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REVIEW ARTICLE

The role of G proteins in transmembrane signalling

Colin W. TAYLOR
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, U.K.

INTRODUCTION

In some transmembrane signalling systems detection of the extracellular stimulus and generation of an intracellular response are properties of the same protein or protein complex. Binding of acetylcholine to the α subunits of the pentameric nicotinic receptor, for example, opens a cation-selective channel formed by parts of each of the receptor subunits, and insulin when it binds to the extracellular domain of its receptor activates a protein tyrosine kinase activity in the intracellular domain of the same protein. Rodbell and his collaborators (Rodbell *et al.*, 1971) were the first to provide evidence for a more complex class of signalling pathway where the sensor and intracellular effector are separate proteins that communicate through a guanine nucleotide-dependent regulatory protein or G protein. The G protein cycles between inactive GDP-bound and active GTP-bound forms. Activation is catalysed by receptors and de-activation is an intrinsic property of the G protein, its GTPase activity. The developments that followed Rodbell's pioneering studies have established that many different receptors regulate many intracellular effectors through a family of closely related G proteins (Citri & Schramm, 1980; Rodbell, 1980; Schramm & Selinger, 1984; Northup, 1985; Levitzki, 1988). Many excellent recent reviews have focused on various aspects of these interactions between receptors, G proteins and intracellular effectors (Casperson & Bourne, 1987; Gilman, 1987; Allende, 1988; Lochrie & Simon, 1988; Neer & Clapham, 1988; Weiss *et al.*, 1988; Chabre & Deterre, 1989; Ross, 1989; Houslay, 1990).

The G protein cycle, at the centre of the conversation between receptors and their effectors, provides one solution to the compromise that cells must make between responding rapidly and being able to respond to very low concentrations of extracellular stimulus. In this review I will consider how different signalling mechanisms are adapted to the cellular processes they control by comparing the properties of the signalling pathways that involve G proteins with the simpler pathways that do not.

STRUCTURE AND FUNCTION IN G PROTEIN SIGNALLING PATHWAYS

The G proteins that transmit information from receptors to their intracellular effector systems belong to a large homologous family of trimeric proteins each with an α subunit that binds guanine nucleotides, and β and γ subunits that are always tightly associated (Casperson & Bourne, 1987; Gilman, 1987; Holbrook & Kim, 1989). Different G proteins are most readily distinguished by their α subunits, though there are also more subtle structural and functional differences in some β and γ subunits (Cerione *et al.*, 1987) (Table 1). Activation of G proteins requires their association with a membrane, the plasma membrane in most cases but the intracellular retinal disc membranes in photoreceptors. Attachment of the α subunit to the cytoplasmic face of the membrane may be mediated by the $\beta\gamma$ complex (Sternweis, 1986) or by fatty acids covalently linked to the *N*-termini of some α subunits (Buss *et al.*, 1987; Lochrie & Simon, 1988; Mumby *et al.*, 1990). For α_s, which lacks covalently bound lipid, the effector itself, adenylate cyclase, may also provide an anchor to the plasma membrane (Arad *et al.*, 1984; Levitzki, 1987). The nature of the attachment is important because G protein activation almost certainly involves dissociation of the α from the $\beta\gamma$ subunits, and if the latter provide the only membrane attachment the active α subunit would leave the membrane. I will return to this aspect later because it has important implications for the rates of communication between receptors, G proteins and effectors.

The receptors that regulate G proteins are also structurally and functionally homologous despite the variety of their stimuli, which range from light, tastes and smells, to the more common extracellular messengers like biogenic amines, proteins and peptides, lipid mediators, and others. The photoreceptor pigment, rhodopsin, is the best characterized of the receptor proteins (Findlay & Pappin, 1986) and has become the model for others. Biophysical measurements of rhodopsin (Chabre, 1985) and the deduced amino acid sequences of other receptors are consistent with the structure shown in Fig. 1. Each receptor is about the same size (40–50 kDa; about 350–500 residues) and probably forms seven membrane-spanning regions linked by three cytoplasmic and three extracellular loops (Wang *et al.*, 1989). The extracellular *N*-terminal tail may contain one or more glycosylated residues and the third intracellular loop and *C*-terminal tail have several serine and threonine residues that are likely, or demonstrated, sites of phosphorylation. The transmembrane sequences are unusual in having many proline and glycine residues. These may form kinks in the helices that help form the ligand-binding pocket buried deep within the transmembrane regions, and they may also play a part in transmitting to the cytoplasmic surface of the receptor the conformational changes that follow ligand binding (Dohlman *et al.*, 1987; Chabre & Deterre, 1989). Ligand recognition too, rather unexpectedly, seems to share common features in the different receptors, with conserved charged residues in the transmembrane segments probably serving as counterions for the positively charged retinal, acetylcholine or adrenaline bound to their respective receptors (Applebury & Hargrave, 1986).

Manipulation of receptor structures is beginning to reveal in more detail the parts of the receptor that recognize the agonist and the G protein. Recognition of the latter appears to involve both the loop that links the fifth and sixth transmembrane regions and part of the *C*-terminal tail (Kubo *et al.*, 1988; Kobilka *et al.*, 1988; O'Dowd *et al.*, 1989) with positively charged residues perhaps playing a major role (Ross, 1989; Huang *et al.*, 1990), but these are unlikely to be the only contacts between receptor and G protein. Mastoparan, a peptide toxin from wasp venom, may mimic this arrangement of positive

Abbreviations used: GTPγS, guanosine 5′-[γ-thio]triphosphate; Gpp[NH]p, guanosine 5′-[βγ-imido]triphosphate; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

Table 1. G protein subunits

The Table summarizes properties of only those G proteins which have been both isolated and for which a function is known (Gilman, 1987; Lochrie & Simon, 1988; Jones *et al.*, 1990). There are many other α subunits with unknown functions and many signalling pathways are known to involve as yet unidentified G proteins.

Subunit	Toxin	Effector	Remarks
α_s	Cholera	Adenylate cyclase (+) L-type Ca^{2+} channels (+)	Large (52 kDa) and small (45 kDa) forms each exist in two forms differing in only a single amino acid residue. All formed by splicing of mRNA from a single gene.
α_{olf}	Cholera	Adenylate cyclase (+)	Closely related to, but distinct from, α_s. Present in olfactory cilia.
α_i	Pertussis	Adenylate cyclase (−) K^+ channels (+)	Three distinct genes encode different α_i subunits that are differentially expressed in different tissues. Most tissues express several forms.
α_{t1}	Cholera and pertussis	Cyclic GMP phosphodiesterase (+)	Present only in rods.
α_{t2}	Cholera and pertussis	Cyclic GMP phosphodiesterase (+)	Present only in cones.
α_o	Pertussis	K^+ channels (+) Ca^{2+} channels (−)	Abundant in nervous tissue. The most abundant α subunit in brain. At least two distinct forms.
β_{35}	No	See text	Almost ubiquitous. No known functional differences between the two closely related forms. Encoded by two distinct genes. Only β_{36} is present in photoreceptors.
β_{36}	No	See text	
γ	No	See text	Ubiquitous. At least three forms, but they have not yet been fully characterized.

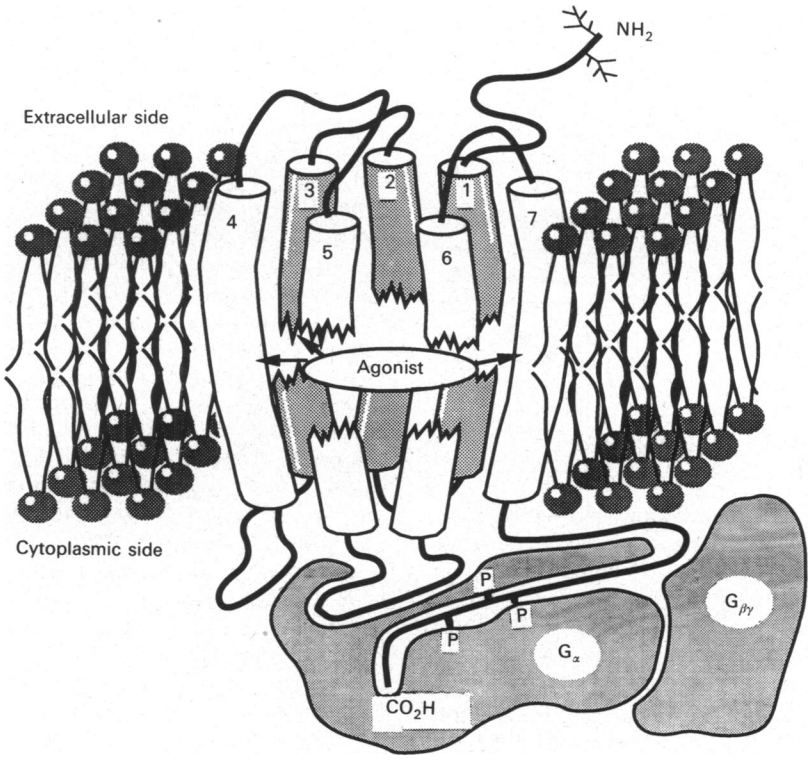

Fig. 1. Shared features of receptors coupled to G proteins

The model illustrates some of the features predicted to be shared by all receptors that interact directly with G proteins. About half the residues are proposed to form seven transmembrane helices and buried deep within them are the conserved residues and presumably others too that form the ligand-binding pocket. Variable numbers of the extracellular residues in the *N*-terminal tail are glycosylated and in the intracellular *C*-terminal tail there are several serine and threonine residues that may be phosphorylated. Some of the regions of the receptor proposed to interact with G proteins are also shown.

