

Regression of cardiovascular remodeling in hypertension: Novel relevant mechanisms

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Abstract

Asymptomatic organ damage due to progressive kidney damage, cardiac hypertrophy and remodeling put hypertensive patients at high risk for developing heart and renal failure, myocardial infarction and stroke. Current antihypertensive treatment normalizes high blood pressure, partially reverses organ damage, and reduces the incidence of heart and renal failure. Activation of the renin-angiotensin system (RAS) is a primary mechanism of progressive organ damage and, specifically, a major cause of both renal and cardiovascular fibrosis. Currently, inhibition of the RAS system [mainly with angiotensin I converting enzyme inhibitors or angiotensin II (Ang II) receptor antagonists] is the most effective antihypertensive strategy for normalizing blood pressure and preventing target organ damage. However, residual organ damage and consequently high risk for cardiovascular events and renal failure still persist. Accordingly, in hypertension, it is relevant to develop new therapeutic perspectives, beyond reducing blood pressure to further prevent/reduce target organ damage by acting on pathways that trigger and maintain cardiovascular and renal remodeling. We review here relevant novel mechanisms of target organ damage in hypertension, their role and evidence in prevention/regression of cardiovascular remodeling and their possible clinical impact as well. Specifically, we focus on the signaling pathway RhoA/Rho kinase, on the impact of the vasodilatory peptides from the RAS and some insights on the role of estrogens and myocardial chymase in cardiovascular hypertensive remodeling.

Key words: Remodeling; Hypertrophy; Rho kinase; Myosin phosphatase target subunit 1; Angiotensin; Angiotensin1-9; Chymase; Angiotensin1-7

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Core tip: Antihypertensive treatment normalizes high

blood pressure, partially reverses organ damage, and reduces the incidence of heart and renal failure. However, residual organ damage and high risk for cardiovascular events still persist. We review here novel relevant mechanisms of cardiovascular damage in hypertension, their role and evidence in prevention/regression of cardiovascular remodeling and their possible clinical impact. We focus on the signaling pathway RhoA/Rho kinase, on the impact of the vasodilatory peptides from the renin angiotensin system and some insights on the role of estrogens and myocardial chymase in cardiovascular remodeling due to hypertension.

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INTRODUCTION

Target organ damage in hypertension causing asymptomatic renal dysfunction, atrial size enlargement along with cardiac hypertrophy and remodeling place hypertensive subjects at a very high risk condition to develop cardiac failure, progressive kidney disease, myocardial infarction and stroke as well. Up-to-date antihypertensive drug therapy reduces to normality elevated arterial blood pressure, does revert organ damage to some extent, and diminishes the occurrence of cardiac and renal disease. However, the clinical impact of all antihypertensive drug classes is not substantially different among most clinical outcomes when the blood pressure effect is equivalent^[1]. Besides, permanent stimulation of the renin-angiotensin axis is a fundamental process of continuing damage to the target organs and a main cause of fibrosis both in the kidney and also in the myocardium. Current pharmacological blockade of the renin-angiotensin axis (primarily with inhibitors of angiotensin I converting enzyme (ACE) or by blocking the angiotensin II receptor) is a most effective antihypertensive strategy for normalizing high blood pressure and for preventing continuing end organ damage^[2]. However, both residual damage in the target organs and consequently, a condition of high hazard for experiencing major clinical events still persist.

Thus, in hypertension, it is most crucial the development of new therapeutic viewpoints, further than only reducing blood pressure to better prevent/decrease target organ damage by aiming to paths triggering and maintaining cardiovascular remodeling and also in the kidney^[2]. Our purpose is to review here three novel mechanisms of target organ damage in hypertension, their role and evidence on regression of cardiovascular remodeling and their possible clinical impact as well. Specifically, we will concentrate on the signaling pathway RhoA/Rho kinase, on the impact of

Table 1 Rho kinase downstream target proteins, some signaling pathways and cellular functions^[6]

ROCK downstream target protein	Signaling pathway	Function
Myosin binding subunit of MLC/MYPT1	MYPT1/MLC	Stress fiber formation
MLC2	MYPT1/MLC2	Mediates calcium sensitization and thereby enhances and sustains contraction in the vascular bed
LIM kinase/cofilin Ezrin/radixin/moesin Adducin	LIM kinase/cofilin	Stress fiber formation

ROCK: Rho kinase; MLC: Myosin light chain; MYPT1: Myosin phosphatase target subunit 1.

the vasodilatory peptides from the renin angiotensin system and on the role of estrogens and the myocardial chymase-angiotensin II pathway in cardiovascular hypertensive remodeling. Interestingly, the 3 aforementioned mechanisms interact strongly with the renin angiotensin system at the cardiovascular level.

RHO KINASE, HYPERTENSION AND CARDIOVASCULAR REMODELING

The small protein Rho (a guanosine triphosphatase) and its target Rho kinase (ROCK), have important functions in blood pressure modulation, by regulating smooth muscle contraction and additionally in cardiovascular remodeling. Agonists of receptors coupled to the G protein in the cell membrane (such as endothelin, angiotensin II, or noradrenalin), growth factors and cytokines activate Rho^[3-6] (Figure 1). Some actomyosin-associated proteins, such as myosin light chain (MLC) phosphatase, myosin light chain 2, LIM-kinase, ezrin radixin-moesin and adducin are considered physiological ROCK substrates^[7-9] (Table 1 and Figure 1). In non-hypertensive people, activation of the classical renin angiotensin system induced by low-salt diet does increase RhoA-ROCK signaling and does stimulate activation of the RhoA guanine exchange factor Arhgef1, which is implicated in vascular tone regulation and in hypertension induced by angiotensin II) in circulating mononuclear cells^[10]. Immediately after Rho activation, this small protein is translocated to the cell membrane where it phosphorylates and activates ROCK (Figure 1), controlling in this way several cellular functions (Table 2), the majority of them related to remodeling. Activated ROCK does phosphorylate MLC phosphatase, which becomes inhibited. This cascade does stimulate tonic contraction of the smooth muscle within the vessels, development of stress fibers, and also cell migration. Thus, activation of both Rho and ROCK has significant effects on numerous cardiovascular diseases^[4-6,11,12], especially in hypertension^[13].

