Biochemical Pharmacology, Vol. 55, pp. 549–556, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00361-4

COMMENTARY

Rethinking Receptor–G Protein–Effector Interactions

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ABSTRACT. Hundreds of different receptors regulate the activity of effector proteins with the assistance of heterotrimeric guanine nucleotide-binding proteins (G proteins). The hypothesis that G protein-coupled receptors (R) govern their effectors (E) indirectly via a shuttling mechanism involving the exchange of heterotrimeric G proteins ($G_{\alpha\beta\gamma}$) or parts thereof (G_{α}, $G_{\beta\gamma}$) between ephemeral R–G and G–E complexes has become firmly established. While there is no direct evidence for the cyclical formation and dissociation of these complexes during signalling, experimental changes in second messenger production, GTPase activity, and the binding characteristics of agonists, antagonists, and guanine nucleotides commonly are believed to reflect perturbations in the equilibria between G protein and the other two components. However, a growing body of evidence seems to argue against the shuttling model. The random, transient association of G protein and receptor is largely inconsistent with the binding of agonists to receptors and the allosteric regulation of that binding by guanine nucleotides. Also, the prevailing paradigm does not readily account for receptor–effector coupling specificity, as the promiscuous interaction of most G proteins with both receptors and effectors *in vitro* is at odds with the general failure of G proteins to be shared among ostensibly congruous signal transduction pathways *in vivo*. The latter paradox would be obviated by the simultaneous interaction of G protein with both receptor and effector. Indeed, various findings indicate that R–G–E complexes do occur. How and where in the cell such complexes are assembled and disassembled should provide important clues to the true mechanism of G protein-linked transduction. BIOCHEM PHARMACOL 55;5:549–556, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. G protein-coupled receptor; guanine nucleotide-binding protein (G protein); effector protein; receptor–effector coupling specificity; G protein shuttle; receptor–G protein–effector complexes

Cellular activity is coordinated via hormones and other messengers that are released from one cell and act via specific receptors on or in a target cell. The largest class of receptors encompasses proteins that span the plasma membrane seven times and utilize heterotrimeric GTP-binding proteins to govern effector proteins, which in turn control intracellular levels of various ions and second messengers. Three protein components, namely receptor (R†), heterotrimeric G protein ($G_{\alpha\beta\gamma}$), and effector (E), together are sufficient to account for the fundamental properties of signal transduction [1–4]. A hormone-induced change in effector activity minimally involves a cycle of five steps, wherein (1) the reversible binding of hormone to a receptor promotes (2) the dissociation of GDP from and (3) the activation of a G protein through the binding of GTP, thus allowing for (4) a change in effector activity that lasts until (5) GTP is hydrolyzed to GDP.

It is widely held that interactions among the various components are transient, and that signal transduction necessarily involves the cyclical formation and breakdown of several different protein complexes [1, 2, 4]. Briefly, it is believed that the agonist-promoted binding of GTP to the G_{α} subunit of a heterotrimeric G protein causes the sequential dissociation of G_{α}-GTP and the stable $G_{\beta\gamma}$ dimer from the receptor. Subsequently, the free G_{α}-GTP and/or free $G_{\beta\gamma}$ dimer independently bind to an effector, altering its activity. After the hydrolysis of GTP to GDP and the dissociation of G_{α}-GDP and/or $G_{\beta\gamma}$ from the effector, the $G_{\alpha\beta\gamma}$ heterotrimer reforms and reassociates with the receptor [1, 2, 4]. The premise of transient protein interactions thus introduces multiple additional steps into the basic mechanism outlined above.

A key feature of the current model of transduction is that receptors and effectors do not come into contact with one another but rather communicate via shuttling G proteins. Various findings indicate that G protein association with and dissociation from both receptors and effectors can occur, but the notion of shuttling is purely speculative. Moreover, few of these observations are unequivocal, and a growing body of evidence exists that is difficult to reconcile with the idea that receptors activate effectors indirectly. Alternative schemes would necessarily imply the existence of hetero–oligomeric complexes containing receptor, G protein, and effector, at least at some instant during transduction.

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† *Abbreviations:* $G_{\alpha\beta\gamma}$, heterotrimeric GTP-binding regulatory protein; G_{α}, GTP-binding α subunit of $G_{\alpha\beta\gamma}$; $G_{\beta\gamma}$, stable complex of β and γ subunits of $G_{\alpha\beta\gamma}$; Gpp(NH)p, 5′-guanylylimidodiphosphate; GTPγS, guanosine-5′-O-(3-thiotriphosphate); R, receptor; and E, effector.