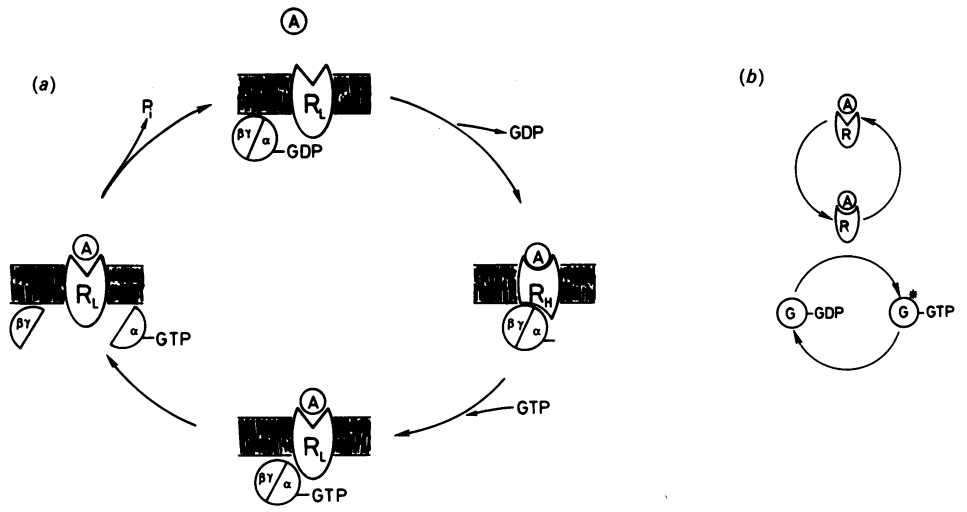

Fig. 2. Receptor interactions with G proteins

(*a*) The interactions between agonist (A), receptor (R), and G protein ($\alpha\beta\gamma$) are described in detail in the text. (*b*) A simplified scheme showing the coupling of the receptor and G protein cycles. At rest the GTPase activity is at least ten times greater than the rate of guanine nucleotide exchange; very little G protein is therefore in the active GTP-bound form. Occupied receptors catalytically activate the G protein by increasing the rate of guanine nucleotide exchange without affecting the intrinsic GTPase activity, and thereby increase the fraction of G protein in the active form. This G protein cycle is coupled to the receptor cycle in which the receptor switches between conformations with high and low affinity for its agonist.

charges in the receptor and thereby activate G proteins (Higashijima *et al.*, 1988). While these approaches may eventually answer the most fundamental question in pharmacology, the nature of receptor activation, we do not have the answer yet. The structures and functions of this family of G protein-linked receptors are described in greater detail in many recent reviews (Findlay & Pappin, 1986; Dohlman *et al.*, 1987; Chabre & Deterre, 1989; Ross, 1989; Strader *et al.*, 1989; O'Dowd *et al.*, 1989).

In contrast to receptors and G proteins, the effectors they regulate appear to have little in common. They include enzymes like adenylate cyclase (Krupinski *et al.*, 1989), polyphosphoinositide-specific phospholipase C (Harden, 1989), cyclic GMP phosphodiesterase (Stryer, 1988) and phospholipase A_2 (Burch *et al.*, 1986), transporters for Mg^{2+} (Erdos *et al.*, 1981) and possibly glucose (Kuroda *et al.*, 1987), and ion channels that gate K^+ (Yatani *et al.*, 1987*a*), Ca^{2+} (Yatani *et al.*, 1987*b*) or Na^+ (Cantiello *et al.*, 1989; Krapivinsky *et al.*, 1989). The molecular details of the interactions between G proteins and effectors are so poorly understood that conserved features of their interactions may not yet have been revealed, but the present evidence suggests that this step in the sequence is probably the least conserved between signalling pathways (see below).

Despite the enormous diversity of receptors, effectors and increasingly of G proteins, the mechanisms that allow transfer of information between them appear to be conserved (Allende, 1988). Although the conservation is impressive, it should not obscure the differences that do exist. For much of this review I will be concerned with the features that are common to all G protein-linked signalling pathways (Fig. 2), but with so many physiological functions controlled by them it comes as no surprise to find cells exploiting the same basic mechanisms in different ways. Some of these differences will be discussed where they throw light on our understanding of the role of G proteins in transmembrane signalling.

Receptor interactions with G proteins

The features common to each of the G protein-linked signalling pathways are shown in Fig. 2. GDP bound to the G protein α subunit normally dissociates only slowly (half time of 1–5 min for G_s and of hours for transducin) (Brandt & Ross, 1985; Gilman, 1987; Stryer, 1988; Chabre & Deterre, 1989), but the concerted actions of intracellular Mg^{2+} and activated receptors catalyse G protein activation by increasing the rate of GDP dissociation from the α subunit and its replacement by GTP. A useful pharmacological tool is the AlF_4^- complex which circumvents the need for GDP dissociation by binding alongside GDP and mimicking the terminal phosphate group of GTP, thereby promoting G protein activation (Bigay *et al.*, 1985).

When an agonist has diffused to within a few molecule diameters of the receptor, the two begin to interact and part of the free energy of binding is used to deform the protein (Jencks, 1975; Burgen, 1981) and switch it to an 'active' conformation, but little is known of the structural changes that accompany receptor activation. The activated receptor has high affinity for a conformation of the G protein in which its α and $\beta\gamma$ subunits are associated and the single guanine nucleotide-binding site of the α subunit is empty (Wessling-Resnick *et al.*, 1987; Chabre *et al.*, 1988). Activated receptors therefore do more than promote release of bound GDP, they also hold open the guanine nucleotide-binding site (Birnbaumer *et al.*, 1980; May & Ross, 1988) and they may even increase its affinity for GTP relative to GDP (Florio & Sternweis, 1989). Although only the α subunit undergoes guanine nucleotide exchange, the $\beta\gamma$ complex plays an essential role in presenting it to the receptor; without it there is little effect of receptors on the α subunit (Fung, 1983; Weiss *et al.*, 1988; Florio & Sternweis, 1989). The ternary complex of agonist, receptor and G protein (DeLean *et al.*, 1980) is normally transitory because a guanine nucleotide binds within milliseconds to the α subunit (May & Ross, 1988), the affinity of the receptor for the G protein is decreased and the two dissociate. However, under experimental conditions, where guanine nucleotides can be omitted, the association between the proteins is long-lived and can be easily measured.

The interactions between receptors and G proteins are analogous to enzyme catalysis; indeed the activated receptors have been described as 'exchange catalysts' by Wessling-Resnick *et al.* (1987). Both enzymes and receptor–agonist complexes bind

tightly to the reactive intermediate or transition state ($G_{\alpha\beta\gamma}$ with no bound nucleotide in the case of the receptor) but not to the substrate or product ($G_{\alpha\beta\gamma}$ with bound nucleotide). I will return to the analogy later.

An agonist must bind more tightly to the active conformation of its receptor. For receptors linked to G proteins, that receptor conformation is the one that binds most tightly to the G protein transition state ($G_{\alpha\beta\gamma}$ with no bound nucleotide). In the absence of guanine nucleotides, therefore, when the association between receptor and G protein is long-lived because the transition state is stable, a fraction of the receptors bind their agonist with high affinity. If a guanine nucleotide (GTP or GDP) is then added, it binds to the vacant site on the G protein α subunit, the transition state is lost, the association between receptor and G protein is weakened and with it the high affinity binding of agonist to the receptor. The analogous change in photoreceptors is the decreased stability of the active form of rhodopsin (metarhodopsin II) in the presence of guanine nucleotides (Pfister *et al.*, 1983).

