reconstituting activity

Treatment of liver membranes	pmol cAMP formed per 15 min	
	Intrinsic adenylyl cyclase activity*	S49 <i>cyc</i> ⁻ reconstituting activity*
Exp. A		
None	6.2 \pm 0.24	12.2 \pm 1.32
2 mM MalNET	0.02 \pm 0.01 [†]	7.3 \pm 0.12
Exp. B		
None	1.1 \pm 0.07	10.6 \pm 0.81
2 mM MalNET	0.03 \pm 0.01 [†]	6.4 \pm 0.31

Liver membranes were treated with MalNET and intrinsic adenylyl cyclase activity of liver membranes and *cyc*⁻ reconstituting activities were measured in the presence of 25 mM MgCl₂, 1 μ M GTP[γ S], and nM glucagon. All assay tubes contained 7–8 μ g of liver membrane protein. Tubes in which *cyc*⁻ reconstituting activity was measured received 31 μ g of *cyc*⁻ membrane protein. S49 *cyc*⁻ reconstituting activity is defined as the difference in activities exhibited by liver membranes in the presence and absence of S49 *cyc*⁻ membranes. *Exp. A.* Control and MalNET-treated liver membranes were suspended in 50 μ l of 1% cholate (3.5–4.0 mg of protein per ml) and thoroughly mixed for 1 min. This cholate suspension was then diluted to 250 μ l with 25 mM NaHepes (pH 8.0), and 10 μ l of this diluted suspension was added to assay tubes. *Exp. B.* All procedures were identical to those in *Exp. A* except that the membrane suspension in 1% cholate were incubated at room temperature (22–24°C) for 20 min.

* Values are means \pm SD of triplicate determinations.

[†] Not significantly different from assays in the absence of any added membrane.

Table 2. Treatment of C^- liver membranes with various effectors on cyc^- S49 reconstituting activity

Additions during treatment	cyc^- reconstituting activity,* pmol cAMP formed per 15 min		
	No additions	10 μ M GTP[γ S]	10 μ M GTP[γ S] + 30 nM glucagon
None	0.61 \pm 0.02	2.61 \pm 0.01	6.3 \pm 0.22
EDTA	0.54 \pm 0.03	2.83 \pm 0.01	7.1 \pm 0.28
GTP[γ S] + EDTA	0.92 \pm 0.08	3.16 \pm 0.15	7.3 \pm 0.17
Glucagon + EDTA	0.52 \pm 0.01	4.36 \pm 0.18	6.8 \pm 0.43
Glucagon + GTP[γ S]			
+ EDTA	0.93 \pm 0.04	4.14 \pm 0.09	6.41 \pm 0.31
MgCl ₂	0.48 \pm 0.03	1.45 \pm 0.05	5.46 \pm 0.27
GTP[γ S] + MgCl ₂	3.72 \pm 0.11	3.92 \pm 0.11	6.86 \pm 0.14
Glucagon + MgCl ₂	0.64 \pm 0.06	1.72 \pm 0.06	5.22 \pm 0.31
Glucagon + GTP[γ S] + MgCl ₂	7.14 \pm 0.44	6.36 \pm 0.32	7.31 \pm 0.62

Treatments were for 10 min in the presence of C^- liver membrane protein at 2.5 mg/ml without or with 2.5 mM EDTA, 25 μ M GTP[γ S], 30 nM glucagon, and 25 mM Mg²⁺. Treated membranes were washed and then assayed for cyc^- reconstituting activity in the presence or absence of GTP[γ S] and glucagon. During the reconstitution, C^- liver membrane protein was 8–9 μ g for all treatment conditions, and cyc^- membrane protein was 26 μ g.

* Values reported are means \pm SD of triplicate determinations.

Table 2 shows the effect of various ligands on the activation of N in C^- liver membranes as assessed by increases in cyc^- S49-reconstituted activity. As demonstrated previously (9), activation of the liver membrane N requires the simultaneous presence of guanine nucleotide and Mg²⁺. Neither ligand by itself produced an active N. Glucagon by itself or with either GTP[γ S] or Mg²⁺ individually was also ineffective in changing the activity of N. However, in the presence of both GTP[γ S] and Mg²⁺, glucagon increased the levels of activated N as seen by a doubling of the cyc^- reconstituted activity over that observed in the absence of glucagon with GTP[γ S] and Mg²⁺ alone. Further, the data in Table 2 show that, once N has been activated, removal of guanine nucleotide results in a relatively slow relaxation of the activated state. However, as shown previously for the activated liver N in detergent solution (9), maintenance of the activated state of the membrane-bound regulatory component was dependent on the presence of high (20–25 mM) concentrations of Mg²⁺ throughout all of the washing procedures (data not shown). Thus, whereas both ligands are required to obtain the active state of N, the presence of only one of them is required for sustenance of this activated state.

