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REVIEW ARTICLE

Activation and attenuation of adenylate cyclase

The role of GTP-binding proteins as macromolecular messengers in receptor–cyclase coupling

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Perspective

Since the discovery that cyclic AMP mediates the action of a large number and variety of hormones (Sutherland & Robison, 1966), there has been considerable interest in trying to understand the molecular mechanisms by which hormone occupancy of specific cell surface receptors results in activation of membrane-bound adenylate cyclase, the enzyme responsible for synthesis of cyclic AMP from ATP. Although the earliest concepts of the adenylate cyclase system suggested that hormones might regulate adenylate cyclase activity via binding at allosteric sites on the catalytic moiety, more recently it has been demonstrated that adenylate cyclase systems consist of separable receptor and catalytic subunits (Orly & Schramm, 1976; Limbird & Lefkowitz, 1977; Haga *et al.*, 1977*a*) and, in addition, a third separable component which confers the multiple regulatory effects of guanine nucleotides on the adenylate cyclase system (for a review focusing on the biochemical properties of the separate components see Ross & Gilman, 1980). The focus of the present review is on these regulatory effects of guanine nucleotides and on the growing body of evidence suggesting that the guanine nucleotide regulatory protein (G-protein) may represent the functional communicator between hormone-occupied receptors and the adenylate cyclase enzyme. The perspective of this review is a historical one; it attempts to summarize the critical observations that established the important role of guanine nucleotides in regulating adenylate cyclase activity and to describe how the further exploration of several apparent enigmas eventually revealed the partial reactions that are involved in receptor–cyclase coupling and hormone-induced transmembrane signaling.

Abbreviations used: G-protein, guanine nucleotide regulatory protein (also known as G/F, G or N); p[NH]ppA, adenosine 5′-[β,γ-imido]triphosphate; p[NH]ppG, guanosine 5′-[β,γ-imido]triphosphate; pp[CH_2]pG, guanosine 5′-[α,β-methylene]triphosphate; GTPγS(= [S]pppG), guanosine 5′-[γ-thio]triphosphate; SDS, sodium dodecyl sulphate; C and R, catalytic and regulatory components of adenylate cyclase; $K_{0.5}$, the concentration of effector which results in apparent 50% occupancy of a given site or apparent 50% maximal stimulation of a given effect (it is not necessarily equivalent to a true K_D or K_m).

The important role of guanine nucleotides

A crucial breakthrough for subsequent studies aimed at understanding receptor–cyclase coupling was the realization that there is an absolute requirement for guanine nucleotides in the stimulation of adenylate cyclase by hormones and drugs. Initially, it was thought that guanine nucleotides were only required to 'enhance' or amplify hormone-stimulated adenylate cyclase. Recognition of the obligatory role of GTP relied on three technological advancements: (1) preparation of membranes washed free of endogenous guanine nucleotide contaminants; (2) the availability of ATP preparations free of contaminating GTP or GDP (e.g. prepared by phosphorylation of adenosine rather than by extraction of skeletal muscle) and (3) inhibition of membrane transphosphorylation of GDP→GTP by utilizing the ATPase-resistant ATP analogue, p[NH]ppA, as a substrate for adenylate cyclase. It has now been demonstrated that guanine nucleotides are required for stimulation of adenylate cyclase by virtually all hormones and drugs investigated to date (Cryer *et al.*, 1969; Rodbell *et al.*, 1971*a*,*b*, 1974, 1975; Londos *et al.*, 1974; Ross *et al.*, 1977; Williams & Lefkowitz, 1977). The $K_{0.5}$ for guanine nucleotide stimulation of adenylate cyclase is in the range of 10^{-8}–10^{-7} M, and typically demonstrates the specificity of p[NH]ppG ≥ GTP > GDP ≫ GMP; adenine and pyridine nucleotides are without a significant effect.

Several lines of experimental evidence have established the triphosphate nature of the guanine nucleotide requirement. Most recently, guanosine 5′-[γ-thio]diphosphate, a transphosphorylase resistant analogue of GDP, has been employed to demonstrate that GDP is a competitive inhibitor of GTP activation of adenylate cyclase (Eckstein *et al.*, 1979). In certain model systems, e.g. the turkey erythrocyte, GDP cannot mimic or even partially fulfill the role of GTP. In other systems, such as rat

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liver plasma membranes, GDP appears to facilitate hormone-stimulated adenylate cyclase activity, although at a significantly lower rate than observed with GTP in the same preparations (Eckstein *et al.*, 1979; Iyengar & Birnbaumer, 1979). However, it is still argued whether this effect of GDP represents a low intrinsic activity of the diphosphate nucleotide or is simply a consequence of membrane-catalysed transphosphorylation of GDP to GTP (Kimura & Nagata, 1979). A second line of evidence suggesting that GTP is the modulator of hormonal responsiveness *in vivo* is derived from studies using mycophenolic acid, an inhibitor of IMP dehydrogenase, to decrease intracellular GTP levels. An 80% reduction in intracellular GTP levels results in a concomitant 40–70% loss in responsiveness to catecholamines in C6 glioma cells (Franklin & Twose, 1977), Erlich ascites tumour cells (Smith *et al.*, 1977) and NRK cells (Johnson & Mukku, 1979) and a 50% decrease in the stimulated production of cyclic AMP by prostaglandin E_1 in NRK cells (Johnson & Mukku, 1979). Re-incubation of cells with guanosine to restore GTP concentrations to normal levels results in the restoration of hormone responsiveness in these cells. The third, and actually the earliest, line of evidence indicating that a triphosphate guanine nucleotide was favoured in activation of adenylate cyclase evolved from studies using the phosphatase and pyrophosphatase resistant analogues of GTP, p[NH]ppG and pp[CH_2]pG, respectively (Londos *et al.*, 1974, 1977; Speigel *et al.*, 1977). It was demonstrated that these analogues were not only capable of facilitating hormone activation of adenylate cyclase but could also stimulate adenylate cyclase independently of hormones in some tissues. Inherent in the results from studies with the hydrolysis-resistant analogues of GTP was the conclusion that, despite the apparent requirement for a triphosphate guanine nucleotide in mediating hormone activation of adenylate cyclase, the terminal phosphate apparently need not be hydrolysed for participation in phosphorylation or pyrophosphorylation reactions.

The kinetics of activation of adenylate cyclase in the presence of the hydrolysis-resistant analogues were characterized by two unique features when compared with the effects of native GTP: (1) the activation of the enzyme proceeded after a considerable lag which could be shortened or eliminated by increasing the concentration of hormone added to the analogue-containing incubation (Londos *et al.*, 1974) and (2) the stimulated activity of adenylate cyclase obtained with hydrolysis-resistant analogues appeared 'irreversible' and was stable to multiple washes of the membranes as well as to solubilization by detergents (Schramm & Rodbell, 1975; Londos *et al.*, 1974; Lefkowitz, 1974; Lefkowitz & Caron, 1975). Both characteristics of p[NH]ppG activation of adenylate cyclase, when explored further, provided new insights into the partial reactions involved in hormone and guanine nucleotide activation of adenylate cyclase.