Administration of ROCK inhibitors reduces blood

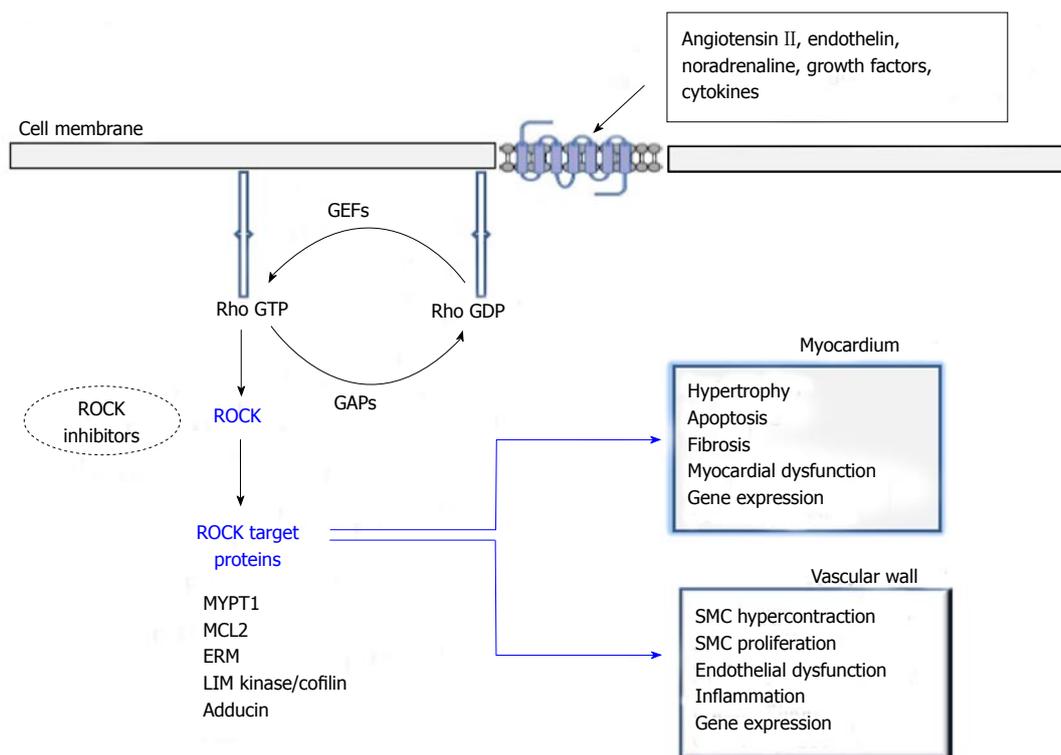


Figure 1 Rho kinase activation and downstream effects on cardiovascular remodeling in hypertension (as well as in cardiovascular disease). GEFs: Guanine nucleotide exchange factors; GAPs: GTPase activating proteins; GTP: Guanosine-triphosphate; GDP: Guanosine-diphosphate; MYPT1: Myosin binding subunit of myosin light chain phosphatase 1; MLC2: Myosin light chain 2; ERM: Ezrin/radixin/moesin; SMC: Smooth muscle cell. Adapted from references^[2,47,144,145].

Table 2 Cellular functions controlled by the RhoA/Rho kinase pathway^[17-9]

Cytoskeletal dynamics and actin organization (formation of stress fibers and focal adhesion complexes)
Cell contraction
Adhesion
Morphology
Motility
Transcriptional regulation (pro remodeling genes)
Cell proliferation and cytokinesis
Differentiation
Apoptosis
Insulin-stimulated insulin receptor substrate-1 phosphorylation
Development

pressure effectively in the rat with spontaneous hypertension (SHR)^[14-17], in the deoxycorticosterone acetate (DOCA) salt hypertensive model in rats^[14,15,18], renal hypertensive rodent^[14], L-NAME hypertensive rats^[19,20] and in also in normotensive rats^[14,15,17,20] which indicates that blood pressure fall by inhibitors of Rho kinase does not depend on the mechanism of hypertension^[21]. Furthermore, the ROCK intracellular signaling cascade is activated in human hypertension^[22,23] where elevated ROCK activity appears to be a consequence derived from up-regulation of the renin angiotensin system and also from higher levels of reactive oxygen species (ROS)^[8,9]. ROCK inhibition decreases smooth muscle contractility by reducing MLC phosphorylation in the smooth muscle cell and by

enhancing endothelial function through reestablishing eNOS activity and NO production^[8,14,18,24-28].

Cardiovascular inflammation and remodeling are also reduced by ROCK inhibition^[8] through: (1) suppressing the levels of cytokines and adhesion molecules such as plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein 1 (MCP-1) and the transforming growth factor 1 in endothelial and in smooth muscle cells^[18,24,29,30]; (2) by inhibiting in endothelial cells ROS production through down-regulation of NADPH oxidase^[24,31,32]; (3) by reducing in smooth muscle cells secretion of cyclophilin A^[33]; and also (4) by augmenting the levels of angiotensin 1-9^[18]. Moreover, ROCK inhibitors delivered in the brainstem reduce blood pressure and sympathetic nerve in hypertensive rodents^[34,35].

In hypertension, there are experimental data available on the significant role of ROCK activation on developing myocardial hypertrophy, remodeling and ventricular dysfunction. In the rodent with salt-sensitive hypertension, cardiac hypertrophy was importantly reduced by using Y-27632, a specific ROCK inhibitor^[36]. In this experimental model, upregulated *RhoA*, *ROCK* gene expression and phosphorylated MLC in the stage with hypertrophy were also inhibited by ROCK inhibition^[36]. Besides, fasudil attenuated cardiac fibrosis possibly throughout inhibition of inflammatory cells myocardial infiltration in hypertensive rats^[37]. Additionally, activated ROCK in the aorta observed in rats with genetically determined elevated levels of

angiotensin converting enzyme and the peptide angiotensin II, is reduced by Fasudil administration causing reduced gene expression that stimulate vascular remodeling (like transforming growth factor 1, PAI-1 and MCP-1) and also enhances oxidative species in the vasculature^[24].

Long-term inhibition of ROCK using fasudil ameliorated diastolic cardiac failure in the Dahl hypertensive rat^[38]. Besides, in rats with left ventricular hypertrophy (LVH) due to pressure overload, inhibition of ROCK with GSK-576371 recovered LV chamber geometry, improved diastolic function and reduced myocardial fibrosis^[39] and recently, long term treatment of DOCA-salt and *N*^ω-nitro-L-arginine methyl ester (LNAME) hypertensive rats with the more potent ROCK inhibitor SAR407899 reduced hypertension and cardiac and renal remodeling in a dose-dependent way in both models^[19]. Interestingly, in DOCA hypertensive rats, blood pressure reduction and protective effects on hypertensive organ damage of SAR407899 were superior compared to amlodipine and also to ramipril^[19] and hearts of hypertensive DOCA or LNAME animals treated with SAR407899 had significantly better systolic left ventricular (LV) function (measured as heart power *in vitro*). Additionally, endothelial-dependent relaxation was significantly and dose-dependently improved after long-term treatment with SAR407899^[19]. An important amelioration of myocardial interstitial fibrosis and expression of collagen genes and of CD3 and CD68 (markers of infiltrating macrophages and leukocytes) in both models was observed, possibly explained by the relevant Rho kinase function in cellular migration and cytokinesis through cytoskeleton modulation^[19].

In more advanced heart disease secondary to hypertension, and evident impairment in cardiac function (both diastolic and systolic), it is very likely that ROCK activity levels be rather similar to Rho kinase activation levels observed in cardiac failure secondary to different mechanisms and its inhibition could produce in this situation similar benefits. In the mouse overexpressing *Gαq*, deletion of *ROCK1* gene did prevent LV chamber dilatation and improved cardiac contractility^[40]. Furthermore, in cardiomyocytes in culture, ROCK activation up-regulates Bax *via* p53 to induce apoptosis^[41]. In the transgenic mouse that overexpress the isoform MYPT2, activation of myosin phosphatase induced LV function decline and remodeling, probably by reducing calcium sensitivity, along with impairing the myofibrillar organization, which is the original report about the functions of both MYPT2 and myosin phosphatase, and the consequences of *in-vivo* cardiac MLC phosphorylation^[42].

Assessing Rho kinase activation in human circulating leukocytes, a possible marker of cardiovascular remodeling and risk

In people diagnosed with metabolic syndrome (MetS), Liu *et al*^[43] reported for the first time significantly increased ROCK activity by 31% through the

measurement of ROCK phosphorylation in circulating leukocytes using the approach of determining myosin binding subunit phosphorylation (MBS). At the same time they observed that plasma concentrations of high-sensitivity C-reactive protein were substantially higher and that circulating levels of adiponectin were significantly lower in MetS subjects as compared with control subjects. Additionally, in this population they found that increased ROCK activation was significantly related with body mass, waist circumference, fasting glucose, high-sensitivity C reactive protein, and additionally with triglyceride levels^[43]. In this clinical study the probability of increased ROCK activity was considerably increased with the amount of MetS components. Experimental findings indicate that insulin resistance promoted by ROCK is implicated in myocardial damage in rats with MetS and that this action of Rho kinase is probably through the IRS-1-PI3-kinase-protein kinase B (Akt) signaling cascade^[44]. In humans, ROCK activation in leukocytes is also enhanced by smoking and does predict endothelial dysfunction^[45].

