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RGS Proteins and Cardiovascular Angiotensin II Signaling: Novel Opportunities For Therapeutic Targeting

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Abstract

Angiotensin II (AngII), as an octapeptide hormone normally ionized at physiological pH, cannot cross cell membranes and thus, relies on, two (mainly) G protein-coupled receptor (GPCR) types, AT_1R and AT_2R, to exert its intracellular effects in various organ systems including the cardiovascular one. Although a lot remains to be elucidated about the signaling of the AT_2R, AT_1R signaling is known to be remarkably versatile, mobilizing a variety of G protein-dependent and independent signal transduction pathways inside cells to produce a biological outcome. Cardiac AT_1R signaling leads to hypertrophy, adverse remodeling, fibrosis, while vascular AT_1R signaling raises blood pressure via vasoconstriction, but also elicits hypertrophic, vascular growth/proliferation, and pathological remodeling sets of events. In addition, adrenal AT_1R is the major physiological stimulus (alongside hyperkalemia) for secretion of aldosterone, a mineralocorticoid hormone that contributes to hypertension, electrolyte abnormalities, and to pathological remodeling of the failing heart. Regulator of G protein Signaling (RGS) proteins, discovered about 25 years ago as GTPase-activating proteins (GAPs) for the Gα subunits of heterotrimeric G proteins, play a central role in silencing G protein signaling from a plethora of GPCRs, including the AngII receptors. Given the importance of AngII and its receptors, but also of several RGS proteins, in cardiovascular homeostasis, the physiological and pathological significance of RGS protein-mediated modulation of cardiovascular AngII signaling comes as no surprise. In the present review, we provide an overview of the current literature on the

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All authors performed literature research and contributed to the writing of the manuscript and the drawing of the figures. A.L. supervised the project, led the writing of, and edited the manuscript.

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involvement of RGS proteins in cardiovascular AngII signaling, by discussing their roles in cardiac (cardiomyocyte and cardiofibroblast), vascular (smooth muscle and endothelial cell), and adrenal (medulla and cortex) AngII signaling, separately. Along the way, we also highlight the therapeutic potential of enhancement of, or, in some cases, inhibition of each RGS protein involved in AngII signaling in each one of these cell types.

Keywords

Adrenal gland; Angiotensin II receptor; Cardiac hypertrophy; G protein-coupled receptor; Hypertension; Regulator of G protein Signaling protein; Signal transduction; Vascular remodeling

1. Introduction

G protein-coupled receptors (GPCRs) represent the most populous and versatile cell membrane receptor superfamily, playing crucial roles in regulation of circulatory physiology and cardiac neurohormonal modulation [1-4]. Both types of angiotensin II (AngII) receptors, AT_1R and AT_2R, belong to this superfamily with the AT_1R primarily coupling to Gα subunits of $G_{q/11}$ subfamily of guanine nucleotide-binding proteins (G proteins) [5]. In contrast, the signaling pathways the AT_2R elicits remain a matter of intense research debate with several studies arguing for lack of G protein activation or at least a very low intrinsic efficacy of this receptor type at doing so [6]. Nevertheless, AT_2R promotes nitric oxide production and is well documented to counteract inflammation, which is part of the physiological role it demonstrates in various tissues as a counter-balancing receptor to the actions of its AT_1R counterpart. [6,7]. Notably, the AT_1R couples also to Gα subunits of other G protein types, like $G_{i/o}$and G_s, in a cell/tissue type-specific manner, mediates signaling through the free Gβγ subunits of activated heterotrimeric G proteins, through monomeric (small) G protein-dependent pathways activated by $G_{12/13}$ proteins, through crosstalk with receptor tyrosine kinases, and, last but not least, signals also through the universal GPCR adapter proteins β-arrestin1 and −2 (Arrestin-2 and −3, respectively) [4,5,8-10]. AngII-stimulated AT_1R regulates several cardiovascular physiological processes, including vascular smooth muscle contraction and blood pressure (and through that, cardiac afterload), cardiac hypertrophy, fibrosis, apoptosis, oxidative stress, and adverse remodeling in general, and it also promotes norepinephrine release from sympathetic neurons and aldosterone synthesis and secretion from the adrenal cortex, thereby exerting a huge impact on the neurohormonal control of cardiac function [3-5]. Therefore, the AT_1R represents a major therapeutic target in cardiovascular pharmacology, directly targeted by angiotensin receptor blocker (ARB) drugs, also known as AT_1R antagonists or sartans, and indirectly by angiotensin converting enzyme (ACE) inhibitors and renin inhibitors [5,11]. In contrast, AT_2R ligands are still in development or in clinical trials for various clinical indications, including cardiovascular ones, but none has reached the market or clinical practice as of yet [12,13].

