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COMMENTARY

G PROTEINS IN CARDIOVASCULAR FUNCTION AND DYSFUNCTION

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The heterotrimeric guanine nucleotide regulatory binding proteins (G proteins) are a family of homologous membrane proteins which are uniquely able to sort and amplify transmembrane signals initiated by agonist binding to cell surface receptors. The agonist specificity and selectivity of these G protein coupled receptors reside in the ligand recognition site at the extracellular domain of the protein, while the interaction with G proteins is via their cytoplasmic domain. Most evidence points to the C-terminus region and particularly the third intracytoplasmic loop as responsible for the coupling of receptors to G proteins [1–4]. The specific amino acid sequences of this area of the receptor, therefore, determine the specific G protein with which the particular receptor will interact. The activated G protein, in turn, interacts with effectors, such as ion channels, phospholipase A_2, phospholipase C, phospholipase D, cGMP phosphodiesterase or adenylyl cyclase to influence one or more cellular functions.

Numerous G protein-linked receptors are present in the cardiovascular system and are involved in regulating contractility of the heart and blood vessels in response to ligands be they drugs, neurotransmitters, neuromodulators or hormones. A comprehensive presentation of these receptors is beyond the scope of this discussion, but several excellent reviews are available dealing with the receptors and with what is known about their G protein linkages [5–8]. In general, there are still many receptor responses for which G proteins have not yet been assigned, and new G proteins are still being discovered. The challenge during the coming years will be to identify receptor-G protein-effector linkages in the heart and blood vessels, to establish their roles in regulating cardiovascular function, and to examine their plasticity.

G proteins are composed of three dissimilar protein units. The α subunit which can bind GTP in exchange for GDP also possesses GTPase activity. The numerous and distinct α subunits which are associated with the various G proteins are highly homologous proteins. The diversities of the β and γ subunits appear to be lower than that of the α subunit; however, the many possible combinations of the three subunits are staggering and each trimeric combination could interact with single or multiple receptors. Classically, effector activation has been attributed to the α subunits and many of the α subunits have established effector linkages. More recently, evidence indicates that $\beta\gamma$ subunits also regulate effectors. The β and γ subunits form a tightly bound lipophilic complex ($\beta\gamma$) which interacts with the α subunit to modulate its action. It has also been proposed that the $\beta\gamma$ dimer may mediate ligand-induced inhibition of adenylyl cyclase [9, 10], activation of phospholipase A_2 [11], and stimulation of K^+ channel activity [12]. The latter, however, has been disputed [13, 14]. The $\beta\gamma$ complex has also been proposed to mediate the interaction of G proteins with the cytoskeleton [15]. Furthermore, the dimer has been shown to facilitate the translocation of receptor-associated kinases to the cell membrane where they can promote the phosphorylation of muscarinic or β-adrenergic receptors [16, 17]. This sequence of molecular events has been proposed to mediate homologous desensitization [17].

Under resting conditions, the G protein is predominantly in its trimeric form with GDP bound to the α subunit. The agonist-occupied receptor initiates a cycle of G protein activation/inactivation which regulates effector function (Fig. 1). Receptor stimulation promotes a conformational change of the G protein and the binding of GTP to the α subunit. This reaction in which GTP exchanges for GDP at the α subunit is dependent on the presence of Mg^{2+}. In the process, the activated α subunit–GTP complex dissociates from the $\beta\gamma$ complex. The conformationally transformed free G_α-GTP can then interact with one or more effector systems, while the $\beta\gamma$ dimer may influence other effectors (see above). The activation of the G protein is terminated when the α subunit hydrolyzes the bound GTP and this is followed by the reassembly of α-GDP with the $\beta\gamma$ complex to form the inactive trimeric form of the protein. Recent data suggest that the deactivation of G protein-mediated signal transduction may, alternatively, be mediated via an inhibitory protein factor [18]. Furthermore, the hydrolysis of GTP by the α subunit has been shown to be facilitated markedly by interaction with its effector enzyme. This indicates the presence of a GTPase activating protein (GAP) for heterotrimeric G proteins in an intact membrane system [19]. It thus appears that the suggested activation/inactivation

