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Review

Regulation of Giα Protein Expression by Vasoactive Peptides in Hypertension: Molecular Mechanisms

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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)/phosphatidyl inositol (PI) turnover, 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 and G proteins 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) and mRNA have been reported in different models of hypertension, whereas Gsα levels are shown to be unaltered. 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 levels of vasoactive peptides including ET-1 and Ang II and growth factors are augmented in hypertension and contribute to the enhanced expression of Giα proteins in hypertension. In addition, oxidative stress due to enhanced levels of Ang II and ET-1 is enhanced in hypertension and may also be responsible for the enhanced expression of Giα proteins observed in hypertension. Furthermore, Ang II- and ET-1-induced transactivation of growth factor receptor through the activation of MAP kinase signaling is also shown to contribute to the augmented levels of Giα in hypertension. Thus, it appears that the enhanced levels of vasoactive peptides by increasing oxidative stress and transactivation growth factor receptors enhance MAP kinase activity that contribute to the enhanced expression of Giα proteins responsible for the pathogenesis of hypertension. In this review, we describe the role of vasoactive peptides and the signaling mechanisms responsible for the enhanced expression of Giα proteins in hypertension.

Keywords: G-Proteins, Adenylyl cyclise, Vasoactive peptides, Growth factor receptors, Oxidative stress, MAP kinase, Hypertension.

Introduction

Guanine nucleotide regulatory proteins (G-proteins) are a family of GTP-binding proteins which regulate a variety of physiological functions, including vascular tone and reactivity, cardiac contractility, proliferation etc. through the activation of a variety of signal transduction systems. These include adenylyl cyclase/cAMP system[1], receptor-mediated activation of phospholipase C and A2[2,3] and a number of hormone- and neurotransmitter-regulated ionic channels[4]. The adenylyl cyclase activity is regulated by two G-proteins; stimulatory (Gs) and inhibitory (Gi) which mediate the stimulatory and inhibitory responses of hormones on adenylyl cyclase respectively[5-7]. G proteins are heterotrimeric proteins composed of α, β and γ subunits[7]. Molecular cloning has revealed four different forms of Gsα, resulting from the differential splicing of one gene[8-10] and three distinct forms of Giα: Giα-1, Giα-2 and Giα-3 encoded by three distinct genes[11-13]. All three forms of Giα (Giα1-3) have been shown to be implicated in adenylyl cyclase inhibition[14] and activation of atrial Ach-K+ channels[15]. The expression of Giα proteins and Gi-mediated inhibition of adenylyl cyclase is increased in various models of hypertension[16-23] .In addition, the levels of several vasoactive peptides, including angiotensin II (Ang II) and endothelin-1 (ET-1) are also increased in hypertension[24-27] which may be the contributing factors in the augmentation of Giα proteins in hypertension. In this review, we describe the role of vasoactive peptides and the signaling mechanisms responsible for the enhanced expression of Giα proteins in hypertension.

G proteins and membrane signaling in hypertension

Altered expression of G proteins and functions has been reported in several pathophysiological conditions, such as hypertrophy, heart failure,

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hypertension, etc[28-36]. We have demonstrated that the expression and functions of Giα-2 and Giα-3 proteins and their genes, but not Goα are enhanced in hearts and aorta from SHR, DOCA-salt, L-NAME-induced and 1 kidney-1clip (1K1C) hypertensive rats (HR)[16-23] and in vascular smooth muscle cells (VSMC) and lymphocytes from 12-week old SHR[22, 37], whereas Gsα protein and its gene are not altered in SHR and is decreased in DOCA-salt HR[16-18,23]. The enhanced expression of Giα-2 and Giα-3 proteins and their mRNA in heart and aorta precedes the development of blood pressure in SHR and DOCA-salt hypertensive rats[38,39]. In addition, the inactivation of Giα proteins in prehypertensive rats (2 week-old SHR) by single injection of pertussis toxin prevents the development of blood pressure[40]. These results suggest that enhanced levels of Giα-2 and Giα-3 proteins and resultant decreased levels of cAMP may be one of the contributing factors for the pathogenesis of hypertension. The levels of vasoactive peptides, such as Ang II, ET-1 and arginine vasopressin (AVP) as well as growth factors that are augmented in various models of hypertension[24-27] may be responsible for the enhanced expression of Giα proteins in hypertension.