The idea that signal transduction involves R–G–E complexes is attractive in that it provides inherent specificity within receptor–effector coupling pathways. The prevailing paradigm falls short in this regard, since experiments with purified components have shown that multiple receptors can activate the same G protein, one receptor can activate different G proteins, different G proteins can activate the same effector, and one G protein can activate different effectors [2, 4]. Also, $G_{\beta\gamma}$ subunits appear to be largely interchangeable among G_{α} subunits as well as among effectors and receptors [4]. Effectors governed by $G_{\beta\gamma}$ thus would be expected to show sensitivity to most receptors, but such evidence has not been forthcoming. Cells typically have multiple types of receptors, G proteins, and effectors, and it is difficult to understand how specific receptor–effector communication would result from a myriad of promiscuous protein interactions. Still, receptor–effector communication does appear to be quite specific in living cells (discussed below). Attempts to reconcile these apparent differences between purified proteins and whole cells have led to the idea that G proteins shuttle between receptors and effectors within restricted *microdomains* [4]. According to this modification of the model, G proteins are corralled together with the "correct" receptors and effectors, while the "wrong" combinations are somehow forbidden. Thus, in addition to numerous steps involving the association and dissociation of various protein complexes, the shuttling model also requires the existence of additional factors, yet unknown, which are not necessary for effector activation but which are essential for receptor–effector coupling specificity. Alternatively, the concept of restricted mobility can be taken a step further, with receptor, G protein, and effector all present within a single hetero-oligomer. The assumption of physical continuity between receptor and effector allows for a much simpler picture of G protein-mediated signal transduction than is possible if G proteins are assumed to shuttle between the other two components.

HISTORICAL PERSPECTIVES

Prior to the identification of G proteins as distinct entities, the *floating receptor model* proposed that receptors and effectors exist independently and combine to form transient receptor–effector complexes [5]. This early model was an attempt to rationalize the disproportionately high levels of adenylyl cyclase stimulation observed at relatively low levels of receptor occupancy by agonist (e.g. Ref. 6) and the findings that the simultaneous maximal stimulation of adenylyl cyclase via two or more receptors could be less than the sum of the individual receptor-mediated maxima (e.g. Ref. 7). Such observations seemed inconsistent with the idea of a single enzymatic moiety being fixed to a single agonist binding site and vice versa. In terms of the floating receptor model, an excess of receptor over effector accounts for the phenomenon of "spare receptors," while competition for effector by multiple types of receptors allows for the observed lack of additivity when different receptors are activated simultaneously. The idea that receptor binding activity and the activity of adenylyl cyclase could be separated and recombined was confirmed by cell fusion experiments [8].

The floating receptor model did not explicitly address the role of guanine nucleotides in signal transduction. It had by then been shown that GTP decreases the binding of agonists to their receptors and enhances the effects of agonists on adenylyl cyclase. The latter effect could be explained by a *collision coupling model*, which proposed that the nucleotide binding site was on the effector and that receptors promoted activation by interacting briefly with the effector and causing nucleotide exchange [9]. However, this model, as formulated, predicts neither an effect of nucleotide on agonist binding nor complex agonist binding profiles (see below).

Further developments pointed to the existence of a distinct GTP-hydrolyzing protein capable of activating adenylyl cyclase in response to GTP or an analogue such as Gpp(NH)p or GTPγS [10]. Also, effects of guanine nucleotides on agonist binding could be observed with β-adrenergic receptors that were functionally uncoupled from adenylyl cyclase, suggesting that complexes of receptor and G protein could exist independently of the effector. Such findings led to the *ternary complex model* [11], which proposed that agonist-occupied receptors couple transiently to and activate G proteins, which then are released to interact with effector proteins.

EFFECTS OF G PROTEINS ON RECEPTOR BINDING

Guanine nucleotides were first observed to influence the binding properties of receptors in cell membranes in the early 1970s, and to date no clear and complete explanation has been found to account for the effects of guanine nucleotides on agonist binding profiles. In competition experiments with radiolabeled antagonists, agonists yield binding profiles with Hill coefficients of less than one. The addition of guanine nucleotides causes a steepening of the curve and an increase in the concentration of agonist required to eliminate the binding of the probe. That the binding profiles of agonists are reflective of their pharmacological properties has long been recognized; however, the underlying mechanism was and remains uncertain [12, 13]. A low Hill coefficient at equilibrium potentially could indicate multiple independent binding sites with differing affinities, cooperative binding, or a two-step mechanism involving the receptor plus a separate, stoichiometrically limiting component [12].

Whereas a single instance of multiple agonist affinities could be explained by receptor heterogeneity, the ratio of high to low affinity binding sites (or high to medium to low affinity) tends to vary from one agonist to the next [13].

This suggests that the differing affinity states are not independent but rather are related to one another. In addition, the effects of guanine nucleotides suggest that at least some of the sites can change their affinity. The ternary complex model was shown to describe agonist binding adequately in several systems [11, 14, 15], and was deemed preferable to the "multi-site" model. Protracted agonist binding profiles thus were ascribed to the effects of an auxiliary component, namely the G protein, on the receptor.

Low affinity agonist binding sites in membranes are presumed to be free receptors (R), while high affinity binding is attributed to receptor complexed with G protein (RG). In general, the ternary complex model dictates that at equilibrium an agonist binding profile will resemble a single uniform population of sites and yield an affinity that lies between the true affinities of the agonist for R and R–G [16]. Depending in part on the affinity of R for G, this model can allow for Hill coefficients of less than one only if [G] does not exceed [R] by more than a factor of about two [16]. Least squares analyses of shallow agonist binding curves in terms of the ternary model thus tend to show that receptor and G protein are present in similar amounts [11, 13–15, 17]. In contrast, more direct methods of measurement have shown that receptors *in vivo* are greatly outnumbered by their G proteins [3], in which case agonists would be predicted to bind to an apparently uniform population of sites (i.e. $n_H = 1$).