At least two possible molecular explanations might account for the hysteresis, or lag, observed for stimulation of adenylate cyclase by p[NH]ppG (Frieden, 1970). First, the lag might be due to a slow isomerization between two activity states of the enzyme, and the role of hormone in reducing the hysteresis would in this case be due to increasing the rate of isomerization to the active state of the enzyme (Birnbaumer *et al.*, 1980*b*). Alternatively, the lag in stimulation by p[NH]ppG could also be accounted for by the time required for endogenous nucleotides to dissociate from the GTP-binding site. Shortening of the lag in p[NH]ppG stimulation by hormone could then result from a hormone-stimulated release of endogenous nucleotides and/or exchange at the GTP binding site (Blume & Foster, 1976). This latter possibility was approached directly by Cassel & Selinger (1977*a*, 1978), who demonstrated that the β-adrenergic agonist isoproterenol was able to stimulate the release of [^{3}H]p[NH]ppG. When [^{3}H]GTP was used as the ligand to identify guanine-nucleotide-binding sites in turkey erythrocyte membranes, the primary nucleotide released was [^{3}H]GDP, suggesting that a hydrolysis step may usually accompany activation and/or deactivation of the adenylate cyclase system (Cassel & Selinger, 1978). Most importantly, however, was the observation that catecholamines could facilitate the release of bound GDP and this was interpreted as representing the mechanism by which hormones accelerate the activation of adenylate cyclase by p[NH]ppG (Cassel & Selinger, 1977*b*, 1978; Lad *et al.*, 1980; Swillens *et al.*, 1979). Consistent with this interpretation are observations that pretreatment of turkey erythrocyte membranes with isoproterenol and GMP prior to extensive membrane washing results in a preparation of adenylate cyclase which is responsive to p[NH]ppG in the absence of hormones without an apparent lag (Lad *et al.*, 1980). GMP was included in the above pretreatment studies presumably to occupy the guanine-nucleotide-binding sites with a stabilizing ligand possessing low affinity, so that subsequently added GTP or p[NH]ppG would easily compete for GMP binding to the important regulatory site.

An interesting, and often overlooked, additional aspect of the catecholamine-stimulated release of GDP or p[NH]ppG release from the nucleotide regulatory site has been the observation that the simultaneous presence of unlabelled guanine nucleotides significantly facilitates hormone-induced release of [^{3}H]guanine nucleotide under incubation conditions for which it can be calculated that [^{3}H]guanine nucleotide rebinding is not likely

(Cassel & Selinger, 1977*b*, 1978). This apparent co-operativity suggests that the regulatory component conferring guanine nucleotide sensitivity to the adenylate cyclase system either has more than one nucleotide binding site, or is multimeric or both.

Despite the clear ability of hormones to facilitate release of GDP or exchange of guanine nucleotides at the regulatory site, there is still argument concerning the rate-limiting step in the activation of adenylate cyclase by hormones (Levitzki, 1980*a*). Birnbaumer and colleagues (Birnbaumer *et al.*, 1980*a*,*b*; Iyengar *et al.*, 1980) postulate that an 'isomerization step' subsequent to occupancy by p[NH]ppG of its appropriate binding site represents the critical rate-limiting step in activation of adenylate cyclase, since the duration of the lag observed for guanine nucleotide activation of adenylate cyclase in rat liver plasma membranes is different for different guanine nucleotide analogues. These authors consider that, if emptying of the binding site were the rate-limiting step, the duration of the lag should be independent of the guanine nucleotide added. Recent kinetic studies of reconstitution of p[NH]ppG-stimulated adenylate cyclase activity to isolated guanine nucleotide regulatory and catalytic components indicate that any isomerization, or conformational change, involved must occur within the regulatory protein itself rather than there being a slow conformational change in the catalytic subunit or a slow association of the regulatory and catalytic components (Strittmatter & Neer, 1980). This tentative conclusion is based on the observation that pre-treatment of the isolated nucleotide regulatory component with p[NH]ppG accelerates the rate of activation by p[NH]ppG of subsequently added catalytic component, an observation that would not be predicted if the rate-limiting step in enzyme activation were distal to the nucleotide protein itself. However, it should be remembered that experiments designed to determine the rate-limiting step of adenylate cyclase activation will be difficult to interpret unequivocally if guanine nucleotides both facilitate their own rate of exchange at GTP-binding site(s) (Cassel & Selinger, 1977*b*, 1978), and also regulate the expression of adenylate cyclase activity.

A second aspect of the kinetics of activation of adenylate cyclase that subsequently provided additional insights into the partial reactions involved in regulation of the adenylate cyclase system was the apparent 'irreversibility' or persistence of activation of p[NH]ppG (Lefkowitz, 1974; Lefkowitz & Caron, 1975; Schramm & Rodbell, 1975; Bennett & Cuatrecasas, 1976). Coupled with the finding that GDP was the major nucleotide released from hormone-treated membranes (Cassel & Selinger, 1978), the 'irreversibility' or the activation of adenylate cyclase by hydrolysis-resistant analogues suggested that the hydrolysis of GTP might terminate the activation of adenylate cyclase. {Actually, 'irreversibility' is a misnomer. Consistent with the ability of hormones to facilitate the exchange of guanine nucleotides at the G-protein, incubation of p[NH]ppG-preactivated membranes with hormone and GTP reduces preactivated catalytic activity to that characteristic of hormone + GTP in certain systems studied (Londos *et al.*, 1977; Cassel & Selinger, 1977*b*; Sevilla & Levitzki, 1977; Sevilla *et al.*, 1976.}

Consistent with the prediction that a GTP hydrolysis function is involved in regulating the adenylate cyclase system, Cassel & Selinger (1976) were able to demonstrate the existence of a catecholamine-stimulated GTPase activity in turkey erythrocyte membranes. Despite a high 'background' GTPase activity, these investigators noted a 30–70% stimulation of hydrolysis by catecholamines. The apparent affinity for GTP and isoproterenol in the GTP-hydrolysis reaction is comparable with the apparent affinities of these agents in modifying adenylate cyclase activity. The putative relationship of the catecholamine-sensitive GTPase to the function of adenylate cyclase was further corroborated by the observation that isoproterenol-stimulated GTPase was inhibited by p[NH]ppG and by cholera toxin (Cassel & Selinger, 1977*a*), both agents that promote an apparently irreversible activation of adenylate cyclase. On the basis of these observations, Cassel *et al.* (1977) postulated that activation of adenylate cyclase involves occupancy by GTP, or a guanosine triphosphate analogue, and that the hydrolysis of GTP to GDP represents the 'turnoff' mechanism by which the catecholamine-sensitive system relaxes. Catecholamine-stimulated GTPase activity has subsequently been detected in frog erythrocyte membranes (Pike & Lefkowitz, 1980) and appears to be correlated with both activation and desensitization of the adenylate cyclase system. Thus, when the frog erythrocyte is desensitized by incubation with 10^{-4}M-isoproterenol, decreases in catecholamine-stimulated adenylate cyclase are paralleled by decreases in both isoproterenol-stimulated GTPase and β-adrenergic receptor binding activities. Interestingly, incubation of turkey erythrocytes with 10^{-4}M-isoproterenol results in a loss of both catecholamine-stimulated adenylate cyclase and GTPase activities but no decrease in receptor binding. GTPase activities sensitive to prostaglandin E_1 (Bitonti *et al.*, 1980), secretin and pancreozymin-related peptides (Lambert *et al.*, 1979) and glucagon (Kimura & Shimada, 1980) have now been observed in membranes from human mononuclear cells, rat pancreas and rat liver, respectively. In the case of the membranes from mononuclear cells (Bitonti *et al.*, 1980), there is an apparently poor correlation