In most cells the number of available G proteins appears to limit the number of receptors that can form a high-affinity complex with an agonist. In fibroblasts, for example, the fraction of muscarinic receptors that form a high-affinity complex with agonist is much reduced if the number of receptors is increased (Mei *et al.*, 1989). Because agonist binding is so inextricably linked with the subsequent events that lead to a response, classifications of receptors that rely upon agonist binding or the responses to agonists are likely to be confused by tissue differences in the transduction elements, variations in the number of G proteins or the intracellular concentrations of guanine nucleotides for example. These classification problems have been the cause of considerable debate (Colquhoun, 1987; Kenakin, 1988; Leff *et al.*, 1990; Mackay, 1990).

Antagonist-occupied receptors cannot bind more tightly than empty receptors to the G protein transition state or they would activate G proteins and would not be antagonists. They may simply fail to alter the existing equilibrium between G protein conformations by binding equally well to each; antagonist binding is then insensitive to guanine nucleotides (Lefkowitz *et al.*, 1976). Alternatively, antagonist-occupied receptors may bind more tightly to other, non-transition state conformations of the G protein, an $\alpha\beta\gamma$-GDP conformation, for example. Guanine nucleotides then stabilize antagonist binding, as has been observed for δ opioid, D_2 dopaminergic, A_1 adenosine and muscarinic receptors (Burgisser *et al.*, 1982; Costa & Herz, 1989). In the first situation the antagonist merely occludes the ligand-binding site of the receptor and prevents an agonist from acting, but when the antagonist-occupied receptor discriminates between G protein conformations and binds more tightly to inactive forms, it will have negative intrinsic activity and will inhibit basal activity (Costa & Herz, 1989).

The extent to which a partial agonist forms a high-affinity guanine nucleotide-sensitive complex with its receptor is intermediate between the effects of full agonist and antagonists and closely correlated with its intrinsic activity (Kent *et al.*, 1980; Evans *et al.*, 1985).

The negatively co-operative interactions between binding of guanine nucleotide and of receptor–agonist complex to the G protein are an inescapable consequence of the processes described above (Burgen, 1981; Fig. 2), but they have further functional advantages that are discussed below.

Activation of G proteins has generally been assumed to be controlled only by plasma membrane receptors responding to extracellular signals, but a recent study suggests that they may also be directly activated by intracellular regulators. GAP-43, a protein found tightly associated with the cytoplasmic face of neuronal growth cone plasma membranes, is itself subject to regulation by various intracellular messengers and has recently been shown to promote guanine nucleotide exchange on the neuronal G protein, G_o (Strittmatter *et al.*, 1990). Although the functional significance of this interaction is not yet clear, it does suggest an additional complexity in G protein signalling pathways: the possibility that both receptors responding to extracellular signals and other proteins responding to intracellular signals may regulate G protein activation.

G protein activation

For the present discussion it is convenient to consider activation of a G protein to be the changes that switch it to a form that regulates its effector. Binding of GTP, or its stable analogues (usually Gpp[NH]p or GTPγS), is the step that leads to G protein activation, and hydrolysis of the GTP by an intrinsic GTPase is involved in inactivation. However, the structural changes in the G protein that underlie the changes in activity are not yet certain. When the α subunit of transducin, the G protein involved in phototransduction, binds GTP (but not GDP), its affinity for the $\beta\gamma$ complex is substantially reduced and the transducin dissociates into $\beta\gamma$ and α–GTP complexes (Navon & Fung, 1987). Indeed, a single $\beta\gamma$ complex can recycle between α subunits, allowing many to be activated by a single bleached rhodopsin (Fung, 1983). Other G proteins, after detergent solubilization, behave in a similar way to transducin: GTPγS promotes G protein dissociation whereas GDP stabilizes the oligomeric form (Higashijima *et al.*, 1987; Gilman, 1987), α-GDP binds more tightly than α-GTP to a $\beta\gamma$ affinity column (Pang & Sternweis, 1989), and in membrane preparations GTPγS or GTP with hormone cause dissociation of α and $\beta\gamma$ subunits (Iyengar *et al.*, 1988; Ransnas *et al.*, 1989). In view of the suggestion that $\beta\gamma$ subunits mediate hormonal inhibition of adenylate cyclase by binding to active α_s-GTP (Katada *et al.*, 1984) (see below), it is noteworthy that active α subunits bind with different affinities to the same $\beta\gamma$ complexes: α_s–GTP, for example, binds more tightly than α_i–GTP to $\beta\gamma$ (Sternweis, 1986; Pang & Sternweis, 1989).

The evidence that G protein activation involves dissociation into α–GTP and $\beta\gamma$ complexes is persuasive. More contentious are suggestions that this dissociation causes the active α subunit to leave the membrane. That would, of course, be unavoidable if an α subunit were anchored to the membrane by only its $\beta\gamma$ complex, but there is presently no evidence that this is the only membrane attachment for any G protein (Mumby *et al.*, 1990). Membranes stimulated with hormones and GTP, or with stable analogues of GTP, do release active α subunits (Rodbell, 1985; Iyengar *et al.*, 1988; Ransnas *et al.*, 1989), but the experimental conditions used (prolonged incubation of dilute membrane suspensions often with stable GTP analogues in unphysiological salt concentrations) probably exaggerate the effect by effectively ensuring that any dissociation of α subunits is irreversible. Under physiological conditions, it seems more likely that there is no significant dissociation of active α subunits from the membrane. More direct evidence comes from the work of Levitzki and his colleagues who have consistently argued from both kinetic analyses (Tolkovsky & Levitzki, 1978*a*; Levitzki, 1986) and the physical association of G_s and adenylate cyclase during substantial purification (Arad *et al.*, 1984) that G_s, at least, remains tightly bound to its effector, and therefore to the membrane, throughout the G protein cycle. Even transducin, the G protein that is least tightly associated with the membrane, may not significantly dissociate from the disc membrane after activation under physiological conditions (Liebman *et al.*, 1987; Uhl *et al.*, 1990), although Chabre (1987) has argued that the α_t–GTP subunit is soluble and becomes a cytoplasmic messenger.

The adverse consequences of active α subunits leaving the

membrane would certainly be less serious in rods than in more typical, less specialized, cells. Almost the entire protein content of rod outer segments is accounted for by the very few proteins involved in phototransduction, among them transducin. Release of transducin α subunits into the very narrow cytoplasmic cleft (15 nm) separating adjacent discs would therefore allow their concentration to become considerable, perhaps 500 μM (Bitensky *et al.*, 1988; Chabre & Deterre, 1989). In more typical cells with far smaller G protein contents and larger cytoplasmic volumes, dissociated α subunits would be massively diluted to less than 1000 times the concentration in rods. In rods it seems that cytoplasmic α subunits could be present at sufficient concentration to allow reasonably rapid rates of interaction with the membrane-associated effector or $\beta\gamma$ complex, but in other cells the enormous dilution of free α subunits would much reduce their rates of interaction with the membrane-bound components.

The distinct effects of guanine nucleotides on the interactions between receptors and G proteins and on G protein activation reflect the processes involved in the conformational switch of the G protein between inactive and active forms. Because receptor–agonist complexes bind tightly to the transition state ($G_{\alpha\beta\gamma}$ with no bound nucleotide) but not to the substrate or product ($G_{\alpha\beta\gamma}$ with bound GTP or GDP), high affinity binding of receptor to G protein or the linked function, high-affinity binding of agonist to the receptor (Fig. 2), are disrupted by GTP, GDP or their analogues. By contrast, whereas GTP promotes G protein dissociation and activation, GDP stabilizes the oligomeric G protein and prevents activation (Higashijima *et al.*, 1987). The distinction is important. If GDP binds to the α subunit the G protein leaves the receptor exactly as it arrived, as $G_{\beta\gamma\alpha\text{–GDP}}$; but when GTP binds, the G protein dissociates into its subunits and can only interact with the receptor again when the subunits reassociate after the GTP is hydrolysed. The G protein dissociation that follows binding of GTP effectively makes the activation process irreversible (Fung, 1987):

$$\text{RA} + \beta\gamma\alpha\text{–GDP} \overset{\text{GDP}}{\rightleftharpoons} \text{RA}\text{–}\beta\gamma\alpha\text{–} \overset{\text{GTP}}{\rightleftharpoons} \text{RA} + \beta\gamma + \alpha\text{–GTP}$$

where R and A are the receptor and agonist, and $\beta\gamma\alpha$ are the G protein subunits. Other experiments provide additional insight into the mechanisms of G protein activation. A mutant α_s, H21a, in which a single amino acid residue is altered, interacts normally with β-adrenergic receptors and stabilizes high-affinity, guanine nucleotide-sensitive binding of agonists, but fails to dissociate into α and $\beta\gamma$ complexes upon binding GTP and fails to stimulate adenylate cyclase. The mutant α_s evidently binds guanine nucleotides normally, but it cannot make the conformational change that usually follows GTP binding and leads to activation (Bourne *et al.*, 1988). Finally, binding of GTP and the conformational change that normally follows can sometimes be separated by excluding Mg^{2+}: GTP binds (and would presumably cause the receptor and G protein to dissociate), but only when Mg^{2+} is restored does the G protein change conformation, dissociate into its subunits and become active (Higashijima *et al.*, 1987).

