Roles of Accessory Proteins for Heterotrimeric G-Protein in the Development of Cardiovascular Diseases

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Signal processing via heterotrimeric G-proteins is one of the most widely used systems for signal transfer across the cell membrane. This signaling system regulates most physiological and pathophysiological processes in mammals and is therefore the primary target of many pharmaceutical agents. The heterotrimeric G-protein signaling system includes the G-protein-coupled receptor (GPCR), heterotrimeric G-proteins, and effectors. The G-proteins are activated by the GPCR to mediate a signal to effector molecules. However, other players in this system that regulate the activation status of heterotrimeric G-proteins independently of GPCR have been identified. Such accessory proteins for heterotrimeric G-protein can provide additional signal input to the G-protein signaling system, or may act as alternative binding partners of G-protein subunits serving as yet unknown roles in cells. It has been reported that this class of proteins is expressed in the cardiovascular system and contributes to signal integration involved in the various diseases. This review provides an overview of the current understanding of accessory proteins for heterotrimeric G-proteins in their 4 functional subsets, including guanine nucleotide exchange factors (GEFs), guanine nucleotide dissociation inhibitors (GDIs), GTPase-activating proteins (GAPs), and Gβγ-interacting proteins, and discusses their roles in the development of cardiovascular diseases. Better understanding of these components may contribute insight into the complex network of molecules governing GPCR signaling in the cardiovascular system. (Circ J. 2013;77: 2455–2461)

Key Words: Activator of G-protein signaling (AGS); Cardiovascular disease; Heterotrimeric G-protein; Regulator of G-protein signaling (RGS); Signal transduction
Heterotrimeric G-protein signaling system includes traditional components, such as the GPCR, heterotrimeric G-proteins, and effectors. GPCRs are required to "activate" heterotrimeric G-proteins to mediate transduction of their signals into the cell. That is to say, drugs that interact with GPCRs control the activation status of heterotrimeric G-proteins, a key step in signal transduction by this system.

**Novel Class of Accessory Proteins: Regulation of G-Protein Signaling by Receptor-Independent Pathways**

Activation and Deactivation of Heterotrimeric G-Proteins

Activated GPCRs induce a conformational change in the Gα subunit and cause GDP release from Gα (Figure). Binding of GTP to Gα destabilizes the Gaβγ complex, leading to a structural rearrangement of Ga-GTP, Gaβγ, and the receptor. Thus, Ga-GTP dissociates from the receptor and Gaβγ. Both subunits, Ga-GTP and Gaβγ, stimulate distinct downstream effector molecules, such as adenyl cyclases, phospholipases, ion channels, and protein kinases. This activation of the downstream pathway is terminated when Ga hydrolyzes GTP to GDP through its intrinsic guanosine triphosphatase (GTPase) activity. Thereafter, Ga-GDP re-associates with Gaβγ, which terminates the interactions between G-protein subunits and other effector molecules.

G-Protein Subunits and Linked Signaling Pathways

Heterotrimeric G-proteins are composed of 3 protein subunits: α, β, and γ. The combination of these subunits is a determinant of the downstream pathway evoked by receptor stimulation. There are 15 Gα subunits comprising 4 major subclasses, including Gα1, Gα16, Gα12, and Gα13.14 The 5 Gβ and 12 Gγ subunits are tightly associated and act as a functional unit. The coupling of GPCRs to these different combinations of subunits generates the diversity characteristic of intracellular signaling.

For example, the Gα subunit stimulates adenyl cyclase, which leads to the formation of cAMP and subsequent phosphorylation of intracellular targets by protein kinase A. Gαi/o subunits, on the other hand, inhibit adenyl cyclase and reduce the concentration of cAMP; however, they also activate phosphoinositide 3-kinase. Gαi activates phospholipase C (PLC) leading to the formation of inositol 1,4,5-trisphosphate and diacylglycerol, promoting Ca2+ release from intracellular stores and activation of protein kinase C.15 Gα12/13 has been shown to regulate small G-protein Rho via RhoGEF.16 Gaβγ also interacts with its own target molecules, including the G-protein-coupled inwardly-rectifying K+ channel (GIRK), G-protein-coupled receptor kinase (GRK), PLCβ, and adenyl cyclases.3,12

Signals evoked in these pathways are regulated to optimize signal specificity and maximize signal efficiency, and integrate diverse stimuli through modification of molecules by processes such as subcellular localization, phosphorylation, and lipid modification.