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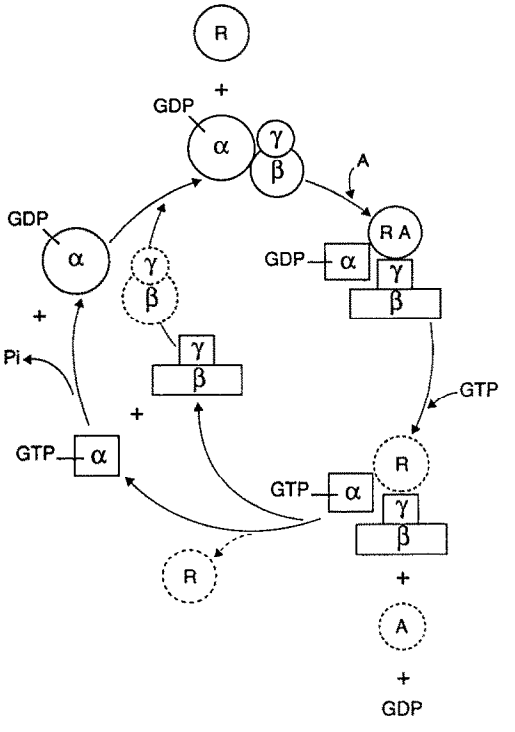

Fig. 1. Schematic representation of the G protein activation/inactivation cycle. G protein activation is initiated by the interaction of agonist (A) coupled receptor (R) with the membrane-associated G protein which stimulates the exchange of GTP for GDP at the α subunit and the dissociation of α-GTP from the $\beta\gamma$ complex, thus allowing each to interact with effector molecules. The termination of the cycle is accomplished by the hydrolysis of GTP by α subunit GTPase and the reassociation of α with the $\beta\gamma$ dimer to form the inactive trimer. α, β and γ designate the three subunits of the heterotrimer. Changes in subunit conformation are depicted by the change from circle to box. Transient or more speculative forms are represented by dashed lines.

cycle of G proteins (Fig. 1) [7], which is predominantly based on elegant studies using artificial reconstituted systems, reveals significant insights into the role of G proteins in transmembrane signal transduction. However, the apparent important influences of various membrane constituents upon this complex system may be even more intricate when the system is examined in biological membranes. Confirmation and refinements of the postulated cycle will have to await studies performed in these native membranes.

Alterations in the effector coupling efficiency of G protein linked receptors have been noted in the cardiovascular system. In some cases these can be ascribed to changes at the receptor level. Changes in either receptor number or covalent modification of the receptor may alter receptor function. Desensitization of G protein coupled receptors have been demonstrated to result from phosphorylation in the area of the third intracytoplasmic loop and/or the C-terminal tail of the receptor [20, 21], and the $\beta\gamma$ complex has been implicated in the regulation of receptor phosphorylation as discussed above. In addition, changes in receptor function may also result from direct phosphorylation of some G proteins or effectors. Evidence is also accumulating to indicate that changes in G protein levels can contribute to alteration in receptor coupling efficiency. To date most evidence suggests that modulation of G_α subunit levels is important in regulating/modulating the physiological consequences of receptor stimulation [22]. As our understanding of the G protein activation/inactivation cycle increases, it seems likely that additional factors will emerge as vital in determining receptor function.