between prostaglandin stimulation of GTPase and adenylate cyclase activities. However, in trying to relate the responsiveness of adenylate cyclase to hormone-stimulatable GTPase activity, it is important to remember that the apparent GTPase activity may represent both a genuine increase in $V_{max.}$ stimulated by hormone, as well as a hormone-facilitated replenishment of GTP at the substrate binding site secondary to hormone-facilitated guanine-nucleotide exchange. The extent to which one or the other of these reactions 'dominates' will no doubt affect the extent to which hormone-stimulated adenylate cyclase activity appears to be related to hormone-modified GTPase activity. For systems in which hormone-sensitive GTPase activity is not easily measured directly, Cassell *et al.* (1977, 1979) have described a kinetic approach by which the rate of cyclase 'turnoff' can be calculated.

Effect of guanine nucleotides on receptor interactions with hormones and agonist drugs

Since it had been observed that hormones and agonist drugs accelerate the rate of interaction of GTP with adenylate cyclase systems, it was expected that guanine nucleotides might modify the kinetics of receptor–hormone interactions. The availability of radioactively labelled hormones and drugs that specifically identify their physiologically relevant receptors allowed the effect of guanine nucleotides on receptor–hormone interactions to be measured directly. The ability of guanine nucleotides to increase the rate of dissociation of hormone from the receptor was first observed with receptors for glucagon in rat liver membranes (Rodbell *et al.*, 1971*a*; Lin *et al.*, 1977). This increased rate of dissociation is paralleled by a decrease in receptor affinity ($K_A = k_{on}/k_{off}$) for hormones. In catecholamine-stimulated adenylate cyclase systems, for which both radiolabelled agonists and antagonists are available to identify the β-adrenergic receptor, it has been demonstrated that guanine nucleotides only decrease receptor affinity for agonists and do not modify receptor–antagonist interactions (Maguire *et al.*, 1976; Lefkowitz *et al.*, 1976). A decrease in receptor affinity for agonists is most easily investigated by studying the competitive binding of unlabelled agonist for radiolabelled antagonist in the presence and absence of added guanine nucleotides. In the absence of added nucleotides, the agonist competition curve is usually abnormally shallow, suggesting a heterogeneity of receptor–agonist interactions. The addition of guanine nucleotides shifts the competition curve to the right, consistent with a reduction in receptor affinity for agonists, and to normal steepness (i.e. pseudo-Hill coefficient approx. 1.0), consistent with receptor–agonist interactions obeying a simple mass action law. This phenomenon has been observed in a number of target membranes. Computer analysis of agonist competition for the binding of [^{3}H]dihydroalprenolol to frog erythrocyte β-adrenergic receptors indicates that the shallow competition-binding curve, possessing an overall higher potency of agonist in the absence of guanine nucleotides, is consistent with occupancy of the receptors by agonists inducing a higher-affinity state of the receptor for a certain fraction of the receptor population (Kent *et al.*, 1980). Guanine nucleotides decrease the fraction of the agonist-occupied receptors in the higher affinity state in a concentration-dependent manner and, at maximal concentrations (e.g. 0.1mM-GTP), convert all of the agonist-occupied receptors to the lower-affinity state, thus providing an explanation for the observation that agonist competition curves are shifted to the right and are of normal steepness in the presence of guanine nucleotides (Kent *et al.*, 1980). Within a particular target system, the extent of the guanine nucleotide-induced shift in receptor affinity is directly proportional to the intrinsic activity of the agonist in stimulating adenylate cyclase (Lefkowitz *et al.*, 1976). The detection of agonist-promoted receptor interactions of higher affinity which are reversed by guanine nucleotides is variable with different target membranes. Thus, guanine nucleotide-promoted shifts in receptor affinity for agonist are more prominent in membranes from S49 lymphoma cells (Ross *et al.*, 1977; Maguire *et al.*, 1976), frog erythrocytes (Lefkowitz *et al.*, 1976), and rat reticulocytes (Limbird *et al.*, 1980) than, for example, in membranes from turkey erythrocytes (Limbird *et al.*, 1979*a*; Lad *et al.*, 1980). However, pre-treatment [e.g., isoproterenol and GMP (Lad *et al.*, 1980)] of turkey erythrocyte membranes under conditions that are intended to decrease contamination with endogenous nucleotides enhances the ability to detect both higher affinity agonist–receptor interactions and GTP-promoted decreases in receptor affinity. Thus, the majority of target systems studied to date appear to be qualitatively similar with regard to the ability of guanine nucleotides to modulate receptor–hormone or receptor–agonist interactions, but may differ quantitatively in this respect, presumably because of different intrinsic rate constants for certain of the partial reactions that occur after the receptor is occupied but before adenylate cyclase is activated.

Regulatory functions of GTP mediated through a separate component

Two important lines of evidence, biochemical and genetic, established that guanine nucleotides exert their regulatory effects via an entity that is separate from the hormonal receptor or catalytic component of adenylate cyclase. The biochemical approach was taken by Pfeuffer (1977), who prepared a biologically active [^{3}H]azido-anilide analogue of GTP that