The manipulations that allow separation of the two processes, guanine nucleotide binding and the conformational change in the G protein that causes activation, provide opportunities to analyse the molecular basis of G protein activation (Bourne *et al.*, 1988), but, as with receptor activation, our understanding of the process is in its infancy.

G proteins and effector systems

Which of the G protein subunits regulates the effector? In retinal rods free α_t–GTP activates cyclic GMP phosphodiesterase by binding to its small inhibitory subunits and relieving the inhibition they impose (Fung & Griswald-Penner, 1989; Chabre & Dettere, 1989), and adenylate cyclase is stimulated by α_s with GTPγS bound (Gilman, 1987). In other signalling pathways the relative roles of α and $\beta\gamma$ subunits in regulating the effector are less clear (Bourne, 1989). While α_i with bound GTPγS can inhibit adenylate cyclase, free $\beta\gamma$ subunits can also inhibit its activity indirectly, probably by binding to, and thereby inactivating, α_s–GTP (Katada *et al.*, 1984), an interaction favoured by the relatively high affinity of $\beta\gamma$ for α_s (see above) and by the large excess (5–10-fold) of G_i over G_s in most membranes (Gilman, 1987).

Regulation of the different classes of K^+ channels, notably the cardiac channel regulated by muscarinic agonists, is controversial with competing claims for α_k ($= \alpha_{i3}$) (Codina *et al.*, 1987) or $\beta\gamma$ (Logothetis *et al.*, 1988) directly regulating the channel. The latter now seems less likely in view of convincing evidence that α_k and related α subunits (α_{i1-3} and α_o) are active (Mattera *et al.*, 1989; Sternweis & Pang, 1990), from evidence suggesting that the effects of $\beta\gamma$ may be mediated by products derived from stimulation of phospholipase A_2 (Kim *et al.*, 1989), and the difficulty it poses in understanding how functionally interchangeable $\beta\gamma$ subunits, freed by activation of any G protein, could specifically convey a signal to an intracellular effector. The latter problem also arises from studies of phospholipase A_2, where biochemical evidence suggests direct regulation by $\beta\gamma$ (Jelsema & Axelrod, 1987; Kim *et al.*, 1989), and of yeast mating factors, where genetic evidence suggest $\beta\gamma$ regulation of an unidentified effector (Whiteway *et al.*, 1989; Nomoto *et al.*, 1990). The same difficulties need not arise from the proposed role of $\beta\gamma$ in mediating inhibition of adenylate cyclase, because there the role of $\beta\gamma$ is not as a direct regulator, but rather to shift the equilibrium between free and associated α_s–GTP.

For other effectors the situation is even less clear: α_s with GTPγS bound activates Ca^{2+} channels (Yatani *et al.*, 1987*b*), but other α subunits are also effective (Hescheler *et al.*, 1987). The G proteins that regulate polyphosphoinositide-specific phospholipase C are particularly elusive, with evidence for regulation by both pertussis toxin-sensitive and -insensitive G proteins (Taylor & Merritt, 1986; Harden, 1989). In *Xenopus oocytes* activated α_o appears to stimulate the enzyme (Moriarty *et al.*, 1990) and less direct evidence from other tissues is consistent with stimulation by the α subunits of unidentified G proteins (Boyer *et al.*, 1989). Earlier claims that the p21 *ras* proteins are the G proteins that couple receptors to polyphosphoinositide-specific phospholipase C have now been refuted (Downward *et al.*, 1988).

For most transmembrane signalling pathways this final step, the conversation between active G protein and effector, is the least understood. In view of the very different structures and functions of the effectors it is perhaps no surprise that it appears also to be the step least conserved between pathways.

The cycle of G proteins switching between inactive GDP-bound and active GTP-bound forms with receptors catalysing the activation and themselves undergoing changes in affinity for their agonists is a common theme in transmembrane signalling. The advantages of such a complex system become clear only when we compare it with simpler transmembrane signalling processes and make the comparisons with the physiology of the tissue in mind.

THE COMPROMISE BETWEEN SENSITIVITY AND RESPONDING QUICKLY

The time courses of events controlled by receptors vary enormously from the milliseconds it takes for acetylcholine to cause postsynaptic depolarization to the hours or days that a growth factor may take to effect a change in cell growth or differentiation. The events that follow receptor activation largely

determine the time courses of these processes, but the early steps in the signalling pathway are also adapted to respond within a time-scale appropriate to the cellular responses they ultimately regulate.

Sensitivity of receptors

For many receptors there is a conflict between the needs to respond to both low concentrations of agonist and to rapid changes in its concentration. Slow dissociation of the agonist from its receptor would provide high-affinity binding and so sensitivity to low agonist concentrations, but would inevitably leave the receptor insensitive to rapid changes in agonist concentration. For receptors linked to G proteins there is an additional problem that comes from having an agonist bound for too long to its receptor: the interaction with G proteins becomes inefficient. The active receptor will initially collide only with inactive G proteins, but with time as more G proteins are activated, an increasing fraction of collisions will be wasted because they will be with already active G proteins. Stickle & Barber (1989) have elegantly demonstrated the problem by showing that a small number of β-adrenoceptors activated for a large fraction of time is a less effective stimulus for adenylate cyclase than is a large number of receptors activated for a smaller fraction of time.

The relatively localized activation of nearby G proteins by receptors may also provide an explanation for an otherwise problematic finding. In lipid vesicles, G_s activated either by addition of stable GTP analogues or by interaction with β-adrenoceptors and guanine nucleotides stimulates adenylate cyclase activity. However, whereas exogenous $\beta\gamma$ subunits attenuate stimulation evoked by the first treatment, they scarcely affect stimulation via receptors (Cerione *et al.*, 1986). One interpretation, that active α_s remains associated with the β-adrenoceptor, is difficult to reconcile with the demonstrated ability of a single β-adrenoceptor to catalytically activate many G proteins (Pedersen & Ross, 1982). An alternative explanation is that α_s and $\beta\gamma$ subunits formed after addition of GppNHp are evenly distributed, but those formed after receptor activation are more locally concentrated around receptors. In the second case more free α_s may be needed to evoke the same stimulation of adenylate cyclase because of the increased local concentration of $\beta\gamma$ and the likelihood of wasted collisions of α_s with adenylate cyclase. In consequence the susceptibility to inhibition by added $\beta\gamma$ may be reduced.

At any agonist concentration a certain *fraction* of receptors are occupied, but the response of the next step in the signalling pathway depends upon the *number* of occupied receptors. The conflict between temporal sensitivity and sensitivity to low agonist concentrations can therefore be satisfied by having receptors of relatively low affinity (hence fast dissociation rates), but to have so many receptors, 'spare receptors', that a maximal response can be evoked when only a small fraction are occupied. In guinea pig ileum, for example, occupancy of less than 0.25% of the muscarinic cholinergic receptors by acetylcholine is sufficient to cause a half-maximal contraction (Kenakin, 1984). This discrepancy between the curves describing receptor occupancy and a later response arises whenever one saturable event controls the next saturable step in the signalling sequence (Strickland & Loeb, 1981; Kenakin, 1984) (Fig. 3*a*). The saturable steps that are unique to the G protein-linked signalling pathways, the receptor–G protein and G protein–effector interactions, are important features because they further exaggerate the dis-