G_s is present both in the heart and in blood vessels. It is positively coupled to adenylyl cyclase, and is thought to be responsible for the β-adrenergic receptor-stimulated increase in cAMP that is associated with increased rate and force of cardiac contraction and with vascular relaxation [23]. There is evidence that in addition to activating adenylyl cyclase which opens cardiac myocyte Ca^{2+} channels by a protein kinase A dependent mechanism, G_s can directly open Ca^{2+} channels independently from cytoplasmic second messenger regulated processes [24, 25]. Direct gating of cardiac Ca^{2+} channels by G_s remains a contentious area, with some investigators arguing that cAMP-dependent phosphorylation is the only signal transduction pathway activated by β-adrenergic receptors [26]. There is some evidence to suggest that G_s may gate Ca^{2+} channels in vascular smooth muscle. Activation of vascular G proteins using NaF or GTP analogs stimulates contraction that is at least partly attributable to Ca^{2+} influx [27, 28]. The contraction is not sensitive to pertussis toxin, suggesting that it is not due to activation of G_i or G_o [29]. Furthermore, the aortic α_1-adrenergic receptor, which stimulates Ca^{2+} influx and contraction but not adenylyl cyclase, appears to activate G_s [30]. G_s may have additional actions including mediation of β-adrenergic inhibition of Mg^{2+} uptake and β-adrenergic inhibition of Na^+ currents in cardiac myocytes [31, 32].

There are four forms of G_s which represent splice variants originating from a single gene [33, 34]. Multiple forms of G_s are frequently present in the same tissue, including the heart and blood vessels [6]. Recombinant versions of the different forms are essentially equivalent in their capacity to couple to adenylyl cyclase or Ca^{2+} channels [35–37], indicating that specificity probably does not reside in inherent differences in the efficiency of coupling to different effectors. Interestingly, recombinant G protein α subunits are substantially less active than their native counterparts [35]. Thus it is possible that native forms of the same α subunits may couple more efficiently to certain effectors even though recombinant subunits do not. Another possibility is that biological membranes may be "wired" in a way that links the different α subunit forms to discrete receptors and effectors. Alternatively, separate domains may exist in the membrane to mediate different functions. This concept is supported by immunolabeling studies which show that G proteins are not evenly distributed in the plasma membrane, but are present in clusters that may represent discrete

functional regions [38, 39]. It will be important to establish whether G proteins in intact biological membranes are present in discrete functional pools or whether they are homogenous. In the former case, important alterations in individual G protein pools could occur in the absence of substantial changes in total G proteins.

G_i is classically associated with inhibition of adenylyl cyclase in a variety of tissues, including the heart and blood vessels [40]. G_i mediates activation of K^+ channels in the heart in response to stimulation of m_2 cholinergic receptors [41]. Three closely related subtypes of $G_{i\alpha}$ proteins have been identified by cDNA cloning and shown to originate from separate genes [42, 43]. They are similar in size (40–41 kDa) and thus are not easily resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). They can, however, be distinguished using selective antibodies [44, 45].

G_o was originally identified in brain where it is present in relatively large quantities [46, 47]. It has also been detected by western blot analysis in the cardiovascular system, although in considerably lower abundance [45]. It is not known to what extent the presence of cardiac and vascular G_o is attributable to localization in peripheral neurons [48]. A variety of functions have been ascribed to G_o in different tissues including stimulation of phospholipase C, inhibition of neuronal Ca^{2+} channels, and stimulation of neuronal K^+ channels [49–51]. It is not known whether G_o couples to these effectors in the cardiovascular system, but there is some evidence for receptor-G_o coupling in the heart and vasculature. G_o copurifies with muscarinic receptors when solubilized atrial or ventricular membranes are stimulated with the cholinergic agonist carbachol and then immunoprecipitated using antibodies selective for the m_2 receptor [52]. Carbachol or serotonin stimulates GTPγS binding to G_o in aortic membranes which is detected by solubilization followed by immunoprecipitation using an antibody selective for G_o [30].