Like all GPCRs, AT_1R acts as a guanine nucleotide exchange factor (GEF) for the Gα subunit of a heterotri meric G protein, in essence separating the Gα subunit from its bound Gβγ dimer thanks to the exchange of guanosine triphosphate (GTP) for guanosine

diphosphate (GDP) on the Gα subunit. Bound to the Gβγ dimer, Gα (and the whole heterotrimer) are inactive, so this Gα subunit "liberation" from Gβγ activates the G protein, allowing Gα (but also Gβγ) to activate or inhibit effectors (e.g., enzymes and ion channels). Nevertheless, regulation of the duration of a GPCR signal is of paramount importance for cellular homeostasis and two major processes at the cell membrane operate to turn off the receptor signal: one is GPCR phosphorylation by GPCR-kinases (GRKs), followed by arrestin binding (homologous or agonist-dependent receptor desensitization) [14]. Other kinases, such as second messenger-dependent kinases, can also terminate the signal of a GPCR (heterologous or agonist-independent receptor desensitization) [14]. The other process, at the level of the active G protein itself, is GTP hydrolysis to GDP by the intrinsic GTPase activity of the Gα subunit [15]. Via GTP conversion to GDP, GDP-bound Gα now regains its affinity for Gβγ and the G protein heterotrimer re-assembles in its inactive state [15]. Unlike the monomeric Ras-like G proteins, which lack intrinsic GTPase activity, Gα subunits of heterotrimeric G proteins do not necessarily need GTPase-activating proteins (GAPs) to hydrolyze GTP [15]. Nevertheless, most Gα subunits (Gαs being a notable exception) also need and do utilize GAPs to get inactivated in time frames fast enough to be compatible with normal cellular function and homeostasis. These GAPs are called "Regulator of G protein signaling (RGS)" proteins, because they all contain a conserved ~120-amino acid-long domain (the "RGS box") that binds the Gα subunit and dramatically accelerates GTP hydrolysis by stabilizing the transition state of this hydrolysis [16-22]. More than 30 different RGS proteins have been identified in mammals, while several other proteins contain non-functional "RGS homology" domains, as well [21,22]. For a comprehensive overview of the RGS protein superfamily, the reader is referred to several other authoritative reviews [17-22]. Interestingly, some RGS proteins, such as RGS4 and RGS2, also interfere with the interaction of active (GTP-bound) Gα subunits or of free Gβγ dimers with downstream effectors, while some others interact directly with GPCRs [16]. By acting as GTPase-activating proteins (GAPs) on Gα subunits, RGS proteins also accelerate free Gβγ signaling termination, since the heterotrimer re-assembles [16,17]. The initial theory that there might be one RGS protein for each Gα subunit in the cell (16 Gα subunits are encoded in the human genome) was quickly dismissed because Now know that, not only do the RGS proteins outnumber the Gα subunits, but also several of them can act upon more than one Gα type/family (e.g., RGS4 inactivates both $G\alpha_{i/o}$ and $G\alpha_{q/11}$ subunits) [17]. Importantly, most (if not all) RGS proteins inactivate their G protein substrates in a cell type- and GPCR type-specific manner, with the identity of the receptor that has stimulated the G protein dictating whether that G protein will be inactivated by which RGS protein. For example, RGS4 inactivates angiotensin II type 1 receptor (AT_1R)-stimulated Gαq but not gonadotropin-releasing hormone (GnRH) receptor-stimulated Gαq subunits [23,24]. This is extremely important pharmacologically, since it endows RGS proteins with remarkable specificity for the receptor-G protein signaling pathways they act upon in each cell, which can be exploited for therapeutic purposes.

In this review, we provide an overview of the current literature on RGS protein-dependent regulation of AngII receptor (essentially AT_1R, since nothing has been reported on AT_2R signaling and RGS proteins), signaling in the cardiovascular system, i.e., in cardiomyocytes, in cardiac fibroblasts, and in vascular endothelial and smooth muscle cells, as well as in

adrenal gland cells (of both adrenal medulla and cortex), all of which are cell types that express AT_1Rs and thus, respond to AngII in a physiologically relevant and important manner. We also highlight the potential of pharmacologically targeting certain RGS proteins for therapeutic purposes based on their roles in AT_1R signaling in specific cardiovascular cell/tissue types.

2. RGS proteins in cardiac AngII signaling

Along with α_1-adrenergic receptors (ARs) and endothelin (ET)-1 receptors, AT_1Rs present in cardiomyocyte membranes are among the most potent pro-hypertrophic signal-mediating GPCRs [2,25]. This is thanks to $G_{q/11}$ protein activation, which, in turn activates phospholipase C (PLC)-β, thereby initiating the classic signaling pathway of the second messengers diacylglycerol (DAG) and inositol 1`, 4`, 5`-trisphosphate (IP_3), which elicit protein kinase C (PKC) activation and intracellular free [Ca^{2+}] elevation (Figure 1) [2,5,25]. Unlike in vascular smooth muscle cells (VSMCs) though (see following section below), where free cytoplasmic [Ca^{2+}] elevation leads to contraction via Ca^{2+}/calmodulin (CaM)-bound myosin light chain kinase (MLCK)-dependent phosphorylation of myosin [26], AT_1R and $G_{q/11}$ protein-coupled receptor-induced cytoplasmic [Ca^{2+}] elevation in cardiomyocytes leads instead to cellular proliferation, inflammation, and pathological hypertrophy/remodeling, mainly via Ca^{2+}/CaM-bound calcineurin-mediated nuclear factor of activated T cells-cytoplasmic (NFATc) activation and Ca^{2+}/CaM-dependent protein kinase II (CaMKII) stimulation [27,28]. Therefore, it follows that RGS proteins acting on $G_{q/11}$ proteins to terminate their signals can have anti-hypertrophic and anti-adverse remodeling effects in the heart. Indeed, RGS4 was among the first RGS proteins to be demonstrated to inhibit phenylephrine (α_1AR), ET1, and AngII-induced cell growth and proliferation of neonatal rat cardiomyocytes, courtesy of its GAP activity on $G\alpha_{q/11}$ (Figure 1) [29]. Subsequently, RGS2 was shown to inhibit AT_1R-dependent pro-hypertrophic signaling in adult isolated murine ventricular myocytes (Figure 1), working in concert with atrial natriuretic peptide (ANP) that activates RGS2 via cyclic guanosine monophosphate (cGMP)-dependent kinase (PKG) phosphorylation [30]. Importantly, RGS2 has been reported to inhibit AT_1R signaling also in cardiac fibroblasts, thereby attenuating AngII-dependent fibroblast activation and cardiac fibrosis (Figure 1) [31]. More specifically, fibroblast RGS2 was transiently upregulated by short term (1 day) AngII treatment but significantly downregulated by long-term (3-14 days) AngII infusion in vivo. RGS2 overexpression reduced AngII-elicited PLCβ activity, cell proliferation, and total collagen production, whereas RGS2 siRNA-mediated knockdown had the opposite effects [31]. Remarkably, none of the other RGS proteins also expressed in cardiac fibroblasts seemed capable of compensating for this role of RGS2 in cardiac fibroblast AT_1R signaling in that study [31]. Thus, RGS2 may block both the pro-hypertrophic (in cardiomyocytes) and pro-fibrotic (in cardiac fibroblasts) AT_1R-induced $G_{q/11}$ protein signaling (Figure 1), which would make it an attractive target for AngII-related therapeutics aimed at combating cardiac adverse remodeling.