We studied the effect of various treatment times on the activation of N by GTP[γ S] in the presence and absence of glucagon at high and low concentrations of Mg²⁺. Fig. 2 shows that activation of the liver N at saturating GTP[γ S] is slow at low Mg²⁺ concentration and fast at high saturating concentrations of the ion. This finding is in agreement with our previous observation in detergent extracts (7). Addition of glucagon resulted in fast activation of N at both concentrations of Mg²⁺ tested.

Fig. 3 shows the effect of various concentrations of Mg²⁺ on the activation of N by 25 μ M GTP[γ S] in the presence and absence of glucagon. In the absence of hormone, progressively increasing concentrations of Mg²⁺ resulted in increasing activation, with maximal obtainable activation being reached with about 25 mM Mg²⁺. Addition of hormone resulted in a dramatic decrease in amount of Mg²⁺ required for full activation. In this experiment, maximal activation was observed at the lowest con-

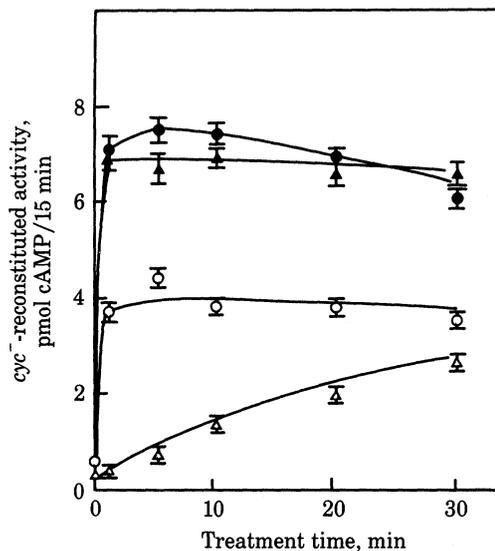


Fig. 2. Effect of the time of treatment of C^- liver membranes with 25 μ M GTP[γ S] at 2 (Δ , \blacktriangle) and 25 (\circ , \bullet) mM Mg²⁺ in the absence (\circ , Δ) and presence (\bullet , \blacktriangle) of glucagon on cyc^- S49 reconstituting activity.

centration of Mg²⁺ used (1 mM). In other experiments not shown here, concentrations as low as 10 μ M free Mg²⁺ were sufficient to sustain full activation in the presence of glucagon and GTP[γ S]. In preliminary experiments using 5 mM EDTA or 5 mM ATP as Mg²⁺ buffers, we have estimated the apparent K_m for Mg²⁺ to be in the range of 0.1–1 μ M in the presence of glucagon. However, even at 20–50 mM Mg²⁺, which provides for maximal effects with GTP[γ S] in the absence of hormone, addition of glucagon resulted, in addition, in increased levels of activation of N. In the experiment shown in Fig. 3, a 43% increase in the extent of activation was observed. In other experiments, up to a 100% increase was observed in the presence of hormone.

In contrast to the marked effect of glucagon on the Mg²⁺ requirement for the activation of N, there is little, if any, effect of the glucagon-occupied receptor on the guanine nucleotide requirement (Fig. 4). This is in agreement with our report that

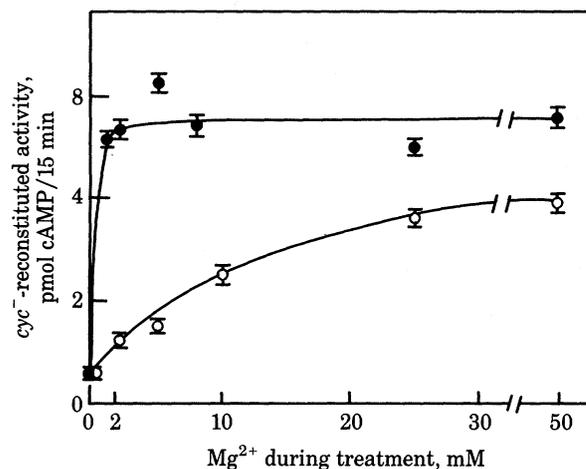


Fig. 3. Effect of various concentrations of Mg²⁺ during 10-min treatment of C^- liver membranes in the presence of 25 μ M GTP[γ S] with (\bullet) and without (\circ) glucagon on cyc^- S49 reconstituting activity. The concentration of Mg²⁺ added as MgCl₂ in excess over 0.5 mM ATP is specified. When no Mg²⁺ was added, the treatment mixture contained 2.5 mM EDTA.