Guanine nucleotides decrease the coupling of G to R in detergent solution (e.g. Ref. 18), and thus supposedly preclude high affinity agonist binding by decreasing R–G coupling. It should be noted, however, that nucleotide-induced decreases in agonist affinity can be observed under conditions where the dissociation of R from G clearly fails to occur [19]. This indicates that decreased agonist affinity and decreased R–G coupling are not simply different manifestations of the same phenomenon. It follows that nucleotide-induced changes in agonist binding need not be equated with R–G uncoupling.

The ternary model fits well to monophasic agonist binding data acquired in the presence of nucleotide, although, curiously, such analyses tend to show nucleotide-dependent decreases in the total number of G proteins (e.g. Ref. 11). In cases where agonists yield complex binding curves in the presence of saturating concentrations of nucleotide (e.g. Refs. 14 and 17), the model appears incomplete, since the dissociation of G protein from receptor anticipated under such conditions should result in a homogeneous population of free receptors in a low affinity state. Alternatively, the presence of two G proteins with differing affinities for the receptor in question could give rise to such binding patterns [20]. In a recent study on cardiac M_2 receptors, each of three nucleotides [GTPγS, Gpp(NH)p, and GDP] exhibited a biphasic dose dependence with respect to the allosteric regulation of carbachol binding [17]. While the effect of each individual nucleotide suggested a system with two G proteins competing for a single receptor, the implied stoichiometry of the two G proteins varied greatly from one nucleotide to the next. Such findings contradict the ternary model. In contrast, the aforementioned effects of GTPγS, Gpp(NH)p, and GDP together could be readily interpreted as nucleotide-induced changes in cooperative interactions within M_2 receptor oligomers [21].

Taken together, analyses of guanine nucleotide-sensitive agonist binding data question whether the ternary complex model constitutes an accurate general description of G protein-mediated signal transduction. An alternative possibility is that the low Hill coefficients that characterize the binding of agonists arise from cooperative interactions among receptors. Accordingly, binding studies on purified M_2 muscarinic receptors have shown that agonists bind with multiple affinities even in the absence of G protein, indicating that complex binding profiles are a property of the receptor *per se* (e.g. Refs. 22 and 23). The idea that ligands bind cooperatively to their receptors has been considered on occasion, and such models have yielded excellent correlations to receptor binding data [21, 23, 24]. Findings indicative of cooperative interactions among receptors include binding data wherein (1) antagonists bind with Hill coefficients of greater than one (e.g. Ref. 24), (2) different radiolabeled antagonists to the same receptor yield widely differing estimates of total binding capacity in the same preparation (e.g. Ref. 23), and (3) agonists at low concentrations increase the binding of antagonists [23].

The existence of dimers or larger receptor oligomers implied by binding data is further supported by biochemical studies on the photoaffinity labeling, radiation inactivation, cross-linking, and hydrodynamic properties of various G protein-coupled receptors (reviewed in Refs. 23 and 25). Also, receptor oligomerization is implied by the finding that two inactive (but complementary) muscarinic/α-adrenergic receptor chimeras could be co-expressed to yield both muscarinic and adrenergic binding as well as effector activation via agonists for either receptor [26]. More recent evidence shows that a peptide derived from the sixth transmembrane segment of the β_2-adrenergic receptor decreases agonist-induced activation of membrane adenylyl cyclase activity via the receptor; moreover, the peptide decreases the amount of receptor found in SDS–PAGE gels running at a position approximately twice the molecular weight of the protein [27]. Similar effects were noted on the apparent molecular size of D2 dopamine receptors in response to peptides derived from either the sixth or seventh transmembrane segment of the receptor [28]. The foregoing arguments should help to dispel the notion that complex agonist binding curves are solely predicated on transient receptor–G protein coupling. The alternative explanation that such binding profiles arise from cooperative interactions among receptors does not, in itself, explain how G proteins and effectors are activated in response to agonists. Thus, the transient interaction of G protein with oligomeric receptor is difficult to rule out completely.

EFFECTS OF RECEPTORS ON G PROTEIN BINDING

If a guanine nucleotide allosterically decreases the affinity of an agonist, then associatively the agonist decreases the affinity of that nucleotide for its binding site [10, 29]. Thus, agonist-activated receptors decrease the binding of GDP, as indicated both by more rapid dissociation of bound GDP from G proteins and by decreases in the affinity of the nucleotide. Agonists increase the respective rate constants of dissociation [30] and association [31] of the GTP analogues Gpp(NH)p and GTPγS, although there is no clear effect on their overall binding affinities (e.g. Ref. 17). The decreased binding of GDP in response to receptor stimulation is thought to facilitate G protein activation by leaving open the binding site for GTP, which is present in cells at relatively high concentrations compared with GDP. Receptors have been found to have little or no direct effect on the rate at which GTP bound to G proteins is hydrolyzed to GDP [1].