Pertussis toxin, which inactivates G_i and G_o, inhibits the effects of some vasoconstrictors, indicating that those receptors couple to G_i/G_o. For example, the α_2-adrenergic receptor-stimulated pressor response is blocked by pertussis toxin, indicating that these receptors rely upon G_i/G_o for contraction. The α_1 pressor response is right-shifted by pertussis toxin with no change in maximal response indicating that this receptor is coupled to both G_i/G_o and to a pertussis toxin-insensitive G protein [53, 54]. The α_1 agonist phenylephrine activates G_i and G_s in blood vessels suggesting that the pertussis toxin-insensitive component of α_1 receptor-stimulated contraction is mediated at least in part by G_s [30]. Other vasoconstrictor receptors also utilize pertussis toxin-sensitive G proteins. Pertussis toxin inhibits acetylcholine-stimulated and serotonin-stimulated arterial contraction as evidenced by right-shifted dose–response curves with reduced maximal contraction [55]. This appears to be due to pertussis toxin inhibition of both G_i and G_o since both are activated by carbachol and serotonin in the aorta [30].

While G_s, G_i and G_o have received the most attention to date, it is likely that additional G proteins, some as yet undiscovered, have important actions in the cardiovascular system. Several novel G_α subunits have recently been identified and shown to be present in the cardiovascular system. These are pertussis toxin-insensitive and thus are candidates for pertussis toxin-insensitive functions in the heart and blood vessels including activation of phospholipase C and contraction in response to certain agonists. They include G_q and the related G_{11} and G_{14} which can activate phospholipase C [56–58], G_{12} and G_{13} [59], and G_h [60]. However, direct demonstrations of receptor coupling to these G proteins in the cardiovascular system have not been reported.

It is becoming apparent that alterations in G proteins do occur during different physiological/pathophysiological states and probably contribute to altered responses associated with these conditions. Alterations in any of the signal transduction elements could contribute to dysfunctions, and it seems that multiple sites can be affected, including the G proteins. It will be important to identify these sites and to learn how they interact to produce an end result. Modulation of G proteins by changing their levels is one such alteration. At present there are several instances where such changes in G proteins have been shown, and some of these are discussed below. Further studies will undoubtedly produce additional examples.

While some studies have shown that modulation of G protein levels occurs, less is known about possible alterations in the functional activity of G proteins. Such studies are methodologically difficult, and the approaches that are currently available have limitations that need to be overcome. For example, reconstitution studies measure the ability of G proteins extracted from membranes to restore activity to preparations deficient in G proteins. There are a variety of concerns about extraction efficiency and about the loss of important interactions with other constituents of the biological membrane as discussed above. Measurements of receptor-stimulated increases in GTPase activity and in nucleotide binding to G proteins do assess G protein function in intact biological membranes, but do not provide a means to resolve the activities of individual G proteins. Toxins or selective antibodies can be used to probe the contribution of individual G proteins to the total GTPase activity or nucleotide binding response, but the level of resolution is not high because the G protein of interest is usually responsible for only a small percentage of the total response. It will be important to develop new ways to measure the function of individual G proteins in intact membranes. One such method combines agonist-stimulated nucleotide binding with selective immunoprecipitation of the individual G proteins [30, 61]. The advantage of this approach is that it measures nucleotide binding to individual G proteins when they are activated in intact membranes.

To date, congestive heart failure is probably the best characterized example of a condition in which a modulation of G protein function is associated with a cardiovascular system dysfunction. Cardiac responsiveness to β-adrenergic stimulation is im-

paired in experimental animals and in humans with congestive heart failure [62]. In some experimental models of heart failure, reduced β receptor number may contribute to the reduced responsiveness [63, 64], but receptor numbers are not decreased in all models of heart failure [65]. It is now apparent that there are alterations in the post-receptor elements that mediate β receptor responses, including alterations in the G proteins. Increased levels of the G_i have been found during heart failure in animals and in humans [66–68]. G_i is associated with inhibition of adenylyl cyclase, so increased G_i may function to counteract β receptor-G_s activation of adenylyl cyclase. It is not clear how this interaction could occur in isolated membranes which presumably lack agonists for G_i-coupled receptors, although there is some evidence that G_i couples constitutively to effectors [69]. Levels of G_s decline in some models of heart failure but not in others, and the available evidence suggest that G_s levels are unaltered in the failing human heart [65, 66, 70].

