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Deregulation of RGS2 in cardiovascular diseases

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1. ABSTRACT

Alteration of G protein-coupled receptor (GPCR) signaling is a salient feature of hypertension and the associated heart diseases. Recent studies have revealed a large family of Regulators of G-protein Signaling (RGS) proteins as important endogenous regulators of GPCR signaling. RGS2 selective regulates $G_{alphaq/11}$ signaling, an essential cause of hypertension and cardiac hypertrophy. Both clinical and animal studies have shown that deregulation of RGS2 leads to exacerbated $G_{alphaq/11}$ signaling. There is an inverse correlation between RGS2 expression and blood pressure, as well as a selective down-regulation of RGS2 in various models of cardiac hypertrophy. The causal relationship has been established in animal studies. RGS2 knockout mice exhibit not only hypertension phenotype but also accelerated cardiac hypertrophy and heart failure in response to pressure-overload. Further *in vitro* studies have shown that RGS2 knockdown with RNA interference exacerbates, whilst RGS2 over-expression completely abolishes the $G_{alphaq/11}$-induced hypertrophy. These findings indicate that deregulation of RGS2 plays a crucial role in the pathogenesis of cardiovascular diseases, marking RGS2 as a potential therapeutic target or biomarker of hypertension or hypertensive heart diseases.

2. INTRODUCTION

Hypertensive heart disease, characterized by hypertrophy of the left ventricles, is a major aetiological condition predisposing to heart failure (HF) (1, 2), a leading cause of cardiac morbidity and mortality in developed countries. Catecholamines and vasoactive peptides such as angiotensin II and endothelin are chronically elevated in patients with hypertensive a heart disease, the increase of which perturbs the G-protein coupled receptor (GPCR) signal transduction and hastens the progression from cardiac hypertrophy to HF (3-6). Hypertension (7) and cardiac hypertrophy (8-11) are indeed characterized by exacerbated $G_{alphaq/11}$ signaling, whereas HF is associated with up-regulated $G_{alphai/o}$ and depressed beta-adrenergic receptor (beta-AR) signaling (12-15).

Upon GPCR activation, guanosine diphosphate (GDP) is exchanged for guanosine triphosphate (GTP) on the G_{alpha} subunit, resulting in the dissociation of the $G_{beta-gamma}$ subunit from the G_{alpha} subunit. Both subunits activate downstream effectors. The intrinsic GTPase activity of the G_{alpha}-subunit catalyzes the hydrolysis of GTP to GDP, thus reconstituting the heterotrimeric G-protein complex and terminating G-protein signaling (16). RGS proteins act as GTPase-activating proteins (GAPs) to accelerate this

process, turning down GPCR signaling via returning G proteins to the GDP-bond heterotrimeric form. In addition to their GAP activity, RGS proteins also bind to certain effecter proteins such as adenylyl cyclase (AC) and the alpha-subunit of G_{alphaS} proteins, resulting in inhibition of their functions (17).

GPCRs have been marked as targets for therapies. Among the most effective clinical treatments are angiotensin converting enzyme (ACE) inhibitors, beta-AR blockers, diuretics, and Ca^{2+} channel blockers (18-21), the administration of which reduces hypertension(22), concomitantly favors the regression of cardiac hypertrophy and fibrosis (23-26); improves the symptoms of heart failure and reduces the 5-year mortality rates (27, 28).

Despite the advances in medication targeting against altered GPCR signaling, cardiovascular diseases remain the leading cause of mortality (29, 30). Thus, identification of novel therapeutic targets for cardiovascular diseases, particularly hypertensive heart diseases, is the current focus of cardiovascular research and medicine. In this regard, recent studies have revealed a large family of endogenous Regulators of G-protein Signaling (RGS) proteins which may be developed as potential therapeutic targets.

The small RGS B/R4 subfamily members, RGS2-5, are the predominant RGS proteins expressed in the cardiovascular system (31-33). RGS3-5 equally regulates both $G_{alphai/o}$ and $G_{alphaq/11}$(34) whereas cardiac myocyte RGS2 displays selectivity for $G_{alphaq/11}$ (34, 35) at least in part via the three unique residues within the G-protein binding domain (36). Given the critical role of $G_{alphaq/11}$ in maladaptive cardiac hypertrophy and remodelling in hypertensive heart disease, RGS2 is gaining increasing attention as an intrinsic suppressor. Both clinical and basic studies have demonstrated that deregulation of RGS2 contributes to the pathogenesis of cardiovascular diseases (36, 37). Clinical studies have exhibited an inverse relationship between blood pressure and RGS2 expression levels: hypertensive patients display a reduction in RGS2 mRNA and protein levels compared with normal subjects (38, 39), whereas patients with Bartter's/Gitelman's syndrome who are characterized by hypotension show increased RGS2 expression (40), highlighting a correlation between deregulation of RGS2 and the pathogenesis of hypertension as well as hypotension. The potential causal relationship between RGS2 deficiency and the development of hypertension and associated heart disease has been substantiated by recent animal studies. First, mice lacking RGS2 have obvious hypertensive phenotype (41, 42). Second, RGS2 deficient mice not only develop hypertension but also exhibit much more severe hypertensive heart disease as manifested by cardiac hypertrophy and heart failure in response to moderate pressure-overload as compared to wild-type controls (43). Additionally, the expression of RGS2, but not RGS3-5, is selectively down-regulated in multiple hypertrophic animal models (44), implying a crucial role of RGS2 deregulation in the development of cardiac hypertrophy. Finally, *in vitro* studies have further established the causal relationship between the reduction of RGS2 and cardiomyocyte hypertrophy (44, 45). These findings indicate that deregulation or malfunction of RGS2 is critically involved in the pathogenesis of cardiovascular diseases, and that RGS proteins in general and RGS2 in particular may serve as novel therapeutic targets or biomarkers of hypertension or hypertensive heart diseases. In this review, we first give an overview of recent advances on the role of $G_{alphaq/11}$-selective RGS2 in cardiovascular diseases. We focus on hypertension and cardiac hypertrophy, which are accompanied with exacerbated $G_{alphaq/11}$ signaling. Finally, we discuss the regulation of RGS2 and its potential pathogenic and therapeutic implications.

3. THE ROLE OF RGS2 AND CARDIOVASCULAR DISEASE

3.1 Deregulation of RGS2 in the vascular system and hypertension

Hypertension is a major risk factor for ischemic heart diseases and a strong predictor of hospitalization for congestive heart failure (CHF) (46). Accumulating evidence shows that RGS2 plays a prominent role in regulating GPCR signaling pathways that directly involved in blood pressure homeostasis. First, RGS2 expression has been detected in vascular smooth muscle, the dysfunction of which contributes to pathogenesis of hypertension (47). Second, RGS2 selectively regulates $G_{alphaq/11}$ signaling, which is activated by many vasoconstrictors including vasopressin, endothelin-1 (ET-1), thromboxane A2 thrombin, and angiotensin II. These observations suggest that the deregulation of RGS2 may promote chronic constriction of the peripheral vasculature, leading to hypertension (41). More importantly, hypertensive patients indeed display a decrease in RGS2 mRNA and protein levels (38, 39). Conversely, patients with Bartter's/Gitelman's syndrome, whom are accompanied with hypotension, have an enhanced expression of RGS2 (40) (Table 1). Further studies of genetic variations in RGS2 in human hypertension populations have been reported (39, 48). Polymorphisms in RGS2 significantly reduce its expression as well as its action in inhibiting angiotensin II receptor type I (AT1)-mediated accumulation of inositol phosphates, thereby hastening the pathogenesis of hypertension (39). That RGS2 is associated with blood pressure regulation is further supported by a recent study showing the association of G/G 1114 RGS2 genotype with the number of neutrally mediated syncope (NMS) episodes, which is also a type of blood pressure dysregulation (49).