**Discovery of Receptor-Independent Accessory Proteins for Heterotrimeric G-Protein**

Other players in this signaling system, which regulate the activation status of heterotrimeric G-proteins independently of the receptor, have been reported. Collaborators and I previously identified such accessory proteins and characterized their effects in biochemical17,18 and signaling systems.18-21 We also examined the role of accessory proteins in the context of signal adaptation of the GPCR system.22 We subsequently focused on identifying accessory proteins for G-proteins that are induced in the myocardium exposed to repetitive transient ischemia23 as well as pressure overload.24 These accessory proteins influence the activation or deactivation of the Gα subunit or form complexes with Gα or Gaβγ distinct from the typical Gαβγ heterotrimer. Accumulating data suggest that accessory proteins for G-proteins provide additional signal input to the G-protein signaling system in the absence of GPCRs or act as alternative binding partners of G-protein subunits serving roles yet to be determined.25,26
Types of Accessory Proteins for Heterotrimeric G-Protein

Accessory proteins for heterotrimeric G-proteins fall into 4 classes according to their action on G-protein subunits.

(1) GEFs for the Ga Subunit This class of accessory proteins stimulates the guanine nucleotide exchange of Ga subunits, such as activated GPCRs, including the activator of G-protein signaling 1 (AGS1),\(^{27–30}\) presenilin 1,\(^{31}\) and PBP/RKIP.\(^{32}\) Of the 4 Ga family members,\(^{14,33}\) GEF proteins preferentially activate the Ga\(_\alpha\) family.

(2) GDIs for the Ga Subunit The majority of this group of accessory proteins share a common structural domain termed the G-protein regulatory (GPR) or GoLoco motif.\(^{35,36}\) The GPR or GoLoco motif is a 20- to 25-amino acid cassette that interacts with Ga\(_{\alpha}\) and transducin (Ga\(_\beta\gamma\)) in the retina.\(^{35,36}\) The interaction of the GPR or GoLoco motif stabilizes the GDP-bound conformation of Ga, which results in inhibition of Ga-mediated signaling.\(^{35,36}\) The GPR or GoLoco motif is found in signaling molecules, including AGS3 (GPSM1), LGN (GPSM2), AGS4 (GPSM3), and WAVE-1, and the GTPase-activating proteins RGS12, RGS14, and Rap1GAP.\(^{35,36}\)

(3) GAPs for the Ga Subunit The proteins of this group interact with the \(\alpha\)-subunit and catalyze GTPase activity.\(^{37}\) The majority of proteins in this group have 120–130 amino acids of the regulator of G-protein signaling (RGS) homology domain. More than 20 mammalian RGS isoforms are expressed in the cardiovascular system.\(^{38}\) Most of the RGS proteins are GAPs for Ga\(_{\alpha}\), but some also have activity toward the Ga\(_{\alpha11},\text{Ga}_{12/13},\) or Ga\(_\alpha\) family members.\(^{39,40}\) To examine the physiological effect of RGS proteins on a particular Ga, knock-in mice expressing RGS-insensitive mutations of the Ga subunits have been characterized.\(^{41,42}\)

(4) Accessory Proteins Interacting With Subunits of G\(\beta\gamma\)

The proteins of this group interact with G\(\beta\gamma\) and cause signaling events independently of GPCR. G\(\beta\gamma\)\(^{13}\) regulates various effector molecules, including GIRK, Ca\(^{2+}\) channels, G-protein-coupled receptor kinase 2 (GRK2), PLC\(\beta\), PLC\(\beta3\), and PLC\(\epsilon\).\(^{3,13}\) It has been shown that effector proteins and the Ga subunit share an overlapping interface on the surface of G\(\beta\gamma\).\(^{13}\) Because G\(\beta\gamma\)-interacting proteins also share a common region on the surface, they would mask and inhibit the G\(\beta\gamma\) signal to other molecules, as does Ga. Thus, the binding of G\(\beta\gamma\)-interacting proteins to this shared site is principally expected to exclude the interactions of other proteins, resulting in the shutdown of G\(\beta\gamma\) signaling. Recently, small molecules targeting the shared interface of G\(\beta\gamma\) have been developed that selectively alter the G\(\beta\gamma\) signaling pathway in cells.\(^{43}\)