Several studies have attempted to determine whether G protein function is altered in heart failure. All are somewhat indirect due to limitations in the methodology that is currently available for investigating G protein function in biological membranes. Reduced adenylyl cyclase response to NaF and forskolin in cardiac membranes during heart failure indicates uncoupling at a site distal to the receptor because these agents are thought to directly activate G proteins and the G_s–adenylyl cyclase complex, respectively. In some models of heart failure, direct activation of the cyclase with Mn^{2+} is unaltered, suggesting that the blunted response to NaF and forskolin is due to reduced G protein function [71]. Other studies show reduced Mn^{2+} activation of adenylyl cyclase in heart failure, and in this case it is possible that the reduced response is attributable to the cyclase rather than the G proteins [68]. Reconstitution studies have attempted to assess more directly whether there may be alterations in G_s function in the failing heart even when G_s levels are unchanged. In these studies the ability of solubilized G_s to stimulate adenylyl cyclase in cyc^- membranes which lack endogenous G_s is examined. Alterations in G_s function have not been detected using this approach [68]. The currently available evidence strongly suggests that alterations in G proteins do occur during heart failure and this contributes to the reduced β receptor responsiveness that is characteristic of the disease. It is also clear that alterations occur at other sites in the signal transduction cascade, including the β-adrenergic receptor and the adenylyl cyclase. Determination of the relative contributions of these alterations, and of other as yet unknown factors, is likely to be an area of intensive investigation during the next several years. Immunological methods for quantitation of individual G proteins have provided a major advance by allowing measurement of G protein levels. New approaches will be needed in order to examine G protein function in intact membranes from the failed myocardium.

G proteins can be modulated in response to prolonged receptor stimulation by agonists. This has been shown in a number of organs and cell types, including cardiovascular tissue, and it is hypothesized that the changes in G proteins that occur during heart failure may be due to prolonged exposure to increased levels of norepinephrine, which does occur during heart failure [72, 73]. Chronic agonist exposure is more classically associated with receptor down-regulation and desensitization [74]. Desensitization of signal transduction elements distal to the receptor has not been widely examined, but this area is receiving increasing attention.

Prolonged treatment of cultured rat cardiac myocytes with norepinephrine results in desensitization of the adenylyl cyclase response to β receptor stimulation, to GTP, and to forskolin. Levels of G_i are increased in these cells, and the reduced response to β receptor stimulation is eliminated by pertussis toxin treatment, suggesting that increased G_i is responsible for the reduced response [75]. Increased mRNA may account for increased G_i because isoproterenol infusion in rats increases mRNA levels for G_{i2} and G_{i3} in the heart [76]. An increase in the amount of G_i appears to be a common adaptive response to stimulation of G_s-coupled receptors. Increased G_i has been detected immunologically or by pertussis toxin ribosylation in a variety of cell types in response to stimulation of several different G_s-coupled receptors or in response to stimulation with forskolin [77–79]. Prolonged stimulation of G_i-coupled receptors can decrease G_i. This has been shown in HT29 cells and adipocytes in response to prolonged stimulation with α_2-adrenergic agonists or A_1 adenosine agonists, respectively [78, 80, 81].

Levels of G_s do not appear to decrease as readily in the heart. Prolonged stimulation with isoproterenol does not alter the amount of G_s in cardiac myocytes or in intact heart [75, 76]. Similarly, surgical ablation of the adrenergic or cholinergic innervation of the myocardium results in increased levels of G_i with no change in G_s [82, 83]. The functional significance of increased cardiac G_i in response to both increased and decreased adrenergic stimulation is not known. It will be important to determine whether all regions of the heart and all forms of G_i are affected similarly. It is possible that stimulation of other G_s-coupled receptors may have more pronounced effects on cardiac G_s levels. G_s level does diminish in response to prolonged exposure to A_1 adenosine agonists and prostaglandin E_1 in adipocytes and NG108-15 cells, respectively [80, 84], so G_s is capable of exhibiting this type of plasticity. It is also possible that functional desensitization of G_s may occur in the absence of altered protein concentration.