The essential role of RGS2 in the regulation of vascular tone has been well demonstrated in mice lacking RGS2. Tang *et al* have shown that mice lacking RGS2 at the age of 3-5 months globally develop modest systemic hypertension and a striking elevation in mean arterial pressure without major cardiac phenotype at resting conditions (42). Furthermore, it has been shown that RGS2 knockout mice are hypertensive and exhibit persistent constriction of the resistance vasculature (41, 42). Consistent with these findings, RGS2-/- mice have more severe hypertension and prolonged vasoconstriction in response to angiotensin II as compared to wild type control animals (41, 42, 50, 51).

Table 1. The expression of RGS2 in hypertension, hypertrophy and heart failure

Diagnosis	Samples	Species	Treatment	Duration of treatment	RGS2 expression compared with control	Reference	Parameters evaluated
$G_{alphaq/11}$* Hypertrophy	Myocardium	FVB mice	TAC[1]	8 wk after TAC[1]	Decrease	(44)	Protein and mRNA
Accelerated hypertension	Aortic media	Fischer 344 rats	Chronic inhibition of NO synthesis with L-NAME	30 days	Decrease	(82)	mRNA
Essential hypertension	Blood mononuclear cells	Human	NA	NA	Decrease	(38)	mRNA
BS/GS[2]	Blood mononuclear cells	Human	NA	NA	Increase	(40)	mRNA
End stage heart failure	Core of AV apex	Human	NA[2]	NA	Increase	(74)	Protein

[1]Transverse aortic constriction, Bartter's and Gitelman's syndromes

Interestingly, Heximer and co-workers have demonstrated that both anesthetized *rgs2*+/– and *rgs2*–/– mice display substantial blood pressure elevation, indicating that that both copies of the *rgs2* gene are essential for normal regulation of blood pressure. Furthermore, they have also reported that the elevated blood pressure in RGS2-/- mice, unlike in many other hypertensive mouse models, is not accompanied by a compensatory fall in heart rate, suggesting that the absence of normal RGS2 function may also cause generalized disruption of cardiovascular reflexes. Taken together, both clinical and basic studies have demonstrated that RGS2 constitutes a requisite for the regulation of normal vascular tone and blood pressure, and that a reduction or malfunction of RGS2 contributes to the pathogenesis of hypertension.

3.1.1. Potential role of $G_{alpha12/13}$

A previous study by Wirth *et al* have shown that both $G_{alphaq/11}$ and $G_{alpha12/13}$ are required for salt-induced hypertension despite only $G_{alphaq/11}$ is necessary to maintain normal blood pressure (52). Given that vasoconstrictors such as angiotensinII, endothelin-1 or throboxaneA2 receptors couple to $G_{alphaq/11}$ and $G_{alpha12/13}$, RGS2 might as well regulates $G_{alpha12/13}$. Further studies are needed to elucidate the effect of RGS2 on $G_{alpha12/13}$.

3.2 Deregulation of RGS2 and cardiac hypertrophy

Hypertension is one of the conditions which increases afterload and is a precipitating cause of cardiac hypertrophy. The myocardium responds to acute overload by undergoing hypertrophy, which was originally described as a compensatory mechanism to reduce wall stress during acute overload (53). However, sustained stress initiates a myopathic transition from a compensatory state to progressive chamber enlargement associated with impaired myocardial vascularization, unfavourable changes in the extracellular matrix composition and fibrosis (54). It is well accepted that pathological hypertrophy is associated with adverse prognosis. Clinical consequences include the development of cardiac arrhythmias, cardiac dysfunction and eventually CHF (1, 55, 56).

3.2.1. $G_{alphaq/11}$ signaling and cardiac hypertrophy

The contribution of G proteins to cardiac hypertrophy and heart failure has been extensively examined in transgenic mouse model systems (9, 11, 57). Despite the finding showing that cardiac hypertrophy can be mediated through vascular actions (58), substantial evidence shows that $G_{alphaq/11}$ in the myocardium is the central player for pressure overload-induced cardiac hypertrophy (9, 59, 60) (Figure 1). Cardiac specific over-expression of $G_{alphaq/11}$ results in the development of cardiac hypertrophy and dilatation, subsequently leads to cardiomyopathy with depressed contractile function (9, 11, 57), the effect of which is blocked by cardiac specific expression of an inhibitor of $G_{alphaq/11}$ signaling (9).

3.2.2. Role of RGS2 in cardiac hypertrophy

Among multiple RGS proteins, RGS2 is selectively down-regulated in hypertrophy animal models (44). The selective down-regulation of RGS2 occurs even before the onset of cardiac hypertrophy (44). Furthermore, Zhang *et al* have demonstrated that over-expression of RGS2 inhibits $G_{alphaq/11}$-mediated cardiac myocyte hypertrophy, whereas gene silencing of RGS2 with RNAi exacerbates cardiac myocyte hypertrophy in response to increased $G_{alphaq/11}$ signaling by the stimulation of ET-1 receptor with endothelin or $alpha_1$-adrenoceptor ($alpha_1$-AR) with phenylephrine (PE) (44, 45). In support of this, it has been shown that RGS2 directly interacts with the third intracellular loop of $alpha_{1A}$-adrenergic receptor (61). Importantly, studies on RGS2 deficient mice have further established a causal relationship between reduced RGS2 expression and cardiac hypertrophy. Specifically, RGS2-/- mice exhibit a very marked hypertrophic response, cardiac dysfunction and premature death in response to pressure overload (43). Although RGS2-/- and its littermates have similar resting cardiac anatomy and functions at the age of 4-5 months in the absence of pressure overload (41), RGS2 deficient mice display a marked dilation following pressure overload, which is accompanied with a significant increase in end-diastolic and systolic dimensions and decline in fractional shortening as well as impaired relaxation compared with littermate controls (43). These results suggest that dysfunction of RGS2 may play a prominent role in the initial events that trigger hypertrophy in response to pressure overload.