Vasoactive peptide receptors and signaling

Angiotensin II receptor and signaling

Ang II, an octapeptide peptide(Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8), is the dominant player of the renin-angiotensin system (RAS) and has potent vasoconstriction effects on VSMC[41]. Ang II is produced both systemically and locally and is also released in cultured VSMC[42].Ang II elicits its physiological effects by interacting with at least two distinct receptor subtypes, designated as AT1 and AT2[7], which are G-protein coupled receptors (GPCR). The presence of AT1 receptor subtype has been shown in rat vascular tissues, however, a small proportion of AT2 receptors are also present in rat aorta[10]. AT2 receptor is expressed in the foetal developmental stage only in healthy individuals, however, its expression at the surface of VSMC[43,44] has been shown under pathological conditions.

The characterization of Ang II receptor subtypes has been made possible by the discovery and development of selective non-peptide Ang II receptor antagonists, namely losartan (AT1-selective) and PD123319 (AT2-selective)[45,46]. Virtually all the known biological actions of Ang II, including vasoconstriction, release of aldosterone, stimulation of sympathetic transmission and cellular growth are exclusively mediated by the AT1 receptor[45,47]. The functional role of the AT2 receptor is not fully understood, but recent studies have ascribed a possible role of AT2 receptors in mediating anti-proliferation, apoptosis, differentiation and possibly vasodilatation[48,49]. Both receptor subtypes have been cloned and pharmacologically characterized[50-52].

Two other Ang receptors have been described, AT3 and AT4 subtypes. The AT3 receptor subtype, initially described in the neuro 2A neuroblastoma cell line[53] is peptide-specific recognizing mainly Ang II. This subtype does not bind nonpeptide ligands, such as losartan or PD123319 and has only been observed in cell lines. The AT4 receptor, which is distributed in heart, lung, kidney, brain and liver binds Ang IV[54], but not losartan or PD123319. Since the pharmacology of AT3 and AT4 receptors has not been fully characterized, these receptors are not yet included in a definitive classification of mammalian AT receptors as defined by the International Union of Pharmacology Nomenclature Subcommittee for angiotensin receptors[55]. Most of the physiological effects of Ang II are mediated through the activation of AT1 receptors that are coupled to several signaling pathways, including adenylyl cyclase/cAMP inhibition through Giα proteins[9,11], MAP kinase[16] and phosphatidylinositol turnover through Gq/11α proteins[23].

ET-1 receptor and signaling

ET-1 is mainly secreted in endothelial cells[56], but is also produced in cultured VSMC[57, 58]. ET-1 interacts with two GPCR subtypes ET_A and ET_B. ET_A receptor appears to be the predominant subtype in VSMC[59] and is coupled to Gq/11α, G12/G13 and Gi proteins, leading to stimulation of phospholipase C/protein kinase C pathway, small RhoA and inhibition of adenylyl cyclase respectively[17,21,38], whereas ET_B is coupled to Gq and Gi[39,40], however, in endothelial cells, its activation releases NO and results in vasorelaxation[60]. There is evidence showing that the expression of ET_B receptor is augmented in pathological conditions, such as essential hypertension[61]. The activation of ET_A receptor also stimulates intracellular signaling pathways, including MAPK/P13K which are similar to those activated by growth factor receptors that possess intrinsic protein tyrosine kinase activity[39]. The activation of GPCR, ET_A/ET_B or AT1 by ET-1 and Ang II, respectively has also been reported to enhance the activation of growth factor receptors, such as epidermal growth factor receptor (EGF-R), platelet-derived growth factor (PDGF-R) and insulin-like growth factor (IGF-R) in a variety of cell types[25,62], the phenomenon known as transactivation[63].

