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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, doe 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 an 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 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.

Table 1 Rho kinase downstream target proteins, some signaling pathways and cellular functions[8]

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.

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

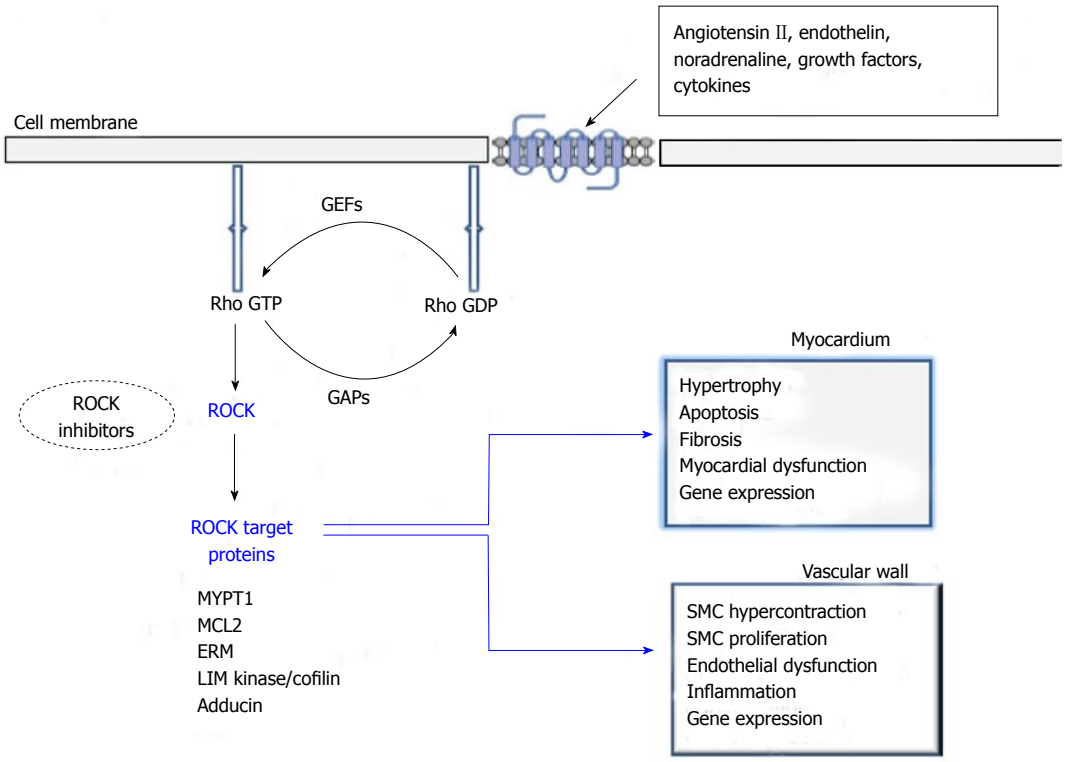

Figure 1 Rho kinase activation and downstream effects on cardiovascular remodeling in hypertension (as well as in cardiovascular disease). GEFs: Guanine nucleotide exchange factors; GAPs: GTPase activating proteins; GTP: Guanosine-triphosphate; GDP: Guanosine-diphosphate; MYPT1: Myosin binding subunit 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[7-9]

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 myocardiac 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 $G\alpha q$, 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

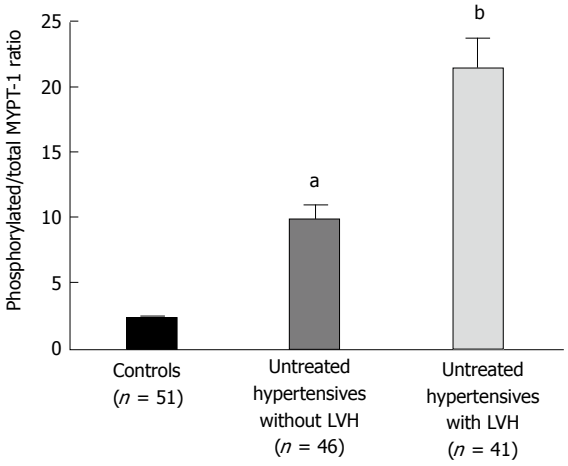

Figure 2 Comparative levels of Rho kinase activity in circulating leukocytes (determined as phosphorylated/total MYPT-1 ratio) in healthy normotensive controls, in untreated hypertensive patients without LVH and in untreated hypertensive patients with left ventricular hypertrophy (Data shown as mean ± 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 hypertesion, 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, wheras 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

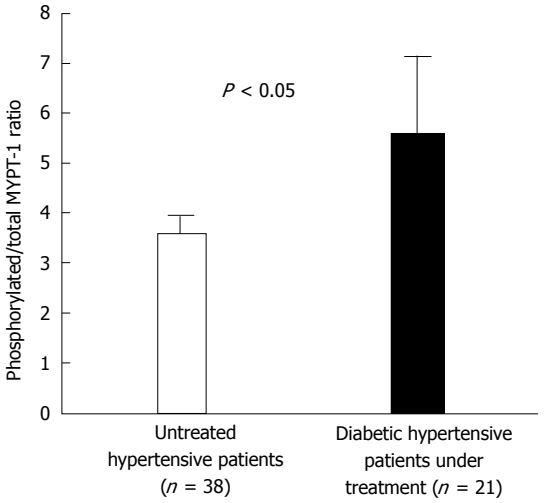

Figure 3 Rho kinase activity in circulating leukocytes (determined as phosphorylated/total MYPT-1 ratio) in untreated hypertensive patients (white bar, mean age 48 years, mean BP 121 mmHg) and in hypertensive diabetic patients under antihypertensive and anti-diabetic pharmacological treatment (black 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 leucocytes) 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 milieus, 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 aions 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 timeth-oligopeptidase which cleave the Pro^{7}-Phe^{8} 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. Neprilysin 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

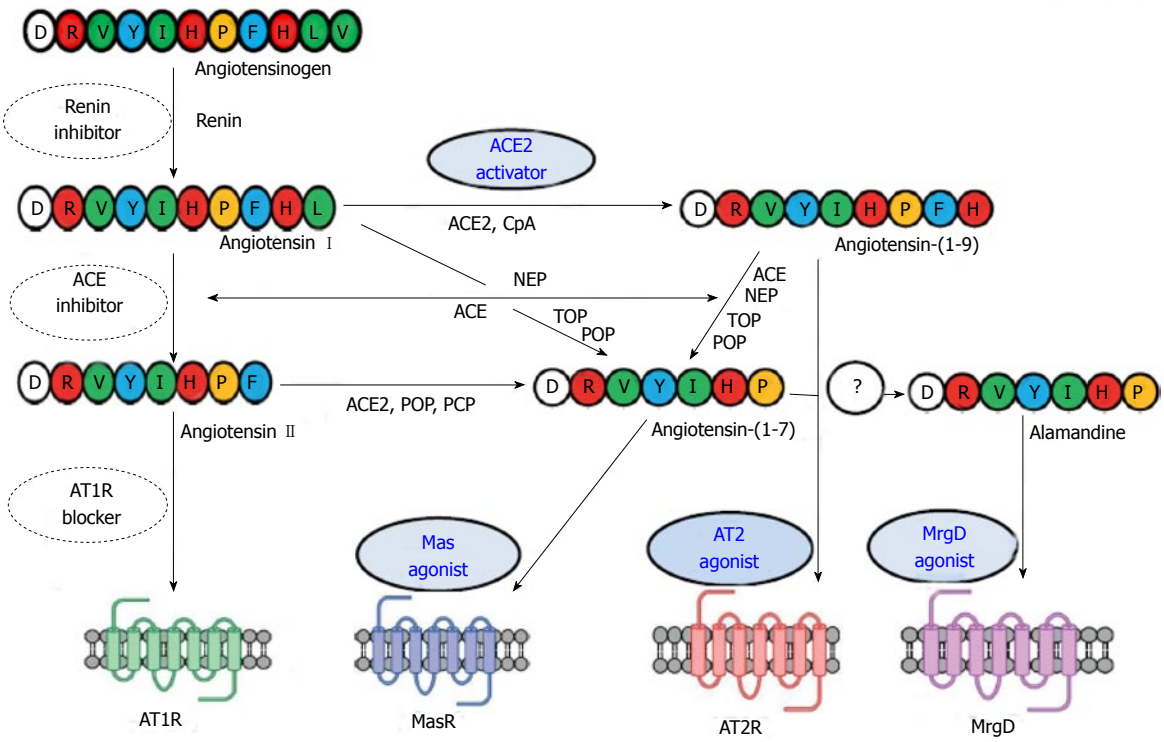

Figure 4 Schematic view of the current renin angiotensin system and sites of possible therapeutic interventions in hypertension and cardiovascular remodeling. Letters in blue: Receptor agonists or enzyme activators; surrounded by interrupted lines: Enzyme/receptor inhibitors. AT1R: Angiotensin II receptor 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 carbopeptidase that was breaking down Ang I forming des-Leu^{10} 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 κB (NF-κB)[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 channels, 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 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 hace 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 port 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 is 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 knowledgement 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 17β-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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REVIEW

RhoA signaling and blood pressure: The consequence of failing to "Tone it Down"

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Abstract

Uncontrolled high blood pressure is a major risk factor for heart attack, stroke, and kidney failure and contributes to an estimated 25% of deaths worldwide. Despite numerous treatment options, estimates project that reasonable blood pressure (BP) control is achieved in only about half of hypertensive patients. Improvements in the detection and management of hypertension will undoubtedly be accomplished through a better understanding of the complex etiology of this disease and a more comprehensive inventory of the genes and genetic variants that influence BP regulation. Recent studies (primarily in pre-clinical models) indicate that the small GTPase RhoA and its downstream target, Rho kinase, play an important role in regulating BP homeostasis. Herein, we summarize the underlying mechanisms and highlight signaling pathways and regulators that impart tight spatial-temporal control of RhoA activity. We also discuss known allelic variations in the RhoA pathway and consider how these polymorphisms may affect genetic risk for hypertension and its clinical manifestations. Finally, we summarize the current (albeit limited) clinical data on the efficacy of targeting the RhoA pathway in hypertensive patients.

Key words: Hypertension; Blood pressure; RhoA; Smooth muscle contraction; Guanine nucleotide exchange factor; GTPase activating protein; Polymorphisms

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Core tip: Studies (primarily in pre-clinical models) indicate that the small GTPase RhoA and its downstream target, Rho kinase, play an important role in regulating blood pressure homeostasis. Herein, we summarize the underlying mechanisms and highlight signaling pathways and regulators that impart tight spatial-temporal control of RhoA activity. We also discuss known allelic variations in the RhoA pathway and consider how these polymorphisms may affect genetic risk for hypertension and its clinical manifestations. Finally, we summarize the current (albeit limited) clinical data on the efficacy of

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INTRODUCTION

Although hypertension is a major risk factor for stroke, myocardial infarction, and kidney failure and contributes to over 350000 deaths annually in the United States[1], we know surprisingly little about its development or the mechanisms by which it promotes cardiovascular disease. A number of antihypertensive drugs are available, but regimens are usually chosen empirically and multiple drugs that target different organ systems are frequently required for effective treatment. One reason for these difficulties is that blood pressure (BP) is a complex trait that is regulated by many organ systems and a large number of humoral factors. Thus, a better understanding of the molecular and genetic mechanisms that control BP under normal and pathologic conditions should lead to novel drug targets and/or to personalized therapies that are more effective and less toxic. Recent advances suggest that RhoA signaling plays a role in the development human hypertension. The focus of this review will be: (1) to highlight the mechanisms underlying RhoA-dependent regulation of BP; (2) to discuss how allelic variations in the RhoA signaling pathway affect genetic risk for hypertension and its clinical manifestations; and (3) to summarize the current (albeit limited) clinical data on the efficacy of targeting this pathway in hypertensive patients.

As a critical regulator of the actin cytoskeleton and acto-myosin contractility, the small GTPase, RhoA, regulates a variety of cellular processes including force development, endocytosis, exocytosis, adhesion, migration, proliferation, and differentiation[2]. Like all GTPases, RhoA is regulated by guanosine triphosphate (GTP) binding and cycles between the active GTP-bound form and the inactive GDP-bound form. When GTP-bound, RhoA interacts with a variety of effector molecules that mediate its effects on the actin cytoskeleton including the Rho-associated coiled-coil domain containing protein kinases (ROCK Ⅰ and Ⅱ), the diaphanous-related formins (mDia1 and mDia2), protein kinase N, citron kinase, rhophilin, and rhotekin. With respect to regulation of BP, Rho kinases are arguably the most important effectors as evidenced by the findings that increased ROCK activity has been observed in spontaneously hypertensive rats and some hypertensive patient populations[3,4] and ROCK inhibitors like Y-27632, Fasudil, and SAR407899 have been shown to reduce BP in hypertensive animal models and patients[5].

RHOA SIGNALING AND BP REGULATION

BP homeostasis is tightly controlled by many organ systems and humoral factors that regulate peripheral vascular resistance, sodium and water balance, and cardiac output. Below we summarize the role RhoA signaling in the regulation of BP highlighting recent findings that implicate this pathway in the development of hypertension.

RhoA and arteriole tone

Vascular resistance is a major determinant of BP and is controlled, in large part, by smooth muscle cell (SMC) contraction within small peripheral arterioles[6-10]. Excitation-contraction coupling in SMC is mediated by the Ca^{2+}-dependent activation of myosin light chain kinase (MLCK), and SMC tension is directly proportional to myosin light chain (MLC) phosphorylation as this enables myosins molecular interaction with actin[11,12]. Interestingly, besides promoting an increase in intracellular Ca^{2+}, many G protein-coupled receptor (GPCR)-coupled contractile agonists including angiotensin Ⅱ (AⅡ), norepinephrine, and endothelin-1 (ET1) also stimulate RhoA activity in SMC and in intact arteries[3,4,13]. Active RhoA leads to Rho-kinase (ROCK)-dependent inhibition of myosin phosphatase and results in elevated MLCK activity and enhanced sensitization to Ca^{2+}[3,14-16]. Importantly, several studies in animal models and patients (described in further detail below) indicate that RhoA-dependent pathways are involved in the increased vascular resistance associated with hypertension[3-5,13,17].

Active RhoA also induces *de novo* formation of actin filaments that are necessary for force development and SMC contraction. Rho-dependent actin remodeling occurs by both ROCK-dependent and independent processes. The Rho effectors mDia 1 and 2 directly catalyze actin polymerization in cooperation with the actin binding protein, profilin, whereas ROCK stimulates actin polymerization by inhibiting the disassembly of actin polymers through LIM-kinase-dependent inhibition of cofilin (ROCK activates LIM-kinase 1 and LIM-kinase 2 by phosphorylation at threonine 508 or 505 respectively within the activation loop[18-22]). ROCK also phosphorylates ERM proteins which enhances their tethering to integral plasma membrane proteins and promotes actin filament stabilization[23].

Recent studies indicate that RhoA signaling also controls SMC contractile gene expression by regulating the nuclear translocation of the Myocardin-related transcription factors (MRTF-A and MRTF-B). Under conditions of low RhoA activity, monomeric (G)-actin binding to the MRTF N-terminus masks a nuclear localization sequence resulting in cytoplasmic sequestration of these serum response factor co-factors. The fall in cytoplasmic G-actin levels that occurs upon RhoA-mediated actin polymerization promotes MRTF

nuclear accumulation and promotes the expression of SM α-actin, SM γ-actin, SM myosin heavy chain, calponin, and SM22[24,25]. Thus, not only does RhoA control SMC contractility, but it also regulates the levels of the SMC-specific contractile proteins that support this function. Moreover, elevated RhoA in endothelial cells impairs endothelial cell-mediated vasorelaxation as it decreases availability of the potent vasodilator, nitric oxide by reducing both eNOS expression and activity[26-30]. In sum, signaling through RhoA enhances Ca^{2+} sensitivity, promotes actin remodeling and induces expression of contractile proteins and these responses are necessary for maintaining sustained SMC contractility and elevated vessel tone (Figure 1).

RhoA and kidney function

The kidneys play a major role in regulating BP by controling sodium excretion and blood volume. In addition, since the kidneys are highly perfused organs receiving up to 25% of total cardiac output, increased contracility of renal arterioles can significantly increase total peripheral vascular resistance. In most vascular beds, arteriolar tone is controlled by automic innervation and circulating hormones. However, in pre-glomerular afferent arterioles, increased kidney perfusion (manifesting as increased renal BP) stimulates SMC contraction through the tubuloglomerular feedback and myogenic responses (see[31] for review). The former mechanism is mediated by increased glomerular filtration and NaCl delivery from the loop of Henle to the macula densa (MD), a cluster of epithelial cells located at the junction between the distal convoluted tubule and the end of the thick ascending limb and adjacent to the abluminal SMCs of the afferent arterioles. Increased NaCl uptake by MD cells results in secretion of ATP and adenosine which stimulate afferent arteriole SMC contraction *via* P2Y4/P2Y6 and A2 GPCRs, respectively. The myogenic response is mediated by the activation of stretch-sensitive cation channels. Together these mechanisms stabilize renal blood flow to protect the sensitive glomerular capillaries from flow-induced trauma. Importantly, afferent arterioles express RhoA, ROCK Ⅰ and Ⅱ[32], and several studies have convincingly demonstrated that the Rho/Rho kinase pathway influences both of these feedback mechanisms in response to increased kidney perfusion[33-37]. The requirement of RhoA is likely due, at least in part, to its necessity for P2Y4/P2Y6 and A2 receptor-dependent contractility. Indeed, ATP (*via* P2Y4/Y6) and adenosine (*via* A2) stimulate RhoA activity in SMC and their pressor responses were prevented by pretreatment with the Rho-kinase inhibitor, Y-27632[32].

Interestingly, recent evidence indicates that RhoA may play an additional role in other cell types within the kidney to impact volume homeostasis. In particular, RhoA activity in tubular epithelial cells can regulate sodium reabsorption and excretion primarily by altering the density and location of epithelial sodium channels (ENaCs) and the sodium-hydrogen exchanger (NHE3)[38]. *In vitro* studies in cultured epithelial cells indicated that the Na^+ current through ENaCs was significantly increased by expression of wildtype or constitutively active RhoA (G14V) and supressed by expression of dominant negative RhoA (T19N). The changes in current correlated with alterations in the density of ENaCs at the PM[39] and mechanistic studies determined that RhoA signaling was essential for intracellular vesicle mediated transport of ENaCs to the apical cell surface[40,41]. RhoA signaling also regulates the activity and subcellular localization of NHE3, a key regulator of sodium absorption in the proximal convoluted tubule. NHE3 associates with ezrin and cortical actin filaments at the plasma membrane and treatment with either the RhoA inhibitor, diphtheria toxin toxin B, or Y-27632 disrupted these interactions and promoted the internalization of NHE3 to submembrane compartments[42,43]. Moreover, Nishiki *et al*[44] showed that spontaneously hypertensive rats exhibited elevated NHE3 activity and a exagerated level of Na^+ reabsorption when compared to normotensive controls and that Na^+ reabsorption was normalized by treatment of the hypertensive animals with Y27632.

RhoA in the central and peripheral nervous system

The central nervous system (CNS) constantly assesses pressure levels in the vasculature and makes necessary signaling adjustments to prevent BP variability. The main mechanism by which the CNS monitors BP is through a rapid negative feedback loop termed the baroreceptor reflex. Baroreceptors are sensory neurons located primarily in the aortic arch and carotid sinuses that continuously respond to pressure-induced streching of the vessels in which they reside. Impulses from baroreceptors are relayed *via* glossopharyngeal and vagus nerves to the nucleus tractus solitarii (NTS) in the brainstem[45], which in turn relays the signal to the rostral ventrolateral medulla[46] and increases or decreases parasympathetic and sympathetic stimulation to the heart and vessels accordingly. Interestingly, the CNS component of this feedback loop has been shown to be dependent on RhoA/Rho-kinase signaling. Rho-kinase inhibitors microinjected directly into the NTS or infection of this structure with an adenovirus expressing a dominant-inhibitory form of Rho-kinase reduces sympathetic nerve activity, heart rate, and BP in normotensive rats and these effects are even more pronounced in spontaneously hypertensive rats[47,48]. Moreover, infusing the ROCK inhibitor, Y27632, into the neural cistern attenuated the BP increase that resulted from AⅡ infusion into the same area of the brainstem[49].