3.2.3. Potential mechanisms underlying RGS2-mediated suppression of hypertrophy

Apart from the converging point on $G_{alphaq/11}$, the exact molecular mechanism underlying RGS2-mediated suppression of hypertrophy remains highly controversial. Zhang *et al* have shown that RGS2 restrains $G_{alphaq/11}$ mediated hypertrophy via suppressing phospholipase C-

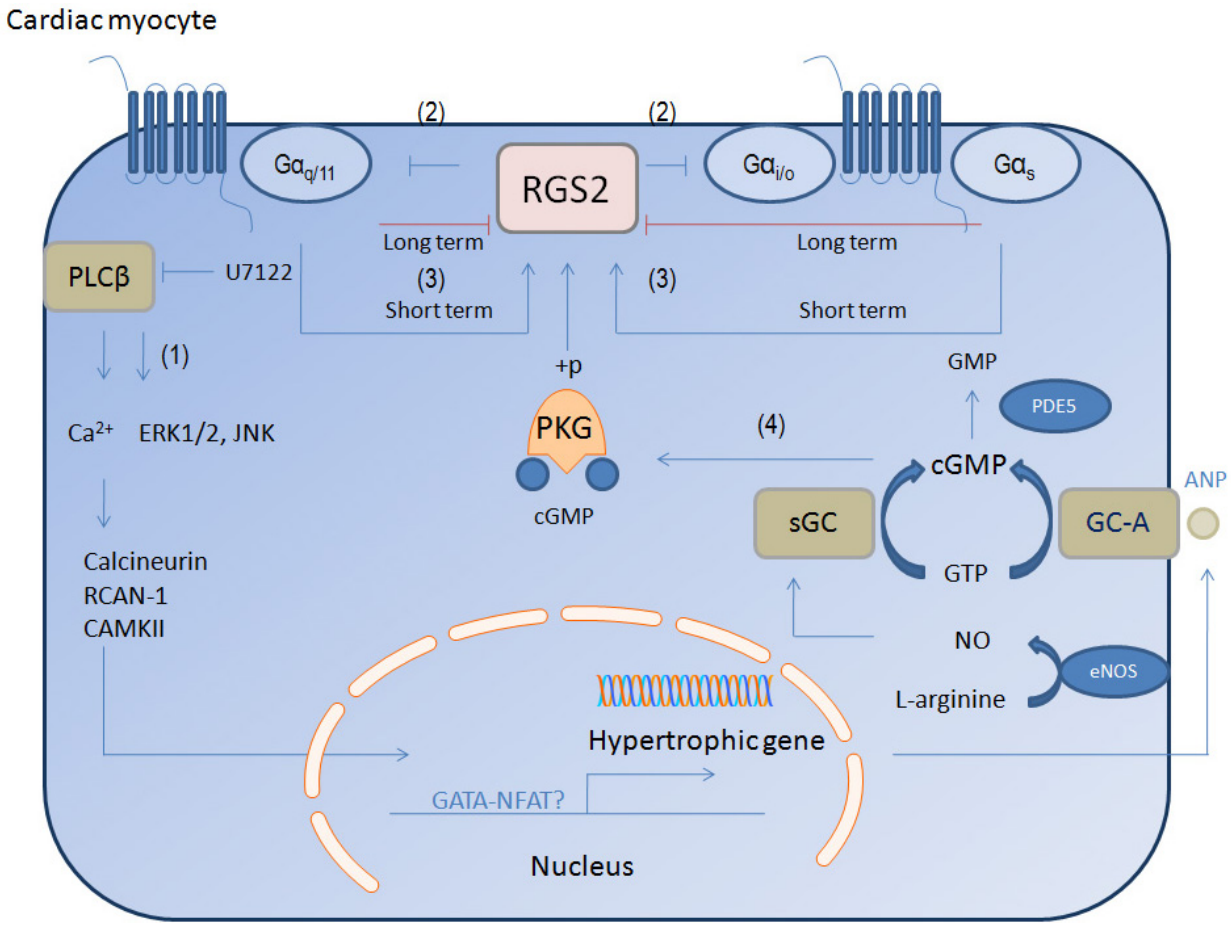

Figure 1. Regulation of RGS2 in the cardiac myocyte and its implication in cardiac hypertrophy.1. Cardiac hypertrophic agonists stimulate $G_{alphaq/11}$-coupled GPCRs and activate phospholipase C-beta (PLC-beta). Subsequent production of IP_3 and elevation of intracellular Ca^{2+} levels not only activate Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), calcineurin (CN) and regulator of CN-1 (RCAN-1), but also MAPK including ERK1/2 and JNK. Activated CN dephosphorylates NFAT, which promotes its nuclear translocation. The cooperation of NFAT and GATA then switches on transcription of the hypertrophic gene program. Of note, the hypertrophic responses were abolished in response to administration of PLC-beta inhibitor, U7122.2. RGS2 negatively regulates $G_{alphaq/11}$ signaling. Association of RGS2 with $G_{alphaq/11}$ increases the GTPase activity of $G_{alphaq/11}$ thus suppressing the $G_{alphaq/11}$-mediated hypertrophic responses. Our lab has recently shown that RGS2 also negatively regulates $G_{alphai/o}$. 3. The expression of RGS2 is biphasically regulated, with a marked up-regulation in response to short term $G_{alphaq/11}$ or G_{alphaS} activation followed by a persistent decline in response to sustained agonist stimulation. 4. The activity of RGS2 is regulated by PKG. PDE5 inhibition-mediated PKGI-activation translocates, phosphorylates and activates RGS2. Apart from PDE inhibition, PKG is also activated by ANP-mediated GC-A activation. Given that PKG phosphorylates both RGS2 and RGS4, RGS2 might be a potential downstream effector of the GC-A/PKG signaling.

beta (PLC-beta) but not MAPK activation (44). However, Takimoto *et al* have recently demonstrated that pressure overload-induced cardiac dilation and hypertrophy in RSG2-/- mice are coupled with exacerbated activation of ERK1/2 and JNK and a concurrent increase in calcineurin, regulator of calcineurin-1 (RCAN-1) and CAMKII activity (Figure 1), which have previously been implicated in maladaptive cardiac remodelling (62). Of note, the aforementioned effects are blocked by the administration of a PLC-beta inhibitor U73122 (43) (Figure 1). Nevertheless, the exact mechanism underlying RGS2-mediated suppression of hypertrophy awaits further investigation.

3.2.4. Potential overlapping role of RGS proteins

Although compelling evidence indicates that reduced RGS2 expression is central in the development of hypertrophy in response to pressure overload and that RGS2 is 10 folds more potent than RGS4 in blocking $G_{alphaq/11}$ activation of PLC-beta (36), recent studies have highlighted a complex and potential overlapping roles of RGS proteins. Over-expression of RGS4 has been shown to inhibit endothelin-1 or PE-induced cardiac myocyte hypertrophy (35, 63) and blunts $G_{alphaq/11}$ stimulated cardiac hypertrophy in mice (64). Furthermore, Kangawa and co-workers have recently demonstrated that RGS4 is required for the cardiac natriuretic peptides mediated anti-hypertrophic effect in the heart (65, 66). However, RGS4

Table 2. The expression of RGS2 in responses to stimulation of $G_{alphaq/11}$/ G_{alphaS}

Agonists	Dose	Time	RGS2 expression	Cell type	References	Parameter evaluated
$G_{alphaq/11}$						
PE[3]	2μM	3h	Increases 20 folds	AVM[1]	(34)	mRNA
		24h	Increases 5 folds	AVM[1]	(34)	
		48h	Increases 2.5 folds	AVM[1]	(34)	
		72h	Basal (unstimulated) to a decrease	AVM[1]	(34)	
PE[3]	10μM	30min-1h	Increases	NVM[2]	(45)	mRNA
PMA[4]	0.16μM	3h	Increases 110 folds	AVM[1]	(34)	mRNA
		72h	Basal (unstimulated)	AVM[1]	(34)	
G_{alphaS}						
ISO[5]/FSK[6]	1nM	2h	Increases	AVM[1]	Unpublished data	Protein
ISO[5]/FSK[6]	1nM	24h	Decreases	AVM[1]	Unpublished data	Protein
ISO[5]	2μM	3h	Increases 4 folds	AVM[1]	(34)	mRNA
		48h	Increases ~1.5 folds	AVM[1]	(34)	

[1]Adult ventricular myocyte, [2]Neonatal ventricular myocyte, [3]Phenylephrine, [4]phorbol 12-myristate 13-acetate, [5]Isoproterenol, [6]Forskolin

over-expressing hearts exhibit rapid cardiac dilation and marked mortality in response to pressure overload (67). Further studies are warranted.