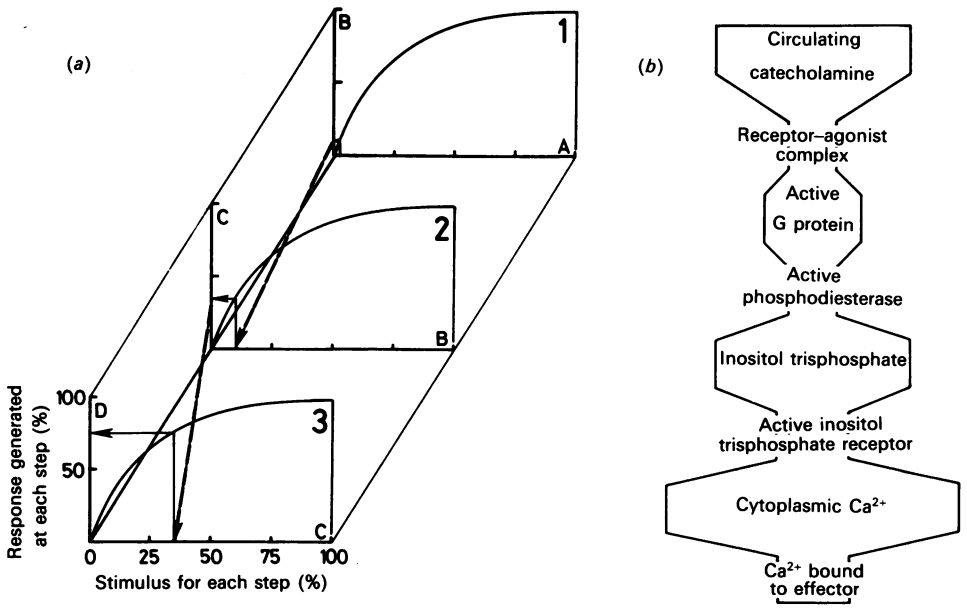

Fig. 3. Amplification in signalling pathways

(*a*) When the product of one saturable process (1) is the stimulus for the next, the later processes (2,3) become considerably more sensitive than the early ones to low concentrations of the initial stimulus. The Figure (modified from Kenakin, 1987) shows how a low concentration of the initial stimulus (A), a hormone for example, produces the first response, an increase in receptor occupancy (B). Occupied receptors (B) now become the stimulus for the next step in the sequence, GTP binding to a specific G protein for example (C) and so on. Because the cell is capable of generating more product from the first step (i.e. receptor–agonist complex) than is needed to fully activate the second step, a relatively small percentage increase in the signal from the first step is enough to provide a relatively large percentage increase in the stimulus to the second step. In this way small changes in fractional receptor occupancy can cause large increases in the fraction of the maximal response finally evoked. (*b*) Too much amplification is dangerous. Cells therefore combine amplification steps with diminution steps in their signalling pathways. The example shows successive steps in part of the α_1-adrenoceptor signalling pathway.

crepancy between occupancy and response curves. 'Spare receptors' are therefore one feature that allows cells to respond to low agonist concentrations without losing sensitivity to rapid changes in agonist concentration.

There is an additional advantage in using 'spare receptors' to set the sensitivity of a tissue to a hormone. Where 'spare receptors' exist, a change in receptor number can substantially alter sensitivity, either between tissues or in the same tissue under different conditions, without preventing a full response to a maximal concentration of agonist.

The interactions between receptors and their G proteins provide an additional mechanism that enhances temporal sensitivity without sacrificing sensitivity to low agonist concentrations. In intact cells, where the concentration of GTP probably exceeds that of GDP (320 μM and 90 μM respectively in hepatocytes; Kleineke *et al.*, 1979), the changes in receptor agonist affinity, reflecting interactions with the G protein transition state, are accompanied by G protein activation. Until the agonist-occupied receptor has activated its G protein, by allowing it to bind GTP, the G protein stabilizes tight binding of the agonist to its receptor. Once the G protein has been activated, it dissociates from the agonist–receptor complex, the receptor reverts to its initial, low-affinity conformation and the agonist is then more likely to dissociate. For many receptors this increased rate of dissociation is substantial for full agonists: 50–100-fold for β-adrenoceptors, α_1-adrenoceptors or muscarinic receptors (Kent *et al.*, 1980; Goodhardt *et al.*, 1982; Evans *et al.*, 1985). Activation of the G protein is therefore effectively coupled to recycling of the receptor (Fig. 2).

It is worth considering the consequences if the receptor were to interact directly with its effector rather than through a G protein. High-affinity binding of the agonist to the receptor would then be stabilized by interaction of the occupied receptor with the active effector. Only dissociation of the two proteins would allow the receptor to recycle to its low-affinity form, but that would, of course, be accompanied by deactivation of the effector. Because tight binding of the active receptor to its effector would be desirable to maximize their productive interaction, the lifespan of the active effector would be limited by the lifespan of the receptor–agonist complex. The results would be more long-lived binding of agonist to its receptor, with consequent loss of temporal sensitivity, and each receptor–agonist complex could stimulate only a single effector molecule, with consequent loss of amplification (see below). There is an additional, more subtle advantage that comes with having a G protein convey the signal between receptor and effector. Only part of the lifespan of the receptor–agonist complex is spent bound to the G protein, and that provides an opportunity for other intracellular systems to read the signal transmitted to the cytoplasm by the receptor (see below). G proteins therefore provide opportunities to increase temporal sensitivity, to increase sensitivity to low agonist concentrations and to build flexibility into the signalling pathways.

For other receptors there are different compromises between temporal and absolute sensitivity. Ligand-gated ion channels like the $GABA_A$, glycine, and nicotinic receptors are receptors that mediate the fastest chemical transmission between excitable cells. These receptors have low affinity for their agonists: the dissociation constant (K_D) of acetylcholine for the active conformation of the nicotinic receptor is probably about 70 μM (Neubig *et al.*, 1982). The receptors can have low absolute sensitivity because they are concentrated at very high density, about 20000 nicotinic receptors/μm^2 at the frog neuromuscular junction (Matthews-Bellinger & Sapter, 1978), and the agonist is released locally and reaches high concentrations. A presynaptic impulse probably causes the acetylcholine concentration to transiently exceed 300 μM in the synaptic cleft, but only for a millisecond or so (Kuffler & Yoshikama, 1975: Changeux *et al.*, 1984). The benefit is fast recognition because rates of agonist association and dissociation are both fast. The receptors are not only able to rapidly detect changes in agonist concentration, they also respond very quickly because the ligand recognition sites and the ion channel are parts of the same preformed complex (see below).

For receptors with intrinsic protein tyrosine kinase activity, like those for insulin or epidermal growth factor (EGF), the problem is different because they must detect very low levels of hormone; the plasma concentration of insulin, for example, is typically in the range 60–600 pM. These receptors generally regulate relatively slow responses: insulin, for example, controls the long-term storage of fuels, protein synthesis and, with other growth factors, processes like gene expression, differentiation and cell growth. The ability to detect sudden changes in hormone concentration is therefore less acute than it is for receptors that control more rapid responses. The affinity of receptor tyrosine kinases for their peptide agonists can therefore be very high, typically around 0.1 nM [Cuatrecasas, 1971, insulin; Carpenter, 1987, EGF; Daniel *et al.*, 1985, platelet-derived growth factor (PDGF); Guilbert & Stanley, 1986, colony-stimulating factor 1]. The rates of agonist dissociation are correspondingly slow (half times of many minutes) (Cuatrecasas, 1971), so slow that receptor–agonist complexes may be internalized more rapidly than the agonist dissociates (Guilbert & Stanley, 1986). Even with such slow agonist dissociation rates, these receptors appear to prolong the lifespan of the active receptor still further by autophosphorylation (see below). These features, high affinity for agonist and autophosphorylation, allow the receptors to detect and respond to either very low concentrations of growth factor (e.g. insulin) or to transient higher concentrations (e.g. PDGF), but within a time scale of minutes rather than seconds or milliseconds as occurs with G protein-linked receptors or ligand-gated channels.

Receptors linked to G proteins typically mediate responses intermediate in their time course between those controlled by ligand-gated ion channels and those controlled by receptor tyrosine kinases. To some extent the affinities of the receptors for their agonists reflect this. Intermediate affinities (1–100 nM) ensure relatively rapid on and off rates that allow both adequate temporal resolution and rapid switching of agonist between receptors (see above). The decreased absolute sensitivity that results is compensated by having 'spare receptors' (made possible by the amplification steps) and by the changes in receptor affinity during the G protein cycle.