A recurrent but generally overlooked property of receptor–G protein interaction is that the agonist-promoted dissociation of radiolabeled guanine nucleotides increases in the presence of unlabeled guanine nucleotides [30–34]. In some systems [30, 34], agonist-promoted dissociation *requires* the addition of unlabeled nucleotide. These data suggest a cooperative effect between two, or more, closely associated nucleotide binding proteins, forming a putative G protein multimer. Accordingly, the effect of the muscarinic agonist carbachol on the binding of guanine nucleotides to cardiac membranes is consistent with a mechanism wherein agonist-induced changes in G protein cooperativity promote the exchange of GTP for bound GDP [25].

G PROTEIN COMPLEXES

Upon purification, receptors are often accompanied by G proteins or G_α subunits. Receptor–G protein co-purification is increased in the presence of agonists, while antagonists and guanine nucleotides generally have the opposite effect (e.g. Refs. 19, 23, 35, 36). In keeping with the accepted model of signal transduction, increases and decreases in R–G co-purification are typically attributed to corresponding ligand-induced changes in the equilibrium $\{R + G \rightleftharpoons RG\}$. This interpretation implies that ephemeral coupling in membranes gives rise to stable protein complexes upon extraction. Interestingly, receptor purification often employs affinity chromatography with resins derived from antagonists. If antagonists truly inhibit R–G association and/or induce R–G dissociation [37], it seems remarkable that they should permit G proteins to remain bound to receptors throughout the extensive dilutions, harsh detergent and salt conditions, and prolonged periods of time required for receptor purification. An alternative explanation for the changes in R–G co-purification noted above is that ligand-induced conformational changes alter the stability of pre-existing complexes and thereby influence the degree of co-purification [23].

In solutions containing millimolar or greater concentrations of Mg^{2+}, GTPγS facilitates the dissociation of heterotrimeric G protein into G_α plus $G_{\beta\gamma}$ [1]. This has led to the conjecture that the GTP-stimulated dissociation of G protein subunits is an essential step in G protein-mediated signal transduction *in vivo*. Notwithstanding the general acceptance of the idea, G protein subunit dissociation does not appear to occur in biological membranes [1, 38], and dissociation with the physiological substrate GTP has been difficult to detect even in detergent solution (e.g. Ref. 39). Indeed, GTP and GDP have been shown to increase the Mg^{2+} requirement for subunit dissociation, while GTPγS decreases it [40]. Also, given that many effectors interact with both G_α-GTP and $G_{\beta\gamma}$ [4], it seems inefficient (not to mention counterintuitive) that G protein subunits should dissociate from each other upon activation by receptor, only to rejoin at the effector. Overall, it appears that G protein subunit dissociation is a phenomenon that is observed only under distinctly non-physiological conditions and, therefore, may not play a significant role in signal transduction *in vivo*.

Relatively little attention has been paid to the stability of G protein–effector interactions. Based on the shuttling model, one would predict that the binding of GTP or an analogue should promote the association of G proteins with their effectors. The effector adenylyl cyclase has been co-purified with its stimulatory G protein, G_s. Contrary to expectation, co-purification of an adenylyl cyclase–G_s complex can be carried out regardless of whether G_s is occupied by GDP or the activating ligand Gpp(NH)p [41]. The latter nucleotide can facilitate subunit dissociation of trimeric G_s under appropriate conditions. In contrast, both $G_{\alpha s}$–Gpp(NH)p and $G_{\beta\gamma}$ copurify with an adenylyl cyclase from bovine brain [42]. These observations argue that G_s does not shuttle back and forth between adenylyl cyclase and a receptor, but rather is stably attached to the effector. Although nucleotide-bound $G_{\alpha s}$ alone is sufficient to activate adenylyl cyclase, trimeric G_s may be the physiological regulator, since several isoforms of the effector have been shown to be regulated by both $G_{\alpha s}$ and $G_{\beta\gamma}$ [43]. Several other effectors are regulated by both G_α and $G_{\beta\gamma}$, including phospholipase Cβ [44, 45] and various ion channels [4, 46], but the stability of G protein–effector coupling has not been established in these systems.

Receptors appear to interact only with heterotrimeric G proteins, since little or no effect is detected with either G_α or $G_{\beta\gamma}$ alone [4]. Similarly, the notion of interactions between heterotrimeric G proteins and effectors is consistent with most or all evidence available. However, the potential role of trimeric G in this case is unclear, since both isolated G_α and isolated $G_{\beta\gamma}$ have been found to modulate various effector activities. On one hand, it is possible that effectors do not bind heterotrimeric G protein *per se*, and it is imaginable that there may be physically separate sites of attachment for G_α and $G_{\beta\gamma}$. On the other

hand, the observed effects of isolated G protein subunits do not dictate that such interactions with effectors are the norm *in vivo*. Also, in the absence of a G protein shuttle, isolated effector binding sites for G_α and $G_{\beta\gamma}$ might not be expected. While much remains to be learned about which regions of receptor, G protein, and effector interact with one another, there is a general lack of overlap between the binding sites on G_α for receptor, $G_{\beta\gamma}$, and effector (see Fig. 2 in Ref. 4), which would seemingly allow for the simultaneous binding of $G_{\alpha\beta\gamma}$ to both receptor and effector.