3.3 Role of RGS2 in the development of heart failure

Hypertension and pathophysiological hypertrophy are well-established risk factors of congestive heart failure (CHF) (2, 68-70). Long-standing arterial hypertension induced cardiac hypertrophy is indeed associated with a marked increase in incidence of heart failure. Kass and co-workers have recently provided strong evidence that RGS2 ablation renders the heart more vulnerable and results in premature death (43) despite the inconsistent expression profile of RGS2 in CHF (32). In response to pressure overload, RGS2-/- mice are acutely decompensated with dilated heart failure and associated with a significantly higher mortality compared with the littermate controls.

It is well documented that enhanced $G_{alphai/o}$-mediated signaling (12, 15, 71), in addition to $G_{alphaq/11}$-dependent signaling, plays a pivotal role in the progression to heart failure. A previous study has shown that RGS2 has no effect on $G_{alphai/o}$-coupled signaling in adult rat cardiac myocytes (34). However, our unpublished biochemical and functional data have shown that RGS2 served as an endogenous negative regulator of $beta_2$-adrenergic receptor ($beta_2$-AR)-activated $G_{alphai/o}$ signaling in rodent cardiomyocytes (Zhu *et al.*, personal communication). Adenovirus mediated RGS2 over-expression abrogated $beta_2$-AR-$G_{alphai/o}$ coupling in cultured cardiac myocytes, which was intact in RGS2-/- mice. In support of this finding, RGS2 has been shown to directly interact with $beta_2$-AR (72). Furthermore, RGS2 may directly inhibit the major myocardial adenylyl cyclase V and VI isoforms (17), the impaired activity of which is a feature of HF. It is likely that the selective down-regulation of RGS2 in hypertrophic hearts or early stage compensated failing hearts leads to increased $G_{alphai/o}$ as well as $G_{alphaq/11}$ signaling, thus contributing to the transition of compensated to decompensated CHF.

The central importance of exacerbated $G_{alphai/o}$ signaling in HF further implies the potential involvement of RGS3-5. While RGS3 and RGS4 have been shown to be increased in myocardium from patients with HF (32, 73), there is also reports argue against the notion (74). The disparity might be, at least in part, owing to the complex aetiology of HF and the dynamic regulation of a multitude of RGS proteins (see below). Taken together, previous studies have provided compelling evidence to support a prominent role of RGS2 deregulation in the development of cardiac hypertrophy, whilst it is possible that other RGS proteins may play a role.

4. REGULATION OF RGS2

4.1 G-protein dependent regulation of RGS2 expression

It has been shown that the regulation of RGS2 expression and activity is both tissue- and receptor-specific. In the cardiovascular system, the gene and protein expression of RGS2 is dynamically regulated by stimulation of a variety of GPCR-coupled signaling pathways (45, 47, 75) (Table 2).

4.1.1. $G_{alphaq/11}$-RGS2-$G_{alphaq/11}$ negative feedback loop

$G_{alphaq/11}$-selective RGS2 has been shown to undergo rapid up-regulation in the myocardium in response to $G_{alphaq/11}$ activation (Figure 1). Acute increase in $G_{alphaq/11}$ signaling evoked by receptor activation or enforced expression of a constitutively active $G_{alphaq/11}$ mutant, $G_{alphaq/11*}$, selectively up-regulate myocardial RGS2 but not RGS3-5 (45). In agreement with this finding, Hao *et al* have showed that the $alpha_1$-AR agonist PE robustly increases RGS2 gene expression by 20 folds. Similarly, administration of phorbol-12-myristate-13-acetate (PMA), a PKC activator, also increases RGS2 by 110 folds over the baseline (34). Similar findings have been reported in vascular smooth muscle cells. Grant *et al* have demonstrated that angiotensin II-mediated activation of AT-1 selectively stimulates the gene expression of RGS2 in vascular smooth muscle cells in a dose- and time-dependent manner (47). It has been suggested that the rapid increase in RGS2 expression in response to the stimulation of a given $G_{alphaq/11}$ coupled-GPCR might serve as a negative feedback loop to facilitate the termination of the receptor signaling.

4.1.2. G_{alphaS}-RGS2-$G_{alphaq/11}$/$G_{alphai/o}$ signaling cross-talk?

Interestingly, Hao *et al* demonstrated that stimulation of G_{alphaS}-coupled beta-AR with isoproterenol

(ISO) also increases RGS2 at both mRNA and protein levels (34) (Figure 1). Similar findings have been obtained with administration of forskolin, which directly activates AC (76). These findings are in agreement with previous notion of cAMP-dependent regulation of RGS2 in other cell types (77). Consistent with these findings, our laboratory has recently shown that administration of ISO increases expression of RGS2 (unpublished results) in rodent cardiac myocytes. Since RGS2 has no GAP activity toward the alpha-subunit of Gs proteins (34), the up-regulation of RGS2 by activation of Gs-coupled signaling is unlikely to represent a negative feedback mechanism, instead highlighting a potential cross-talk between G_{alphaS} and $G_{alphaq/11}$- or $G_{alphai/o}$-mediated signaling pathways in the cardiac myocytes (Figure 1).

4.1.3. Biphasic regulation of RGS2 expression

Evidence has accrued that RGS2 expression undergoes a biphasic regulation, with an initial rise followed by a persistent decline in response to sustained agonist stimulation. To date, most studies have shown that up-regulation of RGS expression is rapid. For instance, stimulation of AT-1 receptor with angiotensin II markedly increases mRNA and protein expression of RGS2 within 1h (47, 75). Likewise, Hao *et al* have also demonstrated that PE-induced up-regulation of RGS2 occurs within the first hour of agonist treatment. Our unpublished data have also shown that administration of ISO for 2h increases RGS2 protein expression in adult mouse and rat cardiac myocytes (Zhu *et al.*, personal communication).

Despite an acute increase following administration of GPCR agonists, RGS2 expression declines in response to sustained stimulation. For instance, Hao *et al* have shown a significant decrease in RGS2 expression at 72h after administration of PE or PMA (34). In support of the finding, our lab has also demonstrated a marked down-regulation of RGS2 protein after 24h ISO or forskolin stimulation in cultured mouse cardiomyocytes (Unpublished results).

