

The role of hormone receptors and GTP-regulatory proteins in membrane transduction

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Cell membrane receptors for hormones and neurotransmitters form oligomeric complexes with GTP-regulatory proteins and inhibit the latter from reacting with GTP. Hormones and neurotransmitters act by releasing the inhibitory constraints imposed by the receptors, thus allowing the GTP-regulatory proteins to interact with and control the activity of enzymes such as adenylate cyclase. This theory may apply generally to membrane signal transduction involving surface receptors.

ADENYLATE CYCLASE, the enzyme that produces cyclic AMP, is part of a complex regulatory system that mediates the actions of hormones and neurotransmitters on their target cells. Structured within the lipid framework of the cell membrane, the enzyme system is composed of at least three classes of components (Fig. 1). Located at the outer membrane surface is the receptor (R) component containing a specific site for binding of hormones and neurotransmitters. At the inner face of the membrane are the catalytic unit (C) and the nucleotide regulatory component (N). The latter contains site(s) for binding GTP and is responsible for mediating the effects of GTP and the various hormones on the activity of C¹. Two types of N units have been distinguished functionally. One mediates stimulation (termed N_s), the other inhibition (N_i) of the adenylate cyclase activity by GTP. As discussed below, each type seems to be linked to separate classes of receptors for hormones and neurotransmitters.

Here I present a theoretical framework for the role of hormone receptors and N units in regulating adenylate cyclase activity. In essence, the theory suggests that the N and R units normally exist separately from C as aggregates or oligomers of an RN complex. In this complex, R inhibits interaction of N with GTP. Hormone binding to R triggers release of the inhibitory constraints imposed on N with resultant enhanced reaction with GTP, followed by breakdown of the oligomers to a monomeric RN complex. The latter reacts with C to form the holoenzyme structure depicted in Fig. 1. Depending on the type of R and N unit attached to C, the holoenzyme exhibits either increased or decreased production of cyclic AMP. In developing this theory, I review evidence for the existence of N_s and N_i and their complexes with R and C, describe the properties of the various components, give recent evidence that RN oligomers exist and discuss the possibility that the theory may be generalised to include R and N units that regulate membrane transduction processes other than adenylate cyclase.

The role of N_s in activation of adenylate cyclase

Before the discovery that GTP is the essential activator of adenylate cyclase² and that hormones enhance the nucleotide's action³, it was thought that hormone receptors interacted directly with the catalytic unit and that fluoride ion, a non-physiological activator, affected the catalytic unit directly⁴. It is now clear that the actions of both hormones (through their

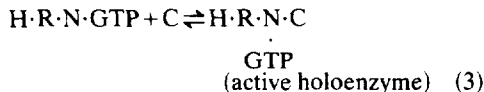
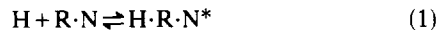
specific receptors) and fluoride ion are mediated through proteins that bind GTP. First identified^{5,6} with GTP-photoaffinity analogues as a heat-stable 42,000 molecular weight protein in detergent extracts of avian erythrocytes, the protein (N_s) has been shown to exist in a variety of cell membranes. Cholera toxin has been particularly valuable for identifying N_s. Long known to stimulate the production of cyclic AMP in animal cells⁷⁻⁹, the toxin potentiates the activating effects of GTP even in the absence of hormones and affects other characteristics of the enzyme that suggest that N_s is its primary site of action¹⁰⁻¹⁴. This was firmly established when it was found that the toxin, which contains in its A₁ subunit an ADP-ribosylating activity, preferentially labels the 42,000-MW protein in the presence of ³²P-NAD (refs 15-17). Cells deficient in N_s by functional criteria also lack the toxin-labelled protein; addition of detergent extracts of membranes containing N_s reconstitutes the ability of hormones, guanine nucleotides, fluoride ion and cholera toxin to stimulate cyclase activity in membranes from cells genetically deficient in N_s but otherwise containing hormone receptors and adenylate cyclase^{18,19}. Thus, judged from several standpoints, N_s is an essential component in the activation of adenylate cyclase. Although its structure remains unknown, N_s must have highly conserved recognition sites that allow it to activate C derived from a variety of cells and to 'couple' with the several types of receptors that mediate the stimulatory actions of hormones.