In addition to RGS2 and RGS4, RGS3 and RGS10 have also been implicated in AT_1R-dependent pro-hypertrophic signaling blockade in cardiomyocytes. RGS3 overexpression in the heart markedly reduced the extent of cardiac hypertrophy, fibrosis, and left ventricular

dysfunction in response to aortic banding in mice (Figure 1) [32]. The underlying mechanism was shown to be inhibition of AT_1R-dependent pro-hypertrophic extracellular signal-regulated-kinase (ERK)1/2 signaling in cultured neonatal rat cardiomyocytes [32]. Therefore, RGS3 also inhibits AngII-dependent pro-hypertrophic and pro-fibrotic signaling in the heart. The exact same results (inhibition of AngII-dependent pro-hypertrophic signaling) with the exact same underlying mechanism (ERK inhibition) were found for cardiac RGS10, as well (Figure 1) [33]. Notably, RGS10 was found markedly downregulated in failing human hearts and in hypertrophic murine hearts [33].

In contrast, two other RGS proteins abundantly expressed in cardiac myocytes, RGS6 and RGS12, seem to facilitate/mediate AT_1R-dependent pro-hypertrophic signaling in the heart (Figure 1). RGS6 is significantly upregulated in failing human hearts and in hypertrophic murine hearts [34]. Mice lacking RGS6 display reduced cardiac hypertrophy, dysfunction, and fibrosis post-aortic banding, whereas transgenic mice with cardiac-specific RGS6 overexpression exhibit exacerbated responses to pressure overload [34]. Moreover, RGS6 facilitated AngII-elicited hypertrophic responses in isolated cardiomyocytes. The mechanism for this somewhat unexpected role of RGS6 in cardiac AT_1R signaling was found to be a direct interaction of RGS6 with apoptosis signal-regulating kinase (ASK)-1, which, in turn, activates downstream p38 mitogen-activated protein kinase (MAPK) & c-Jun-N-terminal kinase (JNK) pro-apoptotic and pro-inflammatory signaling cascades [34]. Similarly, RGS12 was also found to promote cardiac dysfunction and hypertrophy post-aortic banding, as well as facilitate AngII-induced hypertrophic responses in isolated cardiomyocytes [35]. Remarkably, the authors of this study found that ERK1/2 were necessary for the pro-hypertrophic actions of RGS12, indicating that RGS12 somehow mediates, rather than inhibits, AT_1R-stimulated ERK activation in cardiomyocytes [35]. How exactly RGS12 would stimulate ERK activation remains to be elucidated.

In summary, regarding cardiac AngII-dependent signaling, RGS2, −3, −4, and −10 inhibit it, whereas RGS6 and −12 promote it (Figure 1). Thus, pharmacological potentiation of cardiac RGS2, RGS3, RGS4, or RGS10, or inhibition of cardiac RGS6 or −12 might be of therapeutic value in cardiac hypertrophy, fibrosis, and pathological (adverse) remodeling in general.

3. RGS proteins in vascular AngII signaling

Vascular AT_1R has been shown to couple to a variety of different G proteins (G_q, G_{11}, G_i, G_o, G_{12}, G_{13}, even G_s), thereby eliciting a plethora of downstream signaling pathways, including PLC, Ca^{2+} channels, phospholipase D (PLD), phospholipase A_2 (PLA_2), adenylyl cyclase (AC), MAPKs like ERKs, p38 MAPK, and JNKs, the Janus kinase (JAK)-Signal transducer and activator of transcription (STAT) pathway, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, etc. [36,37]. Through these signaling modalities, the AT_1R mediates most pathophysiological effects of Ang II in the vasculature, such as vasoconstriction/hypertension, inflammation, VSMC proliferation, and vascular fibrosis [38,39]. RGS2 was among the first RGS proteins reported to be implicated in negative regulation of VSMC AT_1R signaling (Figure 2) [40]. Indeed, AngII infusion increased blood pressure to a larger extent in RGS2 knockout (KO) mice, compared to