In circulating leukocytes, Hata *et al*^[46] measured the activity of Rho kinase by assessing the relation amid phosphorylated myosin-binding subunit (p-MBS) on myosin light chain phosphatase to the total MBS and also the change on the blood flow in the forearm (FBF) as a pharmacological action of the distinctive Rho kinase inhibitor fasudil using strain-gauge plethysmography in control subjects and also in subjects diagnosed with a cardiovascular illness. Compared to healthy subjects, they found that leukocyte p-MBS/total-MBS ratio was substantially higher (by 90%) in the diagnosed patients^[46]. Besides, they found that the characteristic inhibitor of ROCK fasudil increased FBF by 300% only in their patients with cardiovascular disease, but this was not the case not in the healthy control group^[46]. Moreover, they found an important relationship between leukocyte p-MBS/total-MBS and maximal FBF induced by fasudil in the group with cardiovascular disease ($r = 0.59$), not in the healthy subjects.

Lately, we have evaluated the level of ROCK activation in leukocytes obtained from venous blood, by quantifying the relationship of phospho to total MLC phosphatase 1 (known as MYPT1-P/T) as a potential remodeling marker in untreated hypertensive patients (HT), in HT patients with LVH or with type II diabetes mellitus receiving specific treatment and also in patients with congestive cardiac failure and LV systolic reduced function^[47-49].

In a recent clinical follow up trial with the aim to determine the correlation amid the observed activity of ROCK and a first main cardiovascular event along with hospitalization rates for congestive cardiac failure, the levels of ROCK activity were determined in leukocytes by the technique of Western blot in more than 600 subjects who undertook a health-screening examination^[50]. After a median period of 42 mo of follow-up, 29 deaths were registered (10 of them because of cardiovascular causes), 2 of them were

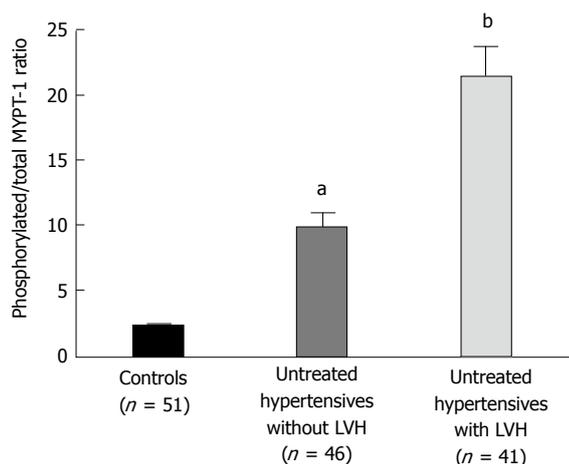


Figure 2 Comparative levels of Rho kinase activity in circulating leukocytes (determined as phosphorylated/total MYPT-1 ratio) in healthy normotensive controls, in untreated hypertensive patients without LVH and in untreated hypertensive patients with left ventricular hypertrophy (Data shown as mean ± SEM). ^a*P* < 0.01 vs Controls; ^b*P* < 0.01 vs untreated hypertensive patients without left ventricular hypertrophy (after significant ANOVA, respectively). Adapted, with permission from reference^[47]. LVH: Left ventricular hypertrophy.

diagnosed with a myocardial infarction, in 20 of them at least one revascularization procedure was performed, 15 developed a stroke, and 17 required hospitalization for congestive cardiac failure^[50]. In the above-mentioned study, once the adjustment for several confounding variables (such as age, gender, known risk factors and other relevant predictors of cardiovascular illness) was performed, ROCK activity remained as a robust independent indicator of a first main cardiovascular event (the hazard ratio was 2.19), of death as a consequence of cardiovascular disease (hazard ratio, 2.57), cerebrovascular accident (hazard ratio, 2.14), and the clinical necessity for revascularization (reported hazard ratio was 2.68)^[50]. The authors concluded that ROCK activity levels determined in circulating leukocytes may be a new marker of cardiovascular events and propose that its inhibition may be a novel therapeutical approach to achieve effective prevention of cardiovascular disease^[50].

Rho kinase activation in subjects with essential hypertension: In a case-control study with the goal of comparing ROCK activation levels in subjects diagnosed with hypertension against a healthy normotensive control group in regard to the existence of LVH assessed by 2D-echocardiography we measured LV mass and dimensions in addition to LV performance and ROCK activation levels in leukocytes from venous blood (by MYPT1-p/t levels through Western blot)^[47]. Compared to non-hypertensive controls, MYPT1-p/t was considerably higher by 450% in the group lacking LVH and by 900% in the group with confirmed LVH by 2D-echocardiography (Figure 2). In contrast with the hypertensive subjects without LVH, MYPT1-p/t was considerable higher (by 200%) in the subjects with

hypertension along with echocardiographic confirmed LVH^[47]. Additionally, in the hypertensive subjects with evidence of eccentric LVH had an MYPT1-p/t relationship remarkably higher (by 400%) compared to hypertensive subjects and no eccentric LVH. Patients having an E/e' ratio measured in the transmitral diastolic flow ≥ 15 showed a substantially higher MYPT1-p/t relationship (26%) as related to the levels in those subjects with a smaller E/e' ratio. This study concluded that ROCK activation levels determined in leukocytes from venous blood are significantly raised up in hypertensive subjects with definite cardiac hypertrophy compared with HT patients without LVH. ROCK activation is additionally increased when eccentric hypertrophy is present. Therefore, in subjects with essential hypertension, ROCK phosphorylation/activation determined in leukocytes from venous blood is correlated to pathological myocardial remodeling and could contribute as one indicator of LVH^[47].

Similarly, Hata *et al.*^[51] recently observed significantly higher Rho kinase activation levels in circulating leukocytes in subjects with essential hypertension when contrasted to healthy individuals by 37%. Besides, in these hypertensive patients under antihypertensive treatment, ROCK activity levels were substantially lowered in patients using calcium channel antagonists compared to patients receiving as antihypertensive treatment inhibitors of the renin-angiotensin-aldosterone axis, thiazides, or β-blockers^[51]. These observations suggest that increased Rho kinase activity associated to hypertension may cause activation of leukocytes along with leukocyte infiltration into the vessel wall, which favors atherosclerosis progression^[51] (as well as remodeling) and suggest the possible clinical relevance of determining in this context the degrees of Rho kinase activation. In a randomized clinical study with the aim of assessing the impact of the specific aldosterone receptor blocker eplerenone, on the endothelial function determined by flow mediated dilatation (FMD) and on Rho kinase activation as well, determined in leukocytes obtained from venous blood in subjects with essential hypertension, 60 patients were received eplerenone, the antagonist of calcium channels nifedipine, or losartan for 48 wk^[52]. They observed that FMD was increased and leukocyte Rho kinase activity was reduced with eplerenone, whereas nifedipine reduced Rho kinase activity but did not modify FMD^[52]. In the aforementioned clinical trial losartan augmented FMD but did not modify Rho kinase activity. In this clinical study, both the blood pressure reducing effect and also the vasodilation levels induced by nitroglycerin were similar with the different three antihypertensive drugs throughout the follow-up^[52].