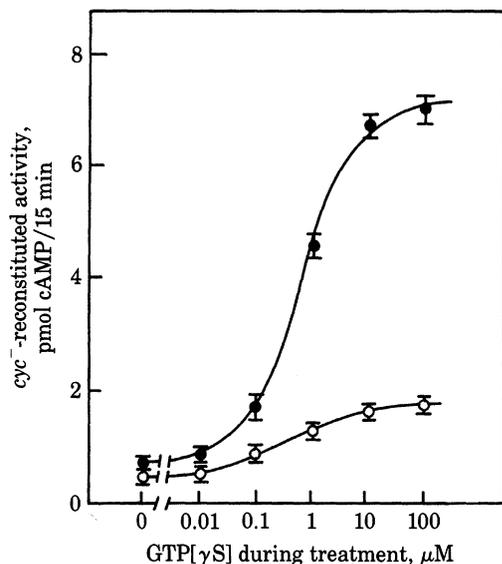


FIG. 4. Effect of various concentrations of GTP[γ S] during 10-min treatment of C^- liver membranes in the presence of 2 mM Mg^{2+} with (●) and without (○) glucagon on cyc^- S49 reconstituting activity.

hormones do not appreciably shift nucleotide concentration–effect curves in intact membranes (18). In other experiments, even at saturating (25 mM) concentrations of Mg^{2+} , no appreciable shift in the concentration of GTP[γ S] required for half-maximal activation was observed upon addition of hormone during treatment (not shown).

DISCUSSION

Site and Mode of Action of Hormone Receptors. We show here that the primary site of action of hormone receptors is at N of adenylyl cyclase. Thus, the liver glucagon receptors examined here and the β -adrenergic receptors in turkey erythrocytes (13) act similarly. In addition, by using N from liver, which, unlike N from turkey erythrocytes, can be activated by guanine nucleotides and Mg^{2+} both in the absence and in the presence of hormone, we were able to examine the effects of hormone-occupied receptors on the interaction of N with these two ligands. We found two simultaneous actions of hormone-occupied receptors on N: (i) a lowering of the apparent K_m for Mg^{2+} for the guanine nucleotide-mediated activation, which is accompanied by an enhancement of the rate of activation by guanine nucleotide; and (ii) an increase in the extent to which N is activated at saturating concentrations of guanine nucleotides and Mg^{2+} . The first of these hormonal effects had been predicted on the basis of extensive kinetic analysis of the intact adenylyl cyclase system in the absence and presence of hormone (2, 4, 12, 19), and the finding in Fig. 1, which shows that hormones accelerate the activation of adenylyl cyclase by guanine nucleotide at limiting Mg^{2+} concentrations and thereby mimic the previously shown action of saturating Mg^{2+} (12). The second effect, that of eliciting an increase in the extent of activation of N, was rather unexpected and may serve as evidence for our previous contention that the action of occupied hormone receptor is to cause a shift in an equilibrium reaction between active and inactive forms of the system (18, 20).

The observation that hormone-occupied receptors activate N primarily by lowering the Mg^{2+} requirement may be of physiological relevance. Cellular guanine nucleotide concentrations are saturating in terms of the requirement for activation of N. However, concentrations of free Mg^{2+} [≈ 0.5 mM (21)] are not

sufficient to support significant activation of this system in the absence of hormone. Because hormonal “stimulation” is associated with reduction of the apparent K_m for Mg to levels well below those found in the cell, allowing the system to become saturated by it, this effect of hormonal stimulation is likely to be responsible for activation of the system in the cellular environment. Hormone-occupied receptors may therefore be considered as “Mg switches.”

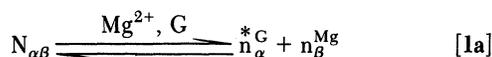
In summary, we show that an increase in the level of hormone–receptor complex results in a reduction in the K_m for a regulatory ligand at the regulatory component of adenylyl cyclase which, in turn, results in an increased level in the activated form of this regulatory component. The signal transduction process therefore appears to be initiated by a V system (increase in abundance of the active species of the hormone–receptor complex) that affects a K system (alteration of Mg^{2+} requirement for activation of N), which in turn leads to a V-type change (increase in abundance of the active species of NC complex) that results in enhanced rates of cAMP synthesis.