While RhoA's effects on the CNS are clear, a heretofore understudied area in this field is the extent to which RhoA regulates neurotransmitter release from the perivascular nerves which are known to play a major role in the control of resistance arteriole tone. While it has long been known that RhoGTPases have an important and conserved function in mediating neuronal

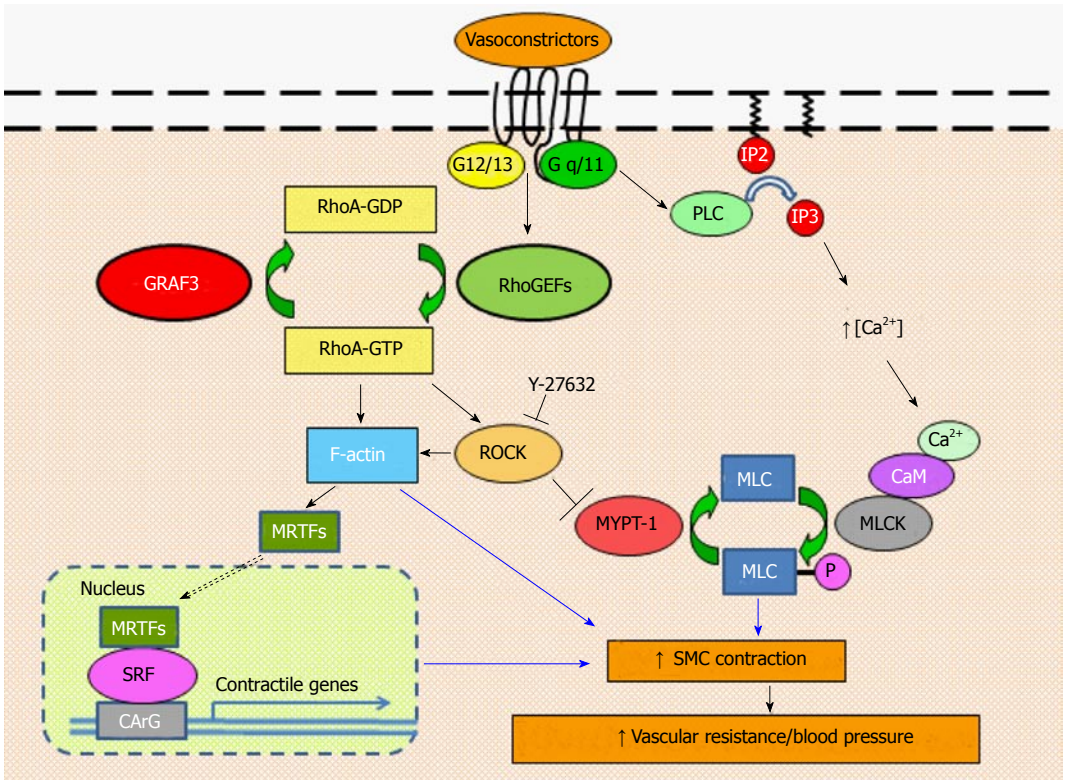

Figure 1 Schematic summarizing RhoA-dependent regulation of vascular smooth muscle contraction and blood pressure homeostasis. Excitation-contraction coupling in smooth muscle cell (SMC) is mediated by the Ca^{2+}-dependent activation of myosin light chain kinase (MLCK), and SMC tension is directly proportional to myosin light chain (MLC) phosphorylation (p) as this enables myosin's molecular interaction with actin. SMC contractility is also regulated by GPCR-coupled contractile agonist-mediated activation of the small GTPase RhoA. Downstream activation of Rho kinase (ROCK) inhibits myosin phosphatase target subunit 1 (MYPT-1), and results in increased levels of pMLC to promote smooth muscle contraction. RhoA also stimulates G-actin polymerization to filamentous actin (F-actin). Actin polymerization increases SMC tension and stimulates myocardin-related transcription factor (MRTFs) nuclear translocation which promotes SRF-dependent transcription of contractile genes. RhoGAPs (such as GRAF3) and RhoGEFs dynamically regulate RhoA activity to achieve blood pressure balance.

survival and death and that tight spatiotemporal control of RhoA is necessary for appropriate neuronal development (neurite outgrowth, growth cone dynamics) and regeneration, to our knowledge no studies have explored the consequence of Rho-kinase inhibition on peripheral nerve structure or function. Future studies to this end are warranted, because some studies in cells and invertebrate model systems indicate that Rho/Rho kinase signaling may limit the release of sympathetic (contractile) agents and promote the release of parasympathetic relaxation factors from motor neurons. For example, Yamaguchi *et al*[50] found that $Ga_{12/13}$-mediated activation of RhoA/ROCK inhibited Ca^{2+} dependent exocytosis of the contractile neurotransmitter dopamine in PC12 cells. In support of these studies, an activating mutation in ArhGEF10, a RhoGEF highly expressed in the peripheral nervous system, was identified in pateints who exhibited slowed nerve conduction velocities[51,52]. On the other hand, Hiley *et al*[53] reported that release of the relaxation neurotransmitter, acetylcholine from cholinergic motor neurons in *C. elegans*, required the regulators of G protein signaling (RGS)-RhoGEF dependent activation of Rho A. Thus, it is formally possible that inhibition of RhoA in perpheral nerves could lead to an increase total peripheral resistance and BP. This concept requires further exploration if RhoA/ROCK inhibitors are to be considered as future anti-hypertensive therapies.

RhoA in the myocardium

Several studies have shown that RhoA signaling has direct effects on cardiac function that increase cardiac output and BP. Transgenic mice that overexpressed either GDIα or dominant negative RhoA exhibited conduction defects and cardiomyocytes isolated from these mice exhibited decreased L-type Ca^{2+} channel currents that likely contributed to the decreased contractility observed *in vivo*[54,55]. Vlasblom *et al*[56] showed that treatment of neonatal ventricular cardiomyocytes with Y27632 reduced the expression and activity of the sarcoplasmic reticulum Ca^{2+} Atpase, SERCA2a, thereby limiting the amount available for Ca^{2+}-induced Ca^{2+} release in the next cardiac cycle. In addition, RhoA-dependent pathways have been shown to be critical for phosphorylation and sensitization of cardiac troponin T complex to intracellular Ca^{2+} levels[57]. Moreover, while not initially thought to be a major mechanism for modulating cardiac contractility, it is becoming clear that cardiac MLC phosphorylation can enhance muscle contractility by increasing Ca^{2+} sensitivity[58] and that MLC phosphatase is a target for Rho kinase-dependent inhibition in the myocardium (like

in SMC). Indeed, Lauriol *et al*[59] showed that cardiac-restricted deletion of RhoA led to decreased contractility and this effect was correlated with decreased MLC activity. Other similarities between RhoA signaling in cardiomyocytes and SMC include the ability of RhoA-mediated signals to promote differentiation/maturation by promoting the expression of contractile genes[60].

CONTROL OF RHOA GTPASE ACTIVITY

Rho proteins act as molecular switches that cycle between an inactive GDP-bound form and an active GTP-bound form and this cycle is under the direct control of three groups of regulatory proteins. Guanine dissociation inhibitors (GDIs) sequester RhoA into an inactive cytoplasmic fraction, guanine nucleotide exchange factors (GEFs) activate RhoA by facilitating exchange of GDP for GTP, and GTPase activating proteins (GAPs) promote RhoA's intrinsic GTPase activity to hydrolyze GTP to GDP and efficiently turn off (or tone down) RhoA-dependent signaling. The GEF and GAP protein families are quite large and structurally diverse and it is likely that additional differences in expression patterns and post translational modification allow for tissue-specific and tight spatio-temporal control of RhoA activity. The following section summarizes the known mechanisms for controlling RhoA activity in SMC.

RhoGEFs and BP control

GEFs activate small GTPases by increasing the GDP dissociation rate by several orders of magnitude which in turn promotes GTP-binding since GTP is in an approximately 10:1 molar excess to GDP in mammalian cells[61]. To date, over 24 different Rho-selective GEFs have been identified. The common functional domain of RhoGEFs is the Dbl homology (DH) domain (also refered to as the RhoGEF domain), which typically serves as both the catalytic site and the major binding interface for RhoA (Figure 2). A pleckstrin homology (PH) domain is almost always found downstream of the DH domain and this unit serves to facilitate membrane binding and cooperates with DH domains to fully activate RhoA[62]. Other common functional domains include the RGS domain that binds large G-proteins to couple the GPCR and Rho signaling pathways and the Postsynaptic Density 95, disk large, Zona occludens-1 (PDZ) domain that binds to Plexin-B1 and Lysophosphatidic acid (LPA) receptor to transmit Semaphorin 4D (57) and LPA signals[63], respectively.

The major contractile agonists that stimulate RhoA activity in SMC include AII, phenylephrine (PE), ET1, and thromboxane A2. The GPCRs for these ligands couple to various Gα subunits, including $G\alpha_{12/13}$ and $G\alpha_{q/11}$, but each has distinct Gprotein coupling properties. For example, PE signals almost exclusively through $G\alpha_q$ and thromboxane through $G\alpha_{12/13}$ while other agonist-receptor interactions lead to more promiscous G protein activation. These heterotrimeric GTPases in turn either directly or indirectly activate a range of RhoGEFS. Therefore, it is likely that each agonist could stimulate somewhat overlapping but distinct set of RhoGEFS to enable fine tuning of the extent and duration of Ca^{2+} sensitization and thus engender precise spatial and temporal control of vessel tone.

RGS family of RhoGEFs (LARG, p115RhoGEF, and PDZRhoGEF)[64] has received a lot of attention in the BP field because these proteins can interact with (and be directly activated by) $G\alpha_{12}$ and $G\alpha_{13}$[65]. Indeed, activaiton of RhoA by many of the aforementioned contractile agonists is mediated, at least in part, by these RhoGEFs. However, studies performed in mice lacking the α subunits of $G\alpha_{q/11}$ or $G\alpha_{12/13}$ in smooth muscle convincingly showed that activation of these RhoGEFs are not simply due to direct binding to $G\alpha_{12/13}$[13]. In fact $G\alpha_{12/13}$ depletion did not affect the pressor effects of AII, or PE, and only modestly reduced the pressor effects of ET1. Instead, depletion of $G\alpha_{q/11}$ completed abrogated PE-induced pressor responses and dramatically attenuated responses to AII. Subsequent studies from Guilluy *et al*[13] identified p115RhoGEF (p115) as the critical GEF that mediates AII-dependent RhoA activity in SMC and small arterioles and showed that smooth muscle specific deletion of p115 rendered mice resistant to AII-dependent hypertension. Interestingly, their mechanistic studies confirmed that p115 activation did not require $G\alpha_{12}$/$G\alpha_{13}$, but instead, was governed by $G\alpha_q$ -mediated, Janus tyrosine kinase-dependent phosphorylation of Tyr738 in the PH domain. Importantly, phosphorylation mimetic and deficient variants at Tyr738 elevated and reduced p115's GEF activity, respectively[13]. As discussed in further detail below, phosphorylation-dependent activation of RhoGEFs has since emerged as a critical regulatory pathway. However, it should also be noted that AII–dependent activation of RhoA in SMC likely involves additional pathways as AII signaling in SMC has been linked to inhibition of p190Rho GTPase activating protein (see below[66]; upregulation of LARG[67], upregulation of PDZ-RhoGEF[68], and $G\alpha_q$/Ca^{2+}/proline-rich tyrosine kinase 2 (PYK2) tyrosine kinase mediated phosphorylation/activation of PDZ-RhoGEF[69]. Indeed, Ying *et al*[69] showed that Ca^{2+}/PYK2-dependent activation of PDZ-RhoGEF was necessary for maximal AII induced RhoA activation.

Interestingly, p115 mutant mice also exhibited a partial reduction in DOCA/salt-induced hypertention but had normal basal BP and normal pressor responses to ET1 and PE; agents that also act through $G\alpha_q$-dependent signaling pathways. Future studies are necessary to determine how PE and ET1/$G\alpha_q$ signals differ from those induced by AII. However, recent studies by the Somlyo laboratory shed some light on this phenomena as they found that there is functional overlap between p115 and LARG. Indeed using genetic mouse models, they found that while the time it took to reach maximal contraction was increased in SMC-

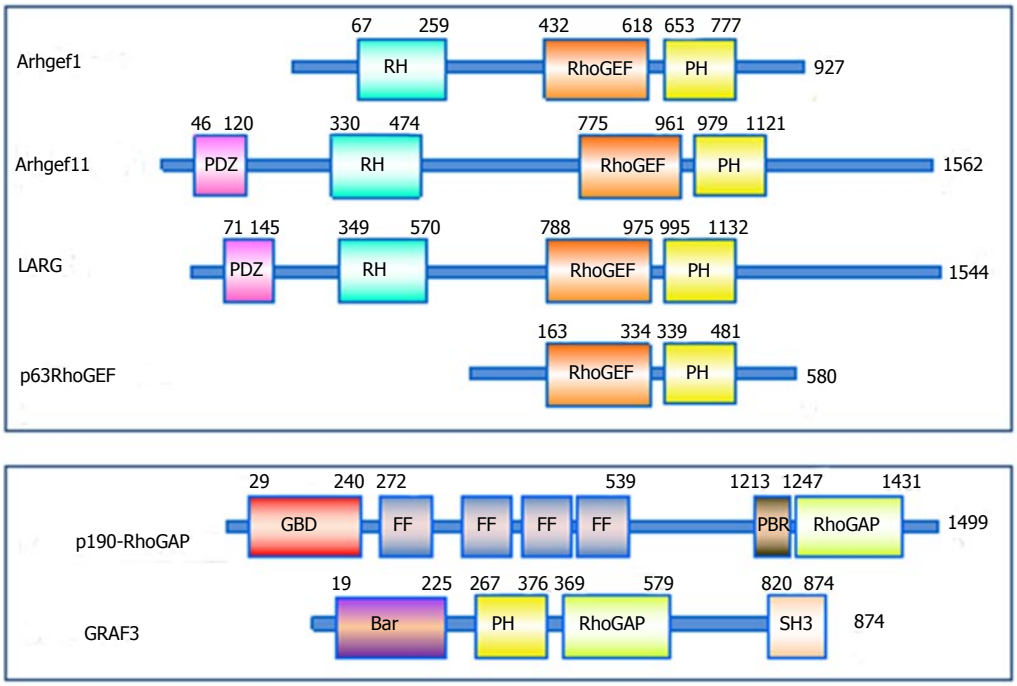

Figure 2 Multi-domain architecture of RhoGEF and RhoGAP proteins known to regulate smooth muscle cell phenotype. The catalytic domain of RhoGEFs is termed a Dbl homology (DH) domain, which serves as the major binding interface with Rho GTPases and catalyzes the dissociation of GDP from the GTPase. Pleckstrin homology (PH) domains are almost always downstream of the DH domain and these units cooperate to fully activate the GTPase. Other functional domains contained in specific RhoGEFs include the RH (Regulators of G protein Signaling Homology) domain and PDZ (Postsynaptic density 95, disk large, zona occludens-1) domain. RhoGAPs are also multi-domain containing proteins. The RhoGAP domain facilitates GTP hydrolysis and inhibits RhoA activity while other domains can regulate RhoGAP targeting and function. For example, BAR (Bin/amphiphysin/Rvs), PH, or polybasic region (PBR) domains direct lipid binding and promote membrane localization. Other domains are involved in protein-protein interactions such as GTP-binding domain (GBD), diphenylalanine motifs (FF) and the SH3 (SRC Homology 3) domains. The amino acid numbers are shown above each protein are based on the human orthologs (http://www.ncbi.nlm.nih.gov/).

specific double knockouts, maximal contraction of smooth muscle from PDZ-RhoGEF and LARG double knockdown tissues was similar to that of the single mutants[70]. Thus it is formally possible that AII (but not ET1 and PE) induced hypertension is blocked by p115 knockout because AII has a relatively reduced capacity to activate LARG. Interestingly, Medlin *et al*[71] identified the vasoconstrictor agonist sphingosine-1-phosphate (S1P) as a potent activator of LARG in cultured vascular SMC. While this pathway has not yet been confirmed *in vivo* the finding that LARG knockout mice are resistant to salt-induced hypertension[4] which leads to volume overload-induced stretching of vessels is consistent with the thesis that LARG may regulate RhoA activity and SMC contractility in response to mechanical forces[72].

Besides activation of the RGS GEFs, several studies have linked $G\alpha_{q/11}$-dependent activation of RhoA to the Trio family of RhoGEFs (Trio, Duet, and p63-RhoGEF)[73,74]. P63-RhoGEF is highly expressed in arterial smooth muscle and it has recently been shown to be important for the early phase of AII-dependent vessel contractilily[75] and for maximal pressor response to other vasoconstrictors such as PE and ET1 that act through $G\alpha_{q/11}$[76]. Moreover, another non-RGS RhoGEF termed lymphoid blast crisis (Lbc) has been shown to be critical for serotonin-dependent activation of RhoA and contractility in vascular SMC[77]. In summary, specific vasoconstrictors can lead to activation of distinct but overlapping sets of RhoGEFs (each with different activation kinetics, catalytic activities, and subcellular locales). A question that warrants further studies is to what extent GEFs might also govern control over the activation of specific RhoA effector subsets by various agonists.

Like p115, PDZRhoGEF and LARG are also activated by tyrosine phosphorylation. Focal adhesion kinase (FAK) as well as its related family member, PYK2 phosphorylate PDZRhoGEF[69,78], while LARG was shown to be activated by FAK[78] and Tec[64,69]. Future identification of the specific sites of phosphorylation will aid in determining if there is a conserved mechanism by which these modifications promote GEF activity.

Control of RhoGEF expression is another important means of regulating RhoA activity in the vasculature. Because p115-RhoGEF, PDZ-RhoGEF, and LARG each play a role in BP regulation in rodents, it is no surprise that these GEFs are expressed in both conductance and resistance arteries of rats and mice[4,68,69,79]. P63RhoGEF is also abundant in the peripheral vasculature[80]. Interestingly, the expression of many of these RhoGEFs fluctuates as BP changes, suggesting that dynamic regulation of their expression is important for BP control. The most comprehensive study performed to date revealed that expression of each of the five RhoGEFs linked to Rho-A dependent vasoconstriction (p115, LARG, PDZ-RHOGEF, p63 RhoGEF, and Lbc)

are all down-regulated in cultured mesenteric artery SMC following treatment with AII for 48 h. Moreover, treatment with the Rho Kinase inhibitor fasudil prevented the AII-mediated suppression of p115, LARG and PDZ-RhoGEF indicating that RGSRhoGEF expression is governed, at least in part, by negative-feedback signaling through the Rho/Rho kinase cascade. A similar decrease in RGSRhoGEF expression was observed in mesenteric arteries from rats treated with AII for 14 d[80]. Whether RhoGEF expression is altered in or contributes to hypertension in animal models is less clear. Ying *et al*[81] reported that aortic expression of all 3 RGSRhoGEFs was higher in aortas from 12 wk old SHR than in normotensive rats. Similarly, a comprehensive microarray analysis revealed that LARG expression was upregulated in DOCA-salt hypertensive mice[82]. In contrast, Hilgers *et al*[68] reported that mesenteric arteries from 14 d AII-treated rats exhibited decreased mRNA levels, but increased protein levels of PDZ RhoGEF. Thus, while it is clear that GEF expression is dynamic, the extent to which elevated expression of these factors contributes to the induction of hypertension and reduced expression to BP normalization is currently unresolved and requires further study.

RhoGAPs and BP control

GAPs inhibit Rho signaling by enhancing the intrinsic ability of Rho to hydrolyze GTP[83,84]. More than 70 RhoGAPs have been identified in eukaryotes that can be divided into 23 subfamilies[85]. Like the RhoGEFs, RhoGAPs are typically large multi-domain containing proteins and their diverse structures allow for dynamic and selective inhibition of small GTPase signaling (see Figure 2). Several Rho-selective GAPs including p190ARhoGAP, ArhGAP1, Myr5, GRAF1, and GRAF3 have been shown to regulate RhoA in cultured vascular SMC[86,87].