EVIDENCE FOR RECEPTOR–EFFECTOR CONTINUITY

Currently, there is no direct evidence against the existence of R–G–E complexes. While physical continuity between receptor and effector has not been demonstrated directly, it is implied in many systems, for example the adenylyl cyclase activity and [^{125}I]human chorionic gonadotropin binding activity in solubilized testicular and ovarian tissues will co-migrate during size-exclusion chromatography [47]. Also, the radiation inactivation of the hormonal stimulation and inhibition of adenylyl cyclase infers large molecular masses, at least as great as what would be expected for receptor, G protein, and effector combined [29]. Numerous experimental findings, outlined below, are difficult to rationalize without assuming that complexes containing both receptor and effector must exist during the process of signal transduction.

In contrast to the promiscuity of purified proteins in reconstituted systems [2, 4], interactions among receptors, G proteins, and effectors in non-transfected systems appear to be specific. In NG108-15 neuroblastoma-glial cells, three different receptors couple to G_i, but no sharing of G proteins among the receptors can be detected [48]. Voltage-dependent Ca^{2+} channels in GH3 cells can be inhibited via agonists to either M_4 muscarinic or somatostatin receptors. In "knockout" experiments, the muscarinic effect was eliminated by preventing the synthesis of $G_{\alpha o1}$, $G_{\beta 1}$, or $G_{\gamma 4}$, while the somatostatin response analogously was eliminated by blocking the synthesis of $G_{\alpha o2}$, $G_{\beta 3}$, or $G_{\gamma 3}$ [49–51]. Similar experimental strategies involving other transduction pathways maintain that specific receptor–G protein–effector pathways tend to be used by cells to the exclusion of other biochemically possible combinations [52–55]. The observed exclusivity of functional receptor–G protein–effector combinations *in vivo* is not obviously consistent with the notion that information is transferred from receptors to effectors indirectly via shuttling G proteins. Rather, these data suggest the existence of R–G–E complexes during transduction.

Several findings imply that β-adrenergic receptors interact with a G_s–adenylyl cyclase complex. For example, the β_2-adrenergic receptor deletion mutant D267-273 is indistinguishable from wild-type receptor with respect to its G protein interactions, yet it is impaired in its ability to stimulate adenylyl cyclase [56]. This contradicts the idea that G_s is the sole purveyor of the hormonal signal to adenylyl cyclase, and instead suggests that the receptor and G protein interact with the effector simultaneously. Similarly, the stable binding of all three subunits of G_s to adenylyl cyclase (noted above) implies either that transduction occurs through a stable R–G–E complex or that receptor interacts transiently with G–E. The latter interpretation is supported by evidence that the receptor acts catalytically in some systems and that the rate of adenylyl cyclase activation by β-adrenergic agonists increases linearly with membrane fluidity. In contrast, analogous experiments with adenosine receptors point to stable receptor–adenylyl cyclase complexes [57]. The isolation of complexes of G_s and receptor under some conditions and G_s-adenylyl cyclase complexes under others suggests that both R–G and G–E may derive from larger, albeit more fragile, hetero–oligomers.

The regulation of phospholipase Cβ1 activity by M_1 muscarinic receptor and G_q similarly appears to occur through an R–G–E complex. The combined effect of the receptor and the effector on the G protein is such that its rapid steady-state rate of GTP hydrolysis cannot be accounted for by its sequential interaction with the two other proteins, which therefore implies that both M_1 and PLCβ1 are acting upon G_q simultaneously [44, 45]. The latter studies were carried out using purified proteins in a reconstituted phospholipid vesicle system. It follows that receptor, G protein, and effector alone are sufficient for R–G–E complexation, and that no other protein components are necessary. Also, in such a system, it may be noted that there is no obvious opportunity for heterotrimeric G_q to dissociate into $G_{\alpha q}$-GTP plus $G_{\beta\gamma}$ during transduction.

MECHANISTIC IMPLICATIONS OF NOT SHUTTLING

Dismissal of the idea that receptors and effectors communicate indirectly via shuttling G proteins implies the existence of R–G–E complexes, but still leaves open the question of how hormonal information is transferred to effectors. Physical continuity between receptor and effector does not *per se* dictate that complexes containing both protein species exist throughout the process of signal transduction. The results of reconstitution experiments utilizing purified components suggests that complexes can form spontaneously, but it seems likely that this process would be subject to regulation *in vivo*. It remains to be determined how, where in the cell, and how frequently R–G–E complexes may form and turn over. The characterization of these events should lead to an improved understanding of the actual mechanism of transduction.