Vasoactive peptides and Gi protein expression

The expression of Giα proteins has been shown to be regulated by various vasoactive peptides. Ang II, ET-1 and AVP are shown to enhance the levels of Giα proteins, whereas natriuretic peptides inhibit the expression of Giα proteins in A10 VSMC[16,64-66]. We have also reported that Ang II as well as ET-1 through the activation of AT1 and ET_A receptor increase the oxidative stress in A 10 VSMC, which through the transactvation of growth factor receptors and MAP kinase signaling increase the expression of Giα proteins and cell proliferation[64,65]. In addition, the implication of other signaling pathways activated by Ang II, including cAMP, phospholipase C (PLC), protein kinase C, intracellular Ca^{2+}, Ca^{2+} channel, calmodulin and calmodulin-dependent protein kinase (CAM kinase) and protein tyrosine kinase is also shown in Ang II-induced enhanced expression of Giα proteins because the inhibitors of these pathways attenuate the enhanced expression of Giα proteins evoked by Ang II[66].

Taken together, it may be suggested that Ang II-induced activation of AT1 receptor through Giα-dependent pathway decreases cAMP levels which per se or/and through the activation of PLC signaling may result in the enhanced expression of Giα proteins. In addition, AT1 receptor activation also increases the levels of intracellular Ca^{2+} through Gq/PLC pathway. The increased levels of Ca^{2+} through the activation of Ca^{2+} calmodulin dependent protein kinase activate tyrosine kinase, which by activating MAP kinase may be responsible for the enhanced expression of Giα proteins in A10 VSMC (Fig. 1). On the other hand, treatment of VSMC with S-Nitroso-N-acetyl-DL-penicillamine (SNAP) and sodium nitroprusside (SNP), nitric oxide (NO) donors and cGMP, the second messenger of NO attenuates the expression of Giα proteins and associated functions (Fig. 2)[67,68].

Role of endogenous vasoactive peptides in enhanced expression of Giα proteins in hypertension

The levels of vasoactive peptides, such as Ang II, ET-1 and AVP as well as growth factors that are augmented in various models of hypertension[69-77] are shown to contribute to the enhanced expression of Giα proteins in hypertension[78,79]. In this regard, the treatment of the SHR and 1K1C HR with captopril, an angiotensin coverting enzyme (ACE) inhibitor that decreases the levels of endogenous Ang II is also shown to result in the attenuation of high blood pressure and the enhanced levels of Giα protein to control levels[30,86]. Similarly, the increased blood pressure and enhanced expression of Giα proteins in L-NAME hypertensive rats is also shown to be restored to control levels by losartan, an AT1 receptor antagonist[80], suggesting the implication of endogenous Ang II in increased levels of Giα proteins and increased blood pressure in L-NAME-induced hypertension also. These treatments (captopril and losartan) are also shown to restore the diminished stimulation of adenylyl cyclase by stimulatory hormones and enhanced inhibition by inhibitory hormones observed in SHRs, 1K1C and L-NAME HR[20,80,81].