bound to membrane proteins with M_r values approx. 86000, 53000, 42000 and 23000 (determined by SDS/polyacrylamide-gel electrophoresis) in pigeon erythrocytes. Primarily the 42000 and 23000-M_r GTP-binding subunits were solubilized by the detergent Lubrol-PX, but only the 42000-M_r species appeared to be associated with the catalytic activity of detergent-solubilized adenylate cyclase in sucrose gradients. Using a complementary biochemical approach, Pfeuffer (1977) designed a GTP-affinity resin to isolate GTP-binding components from the Lubrol-solubilized material. The eluate that did not absorb to the GTP resin contained adenylate cyclase activity that was 70–85% less responsive to NaF and p[NH]ppG than before chromatography. A fraction from the column eluted with p[NH]ppG restored 60–75% of sensitivity to p[NH]ppG and 85% of sensitivity to NaF to the non-adsorbed eluate. These results were confirmed by Speigel *et al.* (1979) using turkey erythrocyte membranes. Taken together, these results indicate that a 42000-M_r subunit binds GTP and, upon reassociation with what is presumably the isolated catalytic moiety, restores not only guanine nucleotide sensitivity but also NaF sensitivity to the adenylate cyclase system. Although Pfeuffer (1977) originally reported that this 42000 M_r G-protein could be removed from the membrane with EDTA, suggesting that this moiety is an 'extrinsic' membrane protein (Steck & Yu, 1973; Yu *et al.*, 1973); this has not been confirmed by others. Instead, the G-protein meets the criteria for an 'intrinsic' membrane protein (Steck & Yu, 1973). Hydrodynamic studies on the detergent-solubilized G-protein have suggested that it (Howlett & Gilman, 1980; Kaslow *et al.*, 1980) binds less detergent than do the catalytic (Neer, 1978; Haga *et al.*, 1977) or receptor (Haga *et al.*, 1977) moieties of the adenylate cyclase system in the same target membrane. These findings are consistent with the hypothesis that the G-protein possesses a relatively small hydrophobic surface area and may therefore not penetrate very far into the biological membrane. Unequivocal determination of the surface area of the G-protein and the nature of its interaction with target membranes, especially as regulated by hormones, GTP and/or NaF, will require studies with purified material in carefully defined reconstituted systems.

Confidence that the 42000-M_r species isolated by Pfeuffer was the G-protein that confers guanine-nucleotide sensitivity to the adenylate cyclase system was bolstered by the almost simultaneous discovery that cholera toxin catalyses the ADP-ribosylation of a 42000-M_r peptide in target membranes (Gill & Meren, 1978; Cassel & Pfeuffer, 1978; Johnson *et al.*, 1978; Moss & Vaughan, 1979). Cholera toxin had long been known to activate adenylate cyclase persistently by a mechanism predicted (Levinson & Blume, 1977), and later demonstrated, to be due to an inhibition of the hydrolysis of GTP in the adenylate cyclase system (Cassel & Selinger, 1977*b*). Thus, following exposure to cholera toxin, GTP behaves like the hydrolysis-resistant analogue p[NH]ppG in producing a persistent activation of adenylate cyclase. Gill & Meren (1978) and others (Cassel & Pfeuffer, 1978; Johnson *et al.*, 1978) demonstrated that incubation of broken cells with cholera toxin and [^{32}P]NAD$^+$ resulted in a transfer of [^{32}P]ADP-ribose to a 42000-M_r peptide. The extent of the covalent modification of this peptide was related to increments in the cholera-toxin-promoted activity of GTP-sensitive adenylate cyclase activity and to decrements in the NaF-sensitive adenylate cyclase activity and the inhibition of catecholamine stimulated GTPase activity. More recently, Lad *et al.* (1980) have argued that cholera-toxin-catalysed ADP-ribosylation also enhances the rate of exchange at the GTP-binding site. If true, this effect would not only contribute to an increase in GTP-sensitive adenylate cyclase but would also explain the increased affinity of toad erythrocyte β-adrenergic receptors for agonists in membranes previously exposed to cholera toxin (Fisher & Sharp, 1978), since the ability of agonist occupying the receptor to induce a high-affinity agonist–receptor complex would be facilitated by decreased contamination of the membranes by endogenous nucleotides.

Perhaps the most important consequence of the discovery of the catalytic function of cholera toxin is the ability to identify specifically the G-protein in functional association with adenylate cyclase systems by using [^{32}P]ADP-ribose as a covalent marker. The precise number of [^{32}P]ADP-ribose moieties incorporated per 42000-M_r subunit is not yet known, but the ratio of mol of [^{32}P]ADP-ribose incorporated per mol of β-adrenergic receptor in several target membranes is 1:1 to 8:1 (Gill & Meren, 1978; Cassel & Pfeuffer, 1978; Johnson *et al.*, 1978; Kaslow *et al.*, 1979; Limbird *et al.*, 1980*b*). This is a considerably more conservative ratio than the 20–100:1 ratio obtained with [^{3}H]-guanine nucleotide binding as an estimate of G-protein quantity (e.g. Speigel & Aurbach, 1974; Lefkowitz, 1975). It is usually presumed that the 'excess' of GTP-binding sites identified by [^{3}H]p[NH]ppG or [^{3}H]GTP represents interactions with GTP-binding proteins involved in biological mechanisms that are unrelated to adenylate cyclase. However, it is equally possible that a large fraction of [^{3}H]p[NH]ppG binding proteins in plasma membranes are, in fact, capable of interacting with the adenylate cyclase system, although the actual number of G-proteins modifying the catalytic activity of adenylate cyclase at any one time may be quite small and may be determined, in part, by the

hormone and nucleotide effectors impinging on the system. This possibility has recently been strengthened in studies demonstrating that the apparently 'excess' G-proteins can restore the sensitivity to guanine nucleotides of isolated catalytic subunits in reconstitution experiments (Strittmatter & Neer, 1980).

Genetic manipulation and reconstitution have provided another avenue for functionally and physically isolating the G-protein, as well as for beginning to identify the nature of its interaction with other components of the adenylate cyclase systems. Mutants of S49 lymphoma cells with functional defects in the adenylate cyclase system are isolated by their resistance to the cytotoxic effects of intracellular cyclic AMP that is elevated in response to isoproterenol or cholera toxin (Daniel *et al.*, 1973; Bourne *et al.*, 1975; Haga *et al.*, 1977*b*). Two categories of mutants have been particularly important for furthering the understanding of adenylate cyclase, the AC^- mutant (discovered and termed cyc^- by Bourne *et al.*, 1975) and the UNC mutants (Haga *et al.*, 1977*b*). Both the AC^- and the UNC mutants possess β-adrenergic and prostaglandin E_1 receptors and the catalytic subunit of the adenylate cyclase system. However, the AC^- mutant lacks a G-protein, at least as far as can be established by using any existing functional or structural criteria (Bourne *et al.*, 1975; Ross & Gilman, 1977*a*,*b*; Johnson *et al.*, 1978). As a consequence, catalytic activity in AC^- membranes is detectable only in the presence of the artificial substrate, Mn^{2+}–ATP, and no responsiveness to guanine nucleotides, NaF, hormones or cholera toxin is demonstrable (Ross *et al.*, 1978). G-proteins from wild-type S49 lymphoma membranes (Ross *et al.*, 1978; Howlett *et al.*, 1979) and other sources (Ross & Gilman, 1977*a*,*b*) restore basal (Mg^{2+}–ATP), NaF-, guanine nucleotide-, cholera toxin- and hormone-sensitive adenylate cyclase activity to AC^- membranes. Two important conclusions can be derived from these reconstitution studies: (1) Mg^{2+}-supported 'basal' activity of adenylate cyclase represents the concerted function of the separate catalytic and G-protein moieties, and (2) the G-protein confers sensitivity to both NaF and guanine nucleotides to the catalytic moiety as suggested in the earlier studies of Pfeuffer (1977).