To our knowledge, GRAF3 is the only RhoGAP that has been implicated in the regulation blood pressure. The founding member of the GRAF (GTPase regulator associated with FAK-1) family, was originally identified by our group[88-90] by screening an embryonic λgt11 expression library for proteins that interacted with the carboxyl-terminal domain of FAK[88]. The GRAF family's three members are defined by an N-terminal BAR (Bin/amphiphysin/Rvs) domain, a phosphatidylserine (PS)-binding PH domain, a central Rho-GAP domain, a serine/proline rich domain, and a C-terminal SH3 domain (Figure 1A). The GRAF1 SH3 domain was shown to specifically bind to a proline-rich region in the carboxy terminus of FAK and this protein-protein interaction was important for directing GRAF1 to the actin cytoskeleton[88]. GRAF1 is expressed predominantly in the brain and striated muscle (cardiac and skeletal), and our studies in GRAF1-depleted *Xenopus* and mice revealed that GRAF1-dependent inhibition of RhoA activity promoted mammalian muscle growth by facilitating myoblast fusion and injury repair[90-93]. GRAF2 is more ubiquitously expressed[94] and could partially compensate for the loss of GRAF1 during myotube formation supporting at least some functional redundancy within this family[92]. Evolutionarily, GRAF3 is the youngest family member and is the most recently annotated. Interestingly, our genome wide analyses of chromatin structure in primary human SMC suggested that this gene was regulated in a smooth muscle-specific fashion. Indeed, we found that GRAF3 was highly and selectively expressed in SMC with particularly high expression in resistance vessels[87]. After validating that GRAF3 functioned as a RhoA-specific GAP in these cells, we considered the possibility that GRAF3 might control BP homoeostasis. Importantly, we found that homozygous GRAF3 knockdown mice showed a consistent and significant elevation in systolic, diastolic, and mean arterial BP (+ 20-30 mmHg). The observation that heterozygous GRAF3 knockdown mice exhibited a 15 mmHg increase in BP strongly supports a dose-dependent relationship between GRAF3 expression and BP. GRAF3-deficient mice exhibited significantly elevated pressor responses following treatment with AII, ET1, or PE and these effects were inhibited by treatment of GRAF3 deficient mice with Y-27632. Accordingly, RhoA activity and myosin light chain phosphorylation were elevated in GRAF3-depleted SMC *in vitro* and *in vivo*[87].

The remarkable SMC-selective expression pattern of this Rho-selective GAP when coupled with the ability of ROCK inhibition to normalize the hypertensive phenotype of GRAF3-deficient animals strongly supports a model in which GRAF3 plays a major role in regulating BP homeostasis by limiting RhoA-mediated SMC contractility in resistance vessels[87]. Interestingly, as discussed in further detail below, a large GWAS revealed that polymorphisms in the GRAF3 gene contribute to BP variation in humans[95,96]. Thus future studies that strive to determine the mechanisms that control variations in GRAF3 expression and/or activity will likely lead to important insights into how to better control BP in the general population.

P190RhoGAP may also play a role in limiting RhoA-dependent arterial tone. p190RhoGAP contains an amino-terminal GTP-binding domain, a large middle domain with multiple protein-protein interaction motifs (diphenylalanine, FF motifs) a polybasic region, and a carboxy-terminal GAP domain[97]. Knockdown of p190RhoGAP in SMC by siRNA increased RhoA/Rock activity[98], and several studies have shown that p190RhoGAP is activated by phosphorylation of Y1105 by cAbl and Src tyrosine kinases[66,99]. p190RhoGAP is a substrate for the tyrosine phosphatase, SHP-2, and SHP-2-dependent dephosphorylation of p190RhoGAP was shown to be important for the initial burst in RhoA activity in SMCs treated AII and ET1[98]. Interestingly, ROCK-dependent phosphorylation at Ser1150 attenuated p190RhoGAP activity creating a positive feedback loop for further RhoA activation[86]. Pho-

sphorylation of several C-terminal residues by ERK also suppresses p190RhoGAP activity during focal adhesion formation[100]. Finally, although not yet shown in SMC, p190RhoGAP has also been shown to be regulated by phospholipid binding[101]. Additional studies will be necessary to determine if p190RhoGAP plays an important role in BP regulation *in vivo*.

Regulation of GDIs

GDIs bind to GDP-bound GTPases and inhibit GDP dissociation. GDI binding also limits translocation of GTPases to the membrane effectively "locking" them in the inactive state. Indeed, studies have shown that GDIs can inhibit RhoA dependent Ca^{2+} sensitization in SMCs treated with α-adrenergic and muscarinic agonists[102]. However, the extent to which RhoGDIs regulate BP or RhoA activity *in vivo* is unclear. One study showed that RhoGDIα knock out mice displayed a salt-dependent increase in BP, but this effect was attributed to an increase in Rac1 activity in the kidney[103]. However, since SMC-specific Rac1 knockout mice were hypertensive and exhibited increased RhoA activity, it will be important to measure RhoA and Rac1 activity in SMC in RhoGDIα knock out mice[104]. RhoGDIs have been shown to bind to and regulate RhoGEFs and RhoGAPs[105], an effect that could indirectly influence RhoA activity and vessel tone. Thus, additional studies will be needed to assess RhoGDIs' role in RhoA dependent blood pressure regulation.

Direct regulation of RhoA

Additional control of RhoA signaling may be imparted by mechanisms that alter RhoA protein levels and/or alter functional post-translational modifications. Notably, protein ubiquitination followed by proteasome-dependent degradation is a major means of fine-tuning protein levels and Chen *et al*[106] reported that RhoA is a direct target of the Rho-BTB/Cullin-3 E3 ubiquitin ligase degradation pathway. Interestingly, the Sigmund laboratory found that Cullin-3 regulated vascular smooth muscle function and arterial BP through a RhoA/Rho-kinase pathway. Moreover, they found that a human hypertension-associated mutation in Cullin-3 in which exon 9 is deleted led to decreased Cullin-3 activity and reduced ubiquitin-mediated Rho A degradation[107,108] (see below for further discussion of these and other genetic variants that influence RhoA signaling and human hypertension). Ubiquitination-dependent regulation of RhoA is also catalyzed by a distinct E3 ubiquitin ligase termed SMAD ubiquitin regulatory factor (Smurf1)[109,110]. Interestingly, Smurf1-dependent degradation of RhoA in endothelial cells has been linked to the development of cerebral cavernous malformation (CCM) a disease that is accompanied by hyperpermeable blood vessels in the brain. CCM results from the homozygous inactivating mutations in one of three *ccm* genes. Crose *et al*[109] demonstrated that *ccm2* bound directly to Smurf1 and that this interaction regulated RhoA degradation, likely explaining the common biochemical defect of elevated RhoA/ROCK signaling and increased permeability observed in *ccm* mutant endothelial cells[109,110]. Whether this class of E3 ligases regulates RhoA in levels in SMC is currently unknown.

Signaling *via* nitric oxide and reactive oxygen species may add another level of spatial/temporal control of RhoA signaling in the vasculature. Levels of ROS increase in the vasculature under a number of pathological conditions including hypertension, and ROS-mediated activation of RhoA has been demonstrated in vascular smooth muscle[81]. Interestingly, Aghajanian *et al*[111] have demonstrated that ROS can mediate direct activation of RhoA by reversible oxidation of reactive cysteines C16/C19 and that this acts in a similar fashion to GEFs in that it leads to nucleotide displacement and increased GTP binding. Because oxidation of RhoA does not impair RhoGEF binding it is possible that ROS-dependent oxidation might prime RhoA for vasoconstrictor-dependent activation. In contrast, the reactive vasodilator, NO has been shown to inhibit RhoA activation *via* post-translational modification. For example, treatment of SMC with the pharmacological NO donor, PAPA-NONOate promoted RhoA S-nitrosylation that reduced GTP binding and therefore inactivated RhoA[112]. NO signaling may also limit RhoA activity in SMC by promoting cGMP-dependent phosphorylation of RhoA on sites that attenuate membrane targeting (and activation) of RhoA[113,114]. However, the role that such post-translational modifications play *in vivo* has yet to be explored.

In summary, RhoA activity in SMC can be dynamically regulated by transcriptional and post-translational mechanisms that alter RhoA protein, its activators, and its inhibitors. Collectively these mechanisms play an important role in precise spatial-temporal control of vessel tone and BP homeostasis. Importantly, while several RhoAGEFs have been shown to be necessary for development of vasoconstrictor-induced hypertension, our recent results in GRAF3-depleted mice demonstrated for the first time that GAP-dependent control of RhoA activity in SMC contributes to the maintenance of basal BP[87].

GENETIC REGULATION OF THE RHOA PATHWAY IN HUMAN HYPERTENSION

Hypertension is a devastating disease associated with significant morbidity and mortality due to detrimental pressure-related effects on the kidneys, heart, lungs, brain, and peripheral vasculature. Hypertension affects roughly 80 million people (approximately 32.6% of adults) in the United States alone and was predicted to be primarily responsible for 25% of deaths worldwide in 2010[115]. Despite the fact that nearly 70 drugs (from 15 distinct classes of compounds) are approved

for treatment of hypertension in the United States, estimates project that reasonable BP control is achieved in only about half of hypertensive patients. This reality coupled with recent projections that the incidence of hypertension will increase to about 41% in the United States by 2030, indicate the urgent need for better screening and treatment modalities[116]. Improvements in the detection and management of hypertension will undoubtedly be accomplished through a better understanding of the complex etiology of this disease.

One way to better predict patient response to therapy is to gain a more comprehensive understanding of the genes and genetic variants that influence BP regulation. Recent projections indicate that up to 60% of BP variation can be explained by genetic factors, but that no single gene exerts a principal effect. Thus, BP is considered to have a complex non-Mendelian mode of inheritance. Indeed a combination of classic positional cloning strategies in families with numerous affected members combined with more recent population-based GWAS studies have led to the identification of 25 rare mutations and 53 SNPs that are predicted to contribute to BP control[117]. The aim of this section of is to highlight variants that impinge on the expression or activity of members of the RhoA signaling axis.

RhoA-related forms of monogenic hypertension

Virtually all known cases of monogenic hypertension are associated with volume expansion resulting from mutations in genes involved in renal salt handling or hormones that affect mineralocorticoid activity. However, although hypertensive patients with Gordon's Syndrome (pseudohypoaldosteronism type IIE) present with salt handling abnormalities, the high BP in these patients is caused by an autosomal dominant mutation in the Cullin-3 gene (see above). Interestingly, this E3 ligase helps target RhoA for proteosomal degradation and *in vitro* studies indicate that increased RhoA/ROCK signaling in vascular SMC may also play a role in Gordon's Syndrome patients[118]. Exclusion of exon 9 abrogates the Cullin-3 dependent interactions between RhoBTB and the E3 ligase and as RhoBTB serves as a chaperone to recruit RhoA to this degradation complex, expression of exon 9-deficient Cullin-3 leads to aberrant RhoA accumulation[107,108].

SNP/EQTLs in RhoA-signaling molecules

Because Rho kinases are major RhoA effector proteins and because both animal and human studies have shown that treatment with Rho-kinase inhibiting compounds lowers BP, a number of case-controlled studies were designed to determine if genetic variants in these genes might influence the development of human hypertension. One group examined the effect of ROCK2 genetic variations on BP in 168 pairs of mono- and dizygotic twins. In this study, four variants were identified in ROCK2, the most notable of which was a nonsynonymous SNP in exon 10 that resulted in a substitution of Thr with Asn at amino acid 431. Importantly, the Asn substitution was associated with increased systemic vascular resistance and BP and was predicted to account for 3%-5% of the BP variance between these patients[119]. Another study in which 18 tag SNPs within the ROCK2 locus were genotyped in 586 normotensive controls and 607 hypertensive Caucasian patients identified a haplotype defined by four SNPs (rs965665, rs10178332, rs6755196, rs10929732) that was recessively associated with a lower risk of hypertension (P = 0.003). However, a subsequent study in a separate population of 1344 Chinese patients with coronary artery disease and hypertension and 1267 ethnically and geographically matched controls did not find an association between this haplotype and either BP or cardiovascular disease[120,121]. Thus, future studies are necessary to determine the relevance of these SNPs with respect to BP control in the general population.

Recent studies have implicated artery stiffness in the pathology of HTN and this parameter has been shown to be a valuable predictor of end organ failure[122-126]. Decreased vessel compliance elevates the mechanical load on the myocardium but also increases peripheral pulse-pressure in the microvasculature resulting in tissue damage in high flow organs such as the brain and kidneys. Until very recently, increased vascular stiffness during aging or the development of HTN was thought to result from changes in extracellular matrix content and composition (*i.e.*, elastin degradation, collagen deposition, *etc*.). However, new studies suggest that the intrinsic mechanical properties of VSMC (including RhoA-dependent formation of force-generating actin filaments, and increased cell adhesion to the extracellular matrix) may also play a role[127,128]. Notably, Liao *et al*[129] identified two SNPs in ROCK2 that were in complete linkage disequilibrium and associated with arterial stiffness in 1483 un-selected patients from a Chinese population in Taiwan. Subsequent, *in vitro* studies revealed that both SNPs were functional. One SNP, rs978906, affected ROCK2 expression by interfering with microRNA(miR)-1183 binding to its 3' UTR, while the other, rs9808232, which was located in a protein-coding region, increased ROCK2 activity[129].

As noted above, S1P is a major upstream activator of RhoA in SMC and has vasoconstrictive effects *in vivo*[71,130]. Interestingly, Fenger *et al*[131,132] assessed the significance of 353 genetic variants contained within exons of genes in the metabolic sphingolipid network. Of these SNPs, 34 and 40 haplotypes were associated with changes in diastolic or systolic pressures respectively in their 2556 subjects. They found that while the BP effects could not be explained by any single gene, several 2-gene interaction pairs were highly correlated with BP variations. S1P is generated from ceramide in a process that involves two critical enzymes ceramidase (ASAH1) and sphingosine 1- kinase (SPHK1) and the most significant of the 2-gene interactions identified were contained in these genes[131,132], further

supporting a role for RhoA signaling in the development of hypertension. It is likely that future gene interaction studies such as these will provide a powerful approach to both predict hypertension risk and possibly inform treatment options.

In the past decade, many GWAS studies have identified common genetic variations in coding and non-coding genomic regions that vary between individuals and are associated with changes in BP and several of these variants occur in genes linked to the Rho signaling cascade. Notably, one GWAS study that used hypertension as a dichotomous trait identified eight loci associated with BP, and two of these variants were located in RhoA-related genes. One of the target genes was the aforementioned RhoBTB1 which functions with the Cullin-3 complex to maintain low RhoA levels[107,118]. Another SNP was found at the rhotekin-2 (RHTKN) locus. Although rhotekin was one of the first identified RhoA effector molecules (it has high affinity to Rho-GTP and is widely used in pull down assays for activated RhoA[133]), how Rhotekin functions at a cellular level is still unclear. Nonetheless this association is provocative and clearly indicates that future studies are warranted. Two separate GWAS for BP variation and hypertension have identified significant association signals in the RhoA-interacting protein, plekstrin homology domain containing family A member 7 (PLEKHA7)[134,135]. PLEKHA7 is highly expressed in the kidney and heart and localizes on the cytoplasmic surface of adherens junctions, where it interacts with junctional proteins cingulin and paracingulin to regulate the activity of Rho family GTPases, including RhoA[136]. While the functional SNP(s) have yet to be identified, the finding that PLEKHA7 is required for the development of salt-induced hypertension *in vivo*, highlights the functional importance of this RhoA-interacting protein in BP regulation[137].

Finally, two separate GWAS for BP and cardiovascular disease endpoints identified a novel BP associated locus containing two SNPs in perfect linkage disequilibrium (rs633185 and rs604723) within *GRAF3* gene (*ArhGAP42*). Both SNPs were associated with a significant reduction in BP with each copy of the minor allele[95,96]. Of extreme importance, as noted above, we reported that mice in which GRAF3 was depleted developed significant hypertension that was RhoA-dependent[87]. Interestingly, the BP locus falls within the first intron of the *GRAF3* gene, indicating that one or both SNPs may affect expression of this Rho-GAP and result in altered SMC contractility. Indeed, data within the Genotype-Tissue Expression database indicated that GRAF3 RNA levels in tibial artery samples were 3-fold higher in patients homozygous for the minor T allele compared to patients homozygous for the major C allele ($P < 1.5e^{-10}$;[138]). Moreover, using allele-specific quantitative RT PCR on RNA isolated from human aortic SMC heterozygous at rs604723, we found that the minor T allele was associated with a significant increase in mRNA expression (Mangum and Mack, personal communication) and we identified a novel cis element by which this allele upregulates GRAF3 transcription. To our knowledge, this is only the second functional SNP identified in a GWAS study that has been linked to a causal gene and pathway (the first being rs5068 located within NPPA/B[117]).

Collectively, these studies will likely have important implications in the future diagnosis and treatment of hypertension. For example, patients predicted to exhibit aberrantly high levels of RhoA signaling may respond better to anti-hypertensive regimens directly targeting vessel tone, compared to those that target blood volume. Moreover, they reveal that the RhoA signaling axis may provide highly selective targets for the treatment of human hypertension and related cardiovascular sequela.

PHARMACOLOGICAL REGULATION OF RHOA AND RHO-DEPENDENT PATHWAYS

Despite the importance of RhoA signaling in the development of hypertension, few treatments are currently available that target this signaling axis. However, some commonly used anti-hypertensives may interfere with RhoA signaling (Figure 3). For example, since RhoA-dependent regulation of vascular tone is a major contributor to AII-mediated increases in BP[13,139], the highly utilized class of anti-hypertensives that target AII (*i.e.*, ACE inhibitors and AII receptor blockers) may exert some of their BP lowering effects by reducing RhoA activation. Moreover, although used to treat high cholesterol, HMG-CoA reductase inhibitors such as simvastatin and atorvastatin also have anti-hypertensive properties[140] and their BP lowering effects have been attributed to their ability to block RhoA signaling. RhoA is known to be modified by covalent attachment of a geranylgeranyl isoprenyl to a C-terminal Cys, and this modification (which is blocked by simvastatin treatment) is required for membrane localization and activation of RhoA[141].

While not yet included in standard of care treatment for hypertension, several pharmacologic agents have been developed for inhibiting Rho kinases. In general, kinases make good drug targets due to the relative ease of targeting specific molecules to the ATP-binding pockets of these enzymes. To date, most of the Rho kinase inhibitors utilized in animal studies and clinical trials target the ATP-binding pockets of both ROCK isoforms[142-144]. Although not clinically used in the United States, studies abroad provide compelling evidence for the use of this therapeutic approach for BP control. One particularly effective ROCK inhibitor, fasudil, is currently used in Japan to treat cerebral vasospasm and clinical trials determined that fasudil was also effective in decreasing peripheral vascular resistance in hypertensive patients[5]. However, despite their wide use in cells and animal disease models, neither

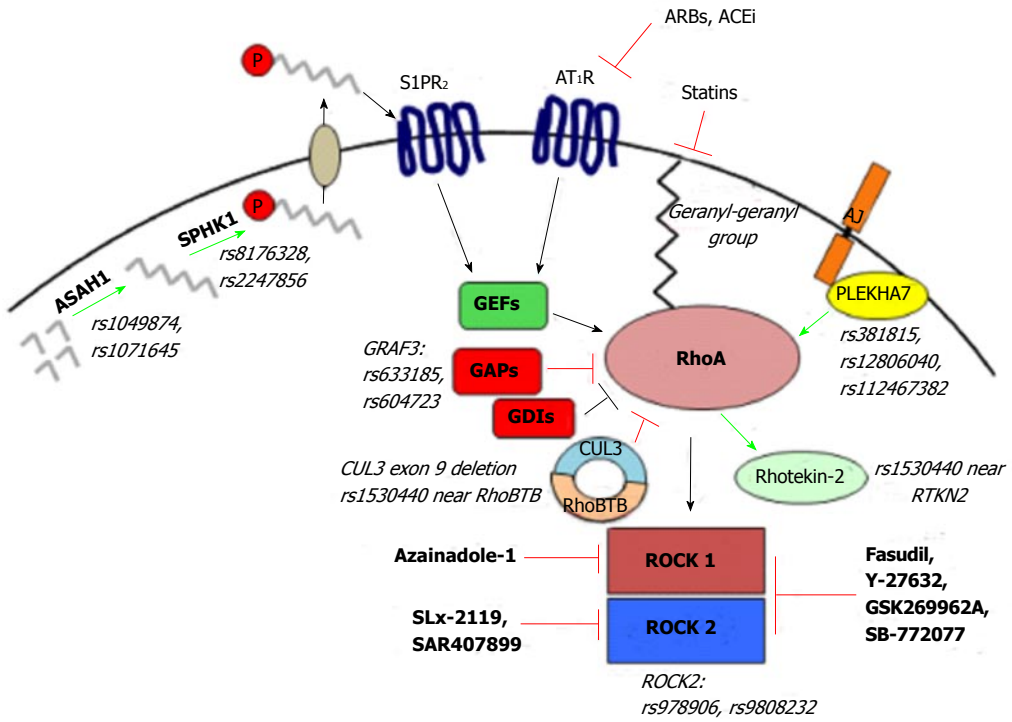

Figure 3 Pharmacologic and genetic regulation of the RhoA signaling axis. Schematic indicating the sites of action of pharmacological inhibitors (bold) of RhoA signaling molecules. Polymorphisms (SNPs/eQTLs) that could influence RhoA signaling are also shown. AJ: Adherens junction; A2R: Angiotensin type II receptor; ARBs: Angiotensin receptor blockers; ACEIs: Angiotensin converting enzyme inhibitors; ASAH1: Acid ceramidase; SPHK1: Sphingosine kinase 1.

fasudil nor Y-27632 exhibit suitable specificity for a therapeutic as they can inhibit the activity of several other kinases including PKC, PKA, and MLCK, at higher concentrations[145,146]. These compounds also suffer from having short half-lives, which is a highly undesirable attribute of a drug designed to treat a longstanding disease[147]. Thus, there is great need for development of additional potent, yet specific, ROCK inhibitors that can be safely used in patients[148]. While a few such compounds have been developed recently with such attributes[149-153], whether any these compounds exhibit the necessary selectivity and pharmacogenetic profiles required for BP management in patients requires further study. Moving forward, given the importance of RhoGEFs and RhoGAPs in the control of SM contractility and BP, we believe that it will be possible to engineer clinically-relevant small molecule regulators of these enzymes that could be used to develop new and effective anti-hypertensive therapies.