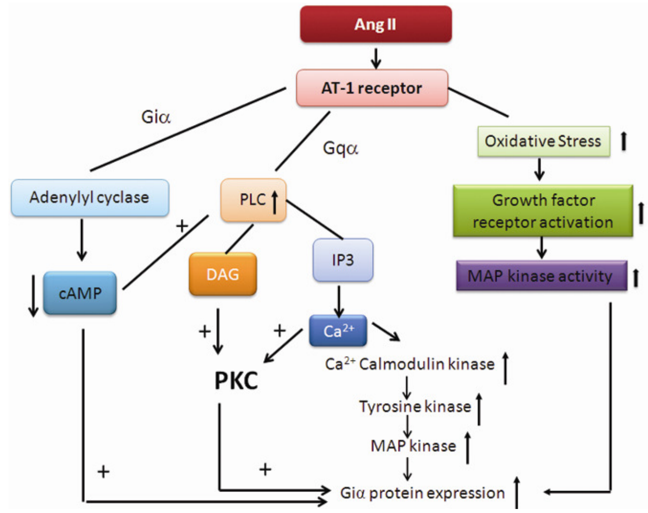

Fig. 1—Schematic diagram summarizing the possible mechanisms of angiotensin II (Ang II)-induced increased expression of Giαproteins [Ang II-induced activation of AT1 receptors through Giα-dependent pathway decreases cAMP levels, which *per se*, and/or through the activation of PLC–PKC signaling may result in the enhanced expression of Giα proteins. In addition, AT1 receptor activation also increases the levels of intracellular Ca^{2+} through the Gq-PLC pathway. The increased levels of Ca^{2+} through the activation of Ca^{2+}-calmodulin-dependent protein kinase further activate tyrosine kinase, which by activating MAP kinase may be responsible for the enhanced expression of Giα proteins in A10 VSMC. Ang II through the activation of AT1 receptor also increases oxidative stress, enhances the activation of growth factor receptors and ERK1/2 signaling that results in the enhanced expression of Giα protein]

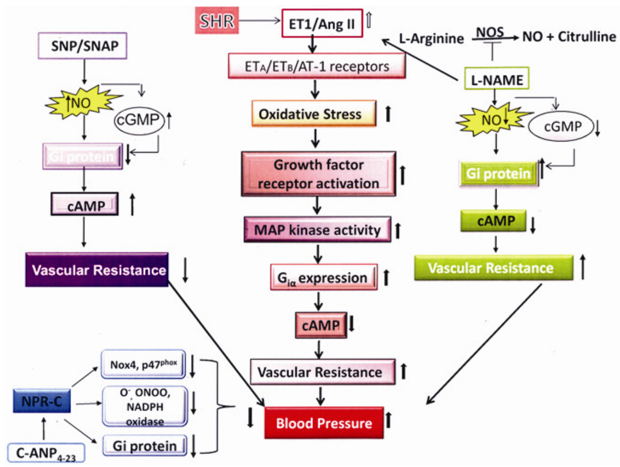

Fig. 2—Possible mechanisms involving angiotensin II, endothelin, oxidative stress and nitric oxide in enhanced expression of Giα protein in hypertension [Giα protein expression is enhanced in genetic (SHR) and experimental hypertension, including L-NAME-induced hypertension. Inhibition of nitric oxide synthase (NOS) by L-NAME activates renin angiotensin system, decreases the level of NO and results in increased expression of Giα proteins. ET-1/Ang II increases oxidative stress which through growth factor receptor activation increases MAP kinase activity and results in the enhanced expression of Giα proteins, leading to hypertension. On the other hand, increased level of NO and cGMP decrease the expression of Giα proteins in VSMC which may be an additional mechanism through which NO decreases BP in L-NAME-induced hypertension. In addition, $C\text{-}ANP_{4\text{-}23}$ through the activation of NPR-C inhibits the enhanced nitroxidative stress and Giα protein expression and results in the attenuation of the development of high BP in SHRs]

In addition, infusion of Ang II in rats that increases blood pressure has also been reported to enhance the levels of Giα proteins[82]. Similarly, nitrendipine and fosinopril treatments have also been reported to have similar effects on Gi proteins and functions in hearts from SHRs[33] and further support the implication of Ang II in enhanced levels of Giα protein in SHR. The implication of enhanced levels of endogenous Ang II and AT1 receptor in enhanced expression of Giα proteins is also reported by the studies, indicating that ACE inhibitor captopril, as well as AT1 receptor antagonist losartan attenuates the enhanced expression of Giα-2 and Giα-3 proteins in VSMC from SHR to Wistar Kyoto (WKY) rat levels. This is further supported by the fact that the silencing of AT1 receptor by siRNA attenuates the enhanced expression of Giα proteins in VSMC from SHR[79]. In addition, the inhibitors of endothelin ET_A and ET_B receptors BQ123 and BQ788 respectively are also shown to restore the enhanced expression of Giα proteins in VSMC from SHR to WKY rat levels[78], suggesting the role of endogenous endothelin that is increased in hypertension in enhanced expression of Giα proteins in VSMC from SHR[78].