Table 1 lists a few of the properties of N_s and C when separate and combined (combinations with R are discussed below). C uses MnATP preferentially as substrate^{20,21}. Only when combined with N_s does C use the natural substrate, MgATP, as effectively as MnATP¹². Association of N_s with C is reversible and is driven by the binding of guanine nucleotides to N_s (refs 6, 22).

Properties and role of RN complexes

In classical theories of hormone action, the binding event leads the receptor to adopt a structure that is favourable for action. The relationship between hormone binding (K_D) and action (K_{act}) on adenylate cyclase systems is complicated by the fact that two ligands, hormone and GTP, are required for action². This complexity is exemplified by studies of the glucagon-sensitive cyclase system in liver membranes²³⁻²⁵. Direct binding studies with labelled glucagon revealed that GTP at concentrations required for activation of adenylate cyclase in the

presence of hormone, converted 90% of the receptors to a state with a higher K_D than K_{act} ; the remaining 10% displayed both the kinetic and thermodynamic properties commensurate with hormonal activation of the enzyme. A similar distribution of glucagon receptor states is seen in intact hepatocytes²⁶ which presumably contain sufficient GTP to interact with N_s at the internal face of the cell membrane. A plausible explanation for the effect of GTP on K_{act} and K_D is the following set of reactions (modified from ref. 27)



in which hormone binding and action are initiated at step (1) with resultant formation of an 'activated' state of $N(N^*)$. Reaction of the latter with GTP (step 2) leads to a complex ($H \cdot R \cdot N \cdot GTP$) which preferentially couples with C to form the activated holoenzyme. The negative heterotropic effects of GTP (decreased hormone binding) derive from a lower-affinity form of RN when occupied by GTP and not complexed with C (step 2). Thus, association of the macromolecular components (R, N, C) enhances the binding affinity of the small ligands (hormone and GTP). In this 'uncoupled' equilibrium model, the final concentration of the activated holoenzyme is a function of the relative concentrations of both the macromolecular components and the small ligands. In the overall equilibrium, all RN complexes have equal potential to form complexes with C but the amount of RNC formed is limited by the concentration of C. In the case of the liver system, the latter may be 10% of R and N.

Evidence from β -adrenergic receptor systems supports the above reaction scheme. For example, agonists but not antagonists promote the negative heterotropic effects of GTP on catecholamine binding²⁸⁻³². This is consistent with an ordered reaction in which hormones promote interaction of N in the RN complex with GTP. Evidence that the negative heterotropic effects derive from the RN complex stems from findings that cells lacking C but containing N_s and R display the negative heterotropic effects of GTP on hormone binding²⁰. Furthermore, RN complexes have been isolated following detergent extraction of membranes and separation from C (or NC); such complexes show the effects of GTP on hormone binding seen with intact membranes^{33,34}. Membranes from cells genetically deficient in N_s fail to show not only responses to hormones and guanine nucleotides, but also heterotropic effects of GTP on

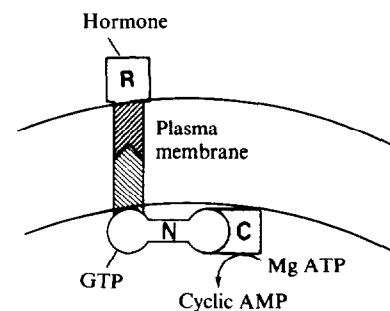


Fig. 1 Schematic representation of the components and organisation of the adenylate cyclase holoenzyme responsible for regulation by hormones and GTP. The receptor (R) is visualised as spanning the plasma membrane and having different segments (indicated by the shaded and cross-hatched areas) which have functions for binding of hormone, attachment to membrane and linkage with the nucleotide regulatory unit (N) that binds GTP. The N unit forms a bridge between R and the catalytic component (C) at the internal face of the membrane.