CONCLUSION: FUTURE POSSIBILITIES FOR PERSONALIZED TREATMENT OF HYPERTENSION

Current anti-hypertensive therapy is often empirically based and involves multiple drug regimens[154,155]- an approach that is moderately effective at best as it frequently contributes to unwanted side effects and intolerance or non-adherence to medication. Accordingly, more effective and specific anti-hypertensive agents are necessary. Moreover, based on the fact that BP is a highly variable trait among individuals, a better understanding of the genetic mechanisms regulating this disease is critical for a more personalized treatment plan for patients. Given the numerous regulatory and counter-regulatory mechanisms modulating the RhoA axis, this central axis provides an excellent opportunity for identifying genetic biomarkers that correlate with different levels of hypertensive risk and drug responses. Indeed, genetic variations in both upstream activators and downstream mediators of RhoA have been linked to BP regulation (Figure 3). Screening for such variants could potentially be used to tailor more effective individualized treatments. For example, one study showed that the BP lowering effects the ACE inhibitors or the angiotensin receptor blockers were more pronounced in patients carying a GG genotype at the -391 RGS2 (Regulators of G-protein signaling 2) locus when compared to responses in GC or CC genotype carriers- while no differences were observed in the responses to calcium channel antagonists[156]. Although RGS2 is known to couple to ATR1, the underlying mechanism by which these polymorphisms lead to altered sensitivity is currently unknown. Genetic differences in pharmacogenetics also play a role in response to anti-hypertensive agents, for example polymorphisms in the gene were associated with reduced BP-lowering effects of the βAR-blocker atenolol[157]. Whether any of the aforementioned Rho-signaling SNPs influence specific responses to or bio-availability of anti-hypertensive treatments remains

a critical unexplored question. The clinical utility of targeting the RhoA pathway should also be further explored.

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MINIREVIEWS

Place of baroreceptor activation therapy in the treatment of resistant hypertension

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Abstract

This mini review describes the development of the therapeutic concept of baroreceptor stimulation over the last fifty years alongside the more recent introduction of it for the treatment of drug - resistant hypertension. The pros and cons of this strategy of treatment over renal sympathetic denervation are also discussed in the light of the results of the studies done in the last decade.

Key words: Resistant hypertension; Treatment; Baroreceptor stimulation; Renal denervation

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Core tip: We herein describe the development of the therapeutic concept of baroreceptor stimulation for the treatment of drug-resistant hypertension. The ups and downs of this treatment strategy are discussed in the light of the results of the studies done in the last decade.

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INTRODUCTION

Baroreceptor activation blunts sympathetic activity and enhances vagal tone, thus rebalancing the neural output to the heart, the vessels, and the kidney in favour of the latter. This treatment strategy, currently defined as baroreceptor activation therapy (BAT), was originally conceived 50 years ago with the ultimate aim of lowering blood pressure (BP): Proof-of-concept studies were first published by Bilgutay *et al*[1] and then by Torresani *et al*[2]. They could nicely document the achievement of a marked reduction in BP in patients who were resistant to the (few) drugs available at

Table 1 Therapeutic strategies in patients with resistant hypertension

Recommendations	Class of recommendation[1]	Level of evidence[2]
In resistant hypertensive patients it is recommended that physicians check whether the drugs included in the existing multiple drug regimen have any BP lowering effect, and withdraw them if their effect is absent or minimal	I	C
Mineralocorticoid receptor antagonists, amiloride, and the alpha-1-blocker doxazosin should be considered, if no contraindications exist	IIa	B
In case of ineffectiveness of drug treatment invasive procedures such as renal denervation and baroreceptor stimulation may be considered	IIb	C
Until more evidence is available on the long-term efficacy and safety of renal denervation and baroreceptor stimulation, it is recommended that these procedures remain in the hands of experienced operators and diagnosis and follow-up restricted to hypertension centers	I	C
It is recommended that the invasive approaches are considered only for truly resistant hypertensive patients, with clinic values ⩾ 160 mmHg SBP or ⩾ 110 mmHg DBP and with BP elevation confirmed by ABPM	I	C

Class of recommendation[1]: Class I: Evidence and/or general agreement that a given treatment or procedure is beneficial, useful, effective; Class II: Conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of the given treatment or procedure; Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy; Class IIb: Usefulness/efficacy is less well established by evidence/opinion. Level of evidence[2]: Level of evidence B: Data derived from a single randomized clinical trial or large non-randomized studies; Level of evidence C: Consensus of opinion of the experts and/or small studies, retrospective studies, registries. ABPM: Ambulatory blood pressure monitoring; BP: Blood pressure; DBP: Diastolic blood pressure; SBP: Systolic blood pressure.

that time. Unfortunately the delivering of this therapy involved an invasive process and, moreover, stimulation was achieved by means of an external device. Accordingly, it turned out to be unpractical out of the hospital. For these reasons, alongside the development of multiple effective BP-lowering medications, BAT was rapidly abandoned.

It took more than forty years for this simple physiological principle to find again its way to clinical application, thanks to the development of implantable devices. The latter were shown to unequivocally lower BP in the DEBUT study[3]. Furthermore, in the Heart Rate Variability sub-study, they were also reported to improve the sympatho-vagal balance[3]. At that time it became readily evident that the BAT not only lowered BP and sympathetic nerve activity to the muscles[4], but was also effective in regressing left ventricular (LV) hypertrophy, in improving LV geometry, and in decreasing BP all around the clock[3].

In June 2010, when the first international meeting on Resistant Hypertension was held in Padua, Italy[5], it seemed, therefore, that BAT was emerging as the "front runner" in the therapeutic armamentarium for the then resurfacing problem of resistant hypertension.

Strong competition in the therapeutic armamentarium

At about the same time the publication of the first study on renal denervation[6] and, immediately after, of the Simplicity HTN-1[7] introduced another strong competitor for BAT in the race for the best treatment of drug-resistant hypertension: Percutaneous renal denervation. This treatment modality, which is undoubtedly less invasive than BAT, was shown to be effective in decreasing BP in carefully selected cases.

In June 2013, based on the evidence provided in the Simplicity HTN-2 trial that was published thereafter[8], the ESC/ESH Guidelines released statements concerning the use of BAT or renal denervation (Table 1)[9]. With a class IIb level of recommendation C these guidelines affirmed that invasive procedures, such as denervation and baroreceptor stimulation, "may be considered" in case of ineffectiveness of drug treatment[9]. In other words, it was explicitly acknowledged that the evidence supporting usefulness/efficacy of these therapies was not well established by evidence/opinion (Class IIb), and that the level of evidence depended on consensus of experts, and/or small studies, retrospective studies and/or registries (Level of evidence C).

Of further importance, with a class I level C the ESC/ESH guidelines recommended that "these procedures remain in the hands of experienced operators and diagnosis and follow-up restricted to hypertension centers". It was further emphasized that these invasive approaches were to be "considered only for truly resistant hypertension".

In March 2014, the scenario changed again with the publication of the first randomized single-blinded, sham-controlled study on renal denervation, the Simplicity HTN-3. While conclusively proving the safety of renal denervation in experienced hands, this study failed on one of its primary endpoints, *e.g.,* the demonstration that renal denervation was more effective than a "sham" procedure in effectively lowering BP[10]. After the publication of this study, the results of a smaller multicenter French study, the DENERHTN[11], added further fuel to the debate. According to this study, when given on top of a carefully planned stepped pharmacologic treatment, renal denervation provided almost identical BP values as placebo at 6 mo follow-up, although the fall of BP, both systolic and diastolic, was greater in the renal denervation arm. However, this was apparently only because the patients in this group had slightly higher baseline values, suggesting the possibility of a "regression toward the mean" effect[11], and raising

questions about the effectiveness of renal denervation in lowering BP. According to the plot depicting the individual BP changes, some patients did show a marked BP reduction and others showed no BP fall whatsoever.

At about the same time, the Prague study[12] also found no evidence for superiority of renal denervation over medical therapy. In this study the patients were randomized to either a multidrug treatment comprising the mineralocorticoid antagonist spironolactone, or to renal denervation. The BP values at 6 mo follow-up were similar in the renal denervation and medical treatment arm. While the long-term results of this study are still awaited, it seems fair to say that it remains to be conclusively proven that renal denervation entails an all-round treatment for all patients with truly resistant hypertension. A head-to-head comparison of BAT and renal denervation is shown in Table 2.

Table 2 Similarities and differences between renal denervation and baroreceptor activation therapy

Features	Renal denervation	BAT
SNS	↓	↓
Invasive	Yes	Yes
Safe	Yes[1]	Yes[1]
BP short-term	↓	↓
BP long-term	?	?
Side	Bilateral	Monolateral
Evidence of success	Delay	Immediate
Reversible	No	Yes
Heart rate effect	↓	↓↓
Metabolic effect	Yes	?
Need for follow-up	Yes	Yes
Logistics[2]	+	+++
Costs	+	+++

SNS: Sympathetic nervous system; BP: Blood pressure; BAT: Baroreceptor activation therapy; [1]Complications rate < 3%; [2]Interventional Radiology *vs* Vascular surgery.

The challenges of proving effectiveness of treatments for resistant hypertension

There is no question that the diagnosis of true drug-resistant hypertension is a difficult one, particularly out of tertiary referral centers, *e.g.,* centers where all diagnostic tools cannot be available[12]. Of note, in the patients initially selected at the French specialized centers that participated in the DENEHRT study, about 50% were excluded because of secondary HT, a rate that is remarkably higher than commonly perceived. This observation suggests that out of the hypertension referral centers under detection of secondary forms of hypertension is a diffuse phenomenon, which recognizes several causes. Nevertheless, the high exclusion rate due to secondary hypertension in the French Study indicates that, in keeping with the guidelines, any invasive procedures for the treatment of HT should be restricted to the centers that are competent in reliably rule out secondary forms of HT. This is not an easy task in patients who are resistant to drug treatment for a very simple reason: these patients are, by definition, on multiple drugs; moreover, they can carry multiple conditions that affect renin-angiotensin-aldosterone system. Thereby, the measurements of renin and aldosterone, the two key biomarkers for the identification of the most common forms of secondary HT, *e.g.,* primary aldosteronism and renovascular hypertension, can be markedly biased. In most cases this problem can hamper the diagnosis.

The current place of BAT

Nowadays BAT devices allow switching on and off the implanted device; therefore, it provides the ideal within-patient design for assessment of the effect on BP. By such strategy the BP-lowering efficacy of BAT has been proven beyond any doubts[13]. Moreover, these features also allowed the demonstration that the effects of BAT are reversible and reproducible over time. Of much importance for the patients with resistant hypertension, who had a BP that was not controlled after renal denervation[14], BAT was shown to work well, albeit only in small subsets of patients.

Finally, BAT can be used in patients who have contra-indications to renal denervation because of unsuitable renal anatomy, previous renal endovascular treatment and/or impaired glomerular filtration rate. These advantages have to be weighed against its invasiveness, which however, has been diminished by the development of the smaller 2nd generation devices, and, more importantly, by the demonstration that unilateral BAT is not inferior to bilateral BAT at least for lowering BP[13]. Decreased invasiveness of the implantation, alongside improved experience of the surgeon and the medical team, will likely result into fewer complications and shorter hospital stay, thus decreasing the costs and increasing the acceptance of the BAT.

Technical aspects: Implantation technique

The system for delivering BAT consists of a carotid sinus lead and a pulse generator. Implantation of the pulse generator is generally performed by a vascular surgeon experienced in a subcutaneous infra-clavicular chest wall pocket, in the fashion of a pacemaker. Electrode implantation is also performed at the same time by a vascular surgeon experienced in carotid artery revascularization by surgical exposure of the carotid sinus through a transverse cervicotomy over the carotid bifurcation. The sinus region is then mapped by temporarily placing the electrode in various locations and applying electrical stimulation to determine the location with the greatest sensitivity to BAT. Sensitivity is measured by observing the hemodynamic changes, *e.g.,* reduction of heart rate and/or BP, associated with increased parasympathetic and/or decreased sympathetic nerve traffic. The electrode is then affixed to the sinus, while the opposite end of the lead is brought to the pulse generator pocket by means of a subcutaneous tunnel[15].

BAT dose is up-titrated over a series of follow-up

visits, much like medications are up-titrated; therapy is initiated at a moderate level in the absence of side effects, then therapy levels are uptitrated as long as the patient can tolerate it, with the objective of achieving full BP lowering at around 3 mo. Because the electrode-baroreceptor interface is unique to each patient, there is no standard dose of the therapy; the focus is therefore to tailor BAT to each individual patient to achieving a therapeutic dose in the absence of side effects[15].

Disadvantages and limitations of BAT

From what presented thus far, it might seem that BAT is the ideal treatment for all patients, but this is likely not the case, in that, as for all new techniques the initial studies aimed at proving the concept, should be followed by larger prospective multicentre studies in order to prove the effectiveness in the long-term control of arterial hypertension and, moreover, in the prevention of cardiovascular events and the improvement of survival. Overall, the main limitations of BAT entails its logistics requirements: The invasive nature of the procedure, the need for a vascular surgery unit, for general anesthesia, and of an outpatient clinic for periodical follow-up visits in order to check and replace generator battery, and/or timely determine if a possible device failure occurred. Finally, the costs, definitely higher than those of renal denervation, render BAT a therapeutic option to be reserved only for few very well selected patients.

CONCLUSION

Available accumulating data indicate that BAT is effective and safe. However, patients are required to follow the precautions that are mandatory for all those with implantable devices, and to stay in contact with the hypertension center for regular check-up and monitoring of the battery status. While these disadvantages can be easily overcome with proper logistic arrangements, whether BAT can reduce CV events in the long run is the key question that could only be answered with a large international multicenter study. What control group would be suitable and ethically acceptable to this end is a critical issue that also needs to be addressed.

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MINIREVIEWS

Roles of catecholamine related polymorphisms in hypertension

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Abstract

The objective of this review is to summarize current data obtained so far in catecholamine-essential hypertension (EH) relationships on a genetic basis. As the major elements driving the sympathetic system's actions, catecholamines modulate a variety of physiological processes and mutations related to the system. This could generate serious disorders, such as severe mental illnesses, stress-induced disorders, or impaired control of blood pressure or motor pathways. EH is idiopathic, and the genetic basis of its causes and substantial interindividual discrepancies in response to different types of treatments are the focus of interest. Susceptibility to disease or efficacy of treatments are thought to reflect genomic variabilities among individuals. Therefore, outlining the available knowledge in functional genetic polymorphisms linked to EH will make the picture clearer and will help to establish future prospects in the field.

Key words: Single nucleotide polymorphism; Catecholamine; Adrenergic receptor; Dopamine receptor; Hypertension; Epinephrine; Norepinephrine

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Core tip: Catecholamines are the major elements of sympathetic system's actions, therefore they also act as important regulators of blood pressure. Polymorphism studies require a tedious approach since there are inconsistencies among the studies due to different ethnical origins, subject size and self discrepancies among individuals. Nevertheless, there are many promising findings and still more fields to investigate. Especially role of genes involved in the biosynthesis and metabolism of catecholamines were relatively missing. This review summarizes the current knowledge about catecholamine-related polymorphisms on the basis of development, prognosis and drug response of essential hypertension and aims to improve better assessment of the disease.

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INTRODUCTION

Catecholamines are the class of molecules containing a catechol ring, which consists of a 2-hydroxyl-attached benzene ring, together with an amine chain. Involving epinephrine (or adrenaline), norepinephrine (or noradrenaline), and dopamine, this class of molecules serves to regulate both metabolic and neural mechanisms in the body and act as important targets for a large group of pharmacological agents.

Adrenaline is a hormone synthesized and released by the adrenal medulla into the bloodstream. Its concentration in plasma can rapidly rise up several-fold under a physical or mental tension, and when a sufficiently high concentration is achieved, it can trigger noradrenaline release from adrenergic terminals, an action indirectly affecting neuronal transmission. Noradrenaline, on the other hand, acts as a neurotransmitter rather than a hormone, though it shares very similar chemical structure with adrenaline, except a methyl group. It is released by neurons in the brain and, similar to adrenaline, it acts through alpha or beta adrenergic receptors. Dopamine is the metabolic precursor of noradrenaline and adrenaline, and it also acts as a transmitter/neuromodulator in the central nervous system through dopaminergic receptors.

As a crucial element of drug actions, catecholamine polymorphisms became a focus of interest in various disorders. Hypertension, one of the most common disease worldwide especially among elderly people, is characterized by high blood pressure (BP) and heart rate, two parameters effectively connected to sympathetic denervation. It is a complex disorder with polygenic and environmental determinants. In the majority of cases, it is idiopathic and there is no clear indication of the source. Therefore, searches for hypertension-related genes, mutations, and polymorphisms will assist in the gene-therapeutic approaches and design of target-based therapies. There are more than 50 genes identified so far through association studies, with the reservation of publication bias from selectivity of positive results and limited genotype-phenotype relation analysis[1].

Since the sympathetic nervous system (SNS) is one of the major mechanisms in the rapid regulation and maintenance of BP, it has been hypothesized that the SNS could have a substantial role in the development of essential hypertension (EH). Catecholamines are the mediators of SNS response, and their release from chromaffin cells or ganglionic neuronal ends would affect myocardiocytes; vascular smooth muscle contraction; blood flow through renal, coronary, and cerebral systems; systolic and diastolic BP, *etc*.

This review will briefly discuss the contribution of genetic polymorphisms in EH by combining known catecholamine-related polymorphisms with the anticipated metabolic and neurologic targets in the regulation of hypertension and will try to accomplish its significance in applications using a combinatorial approach with the available clinical data on that issue. The elements of the SNS, parasympathetic nervous system, and other BP regulators directly or indirectly correlated with catecholamine action and hormonal regulation of BP and heart rate will be overviewed in this perspective based on the current knowledge of the polymorphisms of the relevant elements. It is believed that this compilation will help to integrate current accumulated knowledge on the field, provide a preliminary perspective for the design of future studies, and increase our understanding of the genetic basis of catecholaminergic system components in this one of the most prevalent and complexly structured disorder.

RECEPTOR POLYMORPHISMS

One study has indicated that the long arm of human chromosome 5 contains a cluster of genes presumably involved in BP regulation[2]. This region contains genes encoding the alpha1B (α1B) and beta2-adrenergic (β2) receptors and dopamine receptor type 1A (D1A). The study was conducted with young Caucasians, and showed that this genome region has a significant association with systolic BP. Further studies involved other receptor types as well, with a prominence in β2-adrenoceptors, which will be discussed in detail later.

ALPHA-ADRENERGIC RECEPTORS

The a-adrenergic receptors (α-AR) are classified into two-subtypes: α1 and α2. They are G-protein-coupled receptors, and they activate second messenger systems through the activation of G-proteins (G_q or $G_{i/o}$). α1 mediates vasoconstriction and plays an important role in the regulation of vascular tone, while α2 serves to regulate noradrenaline release from presynaptic terminals.