Growth factor receptors have also been shown to contribute to the enhanced expression of Giα proteins in VSMC from SHR, because the inhibitors of growth factor receptors attenuate the augmented expression of Giα proteins to control levels[78]. The activation of ET_A/ET_B by ET-1 is also reported to enhance the activation of growth factor receptors, such as platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGF-1R) in a variety of cell types[83-85].

We have also shown that the enhanced phosphorylation of EGFR in VSMC from SHR is attenuated by captopril, losartan, as well as by BQ123 and BQ788 to control levels[86] and have suggested the transactivation of EGFR by endogenous Ang II and ET-1 in VSMC from SHR. In addition, we have shown that the enhanced levels of endogenous Ang II and ET-1 contribute to the enhanced expression of Giα proteins in VSMC from SHR through the transactivation of growth factor receptor and MAP kinase signaling (Fig. 2)[78,79]. In addition, SNAP is also found to attenuate the enhanced expression of Giα proteins in VSMC from SHR[87]. These results indicate that the decreased levels of NO in SHR, as well as in L-NAME hypertensive rats may be responsible for the enhanced expression of Giα proteins and suggest that NO-induced decreased levels of Giα proteins may represent an additional mechanism through which NO decreases blood pressure (Fig. 2). Furthermore, $C\text{-}ANP_{4\text{-}23}$ that specifically interacts with natriuretic peptide receptor-C is also reported to attenuate the enhanced expression of Giα proteins in A10 VSMC and VSMC from SHR[88,89]. In addition, we have recently shown that intraperitoneal injection of $C\text{-}ANP_{4\text{-}23}$ to prehypertensive SHR attenuates the increased blood pressure through the inhibition of enhanced expression of Giα proteins and enhances nitroxidative stress[90].

Vasoactive peptides, oxidative stress and Giα protein expression

Elevation of reactive oxygen species (ROS) that cause oxidative stress has been shown to play a major role in the pathophysiology of cardiovascular diseases, such as hypertension, atherosclerosis[91,92]. The implication of Ang II and ET-1 in increased generation of ROS through the activation of NAPDH oxidase has been reported in different models of hypertensive rats including DOCA-salt HR[93,94]. This is supported by the studies showing that ET_A receptor antagonist BQ123 attenuates ET-1-induced enhanced production of superoxide anion ($\bullet O_2^-$) in VSMC from hypertensive rats[95] and AT1 receptor antagonist losartan inhibits the enhanced production of O_2^- anion in VSMC from SHR[22].

It is also reported that ET-1 induces a significant augmentation of O_2^- in a dose-dependent manner in VSMC from ET-1-induced hypertensive rats[96]. We have also shown the implication of AT1, as well as ET_A and ET_B receptors in enhanced oxidative stress exhibited by VSMC from SHR, because the increased production of O_2^- and increased activity of NADPH oxidase in SHR is attenuated by losartan, an AT1 receptor antagonist and BQ123 and BQ788, ET_A and ET_B receptor antagonists, respectively[86]. A role of enhanced oxidative stressin Ang II-induced enhanced levels of Giα proteins is also reported[97]. The studies showing that antioxidants, such as diphenyleneiodonium (DPI) and N-acetyl cysteine (NAC) restore the enhanced levels of Giα proteins in SHR further suggest the implication of oxidative stress in the enhanced expression of Giα protein in SHR[22]. In addition, Ang II is also shown to enhance the levels of Giα proteins and superoxide anion (O_2^-) in A10 VSMC which are restored to control levels by antioxidants[97]. A role of oxidative stress in enhanced expression of Giα proteins is further supported by our studies showing that H_2O_2, an oxidant that induces oxidative stress, enhances the expression of Giα proteins in aortic VSMC[98]