hormone binding to receptors; addition of extracts containing N_s to such membranes restores the ability of GTP to affect hormone binding³². Further evidence that the N_s unit responsible for activation of cyclase by guanine nucleotides, fluoride and cholera toxin is the same as that linked to R can be deduced from findings that cholera toxin affects the actions of GTP on both hormone binding and cyclase activity¹³. Accordingly, there is no need to invoke different regulatory N units linked to R and C, as has been suggested to explain differing properties of guanine nucleotide effects on hormone binding and adenylate cyclase activity^{27,33-37}. The differences can be explained by heterogeneous forms of N associated with the other components (RN, NC, RNC) having different properties with respect to affinities and actions of guanine nucleotides (Table 1).

The evidence cited above for the existence of RN complexes is based on the effects of GTP on hormone binding. On theoretical grounds, reciprocal effects of hormones on guanine nucleotide binding should also be observed. Recent findings^{38,39} that hormones promote the exchange of bound and free guanine nucleotides at the N_s unit provide further evidence that the R and N units are structurally linked. The functional consequences of this linkage on adenylate cyclase regulation are further discussed below.

It is evident that R units not associated with N units are nonfunctional with respect to adenylate cyclase activation by hormones; free R units in membranes have a lower affinity for agonists than do RN units (when unreacted with GTP)²⁸. Perhaps, free R units are formed from dissociation of RN units and are *en route* to endocytotic removal^{40,41}.

Table 2 lists receptors reported to show negative heterotropic effects of GTP on hormone binding and which are presumed, therefore, to represent RN complexes. Note that they fall into three categories: those involved in stimulation of adenylate cyclase (RN_s), those known to mediate inhibition of the enzyme (RN_i), and those which either do not interact with adenylate cyclase or whose function is unknown (RN_x).

Role of RN_i in the inhibition of adenylate cyclase by neurotransmitter and GTP

In contrast to its adenylate cyclase-activating role, GTP can inhibit some adenylate cyclase systems. First observed⁴² and characterised⁴³⁻⁴⁶ in rat adipocyte membranes, recent studies⁴⁷⁻⁴⁹ indicate that this process differs from the components that mediate stimulation by several lipolytic hormones in the same membranes. For example, adenosine promotes inhibition of adipocyte adenylate cyclase by GTP through processes which differ from the GTP-stimulatory process in their differential susceptibility to effects of sulphhydryl agents, proteases, divalent cations, sodium ions and mercurials.

Table 1 Properties of adenylate cyclase components

Components	Properties	Selected refs
C	Preferential reaction with MnATP as substrate	20, 21
NC	MgATP or MnATP as substrate when activated by Gpp(NH)p*, cholera toxin and NAD in presence of GTP, and by fluoride ion	16, 23
R	Low affinity for hormone agonists; no heterotropic effects of GTP	19
RN or (RN) ^o	GTP reduces affinity of R for hormone agonists. Hormones form tight-binding complex	24, 31, 35
RNC	Same as NC but responds to hormones and Gpp(NH)p; binds GDP tightly in absence of hormones. Hormones stimulate exchange of GDP and GTP at N site	38

*Gpp(NH)p is a GTP analogue that is not hydrolysed to GDP by GTPases in membranes.