Rho kinase activity is importantly increased in subjects with very high cardiovascular risk and remodeling hazard, as is frequently observed in subjects with the combined diagnosis of both type 2 diabetes mellitus along with essential hypertension. In a cross sectional clinical study carried through comparing three groups

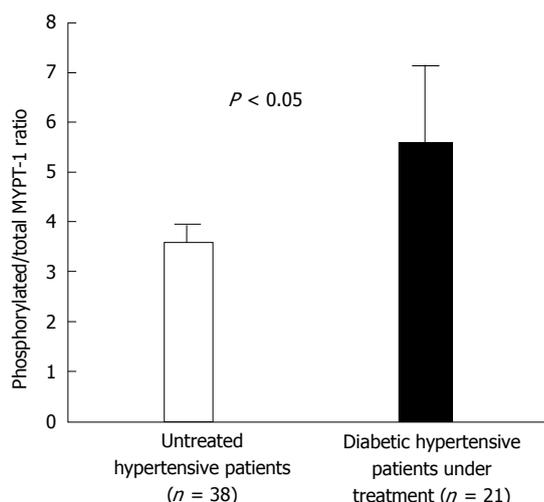


Figure 3 Rho kinase activity in circulating leukocytes (determined as phosphorylated/total MYPT-1 ratio) in untreated hypertensive patients (white bar, mean age 48 years, mean BP 121 mmHg) and in hypertensive diabetic patients under antihypertensive and anti-diabetic pharmacological treatment (black bar, mean age 51, mean BP 111 mmHg). Data shown as mean ± SEM, adapted with permission from reference^[48].

of subjects^[48]: Essential hypertensive patients under no medical treatment, patients with both hypertension and type II diabetes under treatment (with similar degrees of left cardiac mass) and normotensive control subjects, in the patients having the two aforementioned medical conditions, increased ROCK activation (determined in venous blood leukocytes) was found as compared to hypertensive subjects not getting pharmacological antihypertensive treatment^[48] (Figure 3). In this clinical study, in the diabetic hypertensive patients compared to both non-diabetic hypertensives and to normotensive controls, increased levels of oxidative stress were found^[48]. These findings were correlated with reduced arterial compliance and could help to explain the unfavorable vascular remodeling that is commonly detected in hypertensive plus diabetic patients receiving treatment^[48].

In a very recent prospective clinical trial aimed to evaluate the effect of the specific ROCK inhibitor fasudil on diastolic LV function parameters observed in a group of individuals diagnosed with type 2 diabetes presenting with preserved systolic performance, 250 patients with the established clinical diagnosis (62% of them hypertensives), were allocated to receive the ROCK inhibitor (14 d, 30 mg *iv* twice per day) or to placebo^[53]. As planned, echocardiographic parameters were determined before and after 1 mo receiving this treatment. In relationship with the group that was randomized to placebo, in the subjects that were randomized to fasudil, an important reduction in both blood pressure (diastolic) and in echocardiographic late diastolic transmitral flow was observed^[53]. In the aforementioned clinical study, deceleration time, relaxation time (isovolumic), peak early annular diastolic velocity (e'), peak of late diastolic annular velocity, as well as the E to e' ratio also showed an important

recovery by fasudil administration for one month^[53]. Moreover, the Em to Am ratio, both the relaxation (isovolumic) and deceleration times, along with E to e' ratio values observed after receiving treatment with fasudil in the subjects with baseline diminished left ventricular chamber relaxation diverged significantly from what was noticed in the patients presenting with normal left ventricular chamber relaxation. Accordingly, clinical ROCK inhibition by using fasudil improved for the first time left ventricular chamber diastolic function parameters in diabetic patients (the majority of them hypertensives) and normal systolic performance^[53].

Rho kinase increased signaling in subjects with progressive cardiac remodeling: the case of congestive cardiac failure with systolic function decline:

Rho kinase activity is markedly augmented in those subjects with established cardiac failure (CF) due to systolic dysfunction. In a cross sectional study comparing control healthy subjects with patients with chronic and clinically stable CF due to systolic dysfunction under optimal medical treatment, we observed that Rho kinase activation (determined in circulating leukocytes as the MYPT1-P/T ratio) was increased by 100-fold and that it was inversely related with ejection fraction^[49]. Interestingly, in those patients with CF with LV diameter ≥ 60 mm MYPT1-P/T was significantly more elevated than in the CF subjects with LV diameter < 60 mm. Thus, ROCK activity is markedly augmented in patients with stable chronic CF receiving optimal medical treatment, and Rho kinase signaling is robustly related to pathologic LV chamber remodeling and to systolic function decline as well^[49]. Another clinical study examined whether ROCK activation (determined in venous blood leukocytes) is increased in congestive CF and how it is related with the clinical prognosis in 170 patients admitted with this clinical condition^[54]. Patients were prospectively followed up for 14.4 ± 7.2 mo or up to the event of cardiac death. Observed Rho kinase signaling in the patients with congestive CF was significantly higher than that of two control groups. The protein concentrations of both Rho kinase isoforms (ROCK1 and ROCK2) as well as the measured activation of the up-river Rho kinase cascade GTPase RhoA in the congestive CF patients were significantly higher than what was observed in both control groups^[54]. Dyspnea at rest, reduced left ventricular systolic function and impaired renal function were all independent factors predicting Rho kinase activity levels at baseline in the subjects with congestive CF^[54]. By combining Rho kinase activity with N terminal pro brain natriuretic peptide (NT-proBNP) an incremental value in the estimate of long-term mortality (when compared with only the NT-proBNP measurement) was observed. Thus, Rho kinase activity is elevated in these patients with extreme myocardial remodeling, it is also associated with higher mortality and it might be an additional biomarker to congestive CF risk assessment^[54]. In a clinical study to

evaluate whether Rho kinase activity in venous blood leukocytes is elevated in subjects presenting with an established acute coronary syndrome and if Rho kinase activation does predict long-term cardiovascular events, 188 patients with ACS and 61 control subjects were evaluated^[55]. The authors found significantly increased ROCK activity in the two clinical groups (myocardial infarction and unstable angina) when it was compared to control subjects. Besides, patients with both elevated NT-proBNP and Rho kinase activity on admission had a five-fold hazard of a major cardiovascular outcome in relation to the observed hazard in those subjects with low NT-proBNP and low ROCK activity^[55]. Their main conclusion was that by combining Rho kinase activity and NT-proBNP levels a subset of acute coronary syndrome patients at particularly high risk might be identified^[55].

Altogether, these findings strongly suggest that ROCK activation in circulating human leukocytes is directly related to pathological cardiovascular remodeling, from early target organ damage in hypertension to extreme cardiovascular disease. Besides, as this measurement possibly mirrors remodeling it has prognosis value for disease progression, clinical events and conceivably for target organ damage/disease prevention and regression.

IMPACT OF THE VASODILATORY PEPTIDES FROM THE RENIN ANGIOTENSIN SYSTEM ON MECHANISMS IN HYPERTENSION AND CARDIOVASCULAR REMODELING

Remodeling of the cardiovascular structures does occur as a response, not only to modifications in blood arterial pressure or flow but also to variations in the neural and hormonal milieu, where the renin-angiotensin-aldosterone axis exerts a major influence^[56]. The aforementioned neurohormonal system, one of the oldest phylogenetically hormonal systems, is most recognized because of its fundamental role in regulating hydromineral and cardiovascular homeostasis^[56,57]. The renin-angiotensin-aldosterone axis is a fundamental element of CV physiology having a main pathophysiological role by regulating vascular tone, blood pressure, sodium and potassium balance and vascular responses to both injury and inflammation^[58]. Long lasting activation of the renin angiotensin system, throughout both the octapeptide angiotensin II and the mineralocorticoid hormone aldosterone, causes hypertension and at the same time does stimulate prohypertrophy, proinflammation, prothrombosis, and atherogenesis pathways strongly linked with hypertensive organ damage.