Alternative G\(\beta\gamma\)-mediated G-protein activation has been reported.\(^{44,45}\) Nucleotide diphosphate kinase B forms a complex with G\(\beta\gamma\)\(^{44,45}\) and phosphorylates G\(\beta\) at His-266.\(^{44}\) Subsequently, this high-energy phosphate can be transferred to GDP.\(^{44}\) The GTP thus formed induces G-protein activation independently of receptors, as was demonstrated in Ga-mediated cAMP formation in cardiomyocytes,\(^{46}\) mouse embryonic fibroblasts, and the embryonic heart of zebrafish.\(^{46,47}\)
Involvement of Accessory Proteins for Heterotrimeric G-Protein in the Development of Cardiovascular Disease

The accessory proteins for heterotrimeric G-protein involved in the development of cardiovascular disease are shown in the Table. In the following sections, representative proteins are discussed in relation to heart failure, cardiac hypertrophy, ischemic injury, hypertension, and heart rate/arrhythmia.

Heat Failure

AGS1 (DexRas1, RASD1) AGS1, which was initially discovered as dexamethasone-inducible, ras-related cDNA (DexRas), is a direct activator of $G_{aq}$ subunits.27–30 Recently, McGrath et al demonstrated a significant downregulation of AGS1 in the heart after volume overload induced by aortocaval shunt and described its involvement in secretion of atrial natriuretic factor.31 The importance of the biological role of AGS1 on the cardiac endocrine system was emphasized by these results.

RGS4 Increased expression of RGS4 was reported in human hearts in end-stage failure.49–51 RGS4 has GAP activity for $G_{aq}$ and $G_{q}$ and appears to negatively regulate signaling events mediated by $G_{aq}$ subunits. In an animal model, the overexpression of RGS4 did not affect basal cardiac function or the chronotropic response to dobutamine but it significantly reduced the ability of the heart to adapt to an increased afterload induced by transverse aortic constriction.52 The co-overexpression of RGS4 and $G_{aq}$ in transgenic mice delayed the $G_{aq}$-mediated onset of cardiac hypertrophy, the phenotype of which is similar to that of human cardiac hypertrophy.53 The increase in RGS4 in the failing heart may decrease the contractile response; however, this increase reduces the adverse effect of $G_{aq}$ signaling.

Contrasting with these observations, the very low expression of RGS4 in the heart muscle was suggested as a pharmacological target by the study of $RGS4^{−/−}$ mice.54 Subsequently, Cifelli C et al demonstrated higher levels of expression RGS4 in the sinoatrial node compared with the right atrium, suggesting an important role of RGS4 in parasympathetic signaling in the heart, which may influence the prognosis of heart failure.55 Although the overall trend toward protection of the heart by RGS4 remains controversial, the alterations in its expression suggested the potential to alter cardiovascular pathophysiology.

Presenilin-1 The presenilin-1 gene (PSEN1) mutation is responsible for the development of early-onset familial Alzheimer’s disease.56 The C-terminal amino acids of presenilin-1 were shown to regulate activation of the $G_{aq}$ subunit.31 Presenilin-1 is critical for cardiac development,57 and its nonsense mutation is associated with dilated cardiomyopathy.58 It was demonstrated that changes in presenilin levels lead to cardiac dysfunction, owing to aberrant Ca$^{2+}$ channel receptor activities shown in a transgenic Drosophila model of presenilins.59 Gianni et al indicated that protein aggregation and sequence variants in the presenilin-1 gene promoter led to reduced gene and presenilin-1 expression.60