ADRA1

The human α1A-AR is the predominant α1-AR subtype in vascular smooth muscle, the heart, and the liver. Considering its role in smooth muscle contraction, an early study has investigated the role of a previously determined polymorphism in α1A-AR, Arg492Cys, in normotensive and hypertensive black and white American individuals and determined the allele frequency distribution. Arg492 was found to be significantly higher in African-Americans with respect to Caucasians, but the frequency of the variant Cys492 was similar in normotensive and hypertensive individuals[3].

Another α1A-AR polymorphism, Arg347Cys, was examined in a large sample of the Brazilian population (a total of 1568 individuals were involved in the study)[4]. In this study, the Cys/Cys genotype was found to be significantly associated with hypertension (P = 0.06). Moreover, the response to daily treatment with nifedipine, an anti-hypertensive agent, was found

to be related to the same polymorphism in a Chinese population[5]. The later study noted that patients carrying the Cys347 allele of the α1A-adrenoceptor gene (ADRA1A) had a greater systolic BP reduction than did those carrying two Arg347 alleles of the α1A-adrenoceptor gene (32.5 ± 14.0 mmHg *vs* 27.3 ± 15.5 mmHg, respectively, $P = 0.006$) after daily treatment with an oral dose of 30 mg nifedipine gastrointestinal therapeutic system for 16 d, however, diastolic BP reduction was not associated with the Arg347Cys polymorphism. In addition, no significant associations were observed between BP reduction and two other polymorphisms (Arg16Gly and Gln27Glu) of the beta2-adrenoceptor (*β2-AR*) gene.

When the α1B-adrenoceptor gene (*ADRA1B*) was examined for possible polymorphisms, it was found that polymorphisms of this gene are much rare than expected considering its close location to the genes of β2-AR and dopamine receptors (DR)[2,6]. An amino acid addition at position 368 (368Arg) and a substitution (Arg371Gly) were investigated in a small population of 24 male patients with uncomplicated EH (12 Caucasians, 12 African-Americans) and 21 male normotensive (NT), first-degree relatives of the study group (12 Caucasians, 9 African-Americans)[6]. The study was unable to detect a relationship between these polymorphisms and BP levels or response to phenylephrine, an alpha-agonist used as a decongestant.

The role of the α1D-AR subtype in hypertension development was investigated in mice through a salt-induced hypertension model. The study suggested that α1D-AR plays an important role in developing a high BP in response to dietary salt-loading, and that agents having selective α1D-AR antagonism could have significant therapeutic potential in the treatment of hypertension[7]. To our knowledge, there are no studies reporting an association between ADRA1B or ADRA1D gene variants and hypertension.

A recent genome-wide study has strengthened the role of the adrenergic alpha1 receptor (ADRA1) pathway in hypertension and BP regulation. The ADRA1 pathway showed a strong association with diastolic BP ($P_{path} < 0.0007$) and hypertension ($P_{path} < 0.0009$) than systolic BP ($P_{path} < 0.06$). This pathway consisted of genes involved in adrenaline and noradrenaline synthesis, in vascular smooth muscle cell signal transduction leading to intracellular calcium release, and in major regulatory proteins. The study especially stratified the association of α1B-AR (ADRA1B) and the phenylethanolamine N-methyl transferase (*PNMT*) gene, the enzyme that catalyzes conversion of norepinephrine to epinephrine by the transfer of a methyl group[8]. The paper, however, emphasized the fact that neither of the remaining pathways utilizing the PNMT reached pathway significance, nor did the removal of ADRA1 receptor genes affect observed ADRA1 pathway significance, suggesting that none of the elements could be self-sufficient mediators for the observed associations.

ADRA2

The α2A-ARs are mainly involved in neurotransmitter release from sympathetic nerves. They are found on pre- and post-synaptic neurons of the central and peripheral nervous systems and blood vessels, and their involvement in BP regulation has been reported by various studies[9-11]. Yet, studies investigating the relationship between hypertensivity and different polymorphic sites mostly indicate a lack of association in various ethnic populations.

The α2A-ARs act through the G_i/G_o family of G-proteins, and they help to regulate a wide range of physiologic functions, including vascular, cardiac, and metabolic systems, as well as the central and peripheral nervous systems. Agonist binding to receptors causes the receptor to couple with related G-proteins, which in turn initiates effector responses like the inhibition of adenylyl cyclase or the activation of phospholipase C. Pre-synaptic activation of α2-adrenoceptors in sympathetic nerve endings and noradrenergic neurons leads to inhibition of norepinephrine release. Central nervous system activation of post-synaptic α2-adrenoceptors inhibits sympathetic activity, which results in hypotension and bradycardia, as well as sedation. Therefore, α2 agonists could be potent antihypertensive agents. Higher doses of α2-AR agonists, on the other hand, activate smooth muscle receptors in the arterial resistance vessels and could produce hypertension[12].

The BP and other responses to α2-AR agonists and antagonists can show high variability among individuals depending on the population pool. Like other signaling systems, variations can involve different elements through the signaling pathway, like G-proteins or effector enzymes, which will be discussed later. On the receptor side, a single nucleotide polymorphism (SNP) of α2-AR, which results in Asn-to-Lys substitution at amino acid 251 of the third intracellular loop (position 753), was identified in a study conducted by Small *et al*[13]. Subsequently, a total of 376 individuals (125 + 99 NT, 75 + 77 HT for Caucasians and African-Americans, respectively) were genotyped for this locus. The frequency of Lys-251 was 10-fold greater in African-Americans than in Caucasians, but was not associated with EH. Since the third intracellular loop forms the main site of G-protein interaction, the functional role of this substitution was also examined in a cell expression system. There were no detectable changes in ligand binding and basal function, but [^{35}S] GTPgammaS binding was 40% greater in Lys251 form. The findings implicated that this small replacement represented a gain of agonist-promoted function with enhanced inhibition of adenylyl cyclase, activation of MAP kinase signaling, or stimulation of phospholipase C/inositol phosphate pathways.

Based on these observations, it can be said that α1A-AR polymorphisms R347C and R492C significantly contributed to BP regulation. There is an ongoing research related to the other SNPs in α1A as well as

α1B and α1D subtypes and further investigations are needed to accurately assess their roles in hypertension.

β-ADRENERGIC RECEPTORS

The b-adrenergic receptors couple to either G_s or G_i (heterotrimeric stimulatory and inhibitory G-proteins) proteins. β1-ARs are the predominant type in the sympathetic control of heart rate and myocardial contraction. Protein kinase A, activated through the β1-AR → Gs → adenylate cyclase (AC) → cyclic adenosine monophosphate (cAMP) pathway, phosphorylates a set of regulatory proteins in cardiac excitation-contraction coupling, such as L-type Ca^{2+} channels or SERCA proteins. β2-ARs cause smooth muscle relaxation and bronchodilation. Defective β2-mediated vasodilation could result in both increased arterial resistance and reduced venous compliance. β-ARs are effectively used as targets to exogenously administered inhibitory agents, known as β-blockers. The β3 receptor, a relatively novel subtype, is mostly found in brown adipose tissue and plays role in the enhancement of lipolysis in this tissue, and is also responsible for thermogenesis in skeletal muscles.

ADRB1

There are many SNPs identified in the gene of β-ARs corresponding to different parts in structure[14]. Functional SNPs related to BP regulation were Ser49Gly at the N-terminus and Arg389Gly at the C-terminus of β1-AR. Three genetic polymorphisms, one of which belongs to the β1-AR, were investigated in Japanese hypertensive subjects by Shioji *et al*[15]. The polymorphisms alpha-adducin (ADD1/Gly460Trp), β1-adrenoreceptor (ADRB1/Arg389Gly), and G-protein β3 subunit (GNB3/C825T) were screened in 867 males and 1013 females. The ADRB1/R389G polymorphism and hypertensive status in male subjects were close to the significance (P = 0.0702). ADD1/G460W polymorphism was associated with hypertension in female subjects, and the GNB3/C825T polymorphism was not associated with hypertensive status in either male or female subjects. None of the polymorphisms was significantly effective on the disease. The relationship between the two polymorphisms (Ser49Gly and Arg389Gly) and BP or heart rate was also tested in a small group of patients (101 subjects) with EH and left ventricular hypertrophy treated with β1-AR blocker atenolol for 12 wk. Though reduction in heart rate was greater in Gly49 patients compared to the Ser/Ser genotype, there was no significant effect detected on heart rate and BP[16].

ADRB2

At 1998, Timmermann *et al*[17] reported four intragenic variants at the promoter region and N-terminus of the β2-AR in a study involving the offspring of 23 hypertensive and 22 normotensive European families. These mutations were a C→T substitution at -47 in the 5′ cistron causing Arg→Cys exchange, a T→C substitution at -20, and an A→G substitution at +46, resulting in Arg→Gly exchange of amino acid at 16 and a C→G substitution leading to Gln→Glu exchange at amino acid 27. All variants were found to be in linkage disequilibrium, but in particular the position -47 variant was significantly higher in frequency in the offspring of hypertensive parents, and Arg16Gly at +46 was significantly associated with parental hypertension and higher BP in this sample pool. Later studies further supported this relationship, mostly focused on the Arg16Gly and Gln27Glu substitutions, which introduce a change on the extracellular part of the receptor; however, as the data accumulated from then, so did contradictive findings. In order to compare study outcomes, a summary of cumulative data according to the ethnicity, study size, and allele distributions for β2-AR is introduced in Table 1[18-30].

Studies were also conducted to determine if β2-polymorphisms have an effect on response to antihypertensive reagents. Benazepril is an angiotensin-converting enzyme (ACE) inhibitor used primarily in the treatment of hypertension, congestive heart failure, and heart attacks. A study investigated the role of Arg16Gly polymorphism on systolic and diastolic BP s (SBP and DBP) before and after a 15-d benazepril treatment in a Chinese population that consisted of a total of 931 hypertensive subjects, and showed that ADRB2 R16G polymorphism may play an important role in DBP response to benazepril treatment[31].

ADRB3

The most widely studied β3-AR polymorphism is the missense mutation at position 64, which replaces tryptophane at this position with arginine. This polymorphism was found to be linked with high body mass index and obesity[32-34]. A white population (German) with type 2 diabetes carrying the Arg variant had higher BP and was more hypertensive, though they admitted to intense antihypertensive treatment[35]. Likewise, a similar study conducted in a large unselected Southern Italian population involving 979 patients showed that carriers of the Trp64Arg genotype were more often in the upper one-third of abdominal adiposity and were more hypertensive than the Trp64Trp homozygotes[36].

Several polymorphisms previously reported as risk factors in elevated BP and hypertension-ADRA1B, ADRA2A, ADRB1, and ADRB2-were examined in relation to systolic and diastolic BP s and heart rate, both at rest and in response to stress, by McCaffery *et al*[37]. Subjects (350 normotensive individuals) of European-American origin were analyzed for their BP s and adrenergic receptor variants at seven sites. At position 1165 of the *ADRB1* gene (Gly386Arg), G allele carriers showed higher systolic and diastolic BPs compared to homozygotes for the C allele. In addition, the AA genotype at position 145 of the gene (*Ser49Gly*) was found to be associated with SBP and DBP. At position 46 of the *ADRB2* gene (Arg16Gly), GG homozygotes had

Table 1 List of recent studies on β2-adrenergic receptor polymorphisms, blood pressure and hypertension

SNP	Ref.	Ethnicity	Sample size (HT/NT)	Association/ significance	Parameter
Arg16Gly	Kotanko *et al*[18] 1997	African Caribbeans	136/81	Yes	Hypertension
Arg16Gly	Gratze *et al*[19] 1999	Austrian Caucasians	57 NT	Yes	Blood pressure regulation
Gln27Glu Arg16Gly	Candy *et al*[20] 2000	Black South African	192/123	No	Hypertension, blood pressure Left ventricular mass
Gln27Glu Arg16Gly	Bray *et al*[21] 2000	Non-hispanic whites	589 families (> 2000)	Yes	Hypertension systolic, diastolic and mean arterial pressure
Gln27Glu Arg16Gly	Jia *et al*[22] 2000	Caucasians	298/298	No	Hypertension
Gln27Glu Arg16Gly	Xie *et al*[23] 2000	Black or white Americans	356/307	No	Hypertension
Arg16Gly	Herrmann *et al*[24] 2000	Black or white Americans	243	No	Hypertension
T-47C Gln27Glu Arg16Gly	Kato *et al*[25] 2001	Japanese	842/633	No	Hypertension
T-47C Gln27Glu Arg16Gly	Ranade *et al*[26] 2001	Chinese	> 800/> 800	Yes (only for Arg16Gly)	Hypertension
Gln27Glu Arg16Gly Thr164Ile	Tomaszewski *et al*[27] 2002	European (Polish)	638	No	Hypertension
Gln27Glu Arg16Gly Thr164Ile	Pereira *et al*[28] 2003	Brasilian	1576	Yes	Hypertension, blood pressure
Gln27Glu Arg16Gly	Galletti *et al*[29] 2004	Non-selected group-middle aged men	405 HT 563 overweight	No	Hypertension, overweight
T-47C Gln27Glu Arg16Gly	Ge *et al*[30] 2005	Han Chinese	503/504	Yes	Hypertension

SNP: Single nucleotide polymorphism; HT: Hypertensive ; NT: Normotensive.

higher resting DBP and AG heterozygotes had lower SBP than other genotypes.

Considering the close relationship between obesity and high BP, polymorphisms of β2 and β3 were investigated in a group of Japanese subjects (1121 men), selected as overweight or obese but not having diabetes mellitus or hypertension. The findings of the study demonstrated that the Arg64 allele of β3 and the Gly16 allele of β2 could have an indicatory role to predict weight gain-induced BP elevation in obese subjects[38]. Similarly, a total of 437 Chinese subjects, including 149 obese hypertensive patients and 139 non-obese essential hypertensive patients, were genotyped to investigate the association between Trp64Arg, Arg16Gly, and Gln27Glu polymorphisms and the susceptibility to obesity and hypertension in a Chinese population. The data revealed that the frequencies of β3-AR 64Arg and β2-AR 27Glu were significantly higher in obese hypertensive patients than in the non-obese hypertensive population[39].

The distribution of β-receptor polymorphisms was also determined in hypertension-related complications, such as left ventricular hypertrophy and arterial stiffness. In a group of 300 patients, pulse wave velocity and hyperemia was found to be associated with Ser49Gly of β1-AR, while left ventricular hypertrophy was more related to Glu27Gln β2 polymorphism, suggesting that these two polymorphisms have an effect on the development of arterial stiffness and left ventricular hypertrophy in EH[40].

BP response to the β-blocker atenolol administered 50 mg twice a day was examined in association with hypertension-related and closely linked SNPs of the β1-adrenergic receptor (Ser49Gly and Arg389Gly) and the β2-adrenergic receptor (Cys19Arg, Gly16Arg and Gln27Glu), together with G-protein β3-subunit (A3882C, G5249A and C825T) in EH patients. None of the SNPs were found to be associated with EH, except GNB3 SNPs and BP responses in females[41].

β-AR mutations are preeminent in BP regulation and EH compared to other adrenergic receptor subtypes. β-blockers are the well known medications in cardiovascular disorders. A large family of antagonists, such as oxprenolol or pindolol, are in current use to

block or suppress epinephrine or norepinephrine-mediated actions of the sympathetic system. These lower the heart rate, the force of contraction and reduce the BP. Therefore, it is no surprise that primary antihypertensive effects of adrenergic receptors belong to the β-AR family. Arg16Gly and Gln27Glu are likely to be potential genetic factors to consider and worthy of attention. Positive associations were reported in large scale Chinese cohorts, but studies conducted in other populations are rather inconsistent and should be supported with further analyses.

DOPAMINE RECEPTORS

Dopamine is a neurotransmitter with a variety of roles, majorly in the brain, but also throughout the body. In the brain, it mediates reward-motivated reactions, and helps to produce coordinated motor output, neuro-endocrine regulation, *etc*. Thus, several important diseases, like Parkinson's or schizophrenia, are highly interfered with dopamine activity. Outside the brain, it acts as a vasodilator in blood vessels, and in kidneys it controls renal sodium excretion.

Dopamine receptors are classified into two families: D1-like (includes D1 and D5) and D2-like (includes D2, D3, and D4). Both D1- and D2-like receptors mainly exist in the central nervous system, as well as on the smooth muscle of renal arteries, the juxtaglomerular apparatus, and the tubules of the kidney and cardio-pulmonary system. Like adrenergic receptors, they are G-protein-coupled receptors with seven transmembrane domains. Both members of the D1-family, D1 and D5, could interact with stimulatory G_s, but coupling with other members of G-proteins can be different for each subtype. For example, D1 can also interact with G_o, participating in the regulation of ion channels like Ca^{2+}, K^+, and Na^+, while D5 can couple to G_z members.

The activation of AC through G_s will cause activation of protein kinase A, which in turn will phosphorylate target proteins. In kidney proximal tubules, phosphorylation of two proteins by PKA, the Na^+-H^+ exchanger (NHE), and Na^+-K^+ ATPase (NKA) will inhibit their activation and affect sodium transport across tubules.

The relationship between salt intake and the development of hypertension, together with the renal functions of the dopaminergic system, brought dopaminergic receptor polymorphisms into attention in hypertension research. In 2000, Sato *et al*[42] screened 131 Japanese EH subjects for the A-48G polymorphic site in the DRD1 gene and showed that EH patients carrying the G allele had a higher diastolic BP in general. Later, the allele frequencies of two SNPs, A-48G and G-94A, were determined in a larger cohort, consisting of 493 hypertensive Caucasian subjects. In contrast to the study involving Japanese patients, this study was unable to show any correlation with hypertension in this population, reflecting the role of ethnicity in polymorphism-related secondary effects[43].

When renal clearance of sodium was taken into consideration, the DRD1 polymorphisms A-48G, G-94A, and C-800T were shown to have an effect in the reabsorption of sodium, especially from distal tubules. In a multivariate association analysis, it was shown that DRD1-94GG homozygotes had lower reabsorption rates. The transmission of the DRD1 AGC haplotype was found to be associated with lower systolic and diastolic BP in a family-based analysis[44]. In a small Turkish cohort involving 101 EH patients run by our group, we were not able to obtain such a correlation with the same SNPs (A-48G and G-94A), suggesting that further analysis is required to clear the picture[45].

Dopamine D2 receptors act through G_i proteins, and their inhibitory action on AC reduces the noradrenaline release from sympathetic nerve terminals. Rosmond *et al*[46] examined a common polymorphism in the coding region, an NcoI site in exon 6 (position 1128) in relation to BP and personality disorders. They found that NcoI site polymorphism of DRD2 is associated with BP, and that the TT genotype was significantly more frequent in hypertensive subjects (284 randomly selected 51-year-old Swedish men) compared to controls.

Obviously, one big gap to be filled is the relation of dopaminergic system variants with hypertension. So far, just a few SNPs have been explored and shown to be associated with EH. Effects are mostly through salt transport in the renal tubules and A-48G in DRD1, in particular, have confirmative data. There are not many available data concerning dopaminergic receptor subtype polymorphisms and in view of its role in the SNS, this field should be considered more extensively in future studies.

OTHER FACTORS

As mentioned previously, catecholamines are the major contributors of SNS actions and the cathecholaminergic system is an essential component for the performance of SS activities. There are many enzymes involved in the biosynthesis of catecholamines, which occurs in the chromaffin cells of the adrenal medulla and post-ganglionic fibers of the SNS (Figure 1). The removal of secreted molecules requires mainly actions of two enzymes, monoamine oxidase and catechol-O-methyltransferase (COMT), but also other downstream enzymes, like aldehyde dehydrogenase or aldehyde reductase (Figures 2 and 3). Synthesized catecholamines are stored in vesicles, where stabilization of the vesicle core requires other supportive peptides, like chromaganine[47]. There are also presynaptic transporters that help to remove released molecules from the synaptic cleft back to the presynaptic terminal; these transporters are the targets for drugs of abuse[48]. To our knowledge, there is no report affirming the role of transporter polymorphisms in BP levels or hypertension development, except some preliminary studies suggesting a predisposition[49].