Adenylate cyclase in UNC ('uncoupled') membranes is sensitive to NaF, p[NH]ppG and cholera toxin, but is insensitive to stimulation by catecholamines and prostaglandin E_1 (Haga *et al.*, 1977*b*; Kaslow *et al.*, 1979). The UNC mutation appears to be defective in the G-protein, since all functions of the adenylate cyclase system can be restored to normal by reconstitution of the UNC membranes with G-proteins from wild-type membranes (Howlett *et al.*, 1979; Sternweis & Gilman, 1979). Recent evidence suggests that the lesion in the UNC G-protein may be a consequence of an alteration in a post-translational modification. Isoelectric focusing of the [^{32}P]ADP-ribosylated G-proteins from UNC and wild type membranes results in a family of peptides differing by a surface charge of -1, calculated from the pI of the peptides (Schleifer *et al.*, 1980). In all cases, however, the G-protein subunits from UNC membranes have a more negative surface charge than G-proteins from wild-type membranes, which could hypothetically be accounted for by the retention of an additional phosphate group. Other interpretations are, of course, possible, but it is interesting to speculate whether or not the UNC mutant represents the omission of a phosphatase function necessary for receptor–effector coupling in activation and/or desensitization of adenylate cyclase. A role for phosphorylation–dephosphorylation in the regulation of adenylate cyclase has been postulated (Constantopoulous & Najjar, 1973; Najjar & Constantopoulos, 1973), although it has never been directly demonstrated.

Purification of the guanine nucleotide regulatory protein

Gilman and colleagues have recently succeeded in purifying the G-protein from rabbit liver plasma membranes as well as from turkey erythrocyte plasma membranes (Northup *et al.*, 1980; Sternweis *et al.*, 1981). The assay for the G-protein during purification was the functional reconstitution of p[NH]ppG-stimulated and NaF-stimulated adenylate cyclase to AC^- preparations. The G-protein complex isolated from rabbit liver membranes appears to consist of three non-identical subunits of M_r approx. 52000, 45000 and 35000 associated in an unknown stoichiometry. The G-protein from turkey erythrocytes possesses only the 45000 and 35000 M_r species (Sternweis *et al.*, 1981). The possible relationship of these subunits to the overall function of the G-protein is as follows. The 45000-M_r subunit is undoubtedly the same as the 42000-M_r species identified by Pfeuffer with the [^{3}H]-azido-anilide analogue of GTP, and responsible, at least in part, for restoring p[NH]ppG-stimulated and NaF-stimulated activity to the catalytically active preparation, deficient in regulatory proteins, that is obtained after exposure to a GTP–Sepharose resin (Pfeuffer, 1977). The 45000-M_r subunit is clearly a substrate for ADP-ribosylation by cholera toxin both in native membranes and following reconstitution of purified G-proteins into AC^- acceptor membranes. Interestingly, the purified G-protein isolated from membranes is not a substrate for cholera toxin-catalysed ADP-ribosylation, since it requires the presence of additional 'factors' to be covalently modified (Sternweis *et al.*, 1981).

One factor, possessing a Stokes' radius corresponding to a globular protein of M_r approx. 50000, can be solubilized from rabbit liver or turkey erythrocyte membranes, and does not appear to correspond to the active catalytic subunit of adenylate cyclase. The relationship of this membrane-provided factor to the approx. 20000-M_r cytosolic factor previously reported to facilitate ADP-ribosylation catalysed by cholera toxin (Enomoto & Gill, 1980) is not yet known. Nor is it known if the 50000-M_r 'factor' might represent yet another component, not previously identified, of the membrane-bound adenylate cyclase system.

The 52000-M_r subunit of the G-protein is also ADP-ribosylated by cholera toxin (Kaslow *et al.*, 1979; Northup *et al.*, 1980). This particular subunit is not observed in turkey erythrocyte membranes or human erythrocyte membranes treated with [^{32}P]-NAD^+ and cholera toxin (Kaslow *et al.*, 1979) and, as mentioned above, cannot be detected as a subunit of the G-protein purified from turkey erythrocyte membranes by either [^{32}P]ADP-ribosylation or protein staining techniques (Sternweis *et al.*, 1981). Since both human and turkey erythrocyte G-proteins nevertheless restore NaF and guanine nucleotide sensitivity to AC^- acceptor membranes, the 52000-M_r subunit is clearly not essential for G-protein activity. Actually, the characteristics of adenylate cyclase in native turkey erythrocyte membranes or AC^- membranes reconstituted with turkey-erythrocyte G-proteins suggest that the 52000-M_r subunit may be more closely aligned, both physically and functionally, with hormone receptors, or at least with guanine nucleotide exchange reactions. Thus, the intrinsic rate of guanine nucleotide exchange appears to be virtually zero in turkey erythrocyte membranes except in the presence of hormone (Cassel & Selinger, 1977*a*, 1978), and this stubborn exchange is paralleled by a trivial effect of p[NH]ppG and GTPγS on the catalytic activity of adenylate cyclase in turkey erythrocytes unless isoproterenol is simultaneously present to accelerate the rate of guanine nucleotide interaction with the cyclase system (Cassel *et al.*, 1977; Kaslow *et al.*, 1979; Lad *et al.*, 1980). Similarly, AC^- membranes reconstituted with turkey erythrocyte G-proteins demonstrate the same stringent requirement for isoproterenol for stimulation of cyclic AMP synthesis by GTP and GTPγS, in distinct contrast to observations in AC^- membranes reconstituted with wild type S49 G-proteins (Kaslow *et al.*, 1979). To continue this speculation, the 52000-M_r subunit may represent the additional binding site for GTP that provides for guanine nucleotide exchange facilitated by GTP (Cassel & Selinger, 1977*a*, 1978), since it should be remembered that Pfeuffer's [^{3}H]azido-anilide GTP analogue did identify a 53000-M_r component of pigeon erythrocyte membranes (Pfeuffer, 1977). Certainly, reconstitution studies with purified G-proteins differing in their stoichiometry of 52000-M_r: 45000-M_r subunits will resolve some of these questions. Of further interest are the data of Hudson & Johnson (1980), which demonstrate that the peptide map of the 52000-M_r subunits shows considerable overlap with that of the 45000-M_r subunit and, furthermore, that a 45000-M_r product can be obtained by mild proteolysis of the 52000-M_r subunit. These data suggest that the 52000-M_r subunit may be a precursor of the 45000-M_r form.

The role of the 35000-M_r subunit is least understood, but it is considered to be an integral part of the G-protein since it cannot be resolved from the other two subunits except by conditions that inactivate the reconstituting ability of the 45000-M_r and 52000-M_r subunits (Sternweis *et al.*, 1981). Perhaps the 35000-M_r species is critical for interaction of the G-protein with other protein components of the adenylate cyclase system or with the lipid milieu of the target membrane.