During a long period, a main research focus has been production and signaling of Angiotensin II, highlighting both the ACE and renin regarding its

production. With the precise description of both main angiotensin II receptors 1 (AT1R) and 2 (AT2R), most of the issues related to the biochemistry, pharmacology, and physiology of the renin-angiotensin-aldosterone system seemed to be resolved^[57,59]. Extensive studies of the RAAS for a long time, were mainly focused on four classes of drugs targeting the renin-angiotensin-aldosterone system at different levels: Angiotensin-converting enzyme inhibitors (known as -prils), Angs receptor blockers (-sartans), renin inhibitors (-kirens) and mineralocorticoid receptor antagonists (Figure 4). All of them are cornerstones in the treatment of HT. However, there are more recent discoveries extending our understanding of important properties of the renin-angiotensin-aldosterone system. In the most recent decades, increasing evidence has been accumulated indicating an exceeding complexity of the RAAS. Among some novel discoveries is the finding and location of the physiologically important ions of Ang-(1-7)^[60], Ang-(1-9)^[61] and alamandine^[62] acting through identifiable tissue receptors^[60,62,63] (Figure 4).

New vasodilatory peptides in the RAAS and hypertensive remodeling

The therapeutic effectiveness of "classic" blocking the RAAS for treating HT and also related cardiovascular illness has been extensively well established. However, in the most recent decades growing facts and data indicate that the roles and biological functions of the RAAS go beyond the effects initially described. Currently, the amount of biologically relevant end-products of the RAAS is even now increasing, which raises new possibilities to attack through this axis cardiovascular disease, and specifically hypertensive CV remodeling. These facts are particularly accurate for the peptides Ang (1-7) along with Ang (1-9) and lately, for alamandine (Figure 4), and in most of the situations these peptides display biological effects opposing to Angiotensin II.

Ang-(1-7): Production of the active molecule Ang-(1-7) mostly depends on the cleavage of the octapeptide Ang II which is performed by ACE2 (Figure 4). Moreover, Ang-(1-7) can be also formed through hydrolysis of the decapeptide Ang I performed by other peptidases such as prolyl-endopeptidase, neutral-endo-peptidase (NEP), and thimet-oligopeptidase which cleave the Pro⁷-Phe⁸ bond to remove the final three amino acids. The function of the prolyl endopeptidase (PEP)^[64], oligopeptidase (TOP) and of NEP^[65] in the enzymatic hydrolysis from Ang I onto Ang-(1-7) depends on tissue distribution and substrate availability of the enzymes. Nephylisin behaves as an especially active enzyme which has been primarily located in the vessel endothelial cells while the thimet oligopeptidase enzymatic activity is relevant in the cleavage toward Ang-(1-7) occurring within the smooth muscle vascular cells^[66,67]. Ang-(1-7) is also formed from Ang II by ACE2^[68]. The level of Ang-(1-7) is regulated by the action of ACE which

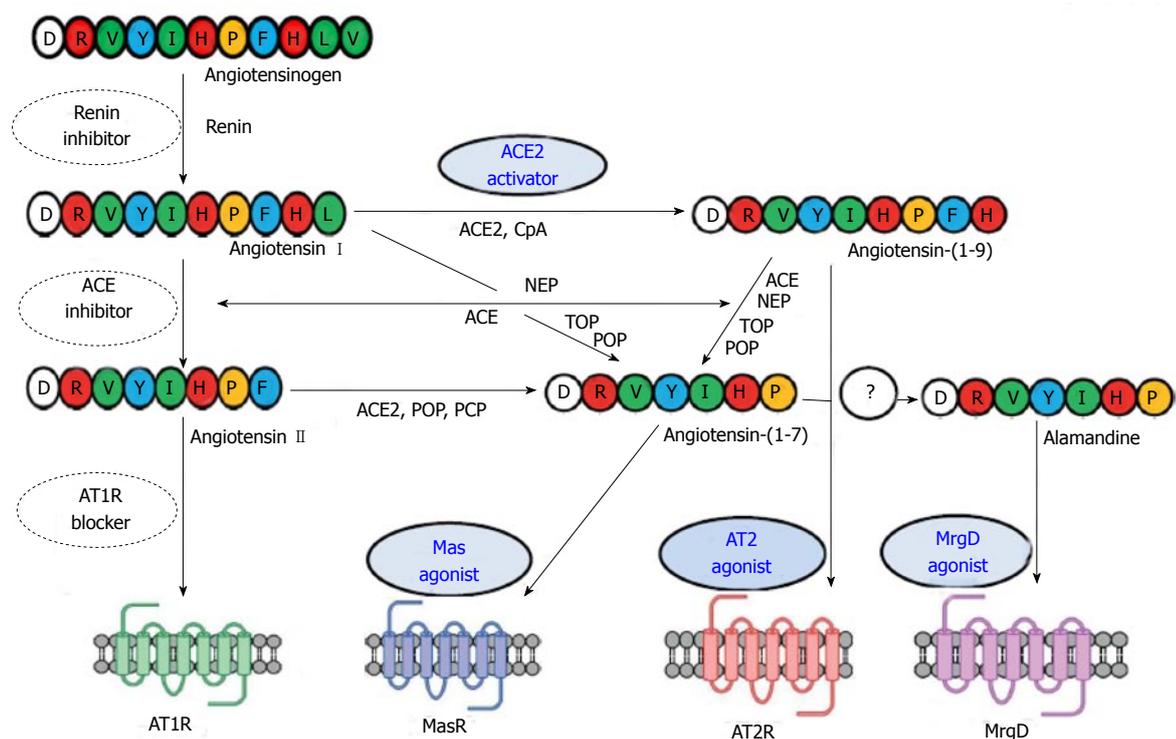


Figure 4 Schematic view of the current renin-angiotensin system and sites of possible therapeutic interventions in hypertension and cardiovascular remodeling. Letters in blue: Receptor agonists or enzyme activators; surrounded by interrupted lines: Enzyme/receptor inhibitors. AT1R: Angiotensin II receptor type 1; AT2R: Angiotensin II receptor type 2; ACE: Angiotensin converting enzyme I; ACE2: Angiotensin converting enzyme 2; MrgDR: Mas-related G protein-coupled receptor; NEP: Neutral endopeptidase; TOP: Thimet oligopeptidase; POP: Prolyl oligopeptidase; PCP: Prolyl carboxypeptidase. Adapted and modified from reference^[146].

hydrolyzes Ang-(1-7) to Ang-(1-5)^[65] (Figure 4).

Ang-(1-7) produces its biological effects and acts throughout its own receptor, the Mas receptor (MasR), one of the membrane receptors coupled to G protein^[8]. This receptor mediates the current known actions of this heptapeptide, as most of them can be prevented by the specific blocker, D-Ala7-Ang-(1-7) (A779)^[8]. The Ang-(1-7) observed effects acting throughout the MasR in the CV system consistently include vasodilation, antihypertrophy, antiarrhythmogenesis, antifibrogenesis and antithrombogenesis^[69-71].

