Chapter 1
Implication of G-proteins in Cardiovascular Disease

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Abstract Guanine nucleotide regulatory proteins (G-proteins) play a key role in the regulation of various signal transduction systems including adenylyl cyclase/cAMP and phospholipase C (PLC)/phosphatidylinositol turnover (PI) which are implicated in the modulation of a variety of physiological functions such as platelet functions, including platelet aggregation, secretion, and clot formation, and cardiovascular functions, including arterial tone and reactivity. Several abnormalities in adenylyl cyclase activity, cAMP levels, G-proteins, and PLC/PKC have been shown to be responsible for the altered cardiac performance and vascular functions observed in cardiovascular disease states. The enhanced or unaltered levels of inhibitory G-proteins (Giα-2 and Giα-3) and mRNA have been reported in different models of hypertension, whereas Gsα levels were shown to be unaltered. These changes in G-protein were associated with functions. The enhanced levels of Giα proteins precede the development of blood pressure and suggest that overexpression of Gi proteins may be one of the contributing factors for the pathogenesis of hypertension. The augmented levels of Giα proteins and associated adenylyl cyclase signaling in hypertension were shown to be attributed to the enhanced levels of vasoactive peptides. In addition, enhanced oxidative stress in hypertension may also be responsible for the enhanced expression of Giα proteins observed in hypertension. The levels of Gqα/11 and PLCβ have been shown to be upregulated in different models of hypertension. On the other hand, the levels of Gsα and not of Giα proteins were decreased in volume- or pressure-overload hypertrophy. The responsiveness of adenylyl cyclase to β-adrenergic agonists was also attenuated. In addition, the levels of Gqα were augmented in hypertrophy and the β-adrenergic receptor levels were decreased. Furthermore, the role of PKC in the development and progression of cardiac hypertrophy was also shown. Similarly, ischemia was shown to be associated with decreased, increased, or unaltered levels of Gsα, with decreased levels of Giα, and with decreased responsiveness of adenylyl cyclase to various stimuli such as β-adrenergic agonists, guanine nucleotides, forskolin, and others. Thus, the altered levels of G-proteins and associated signaling may be responsible for the impaired cardiovascular functions observed in hypertension, hypertrophy, and cardiac failure.
Introduction

Guanine nucleotide regulatory proteins (G-proteins) are a family of guanosine triphosphate (GTP) binding proteins that play a key regulatory role as transducers in a variety of signal transduction systems. These include the adenylyl cyclase/cAMP system (Rodbell et al. 1971), the receptor-mediated activation of phospholipase C and A2 (Cockroft and Gompert 1985; Litosch et al. 1985), and a number of hormone and neurotransmitter-regulated ionic channels (Breiwieser and Szabo 1985; Pfaffinger et al. 1985). G-proteins are heterotrimeric proteins composed of three distinct subunits; $\alpha$, $\beta$, and $\gamma$ (Gilman 1984). The $\alpha$-subunits bind and hydrolyze GTP and confer specificity in receptor and effector interactions (Gilman 1984). The GDP-bound form of $\alpha$ binds tightly to $\beta\gamma$ and is inactive, whereas the GTP-bound form of $\alpha$ dissociates from $\beta\gamma$ and serves as a regulator of effector proteins. All $\alpha$-subunits possess intrinsic GTPase activity and hydrolyze the terminal phosphate of bound GTP to yield bound GDP and free inorganic phosphate ($P_i$). Upon hormone binding and receptor activation, the receptor interacts with the heterotrimeric protein to promote a conformational change and dissociation of bound GDP from the guanine nucleotide binding site. GDP is released and replaced by GTP. Binding of GTP to $\alpha$ induces a conformational change and promotes the dissociation of hormone receptor complex (HR) and the holo G-protein into $\alpha$ and $\beta\gamma$. Both $\alpha$-GDP and $\beta\gamma$-subunits can interact with effectors. This activation cycle is terminated by intrinsic GTPase activity of $\alpha$-subunit. The GDP-bound form of $\alpha$-subunit has high affinity for $\beta\gamma$ and then reassociates with the $\beta\gamma$ dimer to form the heterotrimer in the basal resting state. The family of G-protein $\alpha$-subunits can be subclassified according to functional or structural relationship. More than 20 mammalian G$\alpha$ gene products and several alternatively spliced isoforms have been identified. These can be divided into four major subfamilies according to amino acid homology and are represented by Gs$\alpha$, Gi$\alpha$, Gq$\alpha$/$\alpha_{11}$, and $\alpha_{12}/\alpha_{13}$. The G-proteins Gs$\alpha$ and Gi$\alpha$ are implicated in the regulation of adenylyl cyclase/cAMP signal transduction system.

The hormone-sensitive adenylyl cyclase system is composed of three components: the receptor, the catalytic subunit, and G-proteins—stimulatory (Gs) and inhibitory (Gi). Molecular cloning has revealed four different forms of Gs$\alpha$ having molecular weights of 42, 45, 47 and 52 kDa resulting from the different splicing of one gene (Bray et al. 1986; Robishaw et al. 1986; Murakami and Yasuda 1988). Gs$\alpha$ is positively coupled to adenylyl cyclase and mediates the stimulatory responses of hormones on adenylyl cyclase (Stryer and Bourne 1986; Spiegel 1987). The Gs-mediated activation of adenylyl cyclase results in the increased formation of cAMP. cAMP activates cAMP-dependent protein kinase $\alpha$ that induces the phosphorylation of contractile filaments, sarcolemmal and sarcoplasmic proteins, and regulates intracellular calcium homeostasis (Wankerl and Schwartz 1995). In addition, Gs$\alpha$ was also shown to open the Ca$^{2+}$ channels directly by a cAMP-independent mechanism (Yatani and Brown 1989). In contrast, Gi$\alpha$ protein is associated with adenylyl cyclase inhibition (Stryer and Bourne 1986; Spiegel 1987). Three distinct forms of Gi$\alpha$, namely, Gi$\alpha$-1, Gi$\alpha$-2, and Gi$\alpha$3, have been cloned and encoded by three distinct genes (Itoh et al. 1986, 1988 Jones and Reed 1987). All