At present, the stoichiometry of the three subunits of the G-protein comprising a functional complex is not clear. Hydrodynamic studies of the partially purified G-protein suggest that the complex behaves with an apparent molecular mass of 130000 daltons. Pre-activation with p[NH]ppG or NaF decreases the sedimentation coefficient of the G-protein consistent with a decrease in mass of 40000 daltons (Howlett & Gilman, 1980). However, recentrifugation of this 'smaller' species in the absence of halide or nucleotide effectors results in the re-appearance of the faster-sedimenting form of the G-protein. These observations are puzzling, but suggest either that a dramatic change in shape of the G-protein accompanies activation by NaF or p[NH]ppG or, alternatively, that association–dissociation phenomena accompany activation–deactivation of the G-protein and may play a role in regulation of adenylate cyclase by hormone and nucleotide effectors *in vivo*.

Molecular interactions among protein components of the adenylate cyclase system

Even before the recent availability of techniques for purifying the G-protein, there has been considerable interest in the sequence of molecular events that accompany hormone interaction with the adenylate cyclase system. Limbird and colleagues demonstrated that agonist occupancy of β-adrenergic receptors in frog erythrocyte (Limbird & Lefkowitz, 1978) and rat reticulocyte (Limbird *et al.*, 1980*a*) membranes caused a physical coupling of the β-adrenergic receptor with the G-protein, identified by [^{32}P]ADP-ribosylation catalysed by cholera toxin (Limbird *et al.*, 1980*a*). Thus, occupancy of the receptor by the agonist [^{3}H]hydroxybenzylisoproterenol promotes an increase in apparent size of

the digitonin-solubilized receptor that is not mimicked by antagonist occupancy of the receptor, but is reversed or prevented by guanine nucleotides. The [^{32}P]ADP-ribosylated G-proteins are co-eluted with the receptor only when it is occupied by agonists. The catalytic moiety of adenylate cyclase is not associated with these agonist-promoted receptor–G-protein complexes (Limbird *et al.*, 1979*c*). The co-elution of the β-adrenergic receptor with [^{32}P]ADP-ribosylated G-proteins, whose covalent modification parallels increases in GTP-sensitive adenylate cyclase activity, suggests that the same G-protein, or population of G-proteins, modulates both receptor affinity and catalytic activity. A similar conclusion has been reached on theoretical grounds (Levitzki, 1980*b*) and is corroborated by the parallel restoration of hormone-sensitive cyclase activity and guanine nucleotide modulation of receptor affinity that occurs upon the reassociation of G-proteins with AC$^-$ or UNC mutants of S49 lymphoma cells (Ross *et al.*, 1978; Sternweis & Gilman, 1979). Furthermore, Stadel & Lefkowitz (1981) have provided additional evidence that a single G-protein population modifies both receptor and catalytic functions. These investigators isolated G-proteins associated with agonist-occupied receptors by absorbing the complexes to wheat-germ agglutinin–Sepharose and then eluting the G-proteins with GTPγS. The G-proteins thus eluted allowed adenylate cyclase to be stimulated in solubilized preparations from turkey erythrocytes previously devoid of sensitivity to exogenous guanine nucleotides.

In much the same way that agonist occupancy of the β-adrenergic receptor appears to promote or stabilize receptor–G-protein interactions, the occupancy of G-protein by GTPγS, but not by GDP, promotes formation of a stable complex of G-protein with C (the catalytic component) that can be isolated by sucrose-gradient centrifugation (Pfeuffer, 1979). This coincides with the ability of guanine nucleotides to stabilize the interaction of the G-protein with AC$^-$ and UNC acceptor preparations in reconstitution studies (Ross *et al.*, 1978; Howlett *et al.*, 1979; Sternweis & Gilman, 1979). Concomitant with this interaction of G-protein with C is an increase in the affinity of the G-protein for bound guanine nucleotides (Nielsen *et al.*, 1980; Strittmatter & Neer, 1980).

Fig. 1 provides a schematic diagram of the postulated interactions between the known molecular components of the adenylate cyclase system in the presence of hormones and guanine nucleotides, which is based on the kinetic, molecular and reconstitution studies described throughout this review. Occupancy of the receptor by agonist promotes or stabilizes an interaction between the receptor and the G-protein (Limbird & Lefkowitz, 1978; Limbird *et al.*, 1980*a*). This interaction represents the high-affinity state of the receptor for agonists (DeLean *et al.*, 1980) and provides a molecular basis for the release of GDP from the regulatory protein (Cassel & Selinger, 1977*b*, 1978). Binding of presumably ambient GTP simultaneously dissociates the R–G-protein complex (Limbird *et al.*, 1980*a*), which results in a homogeneous population of dissociated receptors possessing a lower affinity for agonist (DeLean *et al.*, 1980), and promotes or stabilizes the association of the G-protein with C (Pfeuffer, 1979). Synthesis of cyclic AMP continues until GTP is hydrolysed by the GTPase 'turnoff' reaction (Cassel *et al.*, 1977), thus leaving GDP on the G-protein and reducing the affinity of the G-protein–C interaction (Pfeuffer,

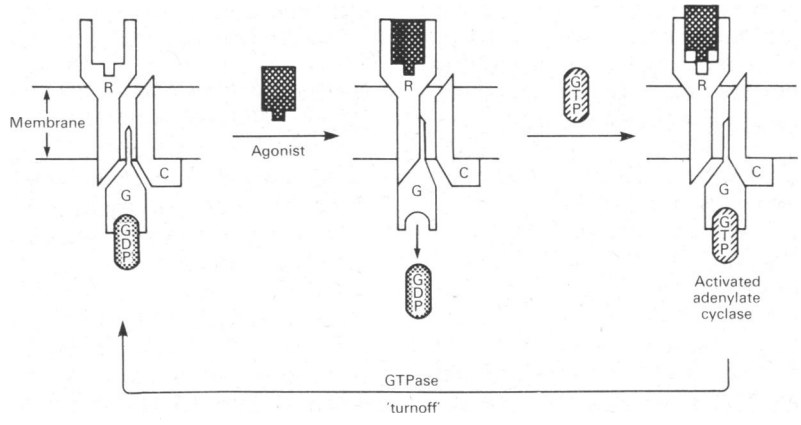

Fig. 1. *Postulated molecular interactions that accompany hormone-stimulated and guanine nucleotide-stimulated adenylate cyclase activity based on data summarized in this review*

R, receptor for hormones and drugs; G, G-protein conferring sensitivity to guanine nucleotides; C, catalytic component.