Angiotensin (1-9): The first observations about Ang-(1-9) (Figure 4) showed a rapid appearance of leucine after the injection of radiolabeled Ang I into dog renal and pulmonary arteries^[72,73], by the enzymatic action of a carboxypeptidase that was breaking down Ang I forming des-Leu¹⁰ Ang I. It is possible to generate Ang-(1-9) from Ang I through the effect produced by several enzymes (carboxypeptidase-type), including cathepsin A and ACE2^[74-76], although at a relatively slow rate compared to the making of Ang-(1-7) starting from Ang II^[77]. Moreover, it was observed that an inhibitor of ACE2 doesn't have an effect on Ang-(1-9) formation, but benzylsuccinate, a CxA inhibitor, does stop the formation of Ang-(1-9) and rises the levels of Ang I in heart membranes^[78]. Alternatively, it is possible to cleave Ang-(1-9) to Ang-(1-7) by the ACE carboxypeptidase or by the effects of other enzymes including prolyl

endopeptidase, NEP and thimetoligopeptidase 1 (TOD)^[79,80]. Recently, it was found that Ang-(1-9) has the capability to be hydrolyzed into the peptide Ang-(2-9) by aminopeptidase A^[81] (Figure 4).

Initially Ang-(1-9) was considered as a biologically non active peptide, operating indirectly by competing with Ang I for the ACE active site, and therefore reducing Ang II levels while increasing Ang-(1-7)s^[76,77,82]. However, increasing evidence has confirmed that Ang-(1-9) does work as a molecule with relevant cardiovascular effects both *in vitro* in addition to *in vivo*, through the AT2R^[61,63,83,84].

Alamandine: Recently a new member of RAAS has been discovered, the heptapeptide Ala-Arg-Val-Tyr-Ile-His-Pro, known as alamandine^[62] (Figure 4). By using mass spectrometry, alamandine was identified as a chemical product of a catalytic hydrolysis of Ang A, an octapeptide, by ACE2^[62]. Alamandine is composed by a sequence of amino acids which is extremely similar to Ang-(1-7). Both peptides diverge only in one amino acid residue (alanine in place of an aspartate residue) located at the amine-terminus. Alamandine may be also synthesized by decarboxylation of the N-terminal aspartate amino acid residue from Ang-(1-7). The enzyme which is in charge of the ultimate reaction remains unknown^[62]. However, Alamandine degradation is not yet completely understood. Nevertheless, it is possible that aminopeptidases have a most relevant

role, since the subtraction of Ala1 could conduct to form Ang-(2-7), deemed as a non-active molecule, although it shows inhibitory activity of ACE^[85]. Other Ang-(1-7)-degrading enzymes, such as NEP or neprilysin, may also participate, given alamandine's similarity to Ang-(1-7)^[86] (Figure 4).

Although Alamandine is rather similar to the peptide Ang-(1-7) and the biochemical effects of both molecules look to be close, alamandine acts through a different receptor, the mas-related G-protein coupled receptor D (MrgD)^[62]. Alamandine produces endothelial-dependent vasodilation in rat and mice aortic rings^[62]. It has recently been observed that oral delivery of alamandine (by including it within HP- β cyclodextrin), produced similar effects to those already observed for Ang-(1-7) such as a long-term antihypertensive effect in SHR and a main reduction of cardiac deposition levels of collagens I and III as well as fibronectin in isoproterenol-treated rats^[62].

Target receptors for the second RAAS arm

Mas receptor and AT2R: the two receptors, the AT2R and the Mas receptor, are G protein coupled receptors (GPCRs) (Figure 4) with their conventional seven transmembrane domains^[60,68]. Interestingly, even though their signaling mechanisms are quite unusual for GPCRs and not completely understood, again, major resemblances have been observed. For both receptors, signaling by activating phosphatases, particularly the Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2, seems to be crucial^[87-90]. In both situations, it has been found that phosphatase activation does interfere, in an inhibitory way, with kinase driven signaling cascades, producing inflammation or hypertrophy involving molecules like the mitogen-activated protein kinases or the nuclear factor κ B (NF- κ B)^[91,92]. Another shared signaling mechanism of importance is the augmented NO synthesis and consequent increase of cyclic guanosine monophosphate (cGMP) levels, which does mediate the vasodilation effects of both receptors^[93-95]. Furthermore, these two receptors are able to develop dimers with the AT1R which results in a functional inhibition of this latter one^[96,97].

The MrgD: MrgD belongs to the GPCRs family and it is associated to the MasR^[98]. The MrgD is located in the myocardium in addition to the vessels wall^[62]. Shinohara *et al.*^[99] found that a small amino acid, β -alanine could internalize the MrgD, and thus be able to induce intracellular influx of calcium and to inhibit production of cAMP in Chinese hamster ovary cultured cells (CHO) that expressed rat, mouse, or human MrgD^[99]. The effect in calcium influx can be understood by the connection of the MrgD with the G-protein α subunit (Gq), and the cAMP suppression does suggest an interaction concerning the MrgD with the inhibitory G protein (Gi)^[99]. Furthermore, MrgD activation through β -alanine also suppressed KCNQ/M-type potassium chan-

nels, and in this way also increased neuron excitability by means of the Gq and phospholipase C (PLC) pathways^[100]. In addition, the incubation of alamandine with CHO cells that are transfected with MrgD induces significant NO release^[62].

It has been proposed that this MrgD receptor is related to pain sensation^[101], sensitiveness to thermic and mechanical stimulation^[102], and tumorigenic activity^[103]. It was also observed that MRGD is able to transduce intracellular signaling of Ang-(1-7)^[104].

Clinical approach to the Ang-(1-7)-MasR, Ang-(1-9)-AT2R and Alamandine-MrgD axis in hypertension and cardiovascular remodeling

Ang-(1-7)-MasR Axis: ACE inhibitors (ACEI) and Ang II receptor blockers (ARB) can affect in part the ACE2-Ang-(1-7) system. Experimental studies in myocardial infarcted rats showed that chronic administration of enalapril prevented myocardial hypertrophy and contractile dysfunction in addition to increased ACE2 activity in plasma and in the ventricular wall^[105].

ACEI increase the Ang I levels, which are hydrolyzed to produce Ang-(1-7), through the actions of both ACE2 and NEP. The arterial pressure reduction effects due to ACEI are also associated to increased excretion of Ang-(1-7), an observation reported in urine collected from subjects with essential hypertension receiving the ACEI captopril during 6 mo^[106]. It's well recognized that ACEI are able to diminish excretion of urinary protein in subjects with established type 2 diabetes mellitus^[107], and interestingly, in the ACE2 knock-out mice the proteinuric blocking effect of ACEI disappears^[108]. At this respect, ARBs may be here markedly effective because elevated Ang II levels as a consequence of them will promote Ang-(1-7) production^[109]. Additionally, the low affinity binding of Ang-(1-7) to the AT1 receptor may allow this peptide to work as an antagonist in the presence of Ang II^[110]. In this regard, normotensive rodents with high ACE and Ang II along with low NEP activity^[111] and Ang-(1-7) concentration^[112] (genetically determined) showed a higher hypertensive response (chronic) after renovascular hypertension induction^[113]. Besides, the inverse correlation observed among the amounts of both Ang II in addition to Ang-(1-7) in the aforementioned rodents, determined increased cardiac fibrous tissue deposition after isoproterenol administration^[114] and also ROCK activation in the aortic wall as well as stimulation of genes that promote vascular remodeling (such as the monocyte chemoattractant protein 1 gene, the transforming growth factor 1 gene, and PAI gene)^[24] and also higher oxidative stress levels in the vessels wall in normotensive rodents^[115].