The close functional linkage between the adenosine receptor (R-site⁵⁰) and N_i in the adipocyte suggests that there is an RN_i complex for adenosine in fat cells. It appears that RN_s and RN_i complexes in the fat cell interact with a common C unit⁴⁹. Opposing regulation by independent types of RN complexes (Fig. 2) may be widespread, particularly in cells that have adenylate cyclase systems governed by the endocrine and neuroendocrine systems. Receptors for such neurotransmitters as opiates, dopamine, catecholamines (α -adrenergic) and cholinergic agents (muscarinic) also mediate inhibition of adenylate cyclase by a GTP-dependent process⁵¹⁻⁵⁴. In common with the adenosine receptor linked to N_i in adipocytes, these receptors are sensitive to sodium ions. Interestingly, in the few cases examined, negative heterotropic effects of GTP on agonist binding are observed with the same membranes showing the GTP dependency of agonist inhibition of adenylate cyclase; sodium effects on agonist binding are also observed (Table 2). These findings suggest, by analogy with the role of RN_s complexes in the stimulation of adenylate cyclase, that RN_i complexes are involved in the inhibitory process.

The structure of RN

The technique of target size analysis has recently been used to examine the size of adenylate cyclase systems in various conditions in their membrane-bound form (for application and theory see ref. 55). Results obtained from liver⁵⁶, adipocytes and turkey erythrocyte membranes are shown in Table 3. The minimal size unit expressing activity is that obtained with MnATP as substrate and no activating ligands; it is presumed to represent C. The size increases significantly as N_sC is produced with pre-activation by guanine nucleotides or fluoride ion. Activation by hormones and GTP which should produce the holoenzyme (RNC), is accompanied by a further increment in size commensurate with this premise. In the case of the liver system, the sizes of the regulatory complexes (RN_s) were estimated from studies of the binding of labelled glucagon in the presence of GTP and from the 'ground-state' cyclase activity, that is the size of components present before coupling. Both studies indicate that the RN_s structure in the liver has a MW in the range $6-13 \times 10^5$, which is three to six times the estimated size of the RN unit associated with the holoenzyme. The conclusion from these findings is that RN_s exists as oligomers when not linked to the C unit. Note in Table 3 that the unit (presumably RN_i) responsible for inhibition of adipocyte adenylate cyclase by adenosine and GTP is significantly larger than the oligomeric RN_s unit that mediates the stimulatory effects of hormones and GTP on the same enzyme system. Although target analysis

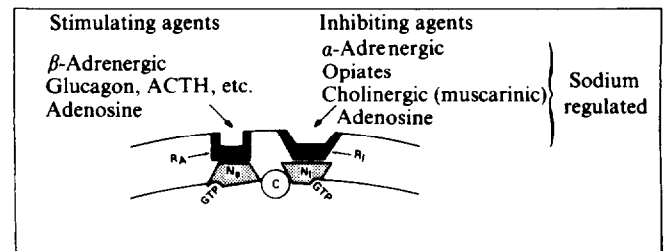


Fig. 2 Schematic representation of dual regulation of adenylate cyclase systems by stimulatory and inhibitory hormones and neurotransmitters. Depicted in the model are two classes of receptor (R) one (R_s) mediating hormone effects through stimulatory nucleotide regulatory units (N_s) and another (R_i) mediating inhibitory effects through linkage with an N_i unit that binds GTP and inhibits adenylate cyclase activity. The $R_i N_i$ units require Na^+ for the effects of the various inhibitory agents on adenylate cyclase (C) activity.

cannot give the structure and composition of the target, it can be inferred from the size differences that the stimulatory and inhibitory processes reflect different structures that are multiples of the RN units comprising the holoenzyme.

Although the sizes of the adenylate cyclase system in turkey erythrocytes display increments with increasing regulatory complexity, this system does not show the oligomeric structures of RN_s ; the functional size remains identical before and after activation with catecholamines and guanine nucleotides. Possibly, R, N and C are already assembled in the holoenzyme structure due to prior activation of the enzyme system during isolation of the membranes. Pre-assembly may explain some notable differences in kinetic behaviour of the turkey and liver adenylate cyclase systems (discussed below). A pre-assembled unit is also consistent with the report⁵⁷ of a linear relationship (rather than the usual hyperbolic relationship) between hormonal activation and receptor occupation in the turkey system.