In action, catecholamines act through their receptors and start signal transduction. As mentioned above,

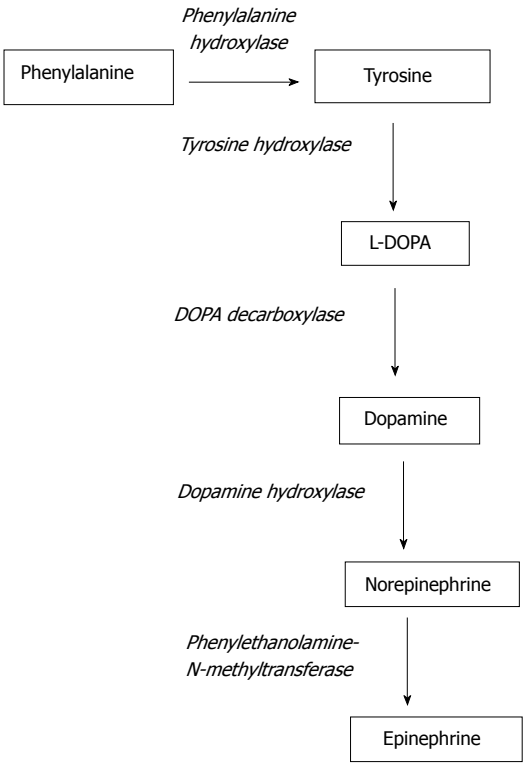

Figure 1 Outline of catecholamine biosynthesis.

catecholamine receptors belong to the GPCR family, and they couple with heterotrimeric G-proteins, finally ending with the activation of protein kinases. There are many proteins taking place on these signal transduction pathways, reflecting the complexity and limited power of association studies. In this large frame of action, the effects of polymorphisms related to the system are too rich to cover in full perspective. In the context of BP and hypertension, however, some important factors are presented by several groups as strong candidates worthy of mention, and will be summarized briefly in the rest of the manuscript.

BIOSYNTHESIS-RELATED FACTORS

The first step in the biosynthesis of dopamine is the formation of L-DOPA, a dopamine precursor, from the amino acid tyrosine by the enzyme tyrosine hydroxylase (TH). TH is the rate-limiting enzyme in catecholamine synthesis. Recent studies have indicated that several polymorphisms of the *TH* gene contribute to BP regulation. Two SNPs at the promoter region of the gene, C-824T and A-581G, were found to be strongly associated with higher BP under stress[50]. In 2010, it was shown that these replacements seriously alter TH promoter activity[51,52]. In accordance with this, Nielsen *et al*[53] reported that the -824T allele increased the relative risk of hypertension by 45%[47]. A study involving 1266 hypertensive subjects searched for the effect of C-824T of TH in hypertension, in addition to the two loci of chromogranin A (CHGA). CHGA is a

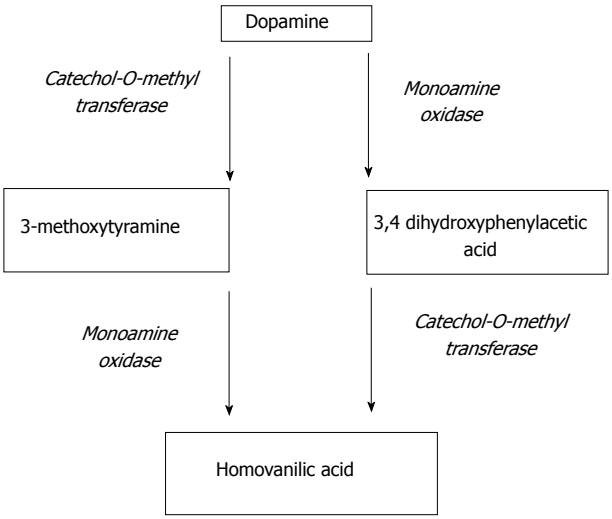

Figure 2 Enzymatic degradation process of dopamine.

peptide located in storage vesicles, and early reports have displayed a strong association between 3'-UTR (C +87T) and EH[54,55]. CHGA polymorphism predicted the risk of developing hypertensive kidney disease in African-Americans. Homozygosity for the minor alleles at T-1014C, T-988G, and G-462A at the promoter region of CGHA exhibited lower stress-induced BP elevations[56].

Another SNP localized to the dopamine hydroxylase promoter, C-970T, was also found to be related with the risk of developing hypertension[57]. More recently, genetic variations of the *PNMT* gene in relation to hypertension were reported in several studies. The distribution of two SNPs, G-367A (rs3764351) and G-161A (rs876493), together with their haplotypes, was screened in 316 pairs of HT and NT patients. Two SNPs' AA haplotypes were found to be less common in hypertensives and therefore suggested to be correlated with the decreased risk of EH in the Han Chinese population[58].

METABOLISM-RELATED FACTORS

The degradation of secreted catecholamines to prevent prolonged stimulation of SNS is very important for the modulation of physiological processes, involving BP and related cardiac functions. Renalase, a novel flavin adenine nucleotide-dependent amine oxidase, is secreted by the kidneys; it helps to reduce the circulating catecholamine concentration. Eight selected SNPs of the renalase gene were genotyped in 503 cases, and three SNPs - rs2576178, rs2296545, and rs2114406 - showed significant associations with EH[59]. The frequency of allele A for rs2576178 in patients with hypertensive and concomitant coronary heart disease was markedly higher. Similarly, the frequency of the C allele of rs2296545 was higher in hypertensives, showing that both genotypes may be contributing to the development of hypertension and chronic heart

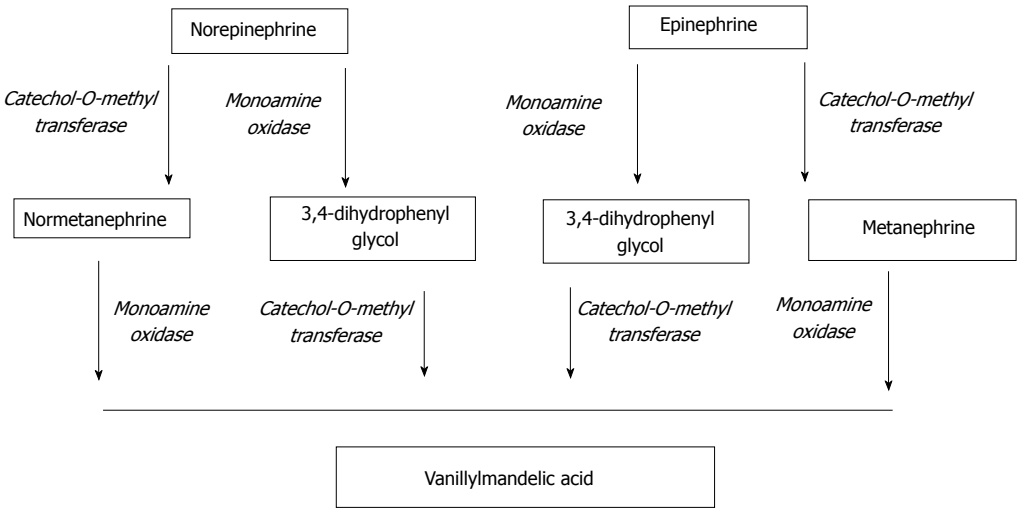

Figure 3 Enzymatic degradation process of epinephrine and norepinephrine.

disease[60].

There were inconsistent results with respect to the *COMT* gene variant *Val158Met* and hypertension. A study on a Japanese population, involving 735 men, showed that the Met allele is associated with higher BP and higher prevalence of hypertension in Japanese men[61]. Another study on a Chinese population, including 215 hypertensive patients, did not detect such a relationship[62]. In the 1995-1997 Word-Trøndelang Health Study (HUNT) group involving 2591 individuals, the Val/Val genotype was found to be more frequent among individuals with hypertension[63].

SIGNAL TRANSDUCTION

A single-base substitution of C825T in exon 10 of the gene encoding the G protein β3 subunit of heterotrimeric Gi proteins (GNB3) was found to be associated with hypertension[64]. This polymorphism leads to alternative splicing of exon 9 and has been associated with enhanced Gi signaling and ion transport[64,65].

Enhanced G-protein-mediated signaling resulting from the truncated C825T form of the G-protein β-subunit may cause high BP as a result of increased NHE activity in tubules, elevated calcium concentration in the cytoplasm, and increased contractility[66].

A large accumulation of studies is present in the literature, strongly supporting the role of C825T in hypertension[64,67-70]. Although the majority of studies has shown an association between the 825T allele and hypertension, there are some contradicting reports, especially in subjects of African and Asian origin, again emphasizing the importance of ethnic origin. Two studies performed by our group also showed that the frequency of the 825T-allele was higher in hypertensive subjects compared to that of controls, and that the difference was statistically significant[71,72].

Recent studies report that the effect of the dopaminergic system in hypertension is mostly due to the impairment of the intrarenal dopaminergic system, and one of the major players is the GRK4, a serine/threonin G-protein receptor kinase, which initiates a desensitization process of the receptor and prevents constitutive activity. Functional polymorphisms of this protein could enhance GRK4 activity, which will reduce dopamine receptor transduction. Increased GRK4 activity also increases angiotensin Ⅱ AT1 receptor activity that is associated with EH[73].

In a small cohort involving 100 EH patients, three gene variants of GRK4 (R65L, A142V, and A486V) were found to be associated with antihypertensive treatment responses[74]. The responses of homozygous double variants of 65L and 142V in particular were much less than the other variants. In another study consisting of 168 Caucasian EH patients, the V allele of the A486V variant was shown to be associated with hypertension and systolic BP[75]. A much larger cohort consisting of 934 whites and African-Americans (44.2%) was also investigated for three proteins of GRK4; it was determined that the 65L allele had a significant effect on systolic BP[76].

Overall, among the synthesis, degredation and signal transduction pathways, there are several replacements possibly involved in the development and progression of hypertension. The most established and well-characterized of these is the C825T mutation in the heterotrimeric G-protein β-subunit. Tyrosine hydroxylase, the primary enzyme for the synthesis of catecholamines, and GRK4, an ezyme with a vital role in salt-transport through regulation of dopamine receptor activity, are conspicuous factors in the assessment of the disease.

CONCLUSION

In the new guidelines released by the European Society of Hypertension (ESH) and the Eurpean Society of Cardiology (ESC), hypertension have been re-

evaluated in the context of its role as a risk factor for cardiovascular diseases[77,78]. Among the previously defined or recently added parameters, individualized therapy approaches are a major concern to estimate overall risk for the patient for the determination and application of the most appropriate treatment and drug regimen[79]. In this perspective, genetic factors need to be well-characterized since they are important contributors of individualized risk assessments.

Regulation of BP coulpatient for the determination could be enhanced and related cardiovascular damage could be reduced if predictors are properly stratified.

As stated above, this review has been restricted to the genetic polymorphisms determined in the catecholamine pathways in relation to BP regulation and hypertension. The selected works contained mostly either positive association studies, unique studies, or a few rare reports in the field of interest. Most of the works in the field are relatively novel, and there is a great number of vacancies to be filled out.

Polymorphism studies always have drawbacks causing them to have inconsistent results, such as ethnicity, sample power, sex, polygenetic factors or linkage effects, and, in the case of drug response studies, periods and consistencies of applied treatments, the reliability of control groups, *etc.* Nevertheless, as will be recognized from the aforementioned reports, the findings are quite remarkable, and a number of studies coincide closely in the outcomes; several variants are highly promising in their potential as predictive markers to estimate the susceptibility of patients to developing hypertension or negative responses to anti-hypertensive drug treatments. As genome-wide association studies are added up, more reliable predictions and their clinical relevance will be achievable, leading the way to more appropriate risk assessments.

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MINIREVIEWS

Management of hypertension: Current state of the art and challenges

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Abstract

Hypertension is a major modifiable cardiovascular risk factor. Hypertension is also recognized as the most important risk factor for global disease burden. It is well established that a sustained reduction in blood pressure by drugs reduces the incidence of cardiovascular morbidity and mortality. In recent years, studies and new guidelines published for the management of hypertension. Awareness, treatment and control of hypertension are very poor, despite the new guidelines. We highlighted the management of hypertension in the light of current literature.

Key words: Hypertension; Therapy; Blood pressure; Cardiovascular risk factor

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Core tip: Hypertension is a major modifiable cardiovascular risk factor. It is well established that a sustained reduction in blood pressure by drugs reduces the incidence of cardiovascular morbidity and mortality. There are several types of drugs that can be used in the management of hypertension. But, the ideal treatment strategy remains uncertain for such a common and treatable disease. In recent years, studies and new guidelines were published addressing management of hypertension. Despite new guidelines, awareness, treatment and control of hypertension are very poor. We highlighted the management of hypertension in the light of current literature.

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INTRODUCTION

Hypertension, a major contributor to cardiovascular

complications and premature death, is a modifiable cardiovascular risk factor[1]. Several studies have demonstrated that high blood pressure (BP) has a strong positive association with cardiovascular morbidity and mortality[2,3]. Hypertension is remarkably common across the world and its prevalence is strongly influenced by age and lifestyle factors[4,5]. Management of hypertension is especially important as hypertension is well recognized as the most important risk factor for global disease burden.

It is well established that treatment of hypertension reduces the risk of cardiovascular morbidity and mortality[6,7]. In contrast, untreated or poorly controlled hypertension is associated with permanent morbidity and mortality. The ultimate goal of antihypertensive therapy is the reduction of cardiovascular morbidity and mortality. There are several types of drugs that can be used in the management of hypertension. Yet, the ideal treatment strategy remains uncertain for such a common and treatable condition. There are new evidences regarding the management of hypertension. More recently, the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) published joint hypertension guidelines in 2013[8]. The panel members who were appointed to the Eighth Joint National Committee (JNC) also published the 2014 JNC report[9]. While these were in agreement on many points with previous guidelines, there were some important differences. This review highlights the management of hypertension in the light of current literature.

NON-PHARMACOLOGICAL THERAPY

Lifestyle changes

All guidelines recommend that the management of hypertension should start with life style modification[8-10]. Several lifestyle interventions have been shown to reduce BP[11,12]. Beside reducing high BP, these strategies are beneficial in managing most of the other cardiovascular risk factors[13]. Lifestyle changes recommended by the current guidelines include several interventions and combination of all these interventions. This has not changed as compared to previous guidelines (Table 1). It is generally believed that BP lowering effect of lifestyle modification is equivalent to drug monotherapy and can also delay drug therapy in patients with stage 1 hypertension.

Table 1 Recommended lifestyle modifications for the management of hypertension

Weight loss (in obese or overweight patients)
Salt reduction
Regular exercise
Moderation of alcohol consumption
Smoking cessation
Increased consumption of vegetables, fruits and low-fat diary products

Renal nerve denervation

The sympathetic nervous system seems to play an important role in resistant hypertension[14]. Two clinical trials (Symplicity HTN 1 and Symplicity HTN 2) have shown the efficacy of renal sympathetic denervation with a post-procedure decline of 27/17 mmHg at 12 mo and 32/12 mmHg at 6 mo, respectively, with few minor adverse events[15,16]. Most recently, results of Symplicity HTN-3 (Renal Denervation in Patients with Uncontrolled Hypertension) trial showed no further reduction in office or ambulatory BP after 1-year follow up[17]. It seems that renal denervation is safe but has no superior BP lowering effects compared with adjustment of drug treatment[18]. In contrast, another more recent study, The Renal Denervation for Hypertension trial, showed that renal denervation plus standardized stepped-care antihypertensive therapy decreases BP more than the same standardized stepped-care antihypertensive therapy alone at 6 mo in patients with well-defined resistant hypertension[19]. So far, conflicting BP lowering effects of renal denervation have been reported. Thus, further studies are needed to reinforce renal denervation as a treatment modality for hypertension.

PHARMACOLOGICAL THERAPY

Despite the non-pharmacological intervention, if BP is still above target, drug therapy should be initiated. There are five major classes of antihypertensive drugs: angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), diuretics, calcium channel blockers and β-blockers. In general, these drugs rarely have serious side effects when appropriately initiated and adequately monitored. We will not focus on the safety profile of these drugs, as it is beyond the scope of this review. According to current ESH/ESC hypertension guideline, grade 1 hypertensive patients with low/moderate cardiovascular risk can initially be treated with monotherapy[8]. On the other hand, JNC-8 panel based their recommendation based on the age of the patients. They recommended initiating therapy to lower BP at systolic BP $\geqslant$ 150 mmHg and diastolic BP $\geqslant$ 90 mmHg for patients aged $\geqslant$ 60 years, and systolic BP $\geqslant$ 140 mmHg and diastolic BP $\geqslant$ 90 mmHg for patients aged < 60 years[9].

MONOTHERAPY

After a long waiting time, recently JNC-8 report (recommendations from only randomized controlled trials) was published. In the same line with 2013 ESH/ESC and National Institute for Health and Clinical Excellence (NICE) hypertension guideline, JNC-8 no longer recommends only thiazide-type diuretics as the initial therapy in most patients. As initial therapy, a thiazide-type diuretic, calcium channel blocker, ACE inhibitor and ARB can be started for uncomplicated hypertension[9]. The 2014 JNC report dismissed β-blockers as first-line therapy. Along the same line, the NICE clinical guideline did not recommend the first-

line use of diuretics and β-blockers[20]. Nevertheless, the 2013 ESH/ESC hypertension guidelines kept all 5 major classes of drugs in their recommendations as first-line regimens because of their opinions that the main benefits of antihypertensive therapy are due to lowering BP *per se* and largely independent of the drugs employed[8].

BP control seems to be more important than a specific agent used to achieve that control. In a recent metanalysis of 18 trials of 23215 Asian patients, a 10 mmHg reduction in systolic BP was associated with a 39.5% reduction in composite cardiovascular endpoints, and a 30% reduction in stroke, regardless of drug class[21]. Similarly, in a more recent metanalysis of trials comparing the renin angiotensin aldosterone system (RAS) inhibitors *vs* other antihypertensive drugs as first-line therapy in patients with primary hypertension, all-cause mortality was similar between these drugs[22]. Still, the choice of drugs may be influenced by other factors as age, ethnicity/race, and other clinical characteristics. Trials in special patient groups (patients with diabetes, coronary artery disease, chronic kidney disease and proteinuria) have proposed that a specific drug group or combinations of certain drugs might be superior to others[8-10]. Thus, patients with special conditions should be considered to start with an appropriate drug based on their comorbidities (Table 2). We think that most hypertensive patients have comorbidities and initiating antihypertensive therapy generally requires compelling indications to select a specific drug group. Otherwise, in absence of comorbidity, it appears that the mere control of BP is more important than the class of antihypertensive drug being used.

RAS BLOCKERS

Based on a large body of evidence, RAS blockers have been used to decrease the incidence of end-organ damage and cardiovascular mortality[23-25]. ACE inhibitors and ARBs can be considered first-line therapy in the management of hypertension, particularly in patients with diabetes mellitus. However, more recent studies showed that ACE inhibitors and ARBs do not have similar effects on cardiovascular outcomes and total mortality.

A metanalysis of 20 clinical trials involving 158998 patients examined the effect of ACE inhibitors and ARBs in patients with hypertension[26]. ACE inhibitors significantly reduced all-cause mortality (HR = 0.90; 95%CI: 0.84-0.97; *P* = 0.04) whereas ARBs did not (HR = 0.99; 95%CI: 0.94-1.04; *P* = 0.683). In a metanalysis evaluating the effects of ACE inhibitors and ARBs on all-cause mortality, cardiovascular events and deaths in patients with diabetes mellitus, ACE inhibitors reduced all-cause mortality (RR = 0.87; 95%CI: 0.78-0.98), cardiovascular mortality (RR = 0.83; 95%CI: 0.70-0.99), and major cardiovascular events (RR = 0.86; 95%CI: 0.77-0.95), whereas ARBs had no benefits on these outcomes[27]. In another metanalysis that included nine randomized controlled trials, no difference was found in total mortality or cardiovascular outcomes for ARBs as compared with ACE inhibitors[28]. According to these evidences, we can conclude that while ACE inhibitors can be used as a first-line therapy, ARBs are preferred for patients who have adverse reactions to ACE inhibitors although there is no agreement among the guidelines.

Table 2 Drugs to be preferred in patients with special conditions

Comorbidity	First-line therapy
Ischemic heart disease	β-blocker (unless contraindicated) Long-acting calcium channel blocker ACE inhibitors (ARBs if ACE inhibitors not tolerated)
Heart failure	ACE inhibitors (ARBs if ACE inhibitors not tolerated) β-blockers Aldosterone antagonists
Diabetes	ACE inhibitors (ARBs if ACE inhibitors not tolerated) β-blockers Calcium channel blockers
Chronic kidney disease	ACE inhibitors or ARBs Loop diuretics rather than a thiazide diuretic (or combination)
Stroke	Diuretic + ACE inhibitors
Asymptomatic organ damage	
Left ventricular hypertrophy	ACE inhibitors, ARBs, Calcium channel blockers
Proteinuria	ACE inhibitors, ARBs

ACE: Angiotensin converting enzyme; ARBs: Angiotensin II receptor blockers.