Signal transduction may occur through three possible mechanisms: (1) the formation of R–G–E complexes, (2) the dissociation of R–G–E complexes, or (3) stable R–G–E complexes. The first possibility allows for a simple rationalization of the non-additivity of combined agonist effects via different receptors [7] as well as the phenomenon of "spare receptors" [6], and recalls the previous *floating receptor* [5]

and *collision coupling* [9] models. Leaving aside their differences with respect to predicted receptor–effector complex stability, both of these early hypotheses postulated that activation of receptor engenders R–E complex formation and subsequent effector activation. Both models, however, may be difficult to reconcile with agonist binding data. In the collision coupling model, the receptor is presumed to interact briefly with a G protein–effector complex; however, the model predicts neither flat agonist binding curves nor sensitivity of the latter to guanine nucleotides. If the transient interaction between R and G–E is presumed to have an effect on agonist binding, then the mechanism is mathematically analogous to the ternary model, the major shortcomings of which are outlined above. Other permutations can be imagined, for example receptor could sequentially engage G protein and then effector, or stable R–G complexes could bind transiently to effectors. The latter scheme seems plausible for soluble effectors such as phospholipase Cβ, which presumably could be recruited from the cytosol. Also, the apparent failure of receptors to share G proteins [48] would be accommodated. Alternatively, the transient coupling of R–G to E seems inconsistent with the observation of stable G–E complexes [41, 42].

In the *disaggregation–coupling model* proposed by Rodbell [29], oligomeric structures containing multiple copies of receptor, G protein, and, possibly, effector are presumed to be inactive and to bind agonists with high affinity. It was suggested that effector might be present in the inactive multimer in some systems but not in others. In either case, agonist binding to some or all of the receptors within the multimer (cf. spare receptors) facilitates the binding of GTP, leading to dispersion into smaller, active hetero–oligomers with low affinity for agonists. In cases where effector is absent from the inactive multimer, disaggregated R–G goes on to activate effector. If effector is present in the multimer, disaggregation and effector activation are presumed to occur together. One feature of this model as originally proposed is that receptor has a presumed damping effect with respect to basal effector activity. However, in contrast to the corollary prediction that basal effector activity should vary inversely with receptor density, subsequent studies with over-expressed receptors have shown that agonist-independent effector activity increases with receptor density (e.g. Ref. 58). Potentially, the disaggregation coupling model could be adjusted to account for such observations, as an inhibitory effect of receptor is not a central aspect of the original.

A third possibility is that R–G–E complexes neither assemble nor disassemble during signal transduction. While this would seem to be the simplest explanation, a complex containing one equivalent each of R, G, and E would be inadequate with respect to observed agonist binding properties as well as the disproportionately high effector activation observed at low levels of agonist occupancy. Both of these problems could potentially be remedied by the assumption of multiple interacting copies of receptor within the hetero–oligomeric complex. Similarly, multiple interacting G proteins might also be present, and furthermore there is scattered evidence suggesting that some effectors may oligomerize [59]. The idea that multiple copies of receptor, G protein, and possibly effector are contained within a complex recalls the presumed "inactive" complex proposed in the disaggregation coupling model [29]. Whether such complexes can be observed and whether dissociation is needed for effector activation are separate questions, however. Finally, any model that is to be taken seriously must somehow account for the apparent "sharing" of effectors among receptors [7]. One possibility is that R–G–E complexes may contain more than one type of receptor. Although the implied mass of such an aggregation might seem extraordinary, the 1300 kDa complexes inferred from radiation inactivation studies [29] would seem to allow for their existence.

Thanks to André De Léan, Roger Sunahara, and Tuli Mukhopadhyay for their constructive comments on the manuscript. The author is supported by a postdoctoral fellowship from the Medical Research Council of Canada.

References

1. Gilman AG, G proteins: Transducers of receptor-generated signals. *Annu Rev Biochem* **56:** 615–649, 1987.
2. Birnbaumer L, Abramowitz J and Brown AM, Receptor–effector coupling by G proteins. *Biochim Biophys Acta* **1031:** 163–224, 1990.
3. Neubig RR, Membrane organization in G-protein mechanisms. *FASEB J* **8:** 939–946, 1994.
4. Neer EJ, Heterotrimeric G proteins: Organizers of transmembrane signals. *Cell* **80:** 249–257, 1995.
5. De Haën C, The non-stoichiometric floating receptor model for hormone sensitive adenylyl cyclase. *J Theor Biol* **58:** 383–400, 1976.
6. Birnbaumer L and Pohl SL, Relation of glucagon-specific binding sites to glucagon-dependent stimulation of adenylyl cyclase activity in plasma membranes of rat liver. *J Biol Chem* **248:** 2056–2061, 1973.
7. Rodbell M, Birnbaumer L and Pohl SL, Adenyl cyclase in fat cells. III. Stimulation by secretin and the effects of trypsin on the receptors for lipolytic hormones. *J Biol Chem* **245:** 718–722, 1970.
8. Orly J and Schramm M, Coupling of catecholamine receptor from one cell with adenylate cyclase from another cell by cell fusion. *Proc Natl Acad Sci USA* **73:** 4410–4414, 1976.
9. Tolkovsky AM and Levitzki A, Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* **17:** 3795–3810, 1978.
10. Ross EM and Gilman AG, Biochemical properties of hormone-sensitive adenylate cyclase. *Annu Rev Biochem* **49:** 533–564, 1980.
11. De Léan A, Stadel JM and Lefkowitz RJ, A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J Biol Chem* **255:** 7108–7117, 1980.
12. Boeynaems JM and Dumont JE, Quantitative analysis of the binding of ligands to their receptors. *J Cyclic Nucleotide Res* **1:** 123–142, 1975.
13. Wong H-MS, Sole MJ and Wells JW, Assessment of mechanistic proposals for the binding of agonists to cardiac muscarinic receptors. *Biochemistry* **25:** 6995–7008, 1986.