4.1.4 Deregulation of G-protein-dependent axis and its pathophysiological implications

It has been suggested that the eventual decline of RGS2 induced by prolonged GPCR stimulation may serve as an important pathological element via loss of control of certain G-protein signaling, in particular, $G_{alphaq/11}$ and $G_{alphai/o}$ signaling. In support of this perception, Zhang *et al* have provided eloquent evidence that there is a selective RGS2 down-regulation with prolonged enhanced $G_{alphaq/11}$ signaling in the hypertrophic heart (44), suggesting that the down-regulation of RGS2 and the subsequent exacerbated $G_{alphaq/11}$ signaling in response to chronic elevated catecholamines in diseases states may, at least in part, contribute to the pathogenesis of cardiovascular diseases including hypertension and myocardial hypertrophy.

4.2 PKG-dependent regulation of RGS2 activity

4.2.1. G-protein-independent regulation of RGS2 by NO-cGMP-PKG signaling cascade in VSMC

Eloquent evidence shows that RGS2 activity can be regulated through a GPCR/G-protein independent mechanism via activation of the NO-cGMP-PKG axis (42). PKGI-alpha, the predominant PKG isoform in vascular smooth muscle cells, has been shown to inhibit vascular contraction by phosphorylating RGS2 at Ser46 and 64, which modestly increases its GAP activity toward $G_{alphaq/11}$ by 2-folds (42), thereby terminating $G_{alphaq/11}$-mediated vasoconstriction (50).

The role of PKG in regulating RGS2 is further supported by the fact that mice lacking RGS2 have an impaired cGMP-mediated inhibition on Ca^{2+} transients elicited by vasoconstrictors (41, 50), and display decreased cGMP-mediated relaxation (42, 50). Furthermore, it has been shown that cGMP stimulation increases the translocation of wild type RGS2, but not its mutant which lacks PKG phosphorylation sites, to smooth muscle cell plasma membranes, a prerequisite for its activity. Similarly, Blumer and co-workers have demonstrated that PKG-dependent plasma membrane association of RGS2 is necessary for RGS2-mediated inhibition of vasoconstrictor- and $G_{alphaq/11}$-triggered PLC activation, Ca^{2+} release, and capacitative Ca^{2+} entry (78).

4.2.2. G-protein-independent regulation of RGS2 by cGMP-PKG-PDE5 signaling axis in the heart

PKG-dependent regulation of RGS2 has also been demonstrated in cardiac myocytes. Takimoto *et al* have recently shown that RGS2 is a dominating effector of PKG in the myocardium in the initial response to pressure overload (43). Activation of PKGI-alpha is required for membrane translocation of RGS2 both *in vitro* and *in vivo*. Briefly, they have shown that activation of PKGI-alpha alone cannot provide anti-hypertrophic protection unless there is RGS2. Further, inhibition of cGMP-selective PDE5 with sildenafil attenuates pressure overload-induced cardiac hypertrophy in a RGS2-dependent manner. It is noteworthy that the protective effect of PDE5 inhibition has been shown as a direct effect on myocardium rather than a secondary effect of vasodilation.

4.2.3. G-protein-dependent regulation of RGS2 by GC-A-cGMP-PKG axis in the myocardium?

Kangawa and co-workers (65) have recently highlighted the importance of guanylyl cyclase-A (GC-A) in inhibiting cardiac hypertrophy via PKG. It has been shown that hypertrophy itself turns on the hypertrophy gene expression including ANP. Importantly, ANP has been shown to exert anti-hypertrophic effect via stimulating GC-A/PKG-mediated activation of RGS4, which restrains $G_{alphaq/11}$-coupled hypertrophy signaling. Given that both RGS2 and RGS4 are activated by PKG1-alpha, it is speculated that RGS2 might also be regulated by the $G_{alphaq/11}$-GC-A-triggered activation of PKG.

4.2.4. Deregulation of the PKG axis – the implications

The aforementioned findings suggest that PKG is crucially involved in regulating RGS2 activity both in the vascular system and in the myocardium, the activation of which attenuates hypertrophy and hypertension via turning down $G_{alphaq/11}$ signaling. While it is unclear whether PKG activity is also altered in pathophysiological states, clinical studies have shown a marked decrease in expression of

endothelial NO synthase (eNOS) and diminished eNOS–mediated NO production in various cardiovascular disease states (79-81). Reduced eNOS-NO signaling is expected to cause a defect of the PKG-mediated RGS2 regulation, thus leading to an exacerbated $G_{alphaq/11}$ signaling and contributing to the progression of cardiovascular diseases.

5. PERSPECTIVES

Expression and function of RGS2 is markedly altered in hypertension and hypertensive heart diseases, which is accompanied with exacerbated $G_{alphaq/11}$ signaling. Given the selective regulation of RGS2 on $G_{alphaq/11}$, RGS2 appears to be an important candidate as intrinsic suppressor of hypertension and cardiac hypertrophy. In addition, the negative regulation of RGS2 on $G_{alphai/o}$ provides new insights to our understanding of the pathogenesis of HF. Taken together, restoring the expression of RGS2 or increasing its GAP activity in particular and the panel of RGS proteins in general would represent a promising direction in treating major cardiovascular diseases such as hypertension and cardiac hypertrophy and resultant CHF. Understanding the pathophysiology and molecular mechanisms of how RGS2 can turn off maladaptive signals should lead to new insights into pathogenesis and reveal novel therapeutic approaches to treat hypertension and improve the structure and function of the failing heart.

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7. REFERENCES

1. D. Levy, M. G. Larson, R. S. Vasan, W. B. Kannel and K. K. Ho: The progression from hypertension to congestive heart failure. *Jama*, 275(20), 1557-1562 (1996)

2. W. B. Kannel, D. Levy and L. A. Cupples: Left ventricular hypertrophy and risk of cardiac failure: insights from the Framingham Study. *J Cardiovasc Pharmacol*, 10 Suppl 6, S135-40 (1987)

3. G. L. Jennings: Noradrenaline spillover and microneurography measurements in patients with primary hypertension. *J Hypertens Suppl*, 16(3), S35-8 (1998)

4. C. Ferrier, M. D. Esler, G. Eisenhofer, B. G. Wallin, M. Horne, H. S. Cox, G. Lambert and G. L. Jennings: Increased norepinephrine spillover into the jugular veins in essential hypertension. *Hypertension*, 19(1), 62-9 (1992)

5. M. Esler, D. Kaye, G. Lambert, D. Esler and G. Jennings: Adrenergic nervous system in heart failure. *Am J Cardiol*, 80(11A), 7L-14L (1997)

6. M. R. Bristow: Mechanisms of Development of Heart Failure in the Hypertensive Patient. *Cardiology*, 92(Suppl. 1), 3-6 (1999)

7. D. M. Harris, H. I. Cohn, S. Pesant, R.-H. Zhou and A. D. Eckhart: Vascular smooth muscle Gq signaling is involved in high blood pressure in both induced renal and genetic vascular smooth muscle-derived models of hypertension. *Am J Physiol Heart Circ Physiol*, 293(5), H3072-3079 (2007)

8. V. LaMorte, J. Thorburn, D. Absher, A. Spiegel, J. Brown, K. Chien, J. Feramisco and K. Knowlton: Gq- and ras-dependent pathways mediate hypertrophy of neonatal rat ventricular myocytes following alpha 1-adrenergic stimulation. *J. Biol. Chem.*, 269(18), 13490-13496 (1994)