Cardiac Hypertrophy

RGS2 RGS2 has unique selectivity for $G_{q}$ rather than $G_{aq}$.61 It was found that RGS2 is selectively downregulated during the early onset of cardiac hypertrophy with enhanced $G_{aq}$ signaling.62 SiRNA-mediated RGS2 knockdown increased phenylephrine- and endothelin-1-induced PLC$\beta$ stimulation and exacerbated the hypertrophic effect.63 It is interesting that no overt changes were observed in the cardiac function of RGS2-deficient mice up to 6 months of age,64 however, knockdown of RGS2 increased Gaq signaling-induced hypertrophy in response to pressure overload with more rapid transition to failure and early mortality.65 It was reported that RGS2 is functionally important and a highly controlled negative regulator of angiotensin II-induced signaling, cell proliferation, and collagen in adult ventricular fibrosis.65 Although cardiomyocyte-specific RGS2 overexpression in transgenic mice in vivo did not lead to attenuation of cardiac hypertrophy induced by pressure overload,66 evidence suggests a beneficial effect of RGS2 in protecting the heart.

RGS5 A role for RGS5 in protecting against cardiac hypertrophy in response to pressure overload was revealed in mice with cardiac-specific transgenic overexpression or global deletion of RGS5.67 Cardiac-specific overexpression of RGS5 attenuated cardiac hypertrophy and interstitial fibrosis, whereas RGS5-deficient mice displayed the opposite phenotype in response to pressure overload, indicating that RGS5 is a crucial component of the signaling pathway involved in cardiac remodeling.67

TFE3 (AGS11) TFE3 (AGS11) was identified as a novel activator of G-protein signaling (AGS) in the hypertrophied mouse heart induced by transverse aortic constriction.34 Although TFE3 is a well-known nuclear transcription factor, it selectively forms a complex with $G_{aq}$ but not $G_{aq}$, $G_{q}$, or $G_{q}$, $G_{16}$ is enriched in hematopoietic tissue; however, it is also expressed in the heart68,69 and induced in response to pressure overload.24 Interestingly, TFE3 translocates $G_{aq}$ to the nucleus in association with increased expression of claudin-14, a key component of membrane structure in cardiomyocytes. Upon pressure overload stress, claudin-14 increases with accompanying upregulation of TFE3 and $G_{aq}$ in the heart. TFE3 transcription factor is an AGS for $G_{aq}$ that appears to form a TFE3-G$G_{aq}$ complex in the nucleus that drives the transcription of claudin-14, suggesting the existence of a novel mechanism of transcriptional regulation by the Ga subunit with a specific AGS protein in response to pathophysiological stress. Induction of claudin-14 is observed in the left ventricle of the hypertrophied heart and is postulated to be a part of the cardiac adaptation response to mechanical stress by pressure overload.

Ischemia-/Hypoxia-Mediated Injury

AGS8 Ischemic injury of the heart is associated with activation of multiple signal transduction systems including the heterotrimeric G-protein system. AG8 was identified as an ischemia-inducible regulator of the $G_{q}$ subunit from a cDNA library of rat hearts subjected to repetitive transient ischemia with the development of collateral vessels.23,70 Suppression of AGS8 blocks hypoxia-induced apoptosis of cardiomyocytes, suggesting that AGS8 is required for hypoxic stress-induced cell death.71 AGS8 forms complexes with the channel protein connxin 43 (CX43) and regulates its phosphorylation in a G$G_{q}$-dependent manner. Interestingly, AGS8 influences internalization of CX43 induced by repetitive hypoxia as well as the level of cell-surface CX43. These data indicate that AGS8 is required for hypoxia-induced apoptosis of neonatal cardiomyocytes, and that AGS8-G$G_{q}$ alters cellular environments to become more sensitive to hypoxic stress by influencing the permeability of small molecules through CX43.