wild type ones, due to increased myogenic tone and vascular reactivity in response to AngII-activated AT_1R in arterial VSMCs [41]. This suggested that RGS2 attenuates the vaso-constrictive signaling of AT_1R in VSMCs. Interestingly, VSMC AT_1R is known to transcriptionally upregulate RGS2 in VSMCs, as a potential negative feedback mechanism. The mechanism seems to be activation of inducible PLA_2 ($iPLA_2$-beta) [42] and subsequent cyclic 3`, 5`-adenosine monophosphate (cAMP)-response element-binding protein (CREB) phosphorylation and activation/nuclear translocation, since (at least the murine) RGS2 gene promoter contains a conserved cAMP-response element (CRE) critical for CREB binding and RGS2 gene transcriptional activation [43]. Additionally, the AT_1R antagonists olmesartan and losartan, including losartan`s active metabolite EXP3174, abrogate AngII-mediated RGS2 upregulation in VSMCs, although this is a little complicated for losartan, which can directly stimulate RGS2 mRNA expression in an AngII/AT_1R-independent manner [44]. Finally, in a very interesting study on the role of RGS2 in AngII-induced atherosclerotic vascular damage and in the protection against it afforded by another AT_1R antagonist, telmisartan, RGS2 genetic deletion was found to significantly increase blood pressure, mortality rates, and aortic aneurysm incidence in response to AngII infusion for 4 weeks [45]. Surprisingly however, RGS2 genetic ablation facilitated telmisartan`s effects on AngII infusion-induced aneurysm incidence reduction, improving survival, reducing enlarged aortic diameter, and suppressing NADPH-mediated reactive oxygen species (ROS) production in response to AngII treatment [45]. Thus, knockout of vascular RGS2 somehow potentiates the protective effects of low dose telmisartan in the vasculature. The reason and underlying mechanism(s) for this unexpected finding remain unknown.

In addition to RGS2, human aortic smooth muscle cells express RGS1, RGS3 (short-, long-, and PDZ domain-containing forms), and RGS4, all of which attenuate AT_1R signaling [46]. RGS1 has also been shown to attenuate the pro-hypertensive effects of AngII treatment in vivo but only reduces α_1AR-dependent, not AngII-mediated, vasoconstriction in aortas of the apolipoprotein E (ApoE)-deficient mouse model of atherosclerosis [47]. In AngII-treated VSMCs, RGS1 only suppressed ERK activation, without affecting JNK or p38 MAPK signaling induced by the AT_1R [47]. Of note, ERKs are known to phosphorylate and inhibit GPCR-kinase-2 (GRK2) [48], a kinase that critically regulates GPCR-induced arterial vasoconstriction [49]. Thus, RGS1 might regulate AngII-dependent vascular tone and blood pressure via inhibition of free $G\beta\gamma$-activated ERK repression of GRK2 desensitizing activity in VSMCs (Figure 2). RGS4 also suppresses ERK1/2 activation as well as vascular endothelial growth factor (VEGF) stimulation of DNA synthesis and p38 MAPK activation induced by AngII in lung epithelial tubulating cells [50]. Moreover, in renal VSMCs, RGS4 suppresses AngII-induced secretion of the macrophage chemoattractant RANTES (regulated on activation, normal T cell expressed and secreted; also known as chemokine (C-C motif) ligand 5, CCL5) [51]. Interestingly, this process is mediated by the AT_2R, rather than the AT_1R, and decreases macrophage density post-injury in VSMCs [51]. Thus, RGS4 inhibits AngII-dependent cytokine signaling and macrophage recruitment during reperfusion specifically in renal VSMCs, without affecting vascular tone (Figure 2) [51].

Finally, RGS5 has been well documented as a negative regulator of AngII-dependent vasoconstriction (Figure 2) in intact arterioles [52], in VSMCs in vitro [53], and in murine mesenteric arteries [54]. In the latter study, the gene promoter of RGS5 was found to

be a peroxisome proliferator-activated receptor (PPAR)γ and -δ transcriptional target in VSMCs, and RGS5 gene expression was shown to mitigate AT_1R-induced PKC activation preserving large conductance calcium-activated potassium (BKCa) channel activity, which tightly controls myogenic tone in the microcirculation [54]. RGS5 gene promoter is a transcriptional target also of PPARβ and, in mice treated with a PPARβ-specific agonist (GW0742), RGS5 upregulation in brain and blood vessels exerted antihypertensive effects, restored sympathetic tone (circulating norepinephrine levels), and improved vascular structure and function upon AngII infusion [55]. Importantly, Holobotovskyy and colleagues demonstrated the essential role RGS5 plays in prevention of AngII-dependent hypertension [56]. In this study, arterial RGS5 expression went down with chronically elevated blood pressure after AngII treatment resulting in profound hypertension, medial hypertrophy, and vascular fibrosis [56]. Mechanistically, RGS5 was found to inhibit PKC activation, which reduced activity of its downstream targets ERK and Ras homology small GTPase (Rho)-associated protein kinase (ROCK) in response to AngII-activated AT_1R in VSMCs [56]. This resulted in enhanced myosin light chain phosphatase (MLCP) activity and reduced MLCK activity, i.e., decreased overall myosin light chain phosphorylation, and hence, lower vascular tone [56]. Thus, RGS5 controls hyper-responsiveness to vasoconstrictors and vascular stiffening and plays a crucial role in modulation of AngII-dependent vascular homeostasis.

In summary, regarding vascular AngII-dependent signaling, RGS1, −2, −3, −4, and −5 all inhibit it, exerting protective effects against hypertension development and vascular adverse remodeling (Figure 2). RGS5 potentiation, which appears feasible with PPAR agonist drugs aiming at treating metabolic disorders, seems the most promising therapeutic strategy against AngII-dependent vascular pathologies (hypertension), followed by RGS2 stimulation, although the latter`s effects in telmisartan-treated animals are somewhat puzzling. RGS4 potentiation seems likely to be particularly effective in renal vasculature only, whereas RGS1- and RGS3-targeted approaches require further research/studies before their potential for hypertension or atherosclerosis treatment can be gauged.