9. S. A. Akhter, L. M. Luttrell, H. A. Rockman, G. Iaccarino, R. J. Lefkowitz and W. J. Koch: Targeting the Receptor-Gq Interface to Inhibit *in vivo* Pressure Overload Myocardial Hypertrophy. *Science*, 280(5363), 574-577 (1998)

10. N. Wettschureck, H. Rutten, A. Zywietz, D. Gehring, T. M. Wilkie, J. Chen, K. R. Chien and S. Offermanns: Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Galphaq/Galpha11 in cardiomyocytes. *Nat Med*, 7(11), 1236-40 (2001)

11. D. D. D' Angelo, Y. Sakata, J. N. Lorenz, G. P. Boivin, R. A. Walsh, S. B. Liggett and G. W. Dorn: Transgenic Gαq overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci USA*, 94(15), 8121-8126 (1997)

12. T. Eschenhagen, U. Mende, M. Nose, W. Schmitz, H. Scholz, A. Haverich, S. Hirt, V. Doring, P. Kalmar, W. Hoppner: Increased messenger RNA level of the inhibitory G protein alpha subunit Gi alpha-2 in human end-stage heart failure. *Circ Res*, 70(4), 688-96 (1992)

13. D. S. Feldman, T. S. Elton, B. Sun, M. M. Martin and M. T. Ziolo: Mechanisms of Disease: detrimental adrenergic signaling in acute decompensated heart failure. *Nat Clin Pract Cardiovasc Med*, 5(4), 208-218 (2008)

14. O. E. Brodde: Beta-adrenoceptors in cardiac disease. *Pharmacol Ther*, 60(3), 405-430 (1993)

15. A. M. Feldman, A. E. Cates, W. B. Veazey, R. E. Hershberger, M. R. Bristow, K. L. Baughman, W. A. Baumgartner and C. Van Dop: Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. *J Clin Invest*, 82(1), 189-97 (1988)

16. M. C. Hendriks-Balk, S. L. M. Peters, M. C. Michel and A. E. Alewijnse: Regulation of G protein-coupled receptor signalling: Focus on the cardiovascular system and regulator of G protein signalling proteins. *Eur J Pharmacol*, 585(2-3), 278-291 (2008)

17. S. Salim, S. Sinnarajah, J. H. Kehrl and C. W. Dessauer: Identification of RGS2 and Type V Adenylyl Cyclase Interaction Sites. *J. Biol. Chem.*, 278(18), 15842-15849 (2003)

18. P. Vantrimpont, J. L. Rouleau, C. C. Wun, A. Ciampi, M. Klein, B. Sussex, J. M. Arnold, L. Moye and M. Pfeffer: Additive Beneficial Effects of Beta-Blockers to Angiotensin-Converting Enzyme Inhibitors in the Survival and Ventricular Enlargement (SAVE) Study. *J Am Coll Cardiol*, 29(2), 229-236 (1997)

19. J. Frank: Managing hypertension using combination therapy. *Am Fam Physician*, 77(9), 1279-86 (2008)

20. C. Rosendorff: Hypertension and coronary artery disease: a summary of the American Heart Association scientific statement. *J Clin Hypertens (Greenwich)*, 9(10), 790-5 (2007)

21. S. Erdine, O. Ari, A. Zanchetti, R. Cifkova, R. Fagard, S. Kjeldsen, G. Mancia, N. Poulter, K. H. Rahn, J. L. Rodicio, L. M. Ruilope, J. Staessen, P. van Zwieten, B. Waeber and B. Williams: ESH-ESC guidelines for the management of hypertension. *Herz*, 31(4), 331-8 (2006)

22. K. Wachtell, B. Dahlof, J. Rokkedal, V. Papademetriou, M. S. Nieminen, G. Smith, E. Gerdts, K. Boman, J. N. Bella and R. B. Devereux: Change of left ventricular geometric pattern after 1 year of antihypertensive treatment: The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study. *Am Heart J*, 144(6), 1057-1064 (2002)

23. M. Hartford, I. Wendelhag, G. Berglund, I. Wallentin, S. Ljungman and J. Wikstrand: Cardiovascular and renal effects of long-term antihypertensive treatment. *Jama*, 259(17), 2553-7 (1988)

24. F. G. Dunn, H. O. Ventura, F. H. Messerli, I. Kobrin and E. D. Frohlich: Time course of regression of left ventricular hypertrophy in hypertensive patients treated with atenolol. *Circulation*, 76(2), 254-8 (1987)

25. M. Shahi, S. Thom, N. Poulter, P. S. Sever and R. A. Foale: Regression of hypertensive left ventricular hypertrophy and left ventricular diastolic function. *The Lancet*, 336(8713), 458-461 (1990)

26. F. G. Dunn, W. Oigman, H. O. Ventura, F. H. Messerli, I. Kobrin and E. D. Frohlich: Enalapril improves systemic and renal hemodynamics and allows regression of left ventricular mass in essential hypertension. *Am J Cardiol*, 53(1), 105-108 (1984)

27. E. J. Eichhorn, C. M. Heesch, J. H. Barnett, L. G. Alvarez, S. M. Fass, P. A. Grayburn, B. A. Hatfield, L. G. Marcoux and C. R. Malloy: Effect of metoprolol on myocardial function and energetics in patients with nonischemic dilated cardiomyopathy: a randomized, double-blind, placebo-controlled study. *J Am Coll Cardiol*, 24(5), 1310-20 (1994)

28. R. Adorisio, L. De Luca, J. Rossi and M. Gheorghiade: Pharmacological treatment of chronic heart failure. *Heart Failure Reviews*, 11(2), 109-123 (2006)

29. A. D. Lopez and C. D. Mathers: Measuring the global burden of disease and epidemiological transitions: 20022030. *Ann Trop Med Parasitol*, 100, 481-499 (2006)

30. A. D. Lopez, C. D. Mathers, M. Ezzati, D. T. Jamison and C. J. L. Murray: Global and regional burden of disease and risk factors: systematic analysis of population health data. *Lancet*, 367(9524), 1747-1757 (2001)

31. L. D. Adams, R. L. Geary, B. McManus and S. M. Schwartz: A Comparison of Aorta and Vena Cava Medial Message Expression by cDNA Array Analysis Identifies a Set of 68 Consistently Differentially Expressed Genes, All in Aortic Media. *Circ Res*, 87(7), 623-631 (2000)

32. C. Mittmann, C. H. Chung, G. Hoppner, C. Michalek, M. Nose, C. Schuler, A. Schuh, T. Eschenhagen, J. Weil, B. Pieske, S. Hirt and T. Wieland: Expression of ten RGS proteins in human myocardium: functional characterization of an upregulation of RGS4 in heart failure. *Cardiovasc Res*, 55(4), 778-786 (2002)

33. H. Cho, K. Harrison, O. Schwartz and J. H. Kehrl: The aorta and heart differentially express RGS (regulators of G-protein signalling) proteins that selectively regulate sphingosine 1-phosphate, angiotensin II and endothelin-1 signalling. *Biochem. J.*, 371(3), 973-980 (2003)