14. Wreggett KA and De Léan A, The ternary complex model. Its properties and application to ligand interactions with the D_2-dopamine receptor of the anterior pituitary gland. *Mol Pharmacol* **26:** 214–227, 1984.
15. Ehlert FJ, The relationship between muscarinic receptor occupancy and adenylate cyclase inhibition in the rabbit myocardium. *Mol Pharmacol* **28:** 410–421, 1985.
16. Lee TWT, Sole MJ and Wells JW, Assessment of a ternary model for the binding of agonists to neurohumoral receptors. *Biochemistry* **25:** 7009–7020, 1986.
17. Green MA, Chidiac P and Wells JW, Cardiac muscarinic receptors. Relationship between the G protein and multiple states of affinity. *Biochemistry* **36:** 7380–7394, 1997.
18. Smith SK and Limbird LE, Solubilization of human platelet α-adrenergic receptors: Evidence that agonist occupancy of the receptor stabilizes receptor–effector interactions. *Proc Natl Acad Sci USA* **78:** 4026–4030, 1981.
19. Matesic DF, Manning DR, Wolfe BB and Luthin GR, Pharmacological and biochemical characterization of complexes of muscarinic acetylcholine receptor and guanine nucleotide-binding protein. *J Biol Chem* **264:** 21638–21645, 1989.
20. Leung E, Jacobson KA and Green RD, Analysis of agonist–antagonist interactions at A_1 adenosine receptors. *Mol Pharmacol* **38:** 72–83, 1990.
21. Chidiac P, Green MA, Pawagi AB and Wells JW, Cardiac muscarinic receptors. Cooperativity as the basis for multiple states of affinity. *Biochemistry* **36:** 7361–7379, 1997.
22. Ikegaya T, Nishiyama T, Haga K, Haga T, Ichiyama A, Kobayashi A and Yamazaki N, Interaction of atrial muscarinic receptors with three kinds of GTP-binding proteins. *J Mol Cell Cardiol* **22:** 343–351, 1990.
23. Wreggett KA and Wells JW, Cooperativity manifest in the binding properties of purified cardiac muscarinic receptors. *J Biol Chem* **270:** 22488–22499, 1995.
24. Mattera R, Pitts BJR, Entman ML and Birnbaumer L, Guanine nucleotide regulation of a mammalian myocardial muscarinic receptor system. *J Biol Chem* **260:** 7410–7421, 1985.
25. Chidiac P and Wells JW, Effects of adenyl nucleotides and carbachol on cooperative interactions among G proteins. *Biochemistry* **31:** 10908–10921, 1992.
26. Maggio R, Vogel Z and Wess J, Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. *Proc Natl Acad Sci USA* **90:** 3103–3107, 1993.
27. Hebert TE, Moffett S, Morello J-P, Loisel TP, Bichet DG, Barrett C and Bouvier M, A peptide from a β_2-adrenergic receptor domain inhibits both receptor dimerization and activation. *J Biol Chem* **271:** 16384–16392, 1996.
28. Ng GYK, O'Dowd BF, Lee SP, Chung HT, Brann MR, Seeman P and George SR, Dopamine D2 receptor dimers and receptor-blocking peptides. *Biochem Biophys Res Commun* **227:** 200–204, 1996.
29. Rodbell M, The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* **284:** 17–22, 1980.
30. Michel T and Lefkowitz RL, Hormonal inhibition of adenylate cyclase. *J Biol Chem* **257:** 13557–13563, 1982.
31. Brandt DR and Ross EM, Catecholamine-stimulated GTPase cycle. Multiple sites of regulation by β-adrenergic receptor and μg^{2+} studied in reconstituted receptor G_s vesicles. *J Biol Chem* **261:** 1656–1664, 1986.
32. Cassel D and Selinger Z, Mechanism of adenylate cyclase activation through the β-adrenergic receptor: Catecholamine-induced displacement of bound GDP by GTP. *Proc Natl Acad Sci USA* **75:** 4155–4159, 1978.
33. Fung RLK and Stryer L, Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc Natl Acad Sci USA* **77:** 2500–2504, 1980.
34. Murayama T and Ui M, [^{3}H]GDP release from rat and hamster adipocyte membranes independently linked to receptors involved in activation or inhibition of adenylate cyclase. Differential susceptibility to two bacterial toxins. *J Biol Chem* **259:** 761–769, 1984.
35. Senogles SE, Benovic JL, Amlaiky N, Unson C, Milligan G, Vinitsky R, Spiegel AM and Caron MG, The D_2-dopamine receptor of anterior pituitary is functionally associated with a pertussis toxin-sensitive guanine nucleotide binding protein. *J Biol Chem* **262:** 4860–4867, 1987.
36. Knezevic I, Borg C and Le Breton GC, Identification of G_q as one of the G-proteins which copurify with human platelet thromboxane A_2/prostaglandin H_2 receptors. *J Biol Chem* **268:** 26011–26017, 1993.
37. Costa T, Ogino Y, Munson PJ, Onaran HO and Rodbard D, Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: Thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. *Mol Pharmacol* **41:** 549–560, 1992.
38. Yi F, Denker BM and Neer EJ, Structural and functional studies of cross-linked G_o protein subunits. *J Biol Chem* **266:** 3900–3906, 1991.
39. Huff RM and Neer EJ, Subunit interactions of native and ADP-ribosylated α_{39} and α_{41}, two guanine nucleotide-binding proteins from bovine cerebral cortex. *J Biol Chem* **261:** 1105–1110, 1986.
40. Basi NS, Okuya S and Rebois RV, GTP binding to G_s does not promote subunit dissociation. *Cell Signal* **8:** 209–215, 1996.
41. Arad H, Rosenbusch JP and Levitzki A, Stimulatory GTP regulatory unit N_s and the catalytic unit of adenylate cyclase are tightly associated: Mechanistic consequences. *Proc Natl Acad Sci USA* **81:** 6579–6583, 1984.
42. Marbach I, Bar-Sinai A, Minich M and Levitzki A, β Subunit copurifies with GppNHp-activated adenylyl cyclase. *J Biol Chem* **265:** 9999–10004, 1990.
43. Sunahara RK, Dessauer CW and Gilman AG, Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* **36:** 461–480, 1996.
44. Berstein G, Blank JL, Jhon D-Y, Exton JH, Rhee SG and Ross EM, Phospholipase C-β1 is a GTPase-activating protein for $G_{q/11}$, its physiologic regulator. *Cell* **70:** 411–418, 1992.
45. Biddlecome GH, Berstein G and Ross EM, Regulation of phospholipase C-β1 by G_q and m1 muscarinic cholinergic receptor. Steady-state balance of receptor-mediated activation and GTPase-activating protein-promoted deactivation. *J Biol Chem* **271:** 7999–8007, 1996.
46. Slesinger PA, Reuveny E, Jan YN and Jan LY, Identification of structural elements involved in G protein gating of the GIRK1 potassium channel. *Neuron* **15:** 1145–1156, 1995.
47. Dufau ML, Baukal AJ, Ryan D and Catt KJ, Properties of detergent-solubilized adenylate cyclase and gonadotropin receptors of testis and ovary. *Mol Cell Endocrinol* **6:** 253–269, 1977.
48. Graeser D and Neubig RR, Compartmentation of receptors and guanine nucleotide-binding proteins in NG108-15 cells: Lack of cross-talk in agonist binding among the α_2-adrenergic, muscarinic, and opiate receptors. *Mol Pharmacol* **43:** 434–443, 1993.
49. Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G and Wittig B, Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature* **353:** 43–48, 1991.
50. Kleuss C, Scherübl H, Hescheler J, Schultz G and Wittig B,