4. RGS proteins in adrenal AngII signaling

RGS proteins, specifically RGS4 and RGS2, have been shown to play major roles in adrenal AT_1R signaling, as well. As a $G_{q/11}$-coupled receptor that increases free intracellular Ca^{2+} levels, AT_1R acts as a secretagogue receptor promoting catecholamine (epinephrine and norepinephrine) secretion from the chromaffin cells of the adrenal medulla but also aldosterone secretion from adrenocortical zona glomerulosa (AZG) cells, via Ca^{2+}-dependent exocytosis [57-60]. Interestingly, AT_1R-dependent Ca^{2+}-dependent exocytosis leading to catecholamine secretion can be mediated not only by G proteins but also by β-arrestin1, a GPCR adapter protein and signal transducer, via a direct interaction of β-arrestin1 with the Ca^{2+} channel TRPC3 (short transient receptor potential channel-3) in adrenal chromaffin cells [61]. Notably, RGS4 is robustly expressed in the adrenal medulla, as well as in the adrenal cortex, and has been reported to inhibit catecholamine secretion from adrenal chromaffin cells [60]. The underlying mechanism is mainly through termination of the $G_{q/11}$ protein signaling of muscarinic cholinergic receptors responding to the physiological stimulus of adrenal catecholamine secretion: acetylcholine [62]. However,

RGS4-mediated termination of AT_1R signaling in chromaffin cells could also contribute to this sympatholytic effect of adrenal RGS4 (Figure 3).

Nevertheless, the main physiological role of adrenal AT_1R is manifested in AZG cells, wherein AngII induces synthesis and secretion of aldosterone via AT_1R activation [57]. AT_1R activation elicits downstream $G_{q/11}$ protein/PLC/Ca^{2+} and β-arrestin1-dependent signaling pathways, both of which result in elevated aldosterone production and secretion [63-66]. The G_q-dependent signaling pathway elicited by the AT_1R in AZG cells and leading to aldosterone production is well documented [63,64]. The second messengers DAG and IP_3, produced by phospholipase C (PLC)-β directly activated by Gαq subunits (but also by Gi/o-derived free Gβγ subunits) [67], ultimately lead to aldosterone secretion, via classic intracellular Ca^{2+} release-triggered exocytosis, and to aldosterone biosynthesis, via ERK activation, which, in turn, stimulate the gene transcription of StAR (steroidogenic acute regulatory) protein [57]. StAR is responsible for mitochondrial uptake of the adrenal steroid precursor cholesterol, which is the rate-limiting step in aldosterone biosynthesis inside AZG cells [68,69]. ERK-induced StAR upregulation is also conferred by β-arrestin1, independently of G proteins [68,69]. Adrenal RGS4 has been shown to get upregulated by low-salt diet and AngII infusion in vivo [70]. In addition, in the human AZG cell line H295R, AngII causes a rapid and transient increase in RGS4 mRNA levels via the CaMK and PKC kinases [70]. Importantly, RGS4 decreases AngII-stimulated aldosterone synthesis and secretion, possibly via downregulation of aldosterone synthase (CYP11B2) expression in these cells [70]. Thus, RGS4 mediates a negative feedback loop in AngII-induced aldosterone production in AZG cells, during which it gets upregulated by the AT_1R in order to inhibit further signaling by this receptor towards enhanced aldosterone synthesis and secretion (Figure 3). Interestingly, identical results have been reported by the same group of investigators for adrenal RGS2, as well [71]. RGS2 was found upregulated by AngII in H295R AZG cells and this upregulated RGS2 in turn reduced AngII-dependent aldosterone synthesis and secretion (Figure 3) [71]. However, it should be emphasized here that these studies were done in AZG cells in vitro only. Whether RGS4 or RGS2 regulate adrenocortical aldosterone secretion in vivo remains to be confirmed. In addition, the precise molecular mechanisms underlying their effects on aldosterone synthase and aldosterone secretion await elucidation in future studies. In any case, the aldosterone-suppressive effect of RGS4 in vitro, coupled with its sympatholytic action in the chromaffin cells of the adrenal medulla in vivo (see above), strongly suggests that adrenal RGS4 (and potentially also adrenal RGS2) plays a central role in regulation of adrenal catecholamine and aldosterone production, both of which go awry in chronic heart failure and in other cardiovascular diseases, leading to enhanced morbidity and mortality due to the elevated circulating levels of these hormones [72-75]. Therefore, adrenal RGS4 could represent a novel molecular target in the design of AngII-related pharmacotherapies for the treatment of chronic heart failure, hypertension, and other heart diseases.

In summary, RGS4 (and to a lesser extent RGS2) dramatically suppresses adrenal AngII signaling towards aldosterone (but also catecholamine) production and secretion. Thus, RGS4 (or RGS2) stimulation to inhibit adrenal AngII signaling could be of therapeutic value in hyperaldosteronism and in diseases characterized by excessive aldosterone levels and sympathetic nervous system activity, such as chronic heart failure, hypertension, etc.