1979). What is emphasized in this scheme is the pivotal role of the G-protein in communicating between the agonist- or hormone-occupied receptor and the catalytic subunit of adenylate cyclase. The obligatory role of the initial R–G-protein interaction as a prerequisite for hormone-stimulated adenylate cyclase activity is indicated by several lines of evidence. First, the AC^- mutant of S49 lymphoma cells does not possess a functional G-protein and is devoid of hormone-sensitive adenylate cyclase activity. Secondly, rat reticulocyte membranes possess an easily demonstrable complex of receptor with G-protein, promoted by agonists, which correlates with the marked stimulation of the adenylate cyclase in reticulocytes by isoproterenol or p[NH]ppG (Limbird *et al.*, 1980*a*). In contrast, this agonist-promoted complex cannot be demonstrated in mature rat erythrocyte membranes, where adenylate cyclase activity is significantly less sensitive to catecholamines or p[NH]ppG than in reticulocyte preparations (Limbird *et al.*, 1980*b*). Thirdly, formation of this complex as an intermediate in stimulation of adenylate cyclase has been demonstrated in experiments where isoproterenol-pretreated turkey and frog erythrocyte membranes are assayed for p[NH]ppG-sensitive adenylate cyclase activity in the presence of the antagonist, propranolol (Stadel *et al.*, 1980). In both preparations, p[NH]ppG stimulates adenylate cyclase to a much greater extent in membranes pre-exposed to agonist. These data are again consistent with the postulate that occupancy by agonist of at least the β-adrenergic receptors promotes the formation of a stable R–G-protein complex that is an important intermediate in the stimulation of adenylate cyclase. Recent reconstitution studies using isolated β-adrenergic receptor and G-protein preparations not only have confirmed the role of agonist in promoting or stabilizing an R–G-protein complex, but also have demonstrated that under certain circumstances R can act catalytically in activating (i.e. facilitating the occupancy of G-protein with p[NH]ppG) at least ten times its own amount of G-protein (Citri & Schramm, 1980). Finally, although the scheme in Fig. 1, and the above data, emphasize the sequential order of events in activating adenylate cyclase, it should be pointed out that many of the partial reactions can be isolated and studied only in cell-free systems, where endogenous guanine nucleotides are removed. *In vivo*, where the concentration of GTP is adequate to prevent accumulation of a long-lived R–G-protein intermediate, the 'collision coupling' model (Tolkovsky & Levitzki, 1978) may represent an appropriate description of the molecular events involved between β-adrenergic receptor occupancy and adenylate cyclase activation.

The ability of agonists to promote the formation of an R–G-protein complex corresponds to the ability of agonist to form a high-affinity state of the receptor (DeLean *et al.*, 1980; Limbird *et al.*, 1980*a*). The coexistence of agonist–R–G-protein and agonist–R complexes in the target membrane provides a molecular explanation for the shallow curves characteristic of agonist competition for antagonist binding (DeLean *et al.*, 1980). The ability of guanine nucleotides to shift agonist competition curves to the 'right' and to normal steepness parallels the ability of guanine nucleotides to dissociate the R–G-protein complex (Limbird *et al.*, 1980*a*), generating a homogeneous population of receptors of lower affinity for agonists. Consistent with the interpretation that an R–G-protein complex represents the high-affinity state of the receptor are observations with AC^- and UNC mutant membranes, where the functional absence of a G-protein (AC) or presence of a defective G-protein (UNC) is associated with the absence of shallow, higher affinity competition curves for agonist. Instead, agonist-competition curves for receptor binding in these mutant membranes are of normal steepness, exhibit the lower affinity characteristic of wild-type membranes in the presence of guanine nucleotides, and are not modulated by the addition of guanine nucleotides to the incubation medium (Ross *et al.*, 1977). Studies with perturbants of the adenylate cyclase system that uncouple the communication of either R to G-protein (Pike & Lefkowitz, 1978; Stadel & Lefkowitz, 1980) or G-protein to C (Limbird *et al.*, 1979*b*; Stadel & Lefkowitz, 1980) provide data that are entirely consistent with observations on the genetic mutants of the S49 lymphoma line. The functional stoichiometry of the components of the adenylate cyclase system implied in Fig. 1 ($R_1:G_1:C_1$) has not yet been documented by biochemical findings, but has been predicted by computer-modelling studies (DeLean *et al.*, 1980). Another complexity of the system, i.e. the stoichiometry of the 52000-, 45000- and 35000-M_r subunits within the functional G-protein complex, has not yet been established but this is likely to be an important regulatory factor that determines the responsiveness of an adenylate cyclase system (Sternweis *et al.*, 1981).

Another experimental approach employed in an attempt to understand the molecular architecture of the adenylate cyclase system is target-size analysis after high-energy radiation inactivation (Houslay *et al.*, 1977; Schlegel *et al.*, 1979). In this technique, membranes are irradiated with high-energy electrons, and the quantity of energy or duration of exposure required to inactivate a measured function (e.g. receptor, binding or cyclase activity) is thought to correlate with the volume or size of the structure required for the measured function in the membrane (Lea, 1955; Kempner & Schlegel, 1977). The presumed appeal of this approach is that the effect of

hormones and guanine nucleotides on molecular associations within the adenylate cyclase system can be evaluated without disruption of the membrane by detergents; however, the use of lyophilized membranes and/or cold (−110°C) conditions for irradiation cannot be assumed not to cause perturbations at the time of analysis that are not detectable in the assay of subsequently rehydrated or thawed samples. Perhaps it is the lack of definition and/or control over these experimental variables that is responsible for the apparently contradictory conclusions drawn from target-size analysis of a single model system, the glucagon-sensitive adenylate cyclase of rat liver. Thus, the findings of Schlegel *et al.* (1979) predict that the regulatory components remain associated with the catalytic subunit when it is in its activated state, whereas the findings of Martin *et al.* (1980) predict that the activation of adenylate cyclase by hormones and p[NH]ppG results in a dissociated catalytic subunit.

Bimodal regulation of adenylate cyclase by GTP

In addition to a crucial role in activating adenylate cyclase, GTP has also been demonstrated to inhibit adenylate cyclase both in the presence and absence of hormones. The bimodal regulation of adenylate cyclase has been studied most rigorously in the fat-cell system (Harwood *et al.*, 1973; Rodbell, 1975; Yamamura *et al.*, 1977; Cooper *et al.*, 1979; Londos *et al.*, 1981).

For systems in which GTP both activates and inhibits adenylate cyclase, the $K_{0.5}$ for GTP activation is usually 10–100-fold lower (higher affinity) than the $K_{0.5}$ for GTP inhibition of adenylate cyclase. The apparent inhibition by GTP at high concentrations is presumably not due to an accumulation of inhibitory GDP, since GDP alone will not support the inhibitory phase (Harwood *et al.*, 1973). Furthermore, the inhibitory phase of GTP regulation is not observed in the presence of p[NH]ppG or after exposure to cholera toxin, suggesting that hydrolysis of the terminal phosphate of GTP may, in fact, be involved as part of the mechanism. A role for GTP as a phosphate donor for phosphorylation of the adenylate cyclase system has, however, not yet been directly demonstrated. The differential effects of trypsin (Yamamura *et al.*, 1977), divalent cations (Cooper *et al.*, 1979) and treatment with mercurials (Cooper *et al.*, 1979) on the activation and inhibition of adenylate cyclase have suggested that distinct regulatory components are responsible for activation and inhibition of adenylate cyclase by GTP. However, since these findings can also be interpreted in terms of differing molecular domains of a single G-protein, or differing consequences of the occupancy of G-protein by increasing concentrations of GTP, it is premature to make claims regarding the molecular nature of the bimodal regulation of adenylate cyclase by GTP until direct biochemical evidence is available.