Receptors and effectors communicate quickly

Having recognized its stimulus, the receptor must elicit a response. That process should be as fast as possible, but speed is of paramount importance for the pathways controlling the fastest physiological responses, the ligand-gated ion channels. There the conformational change that accompanies ligand binding is transmitted rapidly to another part of the same complex to open the ion channel (Colquhoun & Sakmann, 1981). The speed of the process is limited only by the very rapid rates of intramolecular movements of proteins, and these allow the nicotinic receptor to begin to respond within 20 μs of addition of acetylcholine (Katz & Miledi, 1965). For receptor tyrosine kinases activation is a much slower process because, at least for the EGF receptor, stimulation of the kinase activity requires an interaction between two receptors with EGF bound (Yarden & Schlessinger, 1987). The speed of activation is therefore limited by the relatively slow rates of diffusion of large hydrophobic proteins in the lipid bilayer.

With typical lifespans of only a few seconds, receptor–agonist

complexes that activate G proteins must do so rapidly if the initial signal is to be amplified by formation of many active G proteins. Several features of the interaction probably contribute to speeding up the process.

Activation of the G protein by GTP binding causes its immediate dissociation from the receptor, leaving the receptor free to interact with and activate another G protein, or to interact with other intracellular components (see below). This rapid recycling of the active receptor maximizes its catalytic efficiency.

In aqueous solution two proteins may collide in any orientation with only very few collisions bringing their interacting sites into the correct orientation. Fruitful interactions are typically 10000-fold slower for two proteins in solution than the rates of reaction between a protein and its small substrate (Liebman *et al.*, 1987). However, receptors and G proteins interact at the surface of a membrane and not free in solution. The result is to both effectively 'concentrate' the proteins—they are diluted in two rather than three dimensions, and the proteins can be presented to each other in favourable orientations (Liebman *et al.*, 1987). Both processes will increase the rates of productive interaction between receptors and G proteins. A price that should be paid is the relatively slow rate of protein diffusion in the viscous lipid bilayer: a hydrophobic protein in a lipid bilayer diffuses some 100 times more slowly than a hydrophilic protein in aqueous solution. Even that price may not be paid in full.

The membranes of vertebrate retinal discs, enriched in highly unsaturated lipids and deficient in cholesterol, are unusually fluid (Findlay & Pappin, 1986). Indeed, rhodopsin is among the most mobile of integral membrane proteins with a diffusion coefficient some 10 times greater than the integral proteins of most membranes (Poo & Cone, 1974). By contrast, the membranes of *Torpedo* electroplax, which have the densest concentration of nicotinic receptors, are cholesterol-rich and contain many long-chain fatty acids that make them far less fluid (Gonzalez-Ras *et al.*, 1982). Thus, two membranes each highly specialized for a single transmembrane signalling process, differ substantially in their fluidity. The nicotinic receptor, where the response depends upon intramolecular motions, sits in a rigid membrane, but rhodopsin, which must communicate with other proteins, is mobile in a very fluid membrane. The effects of membrane fluidity have also been examined more directly. In turkey erythrocytes an increase in membrane fluidity increases the rate of adenylate cyclase stimulation by β-adrenoceptors but not when the G protein is stimulated directly with AlF_4^- (Hanski *et al.*, 1979).

A comparison between invertebrate and vertebrate photoreceptors hints further at the importance of membrane fluidity in this signalling pathway. Many essential features are similar in vertebrates and invertebrates, indeed the rhodopsins and transducins are functionally interchangeable (Saibil & Michel-Villaz, 1984), though the effector systems are probably different. However, whereas vertebrate rhodopsin is highly mobile, invertebrate rhodopsins, in squid for example, are firmly tethered to microvilli (Foster, 1980; Saibil & Hewat, 1987). It is intriguing, therefore, that whereas a single photobleached rhodopsin may activate up to 500 transducins in vertebrate rods (Stryer, 1986), there is far less amplification in invertebrate photoreceptors (Vandenberg & Montal, 1984); in *Limulus*, for example, only about eight transducins are activated per bleached rhodopsin (Kirkwood *et al.*, 1989). There may be many explanations for this difference, but it is tempting to suggest that the immobility of invertebrate rhodopsin limits its rate of interaction with transducin and thereby limits amplification of the initial signal.

Chabre (Chabre, 1987; Chabre & Deterre, 1989) has presented a different view in which transducin behaves as a 'cytoplasmic' shuttle between membrane-bound rhodopsin and membrane-associated phosphodiesterase. Evidence in favour of this model includes the demonstration that in vertebrates the rate of phosphodiesterase activation depends more upon the viscosity of the cytoplasm than of the membrane. The advantages of restricting interactions between the signalling proteins to the membrane are not lost (because transducin is assumed to remain associated with the membrane), but an additional benefit may be a faster transfer of information between receptor and effector. The G protein (and presumably also the phosphodiesterase) move in an aqueous environment, the cytosol, the viscosity of which is substantially less than even the very fluid membrane of retinal discs.

In light of the conflicting evidence there is clearly a need, as Chabre & Deterre (1989) have commented, to test experimentally the assumption that rates of protein diffusion in the lipid bilayer limit the rates of interaction between the signalling proteins. The need is particularly urgent in more typical transmembrane signalling pathways where the rates would be much slower because receptors and effectors are both generally integral membrane proteins, their concentrations within the membrane are far less than in rods, and the membrane itself is less fluid.

In conclusion, it is becoming clear that in G protein signalling pathways the interactions between the proteins and perhaps the environment in which they operate are adapted to allow rapid transfer of information between them. This rapid communication allows the receptor to generate a response within an appropriate time scale and allows amplification of the signal generated by the fairly short-lived agonist–receptor complex.

AMPLIFICATION

Koshland and his colleagues (1982) have stressed the dangers of excessive amplification in cell signalling. Only a few steps with 1000-fold amplification at each would generate enough intracellular mediator to fill the cell unless there were 'leaks' from the successive amplification steps. Regulation of intracellular Ca^{2+} by one class of α_1-adrenoceptor demonstrates the point. A single occupied receptor probably activates several G proteins (amplification), but every activated G protein is unlikely to interact productively with polyphosphoinositide-specific phospholipase C (diminution). Each activated enzyme catalyses formation of many molecules of inositol trisphosphate (amplification), but these are diluted in the cytosol and only relatively few will bind to their intracellular receptor (diminution); each activated receptor will then allow many Ca^{2+} ions to be mobilized (amplification) (Fig. 3*b*). Amplification is the major theme of this section, but the combination of amplification and diminution steps is important: without the restraint imposed by the latter, amplification would be explosive (Koshland *et al.*, 1982).

Earlier we saw that part of the binding energy of the agonist is used to change the conformation of the receptor, to 'activate' it. Dissociation of the agonist must, therefore, reverse the conformational change and return the receptor to its inactive state: the receptor has no inherent memory of its encounter with the agonist. Yet there are changes in signalling pathways that outlive the lifespan of the receptor–agonist complex and these can contribute substantially to amplification of the initial stimulus. Where does the memory come from?

Autophosphorylation as memory

Binding of insulin to its receptor activates the intrinsic tyrosine kinase activity and this activity is probably the major signal that is relayed to the cell interior and that subsequently leads to other signals and the final cell responses (Rosen, 1985; Czech *et al.*, 1988). Unfortunately, despite intensive searching, neither the

physiological substrates of the receptor tyrosine kinases nor the links between the increased enzyme activity and final responses are known. Such ignorance has prompted Bourne (1988) to speculate provocatively that the only function of the tyrosine kinase activity may be to provide a memory for the receptor. The activated insulin receptor shares with other members of the family of receptor tyrosine kinases an ability to autophosphorylate specific tyrosine residues in the cytoplasmic domain of the receptor itself (Yarden & Ullrich, 1988). The full functional significance of these autophosphorylations is unclear, though they may be important in allowing substrates access to the active site of the kinase domain (Flores-Riveros *et al.*, 1989). For the insulin receptor one consequence is clear: the receptor becomes insensitive to insulin. The tyrosine kinase activity that was first activated by insulin now remains active even when insulin dissociates from the receptor (Rosen *et al.*, 1983). Phosphorylation has provided the receptor with a memory of its earlier encounter with insulin, but the memory was created by the same enzyme activity that provides the signal to the cell interior. The memory is eventually erased when the receptor is dephosphorylated, but the details of that process are still unknown.

Whereas the receptor tyrosine kinases and the signalling pathways that involve G proteins are adapted to remember the formation of active receptors, the nicotinic receptor is deliberately forgetful. Binding of an agonist to the active (open channel) receptor conformation is followed by still tighter binding to long-lived desensitized (closed channel) conformations (Neubig *et al.*, 1982; Cachelin & Colquhoun, 1989). The ligand-gated ion channels therefore respond rapidly and transiently to sudden and substantial changes in neurotransmitter concentration as befits their role as mediators of fast chemical transmission.