5. Conclusions/ Future Perspectives

Despite enormous progress in the field of RGS protein-dependent regulation of AngII receptor signal transduction in the cardiovascular system, there is still a lot of work to be done and a great deal of unknown parameters. The vast plethora (over 30 members) of RGS proteins expressed in cardiac and vascular tissues argues for redundancy of several members or for the action of one isoform being compensated for by another. On the other hand, data from individual isoform knockout mice but also from in vitro cell studies are quite reassuring that no such redundancy exists, because the action of each RGS protein isoform on a particular type of G protein is highly cell type- and receptor type-specific. In other words, a certain RGS protein may suppress the same G protein activated by one GPCR but not others or it may suppress the same G protein activated by the same receptor in one cell type but not others. This essentially means that different RGS proteins affect the same signaling pathway, e.g., AT_1R-elicited Gq protein signaling, in different ways, depending on the cell type and study conditions (e.g., levels of AT_1R expressed). In addition, most RGS proteins do not only act as GAPs for Gα subunits but they also terminate free Gβγ signaling (by inducing re-association of the G protein heterotrimer) and directly modulate the activity of certain GPCRs and second messenger-generating G protein effectors (as reviewed in [16,17,22]). Taken together, not all RGS proteins that can modulate the signaling of a specific G protein activated by a specific receptor in any given tissue necessarily modulate that signaling in the same manner/direction (Table 1). This is probably why certain RGS proteins (e.g., RGS2, RGS4) inhibit AT_1R-elicited $G_{q/11}$ protein-dependent pro-hypertrophic signaling in cardiomyocytes but others (RGS12, RGS6) appear to promote it (Figure 1).

Another important area that needs to see some progress, if RGS protein-targeted pharmacologic therapies are ever to be developed and reach the bedside, is pharmacologic strategies for enhancing or inhibiting specific RGS proteins in cells in vivo. There are several medications or agents that have been reported to upregulate or downregulate RGS protein isoforms, such as PPAR agonists for RGS5 upregulation [55], digoxin for RGS2 upregulation [76], or the neurostimulant para-chloroamphetamine for RGS4 upregulation (inhibition of its degradation) [77]. Thanks to painstaking efforts by several labs, led by the Neubig and Mosberg groups, over the past two decades, various peptide-based and small organic compounds are in the pipeline that directly modulate the GTPase activity of certain RGS proteins, instead of manipulating RGS protein levels (synthesis or degradation) [78-82]. CCG-63802, for instance, acts as an allosteric inhibitor of RGS4, taking advantage of an allosteric (i.e., outside the RGS domain) cysteine-rich pocket of the RGS protein that normally interacts with acidic phospholipids [82]. As more and more pharmaceutical compounds and other molecular (e.g., genetic) tools are discovered or developed to modulate RGS proteins pharmacologically, the field of RGS protein-centered therapeutics is bound to flourish. The remarkable versatility of AngII signaling in the cardiovascular system offers plenty of opportunities for RGS protein-targeted therapeutic interventions awaiting to be materialized.

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Abbreviations:

AngII	Angiotensin II
AR	Adrenergic receptor
AT_1R	Angiotensin II type 1 receptor
AT_2R	Angiotensin II type 2 receptor
AZG	Adrenocortical zona glomerulosa
CaM	Calmodulin
CaMK	Calcium-calmodulin-dependent protein kinase
CREB	Cyclic adenosine monophosphate regulatory element-binding protein
DAG	1`, 2'-Diacylglycerol
ERK	Extracellular signal-regulated kinase
ETR	Endothelin receptor
GAP	GTPase-activating protein
GEF	Guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
G protein	Guanine nucleotide-binding protein
GTP	Guanosine triphosphate
IP_3	Inositol 1`, 4`, 5`-trisphosphate
MAPK	Mitogen-activated protein kinase
MLCK	Myosin light chain kinase
PKC	Protein kinase C
PL	Phospholipase
PPAR	Peroxisome proliferator-activated receptor
RGS	Regulator of G protein signaling
StAR	Steroidogenic acute regulatory protein
VSMC	Vascular smooth muscle cell

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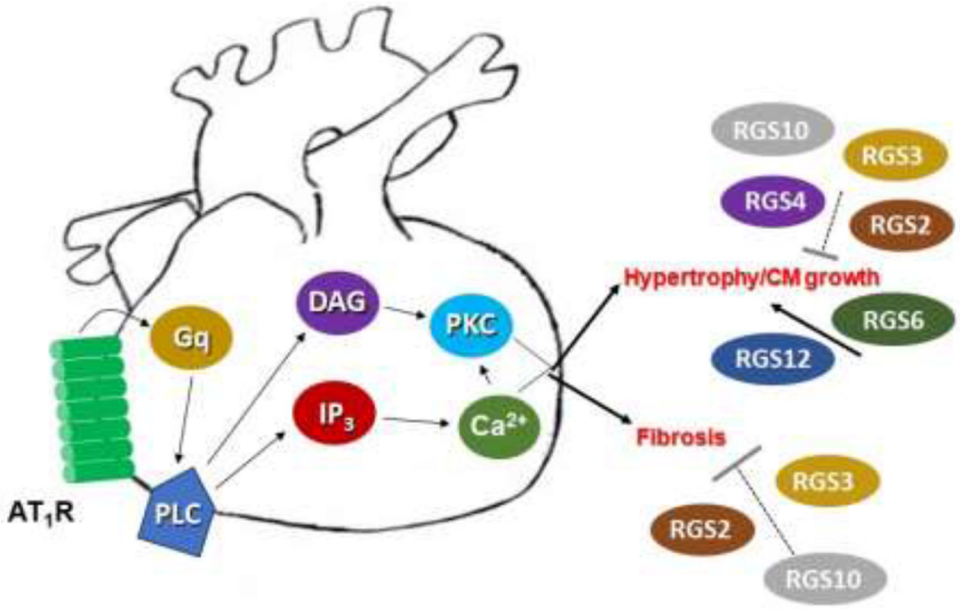

Figure 1. RGS proteins in cardiac AngII signaling modulation.