DIURETICS

Thiazide and thiazide like diuretics (*e.g.,* indapamide, chlorthalidone) remain essential in the management of hypertension. The JNC-7 recommended that thiazide diuretics should be the preferred drugs in most hypertensive patients, either alone or combined with other classes of drugs[10]. Although it is well known that thiazide-type diuretics are effective in reducing BP and preventing cardiovascular disease in hypertensive subjects, it is not clear whether all drugs in this class are equally safe and effective. Recently, the choice of diuretics has emerged as a controversial issue with some evidence favoring the long-acting agent, chlorthalidone, in preference to hydrochlorothiazide. A recent retrospective observational cohort analysis from the Multiple Risk Factor Intervention Trial data set compared the effects of chlorthalidone *vs* hydrochlorothiazide on cardiovascular event rates[29]. Chlorthalidone treatment was associated with significantly fewer cardiovascular events; lower systolic BP, potassium, and total and low-density lipoprotein cholesterol levels; and significantly higher uric acid levels compared with hydrochlorothiazide.

A large observational study with up to five years of follow up reported head-to-head comparative data

on the effects of newly prescribed chlorthalidone *vs* hydrochlorothiazide on cardiovascular and safety outcomes in elderly patients[30]. Chlorthalidone was not associated with fewer adverse cardiovascular events or deaths than hydrochlorothiazide in elderly patients; however, it was associated with a greater incidence of electrolyte abnormalities, particularly hypokalemia.

In a recent metanalysis of 14 trials comparing head to head thiazide-like and thiazide-type diuretics, systolic BP reduction was greater with chlorthalidone and indapamide without more adverse effects[31]. These data suggest using chlorthalidone as preferred thiazide type diuretic for the management of hypertension. On the other hand, hydrochlorothiazide has a dose related BP-lowering effect and greater effect on systolic BP than diastolic BP, thus lowering pulse pressure more than other antihypertensive drugs[32]. We believe that it is too early to reach a final conclusion, as there are no randomized trials that directly compare cardiovascular outcomes in hypertensive patients treated with thiazide-type diuretics *vs* thiazide-like diuretics.

Mineralocorticoid receptors have been shown to play important roles in the pathogenesis of hypertension and hypertension-related cardiovascular outcomes[33-35]. Recent studies have implicated that aldosterone excess as an important pathophysiologic factor in a large fraction of patients with resistant hypertension[36]. Spironolactone can be tried in patients with resistant hypertension requiring three or more drugs to achieve BP control unless contraindicated[20]. Eplerenone may be used as an alternative in patients who experience hormonally related side effects with spironolactone.

We conclude that diuretics remain as leading agents in the management of hypertension. Based on the available data, thiazide-like diuretics (such as chlorthalidone, 12.5 to 25 mg/d) may be preferred to thiazide type diuretics. Moreover, when BP cannot be controlled with other drugs, combining thiazide-like diuretics with ACE inhibitors or ARBs are usually very effective. Combining diuretics with aldosterone antagonists may also be worthwhile in special patient population.

CALCIUM CHANNEL BLOCKERS

Calcium channel blockers have potent BP-lowering effects and have been the most widely used antihypertensive drugs. Several studies have showed that calcium channel blockers had efficacy not only in lowering BP but also in reducing cardiovascular morbidity and mortality in patients with hypertension[37]. In a recent metanalysis of 31 randomized controlled trials, calcium channel blockers reduced stroke more than either placebo (OR = 0.68; 95%CI: 0.61-0.75) or β-blockers (OR = 0.79; 95%CI: 0.72-0.87), but was not different from ACE inhibitors and diuretics[38]. Another Cochrane metanalysis of randomized trials comparing first-line calcium channel blockers with other antihypertensive classes did not find difference among calcium channel blockers, ACE inhibitors or ARBs in terms of all-cause mortality, however, it provided evidence supporting the use of calcium channel blockers over β-blockers in terms of total cardiovascular events, stroke and cardiovascular mortality[39]. Calcium channel blockers are broadly classified into two groups as dihydropyridine and non-dihydropyridine groups. Non-dihydropyridine calcium channel blockers are more negatively chronotropic and inotropic than the dihydropyridine subclasses, and are generally not recommended to use as first-line therapy in the management of hypertension. The NICE guidelines recommend particularly calcium channel blockers as first-line therapy in hypertensive patients aged over 55 years[20]. We conclude that calcium channel blockers may be used as initial first-line therapy particularly in hypertensive patients without compelling co-morbidities or as a component of combination therapy.

β-BLOCKERS

Whether β-blockers should be placed as first-line therapy in the management of hypertension is probably the most controversial issue among major guidelines. Some do not recommended β-blockers as first-line therapy for hypertension[9,20]. But, the 2013 ESH/ESC guidelines continued to recommend β-blockers as one of the first-line anti-hypertensive drugs[8]. On the other hand, the 2014 NICE hypertension guidelines put β-blockers as step 4 drugs. β-blockers can be used as additional therapy to further lower BP, but they may have a special benefit in preventing recurrent coronary artery disease[7].

The class of β-blockers is heterogeneous, and all the drugs in this class may not be the same[40]. Atenolol, metoprolol, carvedilol and nebivolol have different properties in terms of efficacy and side effects. But a recent metanalysis comparing atenolol and non-atenolol β-blockers found that β-blockers had similar effect on cardiovascular end points in hypertensive patients without compelling indications[41]. Only, in the elderly (> 60 years), atenolol was inferior to the other drugs in reducing stroke. We conclude that while β-blockers remain the standard of care for patients with coronary artery disease, particularly after acute myocardial infarction[42], their role in the management of hypertension without coronary artery disease remains controversial.

COMBINATION THERAPY

Combination therapy may have benefit patients through multiple and potentially complementary pharmacologic mechanisms of action. Thus, combining drugs with different classes may be more effective than titrating dose of a single agent. Initiating treatment with a drug combination rather than a single agent is increasingly utilized as a therapeutic strategy. According to the current guidelines, a large majority of patients

require simultaneous administration of two or more antihypertensive drugs to reach the target BP[8-10]. Specifically in patients with stage 2 hypertension, it appears that early combination therapy may lower BP to targets sooner.

Choice of combination therapy

A diuretic, β-blocker, calcium channel blocker and ACE inhibitors (or ARBs) can be combined in the management of hypertension. Amongst the various combinations of antihypertensive drugs, it is generally considered that combining an ACE inhibitor or ARB with a diuretic produces fully additive BP reduction[43]. Combination of antihypertensive agents as initial therapy in stage 2 hypertension can lead to markedly improved BP control in patients as compared with mono-therapeutic regimens[10,44].

Recently, it was noted that the addition of an ACE inhibitor or ARB to a dihydropyridine calcium-channel blocker is increasingly being used[45]. The only trial (ACCOMPLISH) comparing ACE inhibitor-calcium channel blocker combination and ACE inhibitor-diuretic combination found significant superiority of ACE inhibitor-calcium channel blocker over diuretic combination[46]. This combination may also reduce the incidence and severity of edema caused by calcium channel blocker. The combination of ACE inhibitor (or ARB) in addition to diuretic or calcium channel blocker may be used as initial combination therapy. But, it appears that calcium channel blockers are better than diuretics as a component in combination therapy.

Combination of RAS blockers

Currently, the combination of an ACE inhibitor and ARB is not recommended[9]. Recent studies showed that combination therapy did not prove to be superior to the use of an ACE inhibitor or ARB alone in reducing the primary or secondary outcomes. Previously, in the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial, a randomized study of combination therapy *vs* monotherapy in persons at increased cardiovascular risk, no cardiovascular or renal benefits were observed with combination therapy[47,48]. In a recent randomized, controlled study, combination therapy with ACE inhibitors and ARBs provided no benefit to major primary and secondary outcomes in patients with diabetic nephropathy[49]. Furthermore, patients receiving combination therapy had an excess occurrence of hyperkalemia and acute kidney injury. The addition of direct renin inhibitor, aliskiren, to standard therapy with ACE inhibitors or ARBs in patients with type 2 diabetes did not reduce cardiovascular or renal outcomes as compared with placebo. On the contrary, the combination therapy resulted in an increased number of adverse events[50].

CONCLUSION

In conclusion, based on the available data, the amount of BP reduction rather than the choice of antihypertensive drug are the major determinant of reduction in cardiovascular risk in patients with hypertension. But, some hypertensive patients may have compelling indication for a specific antihypertensive drug, which may offer particular benefit independent of BP control. Successful treatment requires identification and reversal of lifestyle factors contributing to treatment resistance; diagnosis and appropriate treatment of secondary causes of hypertension; and use of effective combination regimens. Combination therapy may be necessary in the majority of the patients with hypertension, and current guidelines recommend routine initiation of a combination in patients with stage 2 hypertension. ACE inhibitors, ARBs and diuretics including aldosterone antagonists can result in clinically significant alterations of serum electrolytes and kidney function. Thus, after the initiation of these agents, a chemistry profile should be obtained.

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MINIREVIEWS

Renal venous hypertension

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Abstract

Renal venous hypertension usually seen in young, otherwise healthy individuals and can lead to significant overall morbidity. Aside from clinical findings and physical examination, diagnosis can be made with ultrasound, computed tomography, or magnetic resonance conventional venography. Symptoms and haemodynamic significance of the compression determine the ideal treatment method. This review of the literature discusses normal and pathological developmental aspects of renocaval venous segment and related circulatory disorders, summarizes congenital and acquired changes in left renal vein and their impact on development of renal venous hypertension. Also will be discussed surgical tactics of portosystemic shunting and their potential effects on renal hemodynamics.

Key words: Renal venous hypertension; Nutcracker syndrome; Kidney; Portal hypertension; Splenorenal shunts

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Core tip: Renal venous hypertension characterized by the presence of left renal vein dilatation, varicocele and hematuria. Being a rare cause of hematuria its etiology is diverse but of precise characteristics. Diagnosis is not easy and treatment requires ruling out its precise etiology and considering the intensity of the compression phenomenon because of interventionist attitudes have important implications and are not risk free.

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INTRODUCTION

Renal venous hypertension (RVH) - venous insufficiency caused by inadequate drainage of blood through the renal vein[1]. There are two main reasons in the development of the RVH: Structural abnormalities of

renocaval segment; acquired changes of the left renal vein.

Structural anomalies of renocaval segment and their clinical importance in the development of the RVH

Development of renocaval segment (inferior vena cava, renal, gonadal, adrenal and lumbar veins) - is a complex process in which there is consecutive regression and shifting of three venous structures, such as posterior cardinal, supracardinal and subcardinal veins[2-5]. Inferior vena cava (IVC) and its branches formed from different embryological structures; their segments formed from all three systems mentioned above. The formation of these veins can be impaired at any stage of development[2,3].

Among the developmental abnormalities of the IVC clinical significance in development of the RVH matters - left-sided IVC, with this type of abnormality abdominal aorta compresses IVC at the site of their contact, thus will cause congestion in left renal vein (LRV) and recurrent left sided hematuria[6,7].

Typical abnormality of the right renal vein is anomaly of their quantities, which is due to the fact, that the right renal vein embryogenesis does not undergo significant transformations. Essentially, the significance of these abnormalities in development of RVH negligible[2,3,8].

Clinically significant abnormalities often observed in the LRV, which related to its development. For example, retention of both limbs of the left portion of circumaortic venous ring leads to the formation of the circumaortic LRV, which occurs in 1%-17% of cases according to different authors[2,3,5,8-13]. In this type of anomaly there are pre-aortic and retroaortic limbs. In this situation, the pre-aortic limb usually receives the adrenal, gonadal, and phrenic veins; the retro-aortic limb receives the lumbar and the hemiazygous veins. The retroaortic limb passes obliquely and downward to reach the inferior vena cava at a lower level[8-13]. The clinical significance of this anomaly is that the impeded outflow from the retroaortic limb leads to congestive venous hypertension and increased blood flow in pre-aortic limb[8,9,11-13]. Knowledge of this anomaly is important for the angiographer performing renal and/or adrenal venography. In addition, it is of surgical importance when a left renal transplant and/or splenorenal shunt are considered. As for splenorenal shunt operations, opinions are contradictory. For example, some authors[14] recommend to perform splenorenal shunt, without the risk of RVH, while according to other researchers[15,16] the connection of splenic vein to retroaortic limb leads not only to inadequate drainage portal system with recurrent bleedings, but also the risk of development of RVH.

Another type of abnormality is retroaortic LRV (single or multiple). The retroaortic type of LRV occurs in approximately 2-6.6/cent[17-22]. When the ventral limb atrophies a retroaortic renal vein occurs. In this transformation, there are anatomical prerequisites for disorders of venous hemodynamics - the emergence of congestive venous hypertension, clinically manifested by proteinuria or hematuria and the development of the secondary varicocele[1,5,11,19,23]. Performing splenorenal shunt in this type of LRV is not advisable, since drainage of a large amount of blood from the portal system leads to RVH one hand and recurrent bleeding from gastroesophageal varices on the others[15].

Acquired changes of the LRV

Human body has anatomical preconditions, which may cause significant haemodynamic alterations that may lead to clinical symptoms and significant associated morbidity. The clinical manifestations of this predisposition is nutcracker syndrome[24]. In view of the insufficiency of symptoms during the first decade of life, specified condition practically have not described in pediatric patients, in most cases classified as associated finding. The nutcracker syndrome refers to compression of the LRV between the superior mesenteric artery and abdominal aorta. Obstruction of LRV occasionally causes clinically significant venous hypertension resulting in unexplained left flank, gross haematuria, with formation of periureteric and gonadal varices and varicocele in relatively young and previously healthy patients[25-29]. Other possible symptoms include pelvic congestion, chronic pediatric fatigue syndrome and orthostatic proteinuria[30-38].

Other rare acquired causes of RVH includes renal vein thrombosis, organic renal vein stenosis and arteriovenous fistula[39-41].

Well known that LRV mostly used in performing various types portosystemic shunts for portal hypertension. Issues related to presence of RVH in patients who underwent portosystemic shunting recent years draw increasing attention of researchers[15,16,42,43]. The data about the state of the left kidney after portosystemic shunting operation are very controversial. For example, some authors argue that performing end-to-end splenorenal shunt provides the venous drainage from the portal system to IVC without renal dysfunction[44-48]. But according to other data[14-16,42,43], impeded outflow of the left renal vein leads to not only venous hypertensive nephropathy, but can be cause of insufficiency of created anastomosis and therefore unsatisfactory results of surgical treatment. Furthermore, impeded outflow of LRV results in venous hypertension with the formation of intra- and extrarenal collaterals and/or the development of gonadal vein reflux resulting retrograde flow and has been implicated in the development of varicocele or ovaricocele[49]. According to experimentally induced extrahepatic portal hypertension[50-54] shunting end renal vein to side splenic vein (renosplenic) after ligation of the LRV lateral to the adrenolumbar tributary, leads to haemorrhagic necrosis of the left kidney. Thus, the ureteric, lumbar and pericapsular collaterals cannot adequately drain the left kidney. Ligation of the LRV on the medial side of the adrenolumbar tributary maintained a patent left renal vein in all cases[50,52,53].

Practical experience has shown that performing splenorenal anastomosis with ligation of the LRV proximal to the confluence of the adrenal vein - in one third of cases causes decreasing of renal function (according to the excretory urography), renal infarction, hematuria and proteinuria[52,53].

In addition, in the pathogenesis of the RVH renal arterial blood flow is essential[55]. High pressure in the renal artery in systemic arterial hypertension increases tone of sympathetic-adrenal system, which causes vasoconstriction in the cortex and increases medullary blood flow. Autoregulation mechanisms lead to increasing pressure in the renal venous system, which are the anatomical and functional characteristics of the vascular bed of the kidney. The diversity of intrarenal arteriovenous shunts, venous network ensures acceptance of a large amount of blood in the face of increasing its arterial delivery - this is the pathogenesis of RVH in systemic arterial hypertension[55]. On the other hand, congenital or acquired arteriovenous fistula leads to the restructuring of angioarchitectonics of kidneys and in this case pressure in renal veins increases due to shunting of blood through the abnormal arteriovenous communications. The blood from the arterial bed drains to venous rout bringing extraordinary pressure to the veins. Thus, developed the renal venous hypertension[55,56].

Diagnostics of RVH

In the evaluation of renal hemodynamics, intravascular pressure indicators are most important. Retrograde left renal venography and measurement of the pressure gradient between the left renal vein and the IVC are procedures of choice for diagnosing RVH. Normally, this gradient is determined in a horizontal position from a healthy child was equal to 0.13 ± 0.02 kPa, with individual variations 0.33 ± 0.05 kPa[57,58]. A number of studies indicated that the anomalies of the LRV (usually circumaortic and retroaortic LRV), the pressure gradient increases significantly (up to 0.86 kPa). However, these techniques are invasive and use of such invasive examinations is generally deemed imprudent in children, and non-invasive imaging studies are preferable. Recently progressive development of non-invasive imaging techniques led to that Doppler ultrasound (US) has become the method of choice in the diagnosis of RVH. During the last decade, increased the number of publications describing different ultrasound descriptions of renocaval segment anomalies[1,27-29,34-36,57-60]. Also in details described intrarenal arteriovenous shunts[61-63]. Recent publications dedicated in most cases for nutcracker syndrome[27-29,34-36,57-60]. Kim *et al*[58] suggested that a ratio of the AP diameter, and peak velocity (PV) between the hilar and aortomesenteric portions of the LRV of greater than 5.0 could be used as the cut-off level for the diagnosis of nutcracker syndrome with a sensitivity of 80% and a specificity of 94%. However, it has not yet been confirmed whether these criteria can be applied to children with clinically suspected nutcracker syndrome. In addition, detection of collateral veins around the left renal vein at color Doppler US is a reliable criterion for the diagnosis of nutcracker phenomenon[27]. However, the LRV flow patterns and collateral vein formations associated with nutcracker phenomenon depend on the degree and stage of the phenomenon[58]. In patients with early nutcracker phenomenon, LRV distention and high pressure gradients exist before collateral veins develop. Moreover, in patients with collateral veins, the presence of a distended left renal vein and hypertension of the left renal vein indicate that the nutcracker phenomenon is noncompensatory[58].

Regardless of the incidence angle, the resistances in the renal artery can be evaluated by measuring the resistive index and pulsatility index if the vessel is identified by colour Doppler. Increasing these rates in some cases may be indirect evidence of the venous outflow disturbances from LRV[15,16].

Recently, non-invasive methods such as computed tomography (CT) and magnetic resonance imaging (MRI) have been used in the diagnosis of nutcracker syndrome[10,12,41,64-66]. Performing of the study for our opinion, more appropriate to carry out not only for diagnostic purposes of RVH but also to assess the topographic anatomy course of renocaval segment and their relative position to the vessels of the v. porta and abdominal aorta in the planning of vascular surgery in the retroperitoneal space.

The clinical manifestations of RVH

The clinical presentation of RVH include the development of collateral blood flow and symptoms of renal function disorders[1,5,28,29,67]. The increased venous pressure within the renal circulation promotes the development of collaterals of the renal pelvis, and this plexus of abnormal hypertensive veins causes microhematuria or gross hematuria, orthostatic proteinuria[6,19,30-38]. Other possible symptoms include left flank pain, left-sided varicocele, pelvic congestion, chronic pediatric fatigue syndrome, and gastrointestinal symptoms[1,43,67].

Performing various types of splenorenal shunts using abnormally developed LRV due to portal hypertension can become a reason of unsatisfactory results with recurrent bleeding from gastroesophageal varices[14-16,42,43,68]. In addition, shunting the large amounts of blood from portal vein and its tributaries to abnormally developed LRV manifests as clinical signs of renal venous hypertension[14,16,69].

Different therapeutic methodologies have been used in treatment of RVH. In general, moderate manifestations may be controlled with conservative methods[70]. Nearly all surgical approaches aim to relieve the LRV outflow obstruction[70-85]. Surgical modalities including autotransplantation of the left kidney, LRV bypass with graft interposition and reanastomosis to the IVC anteriorly has been performed with satisfying results[73-75]. Renal autotransplantation may offer maximal efficiency in terms of normalizing renal venous

circulation. In more severe cases with hematuria, significant stenosis of LRV, varicocele, left flank pain and pressure gradient more than 1.33 kPa preferable intervention on LRV. Lot of evidence of the efficacy of endovascular interventions - methods of stenting and balloon angioplasty[76-85]. Initially performed *via* a transperitoneal approach, an external stent can be wrapped around the renal vein to prevent its compression by the mesoaortic clamp. The procedure has now also been performed by laparoscopic surgery. External and internal stenting procedures by either minimally invasive or endovascular approaches are promising treatment options. However, the risk of erosion of adjacent structures and dislodgment of the stent has not been defined yet.