34. J. Hao, C. Michalek, W. Zhang, M. Zhu, X. Xu and U. Mende: Regulation of cardiomyocyte signaling by RGS proteins: Differential selectivity towards G proteins and susceptibility to regulation. *Journal of Molecular and Cellular Cardiology*, 41(1), 51-61 (2006)

35. S. Zhang, N. Watson, J. Zahner, J. N. Rottman, K. J. Blumer and A. J. Muslin: RGS3 and RGS4 are GTPase Activating Proteins in the Heart. *J Mol Cell Cardiol*, 30(2), 269-276 (1998)

36. S. P. Heximer, S. P. Srinivasa, L. S. Bernstein, J. L. Bernard, M. E. Linder, J. R. Hepler and K. J. Blumer: G Protein Selectivity Is a Determinant of RGS2 Function. *J. Biol. Chem.*, 274(48), 34253-34259 (1999)

37. S. P. Heximer, N. Watson, M. E. Linder, K. J. Blumer and J. R. Hepler: RGS2/G0S8 is a selective inhibitor of Gqα function. *Proc Natl Acad Sci USA*, 94(26), 14389-14393 (1997)

38. A. Semplicini, L. Lenzini, M. Sartori, I. Papparella, L. A. Calo, E. Pagnin, G. Strapazzon, C. Benna, R. Costa, A. Avogaro, G. Ceolotto and A. C. Pessina: Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II. *J Hypertens*, 24(6), 1115-24 (2006)

39. J. Yang, K. Kamide, Y. Kokubo, S. Takiuchi, C. Tanaka, M. Banno, Y. Miwa, M. Yoshii, T. Horio, A. Okayama, H. Tomoike, Y. Kawano and T. Miyata: Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens*, 23(8), 1497-505 (2005)

40. L. A. Calo, E. Pagnin, P. A. Davis, M. Sartori, G. Ceolotto, A. C. Pessina and A. Semplicini: Increased Expression of Regulator of G Protein Signaling-2 (RGS-2) in Bartter's/Gitelman's Syndrome. A Role in the Control of Vascular Tone and Implication for Hypertension. *J Clin Endocrinol Metab*, 89(8), 4153-4157 (2004)

41. S. P. Heximer, R. H. Knutsen, X. Sun, K. M. Kaltenbronn, M. H. Rhee, N. Peng, A. Oliveira-dos-Santos, J. M. Penninger, A. J. Muslin, T. H. Steinberg, J. M. Wyss, R. P. Mecham and K. J. Blumer: Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. *J Clin Invest*, 111(4), 445-52 (2003)

42. K. M. Tang, G. R. Wang, P. Lu, R. H. Karas, M. Aronovitz, S. P. Heximer, K. M. Kaltenbronn, K. J. Blumer, D. P. Siderovski, Y. Zhu and M. E. Mendelsohn: Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. *Nat Med*, 9(12), 1506-12 (2003)

43. E. Takimoto, N. Koitabashi, S. Hsu, E. A. Ketner, M. Zhang, T. Nagayama, D. Bedja, K. L. Gabrielson, R. Blanton, D. P. Siderovski, M. E. Mendelsohn and D. A. Kass: Regulator of G protein signaling 2 mediates cardiac compensation to pressure overload and antihypertrophic effects of PDE5 inhibition in mice. *J Clin Invest*, 119(2), 408-20 (2009)

44. W. Zhang, T. Anger, J. Su, J. Hao, X. Xu, M. Zhu, A. Gach, L. Cui, R. Liao and U. Mende: Selective Loss of Fine Tuning of Gq/11 Signaling by RGS2 Protein Exacerbates Cardiomyocyte Hypertrophy. *J. Biol. Chem.*, 281(9), 5811-5820 (2006)

45. M. X. Zou, A. A. Roy, Q. Zhao, L. A. Kirshenbaum, M. Karmazyn and P. Chidiac: RGS2 is upregulated by and attenuates the hypertrophic effect of [alpha]1-adrenergic activation in cultured ventricular myocytes. *Cell Signal*, 18(10), 1655-1663 (2006)

46. W. B. Kannel: Incidence and Epidemiology of Heart Failure. *Heart Fail Rev*, 5(2), 167-173 (2000)

47. S. L. Grant, B. Lassegue, K. K. Griendling, M. Ushio-Fukai, P. R. Lyons and R. W. Alexander: Specific Regulation of RGS2 Messenger RNA by Angiotensin II in Cultured Vascular Smooth Muscle Cells. *Mol Pharmacol*, 57(3), 460-467 (2000)

48. E. L. Riddle, B. K. Rana, K. K. Murthy, F. Rao, E. Eskin, D. T. O'Connor and P. A. Insel: Polymorphisms and Haplotypes of the Regulator of G Protein Signaling-2 Gene in Normotensives and Hypertensives. *Hypertension*, 47(3), 415-420 (2006)

49. M. Lelonek, T. Pietrucha, M. Matyjaszczyk and J. H. Goch: Polymorphism C1114G of Gene Encoding the Cardiac Regulator of G-Protein Signaling 2 May Be Associated with Number of Episodes of Neurally Mediated Syncope. *Archives of Medical Research*, 40(3), 191-195 (2009)

50. X. Sun, K. M. Kaltenbronn, T. H. Steinberg and K. J. Blumer: RGS2 Is a Mediator of Nitric Oxide Action on Blood Pressure and Vasoconstrictor Signaling. *Mol Pharmacol*, 67(3), 631-639 (2005)

51. L. A. Calo, E. Pagnin, G. Ceolotto, P. A. Davis, S. Schiavo, I. Papparella, A. Semplicini and A. C. Pessina: Silencing regulator of G protein signaling-2 (RGS-2) increases angiotensin II signaling: insights into hypertension from findings in Bartter's/Gitelman's syndromes. *J Hypertens*, 26(5), 938-45 (2008)

52. A. Wirth, Z. Benyo, M. Lukasova, B. Leutgeb, N. Wettschureck, S. Gorbey, P. Orsy, B. Horvath, C. Maser-Gluth, E. Greiner, B. Lemmer, G. Schutz, S. Gutkind and S. Offermanns: G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med*, 14(1), 64-68 (2008)

53. W. Grossman, D. Jones and L. P. McLaurin: Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest*, 56(1), 56-64 (1975)

54. A. Diwan and G. W. Dorn, II: Decompensation of Cardiac Hypertrophy: Cellular Mechanisms and Novel Therapeutic Targets. *Physiology*, 22(1), 56-64 (2007)

55. F. H. Messerli and T. Grodzicki: Hypertension, left ventricular hypertrophy, ventricular arrhythmias and sudden death. *Eur Heart J*, 13, 66-9 (1992)

56. B. H. Lorell: Transition from hypertrophy to failure. *Circulation*, 96(11), 3824-7 (1997)

57. U. Mende, A. Kagen, A. Cohen, J. Aramburu, F. J. Schoen and E. J. Neer: Transient cardiac expression of constitutively active Gαq leads to hypertrophy and dilated cardiomyopathy by calcineurin-dependent and independent pathways. *Proc Natl Acad of Sci USA*, 95(23), 13893-13898 (1998)