See text for details. AT_1R: Angiotensin II type 1 receptor; CM: Cardiac myocyte; PLC: Phospholipase C; DAG: Diacylglycerol; IP_3: Inositol 1`, 4`, 5`-trisphosphate; PKC: Protein kinase C. Arrows indicate stimulation, "—I" indicate inhibition.

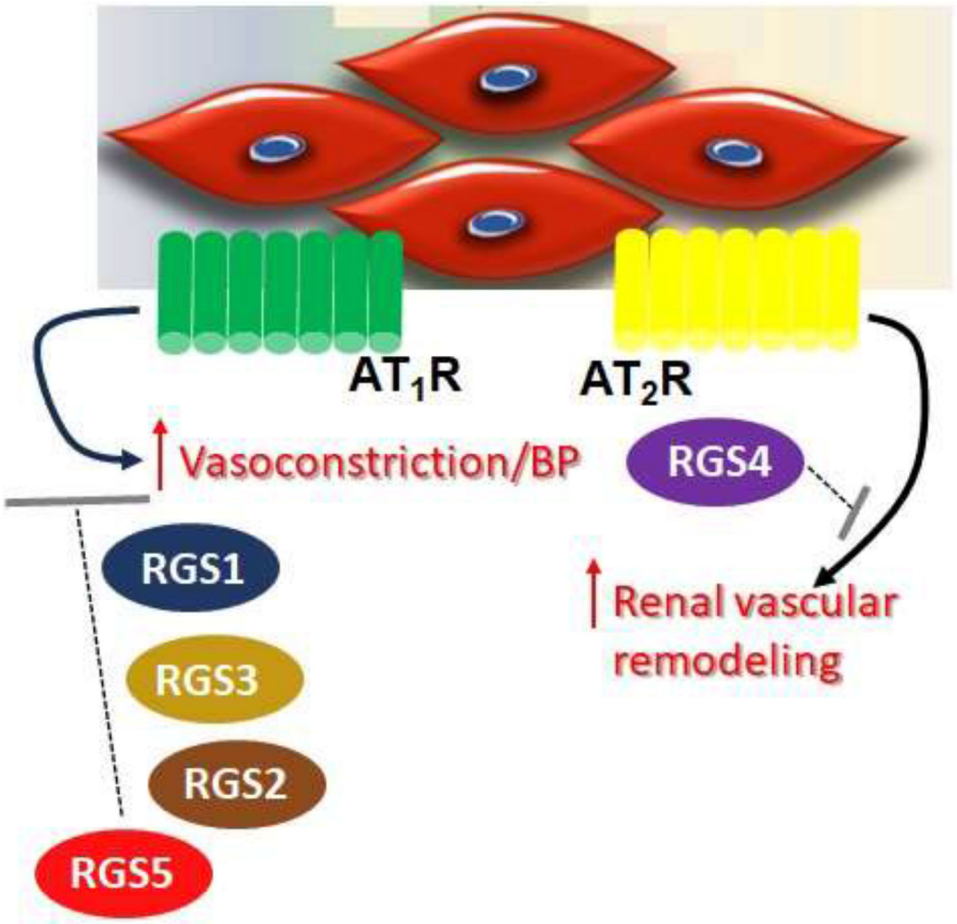

Figure 2. RGS proteins in vascular AngII signaling modulation.

See text for details. AT_1R: Angiotensin II type 1 receptor; AT_2R: Angiotensin II type 2 receptor; BP: Blood pressure; Arrows indicate stimulation, red arrows indicate increase, "—I" indicate inhibition.