G proteins remember

The insulin receptor, and perhaps other members of the family of receptor tyrosine kinases, use the same agonist-regulated enzyme activity both to send a signal to the cell interior and to remember the encounter of the growth factor with its receptor. G protein-linked receptors, however, have no intrinsic ability to catalyse the formation of covalent bonds; they cannot create their own memory of an encounter with an agonist. For these receptors, it is the G protein that remembers.

Our earlier discussion has shown that a transient encounter, probably lasting only a few milliseconds, between an agonist-occupied receptor and an inactive G protein ($G_{\alpha\beta\gamma}$–GDP) leads to activation of the G protein. The receptor leaves the encounter as it arrived with weakly bound agonist, but the G protein leaves activated by its binding of GTP. The G protein has remembered its interaction with the receptor and will forget it only when the bound GTP is hydrolysed by its intrinsic GTPase. There is no evidence to suggest that the GTPase is regulated, though it is a tempting site for regulation (see below). The memory therefore decays inexorably at a rate determined only by the catalytic activity of the GTPase (typically 4 min^{-1}, but ranging from 1 to 15 min^{-1}) (Cassel *et al.*, 1977; Christophe *et al.*, 1981; Higashijima *et al.*, 1987; Gilman, 1987). The memory, with a half-life of several seconds, therefore decays slowly relative to the duration of the initial encounter with the receptor, and at a rate comparable to the rate of dissociation of many agonists from their receptors. Adrenaline, for example, dissociates with half times of about 0.1 and 10 s from the low and high affinity states of α_1- or β_1-adrenoceptors (Goodhardt *et al.*, 1983; Kent *et al.*, 1980). The memory is important because an encounter lasting milliseconds is translated into an effect lasting many seconds. The memory, however, is not so long that temporal sensitivity is lost.

What is gained by entrusting the memory to an additional protein, the G protein, rather than having the receptor or the effector remember? If the G protein is the most mobile protein of the signalling pathway, it makes sense for it also to retain the memory. A relatively short memory, comparable to the lifespan of a receptor–agonist complex, may then be sufficient to allow the G protein to function as a mobile shuttle between receptor and effector without loss of temporal resolution. Less mobile proteins would need a longer memory, with corresponding loss of temporal sensitivity, if they were to communicate as effectively. Having the G protein, rather than other elements of the pathway, remember allows the memory to be long enough for the initial receptor–agonist interaction to be considerably amplified without loss of sensitivity to rapid changes in agonist concentration.

G PROTEINS PROVIDE FLEXIBILITY

Ligand-gated ion channels and receptor tyrosine kinases, receptors with integral effectors, are inherently rather inflexible: agonist binding is tightly linked to a response. The receptors are regulated; nicotinic (Huganir *et al.*, 1986) and EGF (Yarden & Ullrich, 1988) receptors, for example, are phosphorylated by kinases regulated by other signalling pathways, but these modifications pale alongside the versatility afforded the pathways that use G proteins.

Changes in sensitivity

For receptors with integral effectors it is obvious that the receptor is responsible for conveying a signal across the membrane, but the same is true of all transmembrane signalling pathways. The conformational change in the receptor that follows agonist binding is transmitted through the membrane to the cytoplasm where it is detected by another intracellular protein (Chabre, 1987). So far we have been concerned only with G proteins recognizing the cytoplasmic domain of the activated receptor, but those interactions are transient, leaving the domain free to be seen by other intracellular proteins. Two such proteins are rhodopsin kinase (Wilden *et al.*, 1986) and the rather inappropriately named β-adrenoceptor kinase (Strasser *et al.*, 1986). These enzymes bind to and phosphorylate only the active conformation of their substrates, bleached rhodopsin and agonist-occupied G_s- or G_i-coupled receptors (not only β-adrenoceptors) respectively). In their inactive states neither receptor interacts with its kinase. The functional consequence of phosphorylation is homologous desensitization because the phosphorylated receptor binds tightly to another protein, arrestin or an arrestin-like protein, preventing the receptor from interacting with G proteins (Benovic *et al.*, 1987).

Earlier we saw that responses downstream from the receptor may be substantially activated at very low hormone concentrations when only a tiny fraction of receptors are occupied (Fig. 3*a*), while responses more tightly linked to receptor occupancy are less sensitive to hormone. There may be other proteins, besides the receptor kinases, that can read the signal directly from the receptor, but the receptor kinases do highlight the potential benefits. Desensitization of the β-adrenoceptor by cyclic AMP-dependent protein kinase, where the initial receptor signal is substantially amplified before feeding back to regulate the receptor, is up to 100-fold more sensitive to catecholamines than is desensitization by β-adrenoceptor kinase, where the response depends directly on receptor occupancy (Benovic *et al.*, 1989; Lohse *et al.*, 1990).

Rodbell (1985) has discussed the considerable potential for modulation of signalling pathways by covalent modification of G proteins, indeed he has coined the term 'programmable messengers' to emphasize their versatile signalling properties. Phosphorylation of G proteins by, for example, protein kinases

C (Sagi-Eisenberg, 1989; Pyne *et al.*, 1989) or receptor tyrosine kinases (Zick *et al.*, 1986; Valentine-Braun *et al.*, 1986; O'Brien *et al.*, 1987) is likely to be the most important, though perhaps not the only, type of physiological modification. Certainly the bacterial toxins, cholera and pertussis toxins, catalyse a very different modification, they ADP-ribosylate their G protein substrates. The functional significance of covalent modification of G proteins is not always clear, though in human platelets phosphorylation of G_i by protein kinase C has been shown to prevent it from inhibiting adenylate cyclase (Jakobs *et al.*, 1985).

As increasing numbers of G proteins are found to be substrates for covalent modification by endogenous enzymes, it will be interesting to see whether any of the modifications affect the memory of the G protein, its GTPase activity. Cholera toxin has exactly that effect, it abolishes the GTPase activities of its substrates. Regulation of the GTPase activity under more physiological conditions also seems likely. The GTPase activity of G_s measured *in vitro* is sufficient to account for the decay in adenylate cyclase activity (Cassel *et al.*, 1977), but the closing of acetylcholine-regulated K^+ channels (Breitweiser & Szabo, 1988) and decay of the photoreceptor response (Uhl *et al.*, 1990) are too rapid to be explained by the GTPase activities of the relevant G proteins measured *in vitro*. The GTPase activities of the *ras* family of GTP-binding proteins are stimulated by another protein, GTPase-activating protein (GAP) (Trahey & McCormick, 1987), but no such protein has been found to regulate the signal-transducing G proteins, although from analyses of mutant G_s it has been suggested that a similar regulatory function may be in-built in these G proteins (Landis *et al.*, 1989). The GTPase activities of another family of G proteins, the initiation and elongation factors that convey aminoacyl-tRNA to the ribosome during protein synthesis, are stimulated by interaction with their effector, the ribosome. However, there is no evidence to suggest that the GTPase activities of the signal-transducing G proteins are similarly regulated by their effectors. While there is presently no evidence to suggest that the GTPase activity of G proteins is regulated, this property is such a key aspect of their function that it would be quite remarkable if cells had not found a way of controlling it.

The addition of a G protein to a transmembrane signalling pathway provides an enormous increase in flexibility. Cells can read the signal evoked by the extracellular stimulus directly from the receptor or through the G protein, with consequent differences in sensitivity to the stimulus. They may covalently modify the G proteins to give 'programmable messengers', or they may find means of controlling the lifespan of the active messenger by regulating its GTPase activity.

Shared pathways

G proteins provide the first opportunity for signals from different receptors to be integrated and an opportunity to then send the signal on to different effectors. How extensively do cells exploit this signal processing capability of G proteins?

There have been many suggestions that a complex web of interactions links receptors, G proteins and their effectors with signals both converging to shared targets and diverging from shared detectors (Rodbell, 1985; Ross, 1989). Much of the evidence, however, has come from studies of *in vitro* preparations where proteins may be presented with opportunities to interact that are never available in intact cells. The interaction, for example, of an occupied receptor with a G protein is determined by their relative concentrations and the affinity of one for the other, and even a very weak interaction between them may be detected *in vitro* if the two proteins see each other at unrealistic concentrations. The β-adrenoceptor is an example; it interacts most strongly with G_s, but when G_i or transducin are present in sufficient amounts it will also interact with them (Cerione *et al.*, 1985).

Experiments *in vitro* suggest that a single G protein can interact with multiple receptors, but this must be considered alongside our earlier discussion suggesting that in the intact membrane a single receptor has a rather restricted sphere of influence within the membrane (Stickle & Barber, 1989). The considerable structural diversity among even very closely related G proteins (Lochrie & Simon, 1988) cannot yet be adequately related to functional differences, but it too suggests abundant opportunity for receptors to talk more privately to their G protein. There is clearly a need to explore the extent to which within the native membrane different receptors converge to share the same pool of G proteins.