A model for hormone and GTP action on adenylate cyclase

The existence of oligomeric complexes of RN provides a structural basis for the uncoupled equilibrium reactions described above. For illustrative purposes, the model depicted in Fig. 3 shows a tetrameric structure of RN units existing in two

Table 2 Hormone receptors regulated by GTP (the RN complex)

Receptor type	Source	Type of N unit*	Comments	Selected refs
Glucagon	Rat liver	N_s	GTP = GDP; Gpp(NH)p and Gpp(CH) ₂ p less potent	24
Catecholamines (β -receptors)	Several cell types	N_s	Divalent cations promote binding of agonists; only agonist binding affected	28-32
Prostaglandin E	Thyroid, frog erythrocyte	N_s	Binding promoted by Ca^{2+} ions	98, 99
Dopamine	Corpus striatum	N_s, N_x	GTP = GDP > Gpp(NH)p; agonist specific	100, 101
Muscarinic	Canine and rat myocardium	N_i	Methacholine inhibits GTP effects on β -receptor; Na^+ probably affects binding	102, 103
	Neuroblastoma \times glioma cells	N_i	Mg^{2+} enhances agonist binding	105
Catecholamines (α -receptors)	Brain	N_x	GTP = Gpp(NH)p > GDP; Na^+ affects agonist binding	106, 107
	Rat liver	N_x	Agonist specific	83
Angiotensin	Adrenal cortex	N_x	Agonist binding affected by Na^+ in same manner as by GTP	84
Opiates	Brain	N_x	Na^+ affects binding of agonists and antagonists. Two distinct opiate receptors	52, 104, 108

In all cases, addition of guanine nucleotides decreases binding of hormone or neurotransmitter to specific receptors in isolated membrane preparations.

* N_s is N unit linked to stimulation of adenylate cyclase; N_i affects inhibition of the enzyme; N_x is an N unit that is either not related to cyclase activity or has an undetermined relationship to a specific signal-processing system in the cell membrane.

configurations: an unoccupied structure (A) which favours binding of hormones but not GTP, and a hormone-induced or stabilised structure (B) which favours reaction of GTP with the N component (equivalent to N^* in the previous reaction scheme). Reaction with GTP results in dispersion of the oligomer to monomers (at this stage the negative heterotropic effects of GTP occur) which uniquely react with C to form the holoenzyme that converts MgATP to cyclic AMP. In broad terms, this 'disaggregation-coupling' model for hormone and GTP action can be likened to the manner by which cyclic AMP controls through its receptor the activity of protein kinase; the latter involves a change in the association of dimeric regulatory and catalytic subunits⁵⁸. In the case of hormone receptors associated with adenylate cyclase systems, their interaction with N units in the oligomeric state constrains the ability of N to react with GTP, thus preventing the formation of the 'active' form of the regulatory N unit (N_s or N_i). It follows that cyclase systems devoid of receptors linked to N should display high reactivity with GTP. This has been reported⁵⁹ recently with a strain of HeLa cells deficient in β -adrenergic receptors; when the same cells become enriched with receptors, catecholamines are required to restore the level of activity seen with GTP alone in receptor-deficient cells.

The disaggregation-coupling model has several other important features that serve to distinguish it from previous theories of hormone action⁶⁰⁻⁶². First, assuming that the oligomers of RN are unreactive with C, it provides a means for functional compartmentalisation of RN units from C and explains the observed dependency of the reaction on both hormone and GTP. Second, an oligomeric structure of RN allows for homotropic subunit interactions such that minimal occupation by hormones may cause near maximal production of the functional activating 'signal' (the RN-GTP monomer) in the presence of saturating concentrations of GTP; marked activation of adenylate cyclase with minimal occupation of receptors has been observed with several cyclase systems^{24,57,63,64}. Thus, although full occupation of receptor subunits may drive complete coupling of RN with C, coupling may occur even when these units are not occupied with hormone. Third, because the oligomeric RN structure is inert with respect to activation of adenylate cyclase, aggregation of uncoupled regulatory units is a means of 'turning off' the reaction cycle. How 'uncoupling-aggregation' is accomplished and what factors control this part of the cycle remain unknown. Possible candidates are cytosolic factors reported to affect adenylate cyclase activity⁶⁵⁻⁶⁹. Additionally, factors intrinsic to or associated with the plasma membrane may be essential in the aggregation-disaggregation cycle; these factors could include cytoskeletal elements⁷⁰ which may stabilise the aggregated units in the membrane; interestingly, agents that disrupt cytoskeletal structures have been reported^{71,72} to enhance cellular production of cyclic AMP in response to hormones.