Vasoactive peptides, MAP kinase cascade and Giα protein expression

Mitogen activated protein (MAP) kinase are serine/threonine-specific kinase that play an important role in some cellular processes, such as cellular differentiation, growth and apoptosis and the regulation of various transcription factors and genetic expression[99]. The implication of Ang II and ET-1 in the modulation of ERK1/2 and physiological responses has also been investigated by several investigators. Ang II and ET-1-induced increased phosphorylation of ERK1/2 is also reported in A10 VSMC[100]. The modulation of MAP kinase activity by ET-1 is also demonstrated in different cell types, such as cardiomyocytes[101], fibroblasts[102], glomerular mesangial cells[103] and VSMC[104]. The implication of ERK1/2 in the enhanced expression of Giα-2 and Giα-3 protein in VSMC from SHR is also reported[22].

The role of endogenous Ang II in enhanced phosphorylation of ERK1/2 in VSMC from SHR is also shown by the fact that the treatment of VSMC from SHR with captopril and losartan attenuates the hyperphosphorylation of ERK1/2. Furthermore, Iwasaki and collaborators [105] have shown that the ET_A receptor-induced transactivation of EGFR results in the activation of ERK1/2 in aortic VSMC. We have also shown the implication of both ET_A and ET_B receptors in ET-1-induced enhanced ERK1/2 phosphorylation in A10 VSMC, because ET_A and ET_B receptor antagonists BQ123 and BQ788, respectively attenuate the ET-1-induced enhanced phosphorylation of ERK1/2[78,86]. In addition, we have also demonstrated that ET-1-induced increased phosphorylation of ERK1/2 is also attributed to transactivation of EGFR, because AG1478, an inhibitor of EGFR inhibits the ET-1-evoked increased ERK1/2 phosphorylation[78,86]. Besides, the enhanced phosphorylation of ERK1/2 in SHR is shown to be restored to WKY levels by antioxidants[86], as well as by the inhibitor of EGFR[78,86] and it is suggested that the enhanced oxidative stress and EGFR through MAP kinase signaling may contribute to the enhanced expression of Giα protein in SHR. Taken together, it may be suggested that the enhanced levels of endogenous vasoactive peptides through the transactivation of EGFR increase the phosphorylation of ERK1/2 in VSMC from SHR, which contributes to the enhanced expression of Giα proteins in SHR (Fig. 2).

Conclusion

In conclusion, we have discussed the alterations in G-proteins and associated functions in hypertension. We have mainly focused on the role of vasoactive peptides, including Ang II and ET-1 on the modulation of the expression of Giα proteins that regulate cardiovascular functions, including vascular tone and reactivity and cell proliferation. The levels of Giα proteins and mRNA are increased in hearts and aorta from genetic and experimentally induced hypertensive rats, whereas the levels of Gsα are unaltered in genetic and decreased in experimentally-induced hypertensive rats with established hypertrophy. The increased levels of Giα proteins may be a contributing factor in the pathogenesis of hypertension because the enhanced expression of Giα proteins and mRNA precede the development of blood pressure. The role of enhanced levels of Giα proteins in the pathogenesis of hypertension is further supported by our studies showing that inactivation of Giα proteins by pertussis toxin treatment in prehypertensive SHR prevents the development of blood pressure. The levels of vasoactive peptides, including Ang II and ET-1 are increased in hypertension which contributes to the increased expression of Giα proteins in hypertension. In addition, the levels of growth factors and oxidative stress that are also increased in hypertension play a role in the enhanced levels of Giα protein expression. The enhanced levels of endogenous Ang II and ET-1 through increased oxidative stress and transactivation of growth factor receptors and associated MAP kinase signalling may contribute to the enhanced expression of Giα proteins implicated in the pathogenesis of hypertension. On the other hand, natriuretic peptide C-ANP$_{4\text{-}23}$ attenuates the high blood pressure in SHR through the inhibition of enhanced expression of Giα proteins and nitroxidative stress (Fig. 2).

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