In humans, similar relationships have been observed^[116,117]. Particularly, in hypertensive patients having the DD-ACE genotype (with increased ACE levels), the Ang-(1-7) blood levels were reduced by 4 fold as compared to those observed in patients having the II-ACE genotype (and consequently lower ACE levels)^[116]. In these subjects we reported an important effect of the

I/D ACE genotype on circulating NEP enzymatic activity in addition to an interactive effect amid the I/D ACE genotype status and the hypertensive condition^[117].

By blocking the classic ACE-Ang II-AT1R axis a well-recognized and effective anti-hypertensive and antiproteinuric treatment is obtained. More recently, a few patients have received activators of the ACE2-Ang-(1-7)-Mas receptor pathway, which can be separated in two main types: (1) those compounds that augment the enzymatic activity of ACE2 and will impact the system by increasing Ang II inactivation of^[118] and (2) those molecules that increase Ang-(1-7) production and are particularly oriented to stimulate the MasR^[119]. At this time, in the case of ACE2, little molecules have been developed which activate ACE2^[120]. In rats with SHR, a leading ACE2 activator compound (XNT) diminishes BP and does recover ventricular function^[121]. The recombinant human ACE2 has been also developed as a different attempt to use the possible therapeutic capabilities of ACE2. At this respect, it has recently been observed that rhACE2 administration attenuates diabetic kidney damage through a mechanism involving both Ang II reduction and Ang-(1-7) increasing signaling^[122]. AVE 0991 is the first synthetic compound (non-peptide) developed in order to stimulate the MasR^[123]. This molecule is an orally active MasR agonist that imitates the consequences of administering Ang-(1-7) on the kidney, the vessels, and on the heart as well^[124,125]. AVE0991 does considerably prevent organ damage in SHR and also in rats with hypertension induced by L-NAME by preserving cardiac contractility, avoiding hypertension, and by reducing urinary protein excretion^[123]. Two new designed peptides, CGEN-856 as well as CGEN-857, target the other activator of GPCR, and also show high specificity for the Mas receptor^[126].

The Ang-(1-9)-AT2R axis: The first observations regarding the biological actions of ACE2 and Ang-(1-9) counter-regulating the ACE/Ang II axis were made by Ocaranza *et al*^[105] (Figure 4). In myocardial infarction (MI) rats, down regulation of circulating and cardiac ACE2 enzymatic activity is observed in the chronic phase of LV dysfunction and this effect is precluded by enalapril^[105]. When rats with MI or with the sham procedure received the ACEI enalapril for 2 mo, Ang-(1-9) levels in plasma were increased significantly but Ang-(1-7) levels were not modified^[105]. Thus, by taking into account these observations, it was proposed that Ang-(1-9) rather than Ang-(1-7) acts as a counter-regulator of Ang II in this model of heart failure^[105].

Ang (1-9) does regulate cardiac hypertrophy both *in vivo* in addition to *in vitro*^[61,63]. In rats with MI that received vehicle, enalapril, or candesartan during 8 wk, Ang (1-9) did prevent myocardial hypertrophy and increased plasma Ang-(1-9) circulating levels by several fold^[61]. Besides, in those experiments, Ang-(1-9) plasma levels correlated inversely with several markers of cardiac hypertrophy, even by adjusting for reduction of blood pressure^[61]. This observed action was very

specific, since no correlation was found between cardiac hypertrophy with Ang-(1-7), nor with Ang II neither with the bradykinin levels. In other experiments, chronic treatment with Ang-(1-9) to rats with MI by using osmotic pumps diminished circulating levels of Ang II and enzymatic activity of the ACE and also to prevented myocardial hypertrophy^[61]. Since there are available *in vitro* data showing that Ang-(1-9) incubation with ACE does generate Ang-(1-7)^[76], and this peptide negatively regulates hypertrophy^[127,128], the blocker of the Mas receptor A779 was utilized in order to assess whether Ang-(1-7) could intervene in the actions of Ang-(1-9). Even though A779 did augment blood levels of Ang-(1-7) by almost 3 fold, this specific blocker did not alter the Ang-(1-9) suppression effect on cardiomyocyte (CM) hypertrophy secondary to MI^[61]. In experiments using cultured CM incubated with noradrenaline, IGF-1^[61] or Ang II^[63], Ang-(1-9) prevented hypertrophy of cardiac cells and this action was mediated through the AT2R^[63]. In addition, by the same AT2R mediated mechanism, Ang-(1-9) treatment did alleviate streptozotocin (STZ) induced cardiomyopathy dose dependently and did attenuate cardiac dysfunction in rats with diabetes induced by STZ^[129].

Recently, it has been described that long term treatment with Ang-(1-9) significantly reduced HT and hypertensive cardiovascular damage in two experimental models: the Ang II infusion model and the Goldblatt model (2K-1C) as well^[84]. In these experiments, Ang-(1-9) also blunted the modifications in LV systolic function (ejection fraction) in both hypertensive models, without having an effect in the control rats^[84]. Co-administration of Ang-(1-9) together with A779 did not modify the antihypertensive capability of Ang-(1-9) but PD123319, a specific AT2R antagonist, did entirely abolish the favorable effect of Ang-(1-9) on hypertension and on cardiovascular remodeling^[84]. In cultured cardiac rat fibroblasts, we have recently observed that Ang-(1-9) was able to reduce fibroblast proliferation promoted throughout Ang II and also collagen content with no effects on differentiation of fibroblasts onto myofibroblasts^[84]. The biological effects of Ang-(1-9) on hypertensive CV remodeling were corroborated in the rat with spontaneous hypertension which is stroke-prone (SHRSP)^[130]. Those facts demonstrate that activation of the AT2R produced by Ang-(1-9) has a significant myocardial antifibrotic effect that may be associated to a direct effect on cardiac fibroblasts. This preclinical findings suggest a possible clinical approach for cardiovascular complications from hypertension, by stimulating the AT2R using Ang-(1-9) and obtaining in this way antihypertrophic and antifibrotic protective effects.

The AT2R stimulation activates among other mechanisms the NO-cGMP dependent pathway^[131]. This happens through direct or indirect effects *via* bradykinins or by augmented activity or expression of endothelial NOS^[131]. Additionally, AT2R activation might be able to induce relaxation by inverse regulation of the

Rho kinase pathway in the vascular wall^[132].

The release of endothelial vasodilators in response to Ang-(1-9) may be a mechanism involved in the beneficial consequences of Ang-(1-9) observed in hypertensive rodents. In *ex vivo* resistance arteries from Ang II treated rats, it has been observed that Ang-(1-9) does preserve relaxation induced by Ach (which is dependent from endothelium)^[84]. Ang-(1-9) did also augment the concentration of eNOS mRNA in the aortic wall, which is associated to higher plasma concentrations of nitrate. These observed effects of Ang-(1-9) were completely inhibited by using PD123319, which is coherent with the concept that Ang-(1-9) does increase bioavailability of NO through a mechanism mediated by the AT2R^[84]. In keeping with these results by blocking Enos a significantly increased contractile response to Phe in aortic rings of SHRSP chronically treated with Ang-(1-9) infusion has been found^[130]. Furthermore, Ang-(1-9) does stimulate secretion of ANP without modifying the atrial contractility^[83] and this observed effect of Ang-(1-9) is attenuated by using an AT2R antagonist but not when using AT1R nor MasR pharmacologic antagonism. Furthermore, by using inhibitors of phosphatidylinositol 3-kinase (PI3K), nitric oxide synthase (NOS), Akt, or soluble guanylyl cyclase, Ang-(1-9)-induced ANP secretion is blocked. The above-mentioned observations consistently suggest that the Ang-(1-9) peptide does stimulate secretion of ANP throughout the AT2R-PI3K-Akt-NO-cGMP cascade^[83]. The release of arachidonic acid - another potent vasodilator - may be also actively implicated here, in addition to the NO^[133].