Emphasis has been placed recently on regulation of hydrolysis of GTP by a GTPase putatively associated with the N_s unit^{14,73,74}

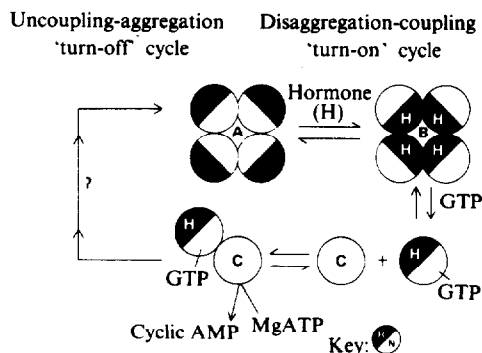


Fig. 3 A model for coupling of the receptor-nucleotide regulatory units (RN) to the catalytic unit (C) of adenylate cyclase and the role of hormone and GTP in this process. See text for detailed description.

Table 3 Functional sizes of adenylate cyclase components

Enzyme source	Components ($MW \times 10^5$)				
	RN_s	C	N_sC	RN_sC	R_iN_i
Rat liver: R = glucagon	6-13*	1.5	2.3	3.5	—
Fat cell (rat)					
R = catecholamine, ACTH	13	†	2.3	†	>13
R_i = adenosine					
Turkey erythrocyte	ND	0.9	1.8	2.5	—
R = catecholamine					

Sizes were determined by target size analysis using high energy irradiation. See ref. 56 for methods for determining size and rationale for assigning components to size. Data for fat cell and turkey erythrocyte data are unpublished. ND, Not detected.

* Estimated both from GTP-sensitive glucagon binding and adenylate cyclase activity of 'ground-state' enzyme.

† Not determined.

and the role of this regulation in turning-off the GTP-activation process. In turkey erythrocyte membranes, catecholamines and cholera toxin affect GTPase activity and GDP is a potent inhibitor that binds tightly to N_s (refs 38, 39). The differences in the kinetic characteristics of the liver cyclase system in its response to GTP and Gpp(NH)p are consistent with a GTPase being involved in its dynamic characteristics⁷⁵. However, GDP has been shown to stimulate liver cyclase activity in the presence of glucagon^{1,36,76}; stimulatory effects of GDP have been reported also in the presence of ACTH on the adipocyte adenylate cyclase system⁷⁷. In view of these findings it is not clear that a GTPase turn-off mechanism^{78,79} (which necessarily requires that GDP binds tightly and acts only as inhibitor) can adequately explain the dynamic properties of all adenylate cyclase systems. Perhaps the difference in the properties of the turkey, liver and adipocyte systems is related to the lack of an oligomeric structure of RN in isolated turkey erythrocyte membranes.

The topographical relationship between the RN oligomers and C in the plasma membrane and the role of membrane lipids in this relationship remain unknown. Studies with phospholipases have shown that hormone action on adenylate cyclase remains intact even after 85% of the membrane phospholipids have been digested; substantial loss in action occurs only when a fraction of the remaining phospholipids is hydrolysed⁸⁰⁻⁸². These findings could mean that RN oligomers and C are in close proximity and possibly in selective domains of interacting phospholipids; the bulk of the phospholipids do not seem to be involved in assembly of the holoenzyme.