Prolonged exposure of blood vessels to high levels of norepinephrine results in desensitization of the α_1 receptor-mediated contractile response [30, 85, 86]. The decreased response is not due to α_1 receptor down-regulation because receptor number and affinity do not change [87]. Decreased contraction is accompanied by decreases in α_1 receptor-stimulated Ca^{2+} efflux and phosphatidylinositol turnover [87, 88]. Desensitization appears to be due to impaired coupling between α_1 receptors and G proteins, as evidenced by a reduction in α_1 receptor-stimulated GTPγS binding to G_i and G_s [30]. Additionally, the concentration–response curve for

NaF-stimulated contraction is right-shifted in these vessels, indicating an alteration in the G proteins [30]. It is not yet known whether the impaired receptor-G protein coupling in these desensitized vessels is secondary to alterations in G protein levels. Alternatively, the receptors and/or the G proteins may be modified in ways that affect function but not levels.

During aging there is a reduction in β-adrenergic responsiveness in the heart and blood vessels. This is evidenced by reduced inotropic and chronotropic responses to β receptor stimulation in the heart and by reduced β receptor-stimulated vascular relaxation [89–91]. Cardiac and vascular β receptor numbers decrease little if at all in the heart and blood vessels during aging, so receptor down-regulation cannot account for the decreased responses [90, 92]. Accumulation of cAMP in response to β receptor agonists is decreased during aging in the heart and in blood vessels, indicating that the site of the alteration is in close proximity to the receptor [92, 93]. There is some evidence for reductions in adenylyl cyclase activity and in responsiveness to cAMP which may help to account for reduced β receptor responsiveness in the heart [94, 95]. The reduced responsiveness is probably not attributable solely to alterations in adenylyl cyclase or cAMP responses. Complementation studies demonstrate a reduced ability of G proteins extracted from senescent heart to restore adenylyl cyclase responses in cyc^- membranes, suggesting that alterations at the G protein level contribute to the loss of β receptor responses during aging [96]. Furthermore, there is an age-related decrease in cardiac β receptor affinity for agonists and a reduction in the ability of guanine nucleotides to affect agonist affinity, which may be due to reduced receptor–G protein interactions [97, 98]. Forskolin and dibutyryl cAMP mediated relaxation is unaltered in the aging aorta, indicating that the decline in β receptor-mediated relaxation is due to an uncoupling between the receptor and the cyclase [99]. These studies strongly suggest that altered G protein function may contribute to the reduction during aging in cardiac and vascular reactivity to β-adrenergic agonists. It will be important to measure G protein levels in the aging cardiovascular system and to more directly assess G protein function.

Hypertension is associated with increased peripheral resistance which is thought to be due in part to structural changes in the vasculature [100]. Additionally, there have been numerous reports of abnormal responses to vasoconstrictor and vasodilator agents during hypertension, suggesting that altered vascular reactivity may also contribute to the increased resistance [101]. Changes in receptor number and affinity have been observed in vascular tissue from hypertensive animals; however, these changes are generally of insufficient magnitude to account for the alterations in contractile responses [102]. There is increasing evidence for enhanced second messenger formation in vessels from hypertensive animals in response to stimulation of vasoconstrictor receptors, suggesting that signal transduction processes may be altered [103–105]. It is not known whether alterations in G proteins contribute to the increased vascular reactivity during hypertension, but this is likely to be an active area of research in the next several years.

Evidence in support of a role for G proteins in cardiovascular regulation, pharmacology and dysfunction is rapidly increasing. Continued work in this area promises to yield significant new information to expand our understanding of the cardiovascular system in health and disease and to provide new therapeutic modalities in treating cardiovascular illnesses.

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