58. J. R. Keys, E. A. Greene, W. J. Koch and A. D. Eckhart: Gq-Coupled Receptor Agonists Mediate Cardiac Hypertrophy Via the Vasculature. *Hypertension*, 40(5), 660-666 (2002)

59. G. Esposito, A. Rapacciuolo, S. V. Naga Prasad and H. A. Rockman: Cardiac hypertrophy: Role of G protein-coupled receptors. *J Card Fail*, 8(6, Part 2), S409-S414 (2002)

60. G. W. Dorn II, H. S. Hahn: Genetic Factors in Cardiac Hypertrophy. *Ann N Y Acad Sci*, 1015, 225-237 (2004)

61. C. Hague, L. S. Bernstein, S. Ramineni, Z. Chen, K. P. Minneman and J. R. Hepler: Selective Inhibition of {alpha}1A-Adrenergic Receptor Signaling by RGS2

Association with the Receptor Third Intracellular Loop. *J. Biol. Chem.*, 280(29), 27289-27295 (2005)

62. J. D. Molkentin, J.R. Lu, C. L. Antos, B. Markham, J. Richardson, J. Robbins, S. R. Grant and E. N. Olson: A Calcineurin-Dependent Transcriptional Pathway for Cardiac Hypertrophy. *Cell*, 93(2), 215-228 (1998)

63. P. Tamirisa, K. J. Blumer and A. J. Muslin: RGS4 Inhibits G-Protein Signaling in Cardiomyocytes. *Circulation*, 99(3), 441-447 (1999)

64. J. H. Rogers, A. Tsirka, A. Kovacs, K. J. Blumer, G. W. Dorn and A. J. Muslin: RGS4 Reduces Contractile Dysfunction and Hypertrophic Gene Induction in Gαq Over-expressing Mice. *J Mol Cell Cardiol*, 33(2), 209-218 (2001)

65. T. Tokudome, I. Kishimoto, T. Horio, Y. Arai, D. O. Schwenke, J. Hino, I. Okano, Y. Kawano, M. Kohno, M. Miyazato, K. Nakao and K. Kangawa: Regulator of G-Protein Signaling Subtype 4 Mediates Antihypertrophic Effect of Locally Secreted Natriuretic Peptides in the Heart. *Circulation*, 117(18), 2329-2339 (2008)

66. K. Tojo, S. Sato, G. Tokudome, M. Ohta, Y. Kawaguchi, O. Sakai, O. Nakagawa and K. Nakao: Stimulation by Corticotropin-Releasing Factor of Atrial Natriuretic Peptide and Brain Natriuretic Peptide Secretions from Cultured Neonatal Rat Cardiomyocytes. *Biochem Biophys Res Commun*, 225(2), 340-346 (1996)

67. J. H. Rogers, P. Tamirisa, A. Kovacs, C. Weinheimer, M. Courtois, K. J. Blumer, D. P. Kelly and A. J. Muslin: RGS4 causes increased mortality and reduced cardiac hypertrophy in response to pressure overload. *J Clin Invest*, 104(5), 567-76 (1999)

68. W. B. Kannel, W. P. Castelli, P. M. McNamara, P. A. McKee and M. Feinleib: Role of blood pressure in the development of congestive heart failure. The Framingham study. *N Engl J Med*, 287(16), 781-7 (1972)

69. G. Esposito, A. Rapacciuolo, S. V. Naga Prasad, H. Takaoka, S. A. Thomas, W. J. Koch and H. A. Rockman: Genetic Alterations That Inhibit *In vivo* Pressure-Overload Hypertrophy Prevent Cardiac Dysfunction Despite Increased Wall Stress. *Circulation*, 105(1), 85-92 (2002)

70. S. V. Prasad, J. Nienaber and H. A. Rockman: G-protein-coupled receptor function in heart failure. *Cold Spring Harb Symp Quant Biol*, 67, 439-44 (2002)

71. R. P. Xiao, S. J. Zhang, K. Chakir, P. Avdonin, W. Z. Zhu, R. A. Bond, C. W. Balke, E. G. Lakatta and H. P. Cheng: Enhanced Gi signaling selectively negates β2-adrenergic receptor (AR)- but not β1-AR-mediated positive inotropic effect in myocytes from failing rat hearts. *Circulation*, 108(13), 1633-1639 (2003)

72. A. A. Roy, A. Baragli, L. S. Bernstein, J. R. Hepler, T. E. Hebert and P. Chidiac: RGS2 interacts with Gs and adenylyl cyclase in living cells. *Cell Signal*, 18(3), 336-348 (2006)

73. V. J. Owen, P. B. J. Burton, A. J. Mullen, E. J. Birks, P. Barton and M. H. Yacoub: Expression of RGS3, RGS4 and Gi alpha 2 in acutely failing donor hearts and end-stage heart failure. *Eur Heart J*, 22(12), 1015-1020 (2001)

74. Y. Takeishi, T. Jalili, B. D. Hoit, D. L. Kirkpatrick, L. E. Wagoner, W. T. Abraham and R. A. Walsh: Alterations in Ca2+ cycling proteins and G?q signaling after left ventricular assist device support in failing human hearts. *Cardiovasc Res*, 45(4), 883-888 (2000)

75. Y. Li, S. Hashim and M. B. Anand-Srivastava: Angiotensin II-evoked enhanced expression of RGS2 attenuates Gi-mediated adenylyl cyclase signaling in A10 cells. *Cardiovasc Res,* 66(3), 503-511 (2005)

76. P. A. Insel and R. S. Ostrom: Forskolin as a Tool for Examining Adenylyl Cyclase Expression, Regulation, and G Protein Signaling. *Cell Mol Neurobiol*, 23, 305-314 (2003)

77. D. J. Pepperl, S. Shah-Basu, D. VanLeeuwen, J. G. Granneman and R. G. MacKenzie: Regulation of RGS mRNAs by cAMP in PC12 Cells. *Biochem Biophys Res Commun*, 243(1), 52-55 (1998)

78. P. Osei-Owusu, X. Sun, R. M. Drenan, T. H. Steinberg and K. J. Blumer: Regulation of RGS2 and Second Messenger Signaling in Vascular Smooth Muscle Cells by cGMP-dependent Protein Kinase. *J. Biol. Chem.*, 282(43), 31656-31665 (2007)

79. L. Kalinowski, I. T. Dobrucki and T. Malinski: Race-Specific Differences in Endothelial Function: Predisposition of African Americans to Vascular Diseases. *Circulation*, 109(21), 2511-2517 (2004)

80. T. Munzel, S. Genth-Zotz and U. Hink: Targeting Heme-Oxidized Soluble Guanylate Cyclase: Solution for All Cardiorenal Problems in Heart Failure? *Hypertension*, 49(5), 974-976 (2007)

81. T. Malinski: Understanding Nitric Oxide Physiology in the Heart: A Nanomedical Approach. *Am J Cardiol*, 96(7, Supplement 2), 13-24 (2005)

82. M. Dupuis, F. Soubrier, I. Brocheriou, S. Raoux, M. Haloui, L. Louedec, J.-B. Michel and S. Nadaud: Profiling of Aortic Smooth Muscle Cell Gene Expression in Response to Chronic Inhibition of Nitric Oxide Synthase in Rats. *Circulation*, 110(7), 867-873 (2004)

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