Regulation of Ang-(1-9) by Rho kinase was assessed by Ocaranza and coworkers for first time in hypertensive DOCA-salt rats, by inhibiting Rho-kinase with fasudil^[18]. In the above mentioned experimental model, it was noticed that over expression of genes promoting cardiovascular remodeling such as transforming growth factor 1, PAI-1 and the MCP-1 molecule were lower by using the specific Rho kinase inhibitor, whereas both ACE2 enzymatic activity and blood levels of Ang-(1-9) were substantially increased. Remarkably, the changes observed in ACE, ACE2 and in the levels of Ang-(1-9) were clearly observed in both experimental groups receiving fasudil (the sham group and the DOCA group with hypertension)^[18]. Thus, this new action of Rho kinase inhibition on ACE2 (gene expression/enzymatic activity) in addition to lowering Ang-(1-9) levels might also conduce to its salutary effects in HT, atherosclerosis disease, and CV remodeling.

AT2R agonists could represent a new class of drugs aimed to preclude and reverse hypertensive CV remodeling. In isolated conductance and resistance vessels, several investigators have shown that CGP42112A (a peptide agonist)^[134,135] and more recently the agonist C21^[136] induce vasorelaxation, consistent with the concept of AT2R opposing to the AT1R. A recent study assessed AT2R-stimulation with C21 on post-MI cardiac function^[137]. Treatment using C21 began

24 h after MI and it was administered during 7 d. The aforementioned treatment with C21 diminished the scar size and this reduction was due to a favorable effect of C21 on myocardial remodeling post MI that also did improve systolic and diastolic cardiac dysfunction. Besides, C21 treatment diminished the content of inflammatory cytokines (IL-1b, IL-2, IL-6) and pro-apoptotic markers (caspase-3, Fas-ligand). Moreover, both the monocyte chemoattractant-protein 1 and levels of myeloperoxidase, a biomarker of oxidative stress, were significantly decreased by C21^[137]. Therefore, AT2R stimulation (direct) did improve cardiac function following an experimental MI throughout both anti-inflammatory and anti-oxidant mechanisms and by a more favorable scar remodeling.

Amelioration of inflammation seems to be a fundamental mechanism of action of AT2R-agonism^[138]. It has been recently observed that AT2R-stimulation with C21 inhibits NF-κB activity and subsequent synthesis of interleukin 6 and other cytokines that promote inflammation by activating tyrosine-phosphatases, serine/threonine-phosphatases and also CYP2C/2J enzymes leading in this way to increased 11, 12-EET synthesis^[91]. 11,12-EET has been shown by Node *et al.*^[139] in 1999 to have anti-inflammatory characteristics by inhibiting NF-κB. The fact that the described action of C21 on IL-6 promoter activity was similar in strength to the effect of hydrocortisone administered at an equivalent dose indicates that the AT2R could be clinically useful as a beneficial target in cardiovascular disease, and also in inflammatory clinical conditions^[140]; an hypothesis which needs to be tested in future experiments.

Alamandine-MrgD axis: Alamandine produces vasorelaxation in phenylephrine-contracted aortic rings and when microinjected into central areas critically involved in BP control, such as caudal ventrolateral and rostral ventrolateral medulla. Alamandine produces a decrease vs increase in BP in the former vs latter, respectively, revealing that it acts locally and centrally in a rather similar manner with respect to Ang-(1-7) (Figure 4). Additionally, oral alamandine administration produces a longstanding antihypertensive effect in SHR rats^[62], suggesting a therapeutic potential for the conditions with underlying cardiovascular remodeling. Furthermore, alamandine has direct effects on remodeling by diminishing cardiac deposition of collagen as well as fibronectin in the rat model of myocardial fibrosis induced by isoproterenol^[62]. Since alamandine is a very new molecule within the RAS, currently there are scant data about its participation in disease, although it has been observed elevated plasma alamandine concentrations in subjects with nephropathy, suggesting that alamandine may be involved in some pathophysiological conditions^[62].

The identification of alamandine and its MrgDR contributes to provide novel insights to the knowledge of the RAS pathophysiology and also opens new

possibilities in order to develop therapeutic approaches oriented to prevent/treat CV remodeling, particularly in hypertension.

POSSIBLE ROLE OF ESTROGENS IN PREVENTING HYPERTENSIVE CARDIAC HYPERTROPHY THROUGH MYOCARDIAL CHYMASE AND ANGIOTENSIN II

Since cardiovascular disease in females is consistently increasing, it becomes relevant to better know the connections among age, gender, and cardiovascular health status in a more precise way. With the assumption that estrogens do prevent pathological cardiac remodeling secondary to pressure overload throughout inhibition of mast cell chymase release, Li *et al.*^[141] recently performed thoracic aortic constriction in intact and in ovariectomized rats (an experimental model resembling postmenopausal hypertension). Three days previously to the aortic constriction surgery, ovariectomized rats began to receive 17 β -Estradiol, an inhibitor of the chymase (or a mast cell stabilizer)^[141]. Their main findings were that both density and degranulation of the mast cells, circulating chymase levels - able to hydrolyze angiotensin I onto the octapeptide angiotensin II - and cardiac transforming growth factor-1 were augmented due to the constriction procedure in ovariectomized rats and that replacement therapy with estrogens significantly diminished the cardiac levels of increased chymase, both degranulation and density of the mast cells, circulating chymase levels and active transforming growth factor-1 in the myocardium as well^[141]. They also observed in this experimental model that estrogens did prevent myocardial hypertrophy and fibrosis^[141] (which is rather similar to cardiac failure with normal systolic performance commonly associated to hypertensive diastolic dysfunction)^[142]. By using the above mentioned experimental model they concluded that the mast cell derived chymase release, which is able to be inhibited by estrogens, is responsible for myocardial protection in the case of adverse cardiac remodeling resulting from transverse aortic constriction^[141].

Novel observations derived from experimental models that emulate the cardiovascular phenotype of women having reduced estrogens levels (such in early ovarian failure or in postmenopause), including the ovariectomized congenic mRen2. Lewis rat and transverse thoracic aortic constriction in ovariectomized rats models provide substantial data in the sense that estrogens actively modulate the tissue renin-angiotensin-aldosterone system, and signaling intracellular pathways related to NOS, in some measure through the G protein-coupled receptor 30 (in the membrane), additionally identified as the G protein-coupled estrogen receptor 1^[143] which might also be connected to the pro remodeling Rho A/Rho kinase signaling pathway^[142].

Current limitations and challenges: More potent ROCK inhibition is a main challenge at this time even though recent preclinical evidence of newer inhibitors in this regard is available^[15,19]. As a consequence, clinical studies with ROCK inhibitors in hypertension will follow. In the field of the vasodilatory peptides from the RAS, Ang 1-9 is effective as antihypertensive and anti-remodeling. However, human data are necessary as well as pharmacodynamic information and means for appropriate delivery. With relationship to the implications of the estrogens-myocardial chymase interaction, from our point of view, more preclinical data are required since the number of studies is small.

CONCLUSION

The discussed evidences in this review about the three aforementioned novel mechanisms of hypertensive myocardial remodeling: the Rho kinase intracellular signaling pathway, the vasodilatory peptides from the RAS and the estrogens-myocardial chymase interaction, open new therapeutic opportunities to effectively get better quality of life, reduce/avoid hypertensive cardiovascular remodeling and residual hypertensive risk.

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