Possible Structural Correlates to the Stimulation of the Regulatory Component and Its Interaction with the Catalyst. Our studies demonstrate that the site of action of hormone-occupied receptor is at the level of N, where it probably alters the affinity of its site for Mg^{2+} , but they do not address the physical nature of such action. However, studies from other laboratories have shown that activation of N results in a decrease in its size as assessed by sucrose density gradient ultracentrifugation (22–24) and gel filtration (25). Activation of N is therefore likely to involve subunit dissociation as the rate-limiting step, especially because high salt concentrations increase the rate of activation of N (25). Indeed, many enzyme systems that display long-term hysteresis such as shown for adenylyl cyclase have subunit association or dissociation reactions as their rate-limiting steps (26). It has been shown that preparations of purified N from rabbit liver (7), turkey erythrocytes (24), and human erythrocytes (unpublished observations) contain two types of subunits. The first type is ADP-ribosylated by cholera toxin and is present either as a single entity of M_r 42,000 or 45,000, such as found in turkey erythrocytes (24) and human erythrocytes (unpublished observations), or as a complex of several peptides of M_r 42,000–45,000 and 50,000–52,000, such as found in liver (7). The other type is of M_r 35,000 and is not ADP-ribosylated by cholera toxin (refs. 7, 23, and 24 and unpublished observations). The M_r 42,000 and 52,000 peptides seem to be closely related (27, 28). It appears, therefore, that N is an oligomer formed of ADP-ribosylatable n_α subunits of M_r 42,000 or 52,000 and non-ADP-ribosylatable n_β subunits of M_r 35,000 and that the decrease in size observed upon activation of N is due to a dissociation reaction in which the n_α subunits separate from the n_β subunits. Recent data from Gilman’s laboratory indicate that addition of n_β to activated N results in a faster deactivation (29). These observations lend further credence to the contention that n_α when associated with n_β cannot activate C. From the photoaffinity labeling studies of Pfeuffer (5), n_α is known to bind the guanine nucleotide. However, the location of the Mg^{2+} site is not yet known. Because hormonal stimulation, while leading to “activation” of N—i.e., putative dissociation of n_α from n_β —leads to no changes in the apparent K_m for guanine nucleotide binding to n_α , but leads to both an increase in the extent of activation and a dramatic reduction in the apparent K_m of the system for Mg^{2+} , it seems possible that Mg^{2+} interacts with the subunit retained by the hormone–receptor complex, subsequent to the subunit dissociation reaction. This subunit is likely to be n_β , which, upon interacting with hormone-occupied receptor, not only increases its affinity for Mg^{2+} but concomitantly reduces its interaction with n_α , thereby fa-

ilitating the activation of the latter by guanine nucleotide. The tentative sequence of events postulated here to lead to activation of adenyl cyclase by a two-step reaction is summarized below both for activation occurring in the absence of hormone (reactions **1a** and **2**) and for activation occurring in the presence of hormone (reactions **1b** and **2**):

1. Activation of N ($N_{\alpha\beta}$) by guanine nucleotide (G) and Mg^{2+} :

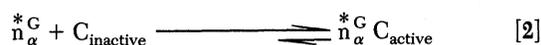
a. In the absence of hormone



b. In the presence of active hormone-receptor complex ($^H R$)



2. Activation of C by activated N subunit (n_{α}^C):



Different equilibrium positions of reactions **1a** and **1b**, the latter being more towards the direction of activation than the former, are postulated on the basis of data reported here showing that hormonal stimulation increases the extent of activation of N. Assignment of n_{α} as the subunit that interacts with C (reaction **2**) is based on ref. 10. Assignment of n_{β} as the binding site of both $^H R$ and Mg^{2+} is speculative but consistent with $^H R$ causing a reduction in apparent K_m for Mg^{2+} (Fig. 3) without affecting the apparent K_m for guanine nucleotides (Fig. 4 and ref. 18). An * over n_{α} indicates that it is active with respect to C activation and an * over n_{β} indicates that it has high affinity for Mg^{2+} . While we recognize that current data do not unequivocally establish the above sequence of events, such a scheme should be useful in designing further experiments aimed at elucidating the interaction of various subunits of N with receptor and C.

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