The clearest evidence that the same G protein may integrate the signals from different receptors in the native membrane is provided by experiments on turkey erythrocytes. Adrenaline, via its stimulation of β-adrenoceptors, stimulates specific binding of GppNHp to G_s, but the GppNHp can then be chased out by subsequent incubation with GTP and adenosine acting through its receptor (Tolkovsky & Levitzki, 1978*b*). Here the G protein is a 'bottleneck' in the signalling pathway and ensures that when a cell has several receptor types and many of each (to provide sensitivity to low agonist concentrations), it need not risk huge increases in intracellular messenger concentration when stimulated by several agonists. However, this early convergence of receptors that share the same intracellular messengers may not be a universal feature. In heart, for example, it seems that different receptors may have independent routes to adenylate cyclase because cyclic AMP formed in response to activation of β-adrenoceptors appears to be a more potent stimulus of cellular activity than is cyclic AMP formed in response to prostaglandin E_1 (Hayes *et al.*, 1979).

If receptors can converge to a single G protein, can they also diverge to activate different G proteins and different effectors? Again, the evidence from experiments *in vitro* is clear: a single receptor may interact with multiple G proteins. Interaction of the β-adrenoceptor with both G_s and more weakly with G_i was discussed above, and the receptor for chemotactic peptide interacts with both G_i and G_o (Kikuchi *et al.*, 1986). The human M_2 and M_3 muscarinic, α_2 adrenergic and 5-HT_{1A} receptors when transfected into intact cells mediate both inhibition of adenylate cyclase and stimulation of polyphosphoinositide-specific phospholipase C (Ashkenazi *et al.*, 1987; Peralta *et al.*, 1988; Fargin *et al.*, 1989; Cotecchia *et al.*, 1990), but the latter occurs only at the highest agonist concentrations and may reflect a very weak coupling, evident only because the receptor/G protein/effector stoichiometry has been distorted by over-expression of the receptor. Under more physiological conditions in adrenal chromaffin cells, an apparently homogeneous class of prostaglandin E_2 receptor couples through G_i to inhibit adenylate cyclase activity and through a different, unidentified G protein to stimulate polyphosphoinositide-specific phospholipase C (Negishi *et al.*, 1989). In a more quantitative approach to the problem, Senogles *et al.* (1990) have recently shown that although D_2-dopaminergic receptors can activate G_{i1}, G_{i2} and G_{i3}, the active receptor has greater affinity for G_{i2} and activates it more effectively. These examples demonstrate that receptors can be promiscuous in their choice of G protein, but they appear to be so only under extreme conditions. There is presently no experimental evidence to suggest that when stimulated with physiologically relevant (i.e. submaximal) concentrations of agonist in their native environment receptors are other than faithful to a single G protein.

There is also evidence, again from experiments *in vitro*, for both convergence and divergence in the later stages of the sig-

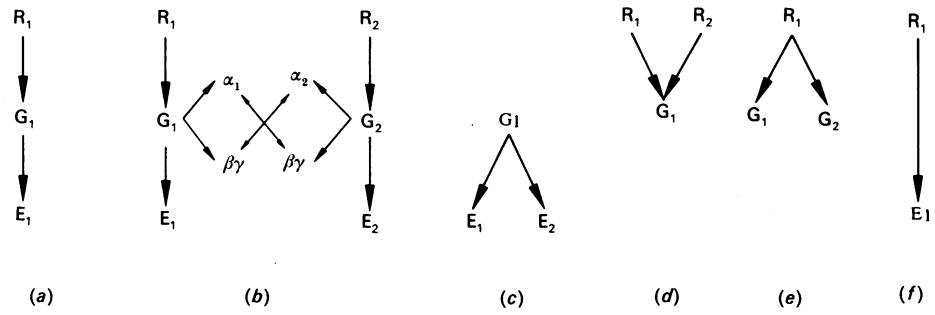

Fig. 4. Convergence and divergence in G protein signalling pathways

The extent to which intact cells exploit the enormous potential for signalling processing as information passes through G protein signalling pathways is largely unknown. The Figure illustrates some of the many possibilities for interactions as information is transferred from receptors (R) to G proteins (G) and effectors (E). Specific examples are discussed in the text.

nalling pathways. A single G protein may interact with multiple effectors: exogenous G_s, for example, can both stimulate adenylate cyclase and open Ca^{2+} channels, and less direct evidence suggests that it may also regulate Mg^{2+} transport (Erdos *et al.*, 1981). There are many examples of different G proteins converging to regulate the same effector. Each form of α_s stimulates adenylate cyclase; α_{i1}, α_{i2}, α_{i3} and α_o each stimulate cardiac K^+ channels (Sternweis & Pang, 1990); both pertussis toxin-sensitive and -insensitive G proteins stimulate polyphosphoinositide-specific phospholipase C in intact cells (Taylor & Merritt, 1986; Cotecchia *et al.*, 1990); and in HL-60 membranes G_i or G_o can both stimulate the enzyme (Kikuchi *et al.*, 1986). There is growing evidence for the existence of multiple closely related isoforms of these intracellular effectors, polyphosphoinositide-specific phospholipase C (Crooke & Bennett, 1989) and K^+ channels (Schwartz *et al.*, 1988) for example. The apparent convergence of multiple G proteins to the same effector may therefore be no more than a reflection of our present inability to detect a more specific interaction between distinct G proteins and particular effector isoforms.

In addition to the sharing of signalling components discussed above, there may also be rather looser interactions determined by the $\beta\gamma$ subunits shared by all G proteins. The likely role of $\beta\gamma$ from G_i interacting with α_s to cause inhibition of adenylate cyclase has already been discussed, but $\beta\gamma$, freed by activation of any G protein, may also have more general effects. Changes in the amounts of free $\beta\gamma$ subunit may affect both the rate of interaction of α subunits with receptors—only the trimeric G protein can be activated by the receptor, and they may influence the equilibrium between free active and bound inactive α subunits. These possibilities too deserve to be addressed in native membranes.

Autonomic control of the heart provides an illustration of some of the ways in which interplay between G protein signalling pathways leads to integrated control of a physiological response (Robishaw & Foster, 1989; Ross, 1989). The opposing effects of catecholamines and acetylcholine on heart rate and force of contraction are in part mediated by their different effects on atrial Ca^{2+} and K^+ channels. Stimulation of cardiac β_1-adrenoceptors activates G_s and the free α_s–GTP subunit directly regulates at least two effectors, adenylate cyclase and voltage-regulated Ca^{2+} channels. The cyclic AMP formed activates its protein kinase and among its many substrates is the L-type Ca^{2+} channel that is also directly activated by α_s–GTP. In concert, phosphorylation of the channel and control by α_s–GTP increase voltage-gated Ca^{2+} currents. Acetylcholine, via muscarinic receptors, activates G_i which dissociates into its α_i–GTP and $\beta\gamma$ subunits. The latter bind to α_s–GTP and relieve its direct and indirect effects on Ca^{2+} channels and may also activate phospholipase A_2, leading to opening of K^+ channels and consequent hyperpolarization of the membrane. The same effect may be caused by the α_i–GTP subunit interacting directly with the K^+ channel. Cholinergic stimulation thereby opposes the effects of adrenergic stimulation by reducing Ca^{2+} currents. This example highlights the rather conservative nature of the interactions between different elements of the signalling pathways: α_s–GTP, for example, diverges to regulate two effectors, but the final result is similar for each.

The homologous structures of receptors and G proteins provide abundant opportunities for different G protein signalling pathways to share various elements. The extent to which cells exploit these shared components to both integrate and further process the signals provided by receptors remains largely unknown. While the potential is enormous, it seems likely that tight cellular control requires that signalling pathways are not too promiscuous and we may perhaps expect converging signals to be a more common feature than diverging signals.

CONCLUSIONS

Every transmembrane signalling pathway has evolved to strike a compromise between detecting very low concentrations of extracellular stimulus and detecting very rapid changes in its concentration. Exactly where the balance lies depends upon the cellular responses that the pathway controls: the receptors for neurotransmitters sacrifice absolute sensitivity to gain sensitivity to rapid changes in neurotransmitter concentration, while the converse is true for growth factor receptors. The receptors that interact with G proteins strike an intermediate compromise, with the G protein itself playing an important role in allowing receptors to respond both rapidly and to relatively low agonist concentrations.

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