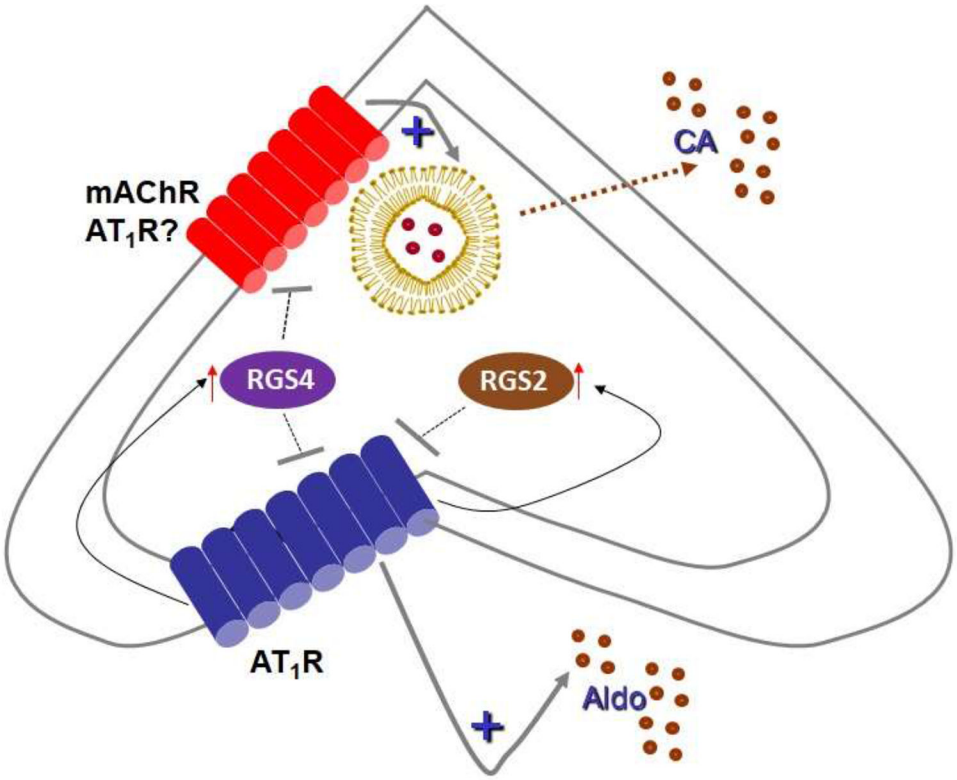

Figure 3. RGS proteins in adrenal AngII signaling modulation.

RGS4 inhibits cholinergic (and potentially AngII-dependent) catecholamine secretion from the adrenal medulla, while both RGS4 and RGS2 inhibit AngII-dependent aldosterone secretion from the adrenal cortex. AT_1R upregulates RGS4 and RGS2 in AZG cells as a negative feedback mechanism. See text for more details. mAChR: Muscarinic cholinergic receptor; Aldo: Aldosterone; CA: Catecholamine (norepinephrine or epinephrine); "?": Action on AT_1R not yet verified experimentally. Arrows indicate stimulation or increase, "—I" indicate inhibition.

Table 1:

RGS proteins and cardiovascular AngII signaling

Tissue or Cell type/ ATR type	RGS protein	Signaling mechanism	Effect in vivo	Refs.
CM AT_1R	RGS4	↓ $G_{q/11}$-Ca^{2+}-MAPK/ERK activation	↓ Cardiac hypertrophy/ remodeling	29
CM AT_1R	RGS2	↑ ANP-cGMP-PKG-dependent inhibition of $G_{q/11}$-Ca^{2+} signaling	↓ Cardiac hypertrophy/ remodeling	30
CF AT_1R	RGS2	↓ $G_{q/11}$/PLCβ-dependent collagen production	↓ Cardiac fibrosis	31
CM AT_1R	RGS3	↓ $G_{q/11}$-Ca^{2+}-MAPK/ERK activation	↓ Cardiac hypertrophy/ remodeling	32
CM AT_1R	RGS10	↓ $G_{q/11}$-Ca^{2+}-MAPK/ERK activation	↓ Cardiac hypertrophy/ remodeling	33
CM AT_1R	RGS6	↑ ASK1/p38 MAPK/JNK activation	↑ Cardiac hypertrophy/ remodeling	34
CM AT_1R	RGS12	↑ MAPK/ERK activation	↑ Cardiac hypertrophy/ remodeling	35
VSM AT_1R	RGS2	↓ $G_{q/11}$-Ca^{2+}-MAPK/ERK activation; AT_1R upregulates RGS2 via $iPLA_2$/CREB	↓ Vascular tone/BP	41-43
VSM AT_1R	RGS1	↓ Free Gβγ/ERK activation/↑ GRK2 activation (?)	↓ Vascular tone/BP	47
(Renal) VSM AT_2R	RGS4	↓ RANTES	↓ Cytokines/Macrophage recruitment/Injury-triggered inflammation	51
VSM AT_1R	RGS5	↓ PKC-ERK-ROCK activation/↓ MLC phosphorylation/↑ K^+ channel activation; PPARβ,–γ,–δ upregulate RGS5	↓ Vascular tone/BP	52-56
Chr Cell mAChR (AT_1R?)	RGS4	↓ $G_{q/11}$-PLCβ-Ca^{2+}-dependent exocytosis	↓ CA secretion	60
AZG AT_1R	RGS4	↓ AS expression/↓ $G_{q/11}$-PLCβ-Ca^{2+}-dependent exocytosis (?); AT_1R upregulates RGS4 via CaMK-PKC	↓ Aldosterone production/ secretion	70
AZG AT_1R	RGS2	↓ AS expression/↓ $G_{q/11}$-PLCβ-Ca^{2+}-dependent exocytosis (?); AT_1R upregulates RGS2 via CaMK-PKC	↓ Aldosterone production/ secretion	71

ANP: Atrial natriuretic peptide; AS: Aldosterone synthase (CYP11B2); ASK1: Apoptosis signal-regulating kinase-1; AZG: Adrenocortical zona glomerulosa; BP: Blood pressure; CA: Catecholamine; CaMK: Calcium/calmodulin-dependent protein kinase; cGMP: cyclic guanosine monophosphate; CF: Cardiac fibroblast; Chr: Chromaffin; CM: Cardiac myocyte; CREB: Cyclic adenosine monophosphate response element-binding protein; ERK: Extracellular signal-regulated protein kinase; GRK2: G protein-coupled receptor kinase-2; $iPLA_2$: Inducible phospholipase A_2; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; mAChR: Muscarinic acetylcholine receptor; MLC: Myosin light chain; PKC: Protein kinase C; PKG: Protein kinase G; PLC: Phospholipase C; PPAR: Peroxisome proliferator-activated receptor; RANTES: Regulated on activation, normal T cell expressed and secreted; ROCK: Rho-associated protein kinase; VSM: Vascular smooth muscle; (?): Effect unknown; (↓): Inhibition or suppression; (↑): Stimulation or potentiation.