Different β-subunits determine G-protein interaction with transmembrane receptors. *Nature* **358:** 424–426, 1992.
51. Kleuss C, Scherübl H, Hescheler J, Schultz G and Wittig B, Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. *Science* **259:** 832–834, 1993.
52. Goetzl EJ, Shames RS, Yang J, Birket FW, Liu YF, Albert PR and An S, Inhibition of human HL-60 cell responses to chemotactic factors by antisense messenger RNA depletion of G proteins. *J Biol Chem* **269:** 809–812, 1994.
53. Liu YF, Jakobs KH, Rasenick M and Albert PR, G protein specificity in receptor-effector coupling. *J Biol Chem* **269:** 13880–13886, 1994.
54. Tang T, Kiang JK, Côté TE and Cox BM, Antisense oligodeoxynucleotide to the G_{i2} protein α subunit sequence inhibits an opioid-induced increase in the intracellular free calcium concentration in ND8-47 neuroblastoma × dorsal root ganglion hybrid cells. *Mol Pharmacol* **48:** 189–193, 1995.
55. Macrez-Leprêtre N, Kalkbrenner F, Schultz G and Mironneau J, Distinct functions of G_q and G_{11} proteins in coupling α_1-adrenoceptors to Ca^{2+} release and Ca^{2+} entry in rat portal vein myocytes. *J Biol Chem* **272:** 5261–5268, 1997.
56. Hausdorff WP, Hnatowich M, O'Dowd BF, Caron MG and Lefkowitz RJ, A mutation of the β_2-adrenergic receptor impairs agonist activation of adenylyl cyclase without affecting high affinity agonist binding. Distinct molecular determinants of the receptor are involved in physical coupling to and functional activation of G_s. *J Biol Chem* **265:** 1388–1393, 1990.
57. Braun S and Levitzki A, Adenosine receptor permanently coupled to turkey erythrocyte adenylate cyclase. *Biochemistry* **18:** 2134–2138, 1979.
58. Chidiac P, Hebert TE, Valiquette M, Dennis M and Bouvier M, Inverse agonist activity of β-adrenergic antagonists. *Mol Pharmacol* **45:** 490–499, 1994.
59. Tang WJ, Stanzel M and Gilman AG, Truncation and alanine-scanning mutants of type I adenylyl cyclase. *Biochemistry* **34:** 14563–14572, 1995.