Generalisations and problems

If the notion is accepted that the effects of GTP on hormone binding to receptors are due to disaggregation of oligomers of receptors linked to N units, then the theory cited above may apply generally to all hormone-regulated systems that illustrate these effects, even those N units (N_s) not linked to adenylate cyclase. The latter include such GTP-affected receptors as angiotensin receptors in the adrenal medulla⁸⁴ and α -adrenergic receptors in liver⁸³ (see Table 2). Recent findings⁸⁵ that insulin activates a cyclic AMP-independent protein kinase in sarcolemma membranes by a process regulated by GTP is an interesting example of a potential N_s -mediated process unrelated to adenylate cyclase.

The possibility that different N units mediate the actions of hormones raises interesting new questions. Can the properties of the same receptor be modified by interaction with different types of N units? What determines the interactions of receptors with a particular type of N unit? The pertinence of these questions is exemplified by reports that catecholamines, acting through α -adrenergic receptors (by pharmacological criteria), both stimulate⁸⁶ and inhibit^{51,54} cyclic AMP production, and induce effects unrelated to adenylate cyclase activity⁸³. Other hormones exerting multiple effects on membrane processes

include adenosine⁴⁸⁻⁵⁰, dopamine^{53,87-89}, opiates⁹⁰⁻⁹², vasopressin^{93,94} and serotonin⁹⁵. Pharmacological studies (specificity, potency, antagonist or hormone binding to receptors) alone may not identify the type of receptor mediating these processes. Heterotropic effects of GTP on agonist binding indicate linkage of receptors to an N unit but do not identify the type of N unit. I have noted in Table 2 that certain RN complexes are affected by sodium ions, others by divalent cations, and that guanine nucleotides have different potencies on agonist binding. Perhaps such differences can be used to classify the N unit associated with the receptor. Clearly, what is necessary in future research in this area is to develop assay methods that identify unequivocally each type of N unit. Testing of biological effects is not necessarily the means of determining the type of RN unit. A bizarre example of problems encountered is the action of cholecystokinin on pancreatic acinar cells⁹⁶. In the cell the hormone stimulates zymogen secretion, calcium release and cyclic GMP production, but not the production of cyclic AMP; after breakage of the cell, the hormone stimulates adenylate cyclase in a GTP-dependent fashion.

Although other explanations are possible, these findings raise the possibility that 'redistribution' of receptors and N units occurs on cell breakage owing to the breakdown of stationary domains that normally segregate these units in the cell. 'Lateral domain redistribution' might be a physiologically regulated process due either to changes in the structural relationship of the cytoskeleton to the ordered domains or to propagated disturbances in the membrane structure. A possible example of the

latter is the report⁹⁷ that catecholamines, acting through a β -adrenergic receptor, stimulate a phospholipid methylating enzyme by a process which is affected by GTP but which does not involve the production of cyclic AMP; associated with methylation is an apparent 'flip' of the internally methylated lipids to the outer face of the membrane and a change in lipid microviscosity. Pleiotropic effects of the hormone could thus be generated by localised changes in lipid structure being propagated laterally and modifying the postulated domain structures of the membrane. In any event, these findings question the commonly held view that β -adrenergic receptors are singularly linked to stimulation of adenylate cyclase. Moreover, the dependency of the hormone effect on GTP suggests again the versatility of the N units in mediating the actions of hormones.

In conclusion, the classical notion of receptor alone controlling the events related to hormone and neurotransmitter action on adenylate cyclase is no longer valid. The constraining role of hormone receptors postulated here differs from the role of the receptor postulated in other theories of hormone action. More importantly, the theory places in perspective the role of another set of regulatory proteins (the N units) hitherto given relatively scant attention, particularly with regard to their apparent multiple and fundamental roles in the regulation of membrane-associated processes. I hope this rather brief article will stimulate investigation of the new problems to be faced in ascertaining both the structures of the GTP-regulatory proteins and how they function in the transduction of hormone binding at cell membrane receptors into physiological action.

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