Hormonal attenuation of adenylate cyclase activity

A rapidly developing research field is the exploration of the mechanism(s) by which hormones inhibit or attenuate adenylate cyclase. Examples of attenuating systems are muscarinic attenuation of catecholamine-stimulated adenylate cyclase in mammalian myocardium (Jakobs *et al.*, 1979), opiate, muscarinic and α-adrenergic attenuation of prostaglandin E_1-stimulated adenylate cyclase in NG 108-15 cells (Sharma *et al.*, 1977; Nathanson *et al.*, 1978; Sabol & Nirenberg, 1979) and α-adrenergic attenuation of prostaglandin E_1 stimulation of adenylate cyclase in human platelets (Jakobs *et al.*, 1979; Steer & Wood, 1979). It should be stressed that receptors that are distinct from those coupled to activation of adenylate cyclase are involved in attenuation of catalytic activity. In a recent review on hormonal inhibition of adenylate cyclase, Jakobs (1979) tabulated the generalizations that can be drawn from the present data. (1) Hormone-induced inhibition of basal or hormone-stimulated adenylate cyclase is never complete, and 40–60% of the control activity is typically retained in the presence of attenuating hormones. (2) GTP is required for the attenuation of adenylate cyclase. The $K_{0.5}$ for GTP in attenuating functions is 10–100-fold higher than the $K_{0.5}$ for GTP in activating functions in the same target membranes. For membrane systems in which GTP has bimodal effects on the cyclase, hormone-induced attenuation is apparent only in the presence of GTP concentrations that lead to inhibition of adenylate cyclase. (3) Attenuation of adenylate cyclase cannot be detected in the presence of GTPase-resistant analogues of GTP, e.g. p[NH]ppG. (4) Guanine nucleotides reduce the attenuating receptor's affinity for agonist in all systems studied to date. (5) Na^+ ($K_{0.5}$ approx. 40mM; $Na^+ \simeq Li^+ > K^+$) is either required for, or enhances, the apparent attenuation of adenylate cyclase in most of the systems studied. One role for Na^+ appears to be elimination of the GTP inhibitory phase of nucleotide regulation, so that inhibition of adenylate cyclase by the attenuating hormone is more apparent (Londos *et al.*, 1981).

Despite the differences between the activating and attenuating hormone systems, the requirement for GTP in hormonal attenuation of adenylate cyclase and the ability of GTP to modulate attenuating receptor affinity for agonists is reminiscent of the effects of guanine nucleotide on activating systems. The similarities observed for the effects of GTP in activating, and attenuating, adenylate cyclase naturally suggest the possibility that similar molecular interactions may account for the effects of guanine nucleotides.

Computer analysis of the modulation of the affinity of human platelet α-adrenergic receptors for agonists by guanine nucleotides suggests that a ternary complex of agonist–receptor–G-protein exists for the human platelet α-receptor similar to that described for β-adrenergic receptors coupled to activation of adenylate cyclase (Hoffman *et al.*, 1980). Furthermore, agonist occupancy of the human platelet α-adrenergic receptor appears to stabilize these receptor–effector interactions occurring in the membrane to solubilization by detergent, since agonist–receptor complexes can be resolved from unoccupied or antagonist-occupied α-adrenergic receptors by their faster sedimentation in sucrose gradients (Smith & Limbird, 1981). Despite the suggestion from these preliminary data that a similar sequence of molecular events may accompany both activation and attenuation of adenylate cyclase, an important question still remains, and that is whether the same, or distinct, G-proteins confer activating and attenuating signals to the adenylate cyclase enzyme.

Summary of future questions

Even without purified components of the adenylate cyclase system, data emerging from a number of experimental approaches in several target systems suggest that a qualitatively similar mechanism for activation of adenylate cyclase exists in all target tissues, especially those tissues regulated by β-adrenergic catecholamines. Thus, the flow of information appears to proceed from the receptor through the G-protein to the catalytic moiety, and a single population of G-proteins appears to modify both receptor–agonist interactions and the catalytic activity of adenylate cyclase. Model systems once felt to represent an 'exception' to the typical scheme envisaged for regulation of the adenylate cyclase system by hormones and guanine nucleotides, e.g. the turkey erythrocyte, are now thought instead to represent an extreme on a continuum. Thus, the lack of immediate effects of p[NH]ppG on catalytic activity and receptor–agonist interactions in turkey erythrocyte membranes appears to result from a hormone-dependent guanine nucleotide exchange reaction at the G-protein rather than the more typical hormone-facilitated exchange reaction (Lad *et al.*, 1980). Consequently, it now seems reasonable to conclude that all systems utilize a qualitatively similar sequence of events to transmit hormone occupancy to catalytic stimulation, but that they differ quantitatively, presumably as a result of differing rate constants governing the partial reactions involved in receptor–cyclase coupling.

In the future, the scheme outlined in Fig. 1 may prove too great an oversimplification, and the multiple possible partial reactions outlined by Ross & Gilman (1980) may have to be more rigorously explored. The defined reconstitution of purified components that comprise the systems for activating and attenuating adenylate cyclase, a goal that will not easily be attained, will no doubt allow the unequivocal resolution of the following questions.

(1) What is the molecular stoichiometry of a functioning adenylate cyclase system, and do changes in the overall stoichiometry of the system and/or in the subunit stoichiometry within the G-protein complex alter the extent of responsiveness of adenylate cyclase to different effectors? (2) What are the molecular inter-relationships of the components of activating and attenuating adenylate cyclase systems? (3) Does the requirement for a hydrolysable guanine nucleotide triphosphate in the inhibition by GTP of adenylate cyclase, in hormonal attenuation of adenylate cyclase and in desensitization of adenylate cyclase in certain cell-free systems (Bockaert *et al.*, 1976; Ezra & Salomon, 1980) reflect an underlying shared molecular mechanism for these three processes? (4) What is the molecular basis for desensitization of adenylate cyclase? (5) What is the relationship between protein-mediated events in receptor–cyclase coupling and lipid-mediated events, if any? (6) What other catalytic activities might components, or component complexes, of the adenylate cyclase system possess (e.g. methyltransferase, phospholipase or GTPase functions)? (7) What is the mechanism for regulation of adenylate cyclase by Mg^{2+} (Cech *et al.*, 1981) and other divalent cations and for activation of the adenylate cyclase system by NaF? (8) Finally, does the ubiquitous association of GTP-binding proteins with the regulatory function of GTP hydrolysis (Caskey *et al.*, 1972; Karr *et al.*, 1979; Shinozawa *et al.*, 1979) suggest that the adenylate-cyclase-coupled G-protein may represent a macromolecular cue that synchronizes hormone-stimulation with other, apparently unrelated, cellular functions?

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