RGS Proteins

The protective role of RGS proteins was suggested in knockout mice expressing RGS-insensitive mutants of the Ga subunit.49 In an ischemia-reperfusion study with Langendorff-perfused hearts, the infarcted area was smaller and mechanical
recovery was enhanced in the knock-in mouse expressing RGS-insensitive Gα, suggesting a role in Gα-mediated cardioprotection and its negative inhibitor RGS proteins.72

Hypertension
RGS2  RGS2-deficient mice are hypertensive and have altered GPCR signaling in a number of tissues.63 RGS2 is suggested to be the most potent negative regulator of Gα, signaling.64 In addition, it has been suggested that RGS2 is involved in the signaling pathway of nitric oxide (NO)-mediated vasodilation.73–75 Genetic variations in RGS2 have been observed in Japanese individuals with hypertension or normotension, with an insertion/deletion variant at 1891–1892 in the non-coding region of the gene present in patients with hypertension.76 The importance of this insertion/deletion variant was confirmed by another group,77 who demonstrated that this variant was present in black patients with hypertension.

RGS5  RGS5-deficient mice exhibit a phenotype of persistent low blood pressure.78,79 This was shown to be accompanied by increased blood vessel diameter but no change in vessel wall thickness.78 However, it was recently suggested that RGS5 expression is downregulated with chronically elevated blood pressure in rats.80 A knockout mouse model showed that loss of RGS5 results in hypertension.81 Loss of RGS5 signaling also correlates with hyperresponsiveness to vasoconstrictors and vascular stiffening.82 Although the overall direction of blood pressure change induced by RGS5 is controversial, a polymorphism in RGS5 associated with hypertension was reported in an African-American population,83 suggesting the importance of RGS5 in human hypertension.

Heart Rate/Arrhythmia
RGS Proteins  Heart rate is regulated by sympathetic and parasympathetic inputs mediated by adrenergic and muscarinic receptors. Activation of signaling pathways involving Gα acts to slow the heart rate via modulation of ion channels. Because mice overexpressing RGS-insensitive Gα were tachycardic during the daytime, RGS protein was thought to be important in the Gα-mediated parasympathetic control of heart rate.41

RGS4 and RGS6 are highly expressed in the sinoatrial node (SAN)85,86 and atrioventricular nodes.87 Although both RGS proteins can negatively regulate Gαs subunits, only RGS6 has the capacity to form a complex that appears to contribute to the inactivation of the inwardly-rectifying potassium channel (IKACH).83 Both RGS4- and RGS6-null mice show an increased bradycardic response to carbachol, suggesting that both these RGS proteins are important for parasympathetic tone in the SAN to prevent severe bradycardia.55,83

In the atria, there is limited information regarding which RGSs might be important.84 RGS10 mediates β-adrenergic receptor effects on the GIRK current in rat atrial cells.85 Inducible atrial flutter has been shown in the RGS2 knockout mouse.86 RGS5 is also reported to be an important regulator of arrhythmogenesis in the mouse atrium. RGS5-deficient mice display enhanced susceptibility to atrial tachyarrhythmia that was associated with repolarization abnormalities.87

As noted before, the role of RGS proteins in mediating parasympathetic tone to cardiomyocytes has been established; however, their role in autonomic traffic via the central autonomic system requires further investigation.

Conclusion and Perspectives
Recent findings indicate the involvement of accessory proteins for heterotrimeric G-protein in the development of cardiovascular disease. The effect of these proteins has become clear and is compelling. Although heterotrimeric G-protein is a general molecular switch in signaling systems, accessory proteins regulate unique pathways mediated by G-protein in cell. As discussed, some accessory proteins for heterotrimeric G-protein are essential in the regulation of the cardiovascular system. However the roles of these proteins were not recognized until their discovery as accessory proteins for heterotrimeric G-protein. Discovery of additional novel accessory proteins for G-proteins and elucidation of their precise functions in signaling systems are necessary for further understanding of signal integration in the cardiovascular system.

In contrast to the accumulated data supporting their roles in animal models, the relationships of accessory proteins to human disease have been only partly demonstrated so far. For example, roles for RGS gene polymorphisms have been reported in hypertension,86,78,79 schizophrenia,80 and cancer.89 The discovery of additional novel accessory proteins has the potential to reveal unexpected regulatory pathways that may contribute to the development of novel therapeutic approaches for human diseases.

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