However, surgical treatment methods have certain disadvantages. Thus, venous vascular suture can be considered as a potential source of thrombosis[72,83]. Postoperative complications may even lead to nephrectomy[84]. Even traditionally performed safe operations intravascular stents placement - can have few complications[79-82].

CONCLUSION

There are reasonable basis for research on the status of renocaval segment for modern pediatric surgeons, urologists, specialists concerned in portal hypertension, liver kidney transplant surgeons. The presence of RVH should be considered on the basis of a thorough clinical examination in patients with hematuria, left flank pain, varicocele, and symptoms of pelvic venous congestion. Dilatation of LRV and its tributaries, anomalies, additional communications observed on ultrasonography, computed tomography CT, or MRI should alert the physician to consider the diagnosis. If the symptoms merit, in particular if cystoscopy demonstrates left ureteral hematuria, selective left renal venography with pullback determination of renocaval pressure gradient is the diagnostic test of choice and should be performed in all patients. At the same time, complexity of revealing the causes of RVH with above mentioned methods, it is feasible to study the role of arterial blood, not only because of their lack of data, but also well-known factors associated with abnormal blood supply, and it is widely performed operations of decompression of the portal system through the LRV. Despite numerous studies, reasonableness of performing various types of splenorenal shunts in portal hypertension with prerequisites for RVH remains debatable. Finally, it is not enough studied phenomenon of nutcracker syndrome after surgical and congenital splenorenal shunts.

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REVIEW

Role of adipocytes in hypertension

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Abstract

Although it has known for some time that obesity is associated with salt sensitivity and hypertension, recent data suggests that the adipocyte may actually be the proximate cause of this physiological changes. In the following review, the data demonstrating this association as well as the potentially operative pathophysiological mechanisms are reviewed and discussed.

Key words: Hypertension; Oxidant stress; Natriuresis; Heme oxygenase; Nitric oxide; Renal function; Obesity

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Core tip: Hypertension is a growing problem worldwide, and the problem is exacerbated by the growing obesity epidemic. This review looks into the complex relationship between these two diseases, outlining what current literature reports for treatment methods, hypotheses on cause, and potential cross talk between the two.

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INTRODUCTION

Hypertension is defined as elevated blood pressure-typically a systolic blood pressure of ⩾ 140 mmHg or a diastolic pressure ⩾ 90 mmHg (or both) in a relaxed state[1-3]. Currently, 29% (or 70 million) of Americans have been diagnosed with hypertension[4,5]. There are several stages of hypertension, as well as salt sensitive and salt resistant types of hypertension[6]. Hypertension may complicate and/or worsen other diseases such as diabetes, cardiovascular disease, chronic kidney disease, and obesity[2,7-9]. Obesity itself has reached pandemic proportions; according to the World Health Organization (WHO), over 500 million adults (10%-14% of world population) were obese in 2008, and this number keeps increasing[2,8,9]. As of 2014, this number has jumped to 600 million. There is an association between hypertension and obesity, but the mechanism(s) by which obesity predisposes to hypertension in humans

has not be clearly established. In this review, we will explore some aspects of this important relationship.

THE THREE KNOWN TYPES OF ADIPOSE TISSUE

Currently there are three known types of adipose tissue, each with it's own specific characteristics: White, brown, and a mixture type, known as beige (or "brite"). The main purpose of adipose, regardless of the type, is to store excess energy that can be released as needed. The way the energy is stored varies between types. White adipose is the type one would think about when thinking about typical obesity. White adipocytes (WAT) are characterized by a spherical shape, a large lipid droplet that takes up 90% of the volume of the cell, very few mitochondria, and a flattened peripheral nucleus[10-15]. WAT can release triglycerides during a time of energy crisis in the body. WAT can be found virtually anywhere on the body, but are mainly located in subcutaneous abdomen, viscera, retroperitoneal, inguinal, and gonadal areas[10,16-19]. White adipocyte cells are known to secrete several kinds of proteins, such as inflammatory factors and the protein leptin[18-23].

Leptin is known as the satiety protein; when released, it inhibits feelings of hunger[13,14]. The antagonist of leptin is ghrelin; this is thought to be one of the hunger hormones[13,18,19,23]. It has clearly been shown by Sennello *et al*[24] and Friedman *et al*[25-27] that patients with an inability to produce leptin develop profound hyperphagia and obesity. However, in most obese subjects, leptin levels are high[25,26]. In these subjects, it is thought that as leptin levels are chronically elevated, responses to leptin are diminished[25]. Despite the high amount of energy already stored, the body ignores satiety signals and thinks it requires more energy to store; this higher level of leptin is consistent with a higher amount of adipocytes which are believed to be the primary source of leptin[25,26,28,29]. This hormone and protein releasing function therefore places white adipose tissue as an endocrine organ[14,30,31].

In addition to leptin, adipocytes also release other hormones and peptides, including tumor necrosis factor α (TNFα) and interleukin-6 (IL-6)[32-35]. These are inflammatory cytokines, and the increased levels are indicative of inflammation in the body[32-34]. Whether this inflammation leads to increased reactive oxygen species (ROS) production creating oxidative stress or oxidative stress from signaling leads to inflammation is currently unclear.

Brown adipocytes (BAT) are a bit different from WAT; they are polygonal in shape, contain fewer and smaller lipid molecules, have abundant mitochondria, and a central round nuclei[10,11,36]. BAT are mainly found in the subscapular region of rodents and human infants[10,37]. Whereas WAT use lipids to store energy, BAT store energy in the form of fat and break them down to produce heat in a process known as non-shivering thermogenesis[38-40]. Thermogenesis is the production of heat in an organism; non-shivering thermogenesis occurs in the brown adipose tissue because of the presence of thermogenin[41-44]. Thermogenin (also known as uncoupling protein 1) allows the uncoupling of protons moving down their gradient from adenosine triphosphate (ATP) synthesis; this energy is then dissipated as heat[43,44]. Free fatty acids from the brown adipose tissue remove any proteins that could inhibit thermogenin[41,42]. Thermogenin then causes an influx of H^+ into the mitochondrial matrix, bypassing the ATP synthase normally used to make ATP[45-49]. This uncouples oxidative phosphorylation, and the energy normally used to convert adenosine diphosphate (ADP) to ATP is release as heat[46,50-52]. Interestingly, thermogenesis can also be produced ion pump leakage[50,53,54]. It is thought that a leaky ion pump in mitochondria releases H^+ ions; the intensity of heat is proportional to the amount of H^+ released during this process[41,43]. The ability of BAT to turn excess energy into heat is a property the WAT lack[4,55-57]. Circulating factors, such as irisin, FGF-21, and natriuretic peptides play a role in regulating BAT[4,55-57]. It is thought that these factors can encourage proliferation of BAT, and increase the amount of present beige adipocytes[55,56,58].

Beige adipocytes are a combination of brown and WAT[4]. Beige adipocytes are born through a browning process; WAT become more like BAT, the one large lipid droplet becomes many, and uncoupling protein 1 becomes expressed, and thermogenic activity increases[5,56,58]. All three types of adipocyte cells, along with muscle cells, come from the same precursor cell-a mesenchymal stem cell[4,5]. The expression of different genes at different points during the life cycle of these cells determines their fate[4,5], see Figure 1. The potential for obese adults to spontaneously form beige adipocytes from their WAT is unclear, but it brings to mind the possibility that such a phenomenon is possible.

THE RELATIONSHIP BETWEEN HYPERTENSION AND OBESITY

Obesity can increase the susceptibility to metabolic syndromes, cardiovascular diseases, type 2 diabetes, cancer, and hypertension[2,7-9]. Although some patients with hypertension are not obese, and *vice versa*, there is a strong correlation across populations[59]. The interactions between obesity, salt sensitivity and hypertension are shown schematically in Figure 2. When blood pressures reach the hypertensive range, there is almost always small vessel disease of the arterioles, or arteriolosclerosis, as well as kidney damage[60-62]. This strongly suggests that there are both vascular and renal components to the disease[63]. Hypertension has genetic and environmental factors in addition to those associated with obesity[4,29,64,65]. Salt sensitive hypertension refers to an increase in blood pressure related to an increase in salt (specifically sodium) intake[6,66,67]. Some workers in this field believe that all hypertension reflects either excessive sodium intake or

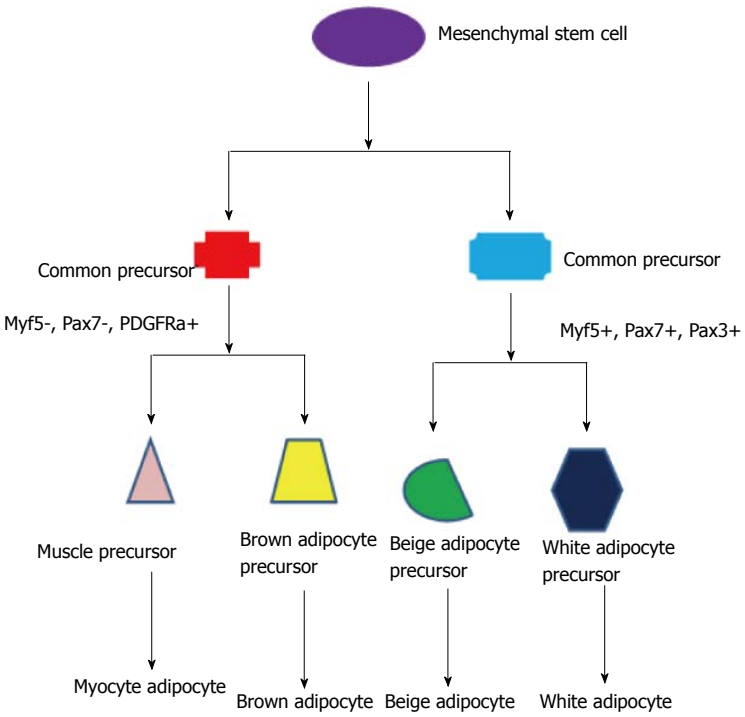

Figure 1 Schematic demonstrating links between three basic type of adipocytes and muscle. All cells come from the same common cell, mesenchymal stem cell. Presence or absence of certain factors during development, such as Myf5, Pax 3 and 7, PDGFRa and myogenin can affect the final product of the cell. Myf5: Myogenic factor 5; Pax 7: Paired box protein 7; Pax 3: Paired box protein 3; PDGFRa: Platelet-derived growth factor receptor, alpha polypeptide.

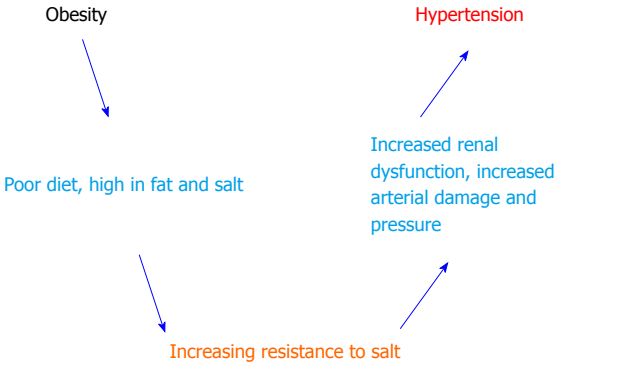

Figure 2 Schematic demonstrating potential relationship between obesity and hypertension.

some form of renal salt sensitivity, but this is admittedly still controversial[63,68-70].

PRESENT THEORIES LINKING OBESITY TO HYPERTENSION

Obesity appears to be associated with or complicated by "increased sympathetic nervous system (SNS) activity, activation of the renin-angiotensin aldosterone system (RAAS), and physical compression of the kidneys by extra-renal fat and by increased intrarenal extracellular matrix"[71-73]. This physical compression can directly activate the RAAS which, in turn, leads to increased SNS outflow as well as increased circulating concentrations of angiotensin Ⅱ, a well-known vasoconstrictor and aldosterone, an anti-natriuretic hormone. The net affect is sodium retention and increased blood pressure[63,73-75]. Leptin levels, as discussed above, appear to be increased in obese patients. This hormone through several biochemical mechanisms, affects appetite as well as SNS outflow, and can cause increases in blood pressure[71,74]. The duration of obesity also plays a role; the longer one is obese, the more renal damage occurs which further impairs pressure natriuresis, exacerbating hypertension[63,71,72,76,77].

Another potentially contributing factor is obstructive sleep apnea (OSA), which is more than just another co-morbidity of obesity. OSA is much more common in people who are overweight or obese[1,2]. OSA occurs when the airway becomes blocked or constricted and can cause snoring, and lapses in breathing that are common to sleep apnea[2,78,79]. Untreated sleep apnea can lead to increases in blood pressure, obesity, heart attack risk, and diabetes, among other problems[2,7,75,80-82]. In addition to increasing the risk for hypertension, OSA can lead to other problems. Hypoxia, or lack of oxygen that occurs when breathing is stopped or obstructed, is also a risk factor for generating ROS and increasing oxidant stress and SNS activity[1,2,78,82].

Recent work suggests that the adipocyte itself could play an important role in hypertension. Research has shown that high dietary sodium can increase the white adipocyte mass as well as leptin levels in rats[82], and blood pressure was significantly increased as well. The increase in adipocyte mass has a cascading effect; the mass increases, the release of additional adipokines occurs, and these lead to an increase in inflammation. This inflammation causes further exacerbation of disordered metabolism and insulin resistance[82-85]. These interactions are shown schematically in Figures 3 and 4.

CURRENT TREATMENTS OF OBESITY AND THEIR EFFECTS ON HYPERTENSION

Currently, there are several treatment methods to deal with

Figure 3 Visual representation of factors contributing to the complex pathophysiology linking adipocyte biology and hypertension.

obesity, and as it is so closely related with hypertension, treatments for the two can often overlap[86-88]. The treatments can be broken up into several categories; lifestyle changes (including nutritional changes and exercise addition), drug therapy, and surgical methods[89]. It has been shown that a reduction in a patient's weight by 5%-10% is enough to reduce their risk of cardiovascular complications, including hypertension[89-91]. When looking at the drug treatment route, it is important to consider however that some drugs are not recommended for patients who have pre-existing conditions, such as hypertension or diabetes. Sibutramine, for example, has been associated with small increases in blood pressure and heart rate, and is not recommended for patients suffering from hypertension[89,92]. Some drugs that are used to treat hypertension can be used as a weight loss agent, such as the drug orlistat[89,93]. These drugs can work on multiple levels; some are known as feeder modulators, and change the way the patient receives signals that the body needs food[89,94]. Some effect the formation of agents such as angiotensin Ⅱ and nitric oxide synthase (NOS)[89,95,96]. Still others work at the molecular level and effect the afferent signaling that can lead to obesity[89,95]. Serotonin drugs have been found to be an effective treatment of obesity, but the downside is they can cause an increased risk of primary hypertension because of their effects on vascular smooth muscle[97]. If we look at surgical approaches, the benefits of surgery on hypertension itself and the abnormal hormonal milieu appear to be huge, at least over the first year or so[98].

REDOX REACTIONS AND THEIR RELATION TO OBESITY AND HYPERTENSION

In addition to salt intake and obesity, nitrous oxide synthase and heme oxygenase (HO) both play a role in the cause and treatment of hypertension[99-101]. Obesity leads to an imbalance in the circulating level of nitic oxide (NO); this is due to increased oxidative stress and decreased NO production[100,102]. Decreasing the availability of the NO can predispose an individual to hypertension[99-101]. NO contributes to vasodilation, which is the relaxation of the vasculature[83,99-101]. If there is less NO present (because of a decrease in NO synthase), vasoconstriction can occur, which can exacerbate the damage of increased pressure from the other factors related to hypertension[99-101]. Human adipose tissue expresses angiotensinogen, angiotensin-converting enzyme (ACE) as well as angiotensin type 1 (AT1), and AT2 receptors[102-105]. The role of angiostatin is not well known, but it has some kind of redox purpose; it appears to involve inhibition of endothelial cell migration, proliferation and induction of apoptosis[65,99-101]. There is a link between NO synthase dysfunction and the ACE enzyme in the obese population[99-101]. Excessive NO formation by the inducible member of the NOS family (iNOS or Nos2) has been shown to cause nonspecific tissue damage; it is thought to be involved in the pathogenesis of inflammatory and autoimmune diseases[106-108]. By inhibiting this inducible factor, obesity still occurs but the pathologies associated are reduced[107]. A similar study showed that even though mice protected from pathologies associated with iNOS inhibition, they are still subjected to increased blood pressure and increased ROS[109,110]. iNOS is associated with increased inflammatory responses, which is related to the cascade of responses associated with obesity and hypertension[33,34,111,112]. It is known that increased NO can induce cellular stress, which can exacerbate the current problems present.

Similarly to NO synthase, HO has a role in amelioration of hypertension[1,100,104,107,113]. An increase in HO expression can cause reductions in ROS, or ROS. Increases in ROS, also called oxidant stress, are believed to be important in the progression of hypertension and associated cardiovascular diseases[65,81,87,93]. The isoform HO-1 is the inducible form of HO, and when induced it can cause a decrease in weight, and therefore a decrease in obesity[113-115]. HO does this by changing the phenotype of the adipocyte[113,116]. HO-1 can interact with NO in several ways, one of which is through Ang Ⅱ[99,117,118]. Increased Ang Ⅱ production causes an increase in ROS, which may inhibit the action of NO[100,119]. This can also increase salt reabsorption. When HO-1 expression is increased, the increases in Ang Ⅱ levels are attenuated; this decreases Ang Ⅱ's downstream signaling effects[99,117,118]. Induction of HO-1 can also reduce the renal vasculature resistance that

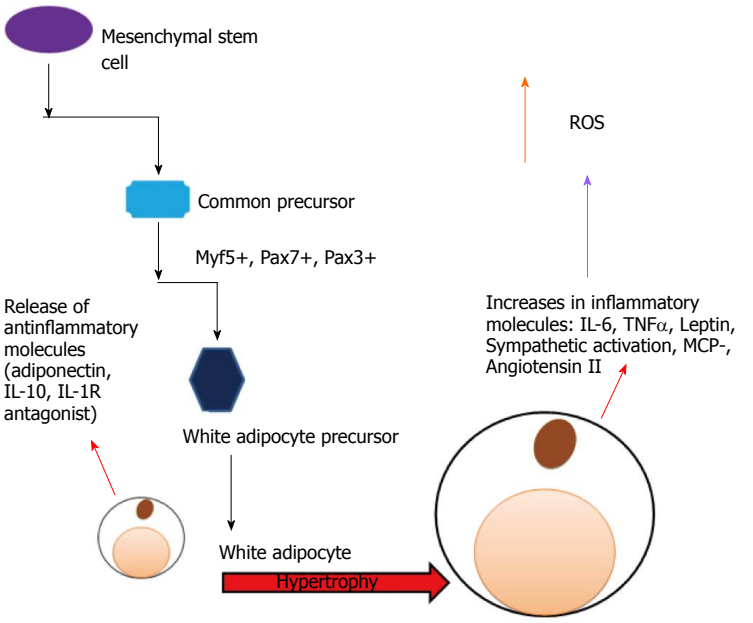

Figure 4 Small adipocytes release anti-inflammatory agents such as adiponectin, interleukin 10, and interleukin 1 receptor antagonist. Hypertrophy of small adipocytes into large adipocytes changes the biochemical release products, into inflammatory markers [interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and monocyte chemotactic protein 1 (MCP1)] and other chemicals such as leptin and angiotensin II that contribute to the disease. Increased activation of the sympathetic nervous system also contributes. IL-10: Interleukin-10; IL-1R: Interleukin 1 receptor; Myf5: Myogenic factor 5; Pax 7: Paired box protein 7; Pax 3: Paired box protein 3; ROS: Reactive oxygen species.

is increased with Ang Ⅱ level increases[99,117,118].

Several studies have shown that induction of HO-1 not only decreases weight and obesity, but it can also prevent the development of hypertension, even if its expression is limited to adipocytes[1,120]. It is not clear if blood pressure is lowered through the indirect effects on the vasculature, kidney, or through the release of other enzymes and factors. If induction of HO-1 is done at any step in the pathway described above, what are the specific effects? If induced during any stage of hypertension, will effects still be seen, or does it need to be induced early in obesity?

Abraham *et al*[1], look specifically at the role of HO-1 and the effects it can have on various aspects of obesity. One study specifically examines adipocyte dysfunction; induction of HO-1 can reverse adipocyte dysfunction and to an extent reverse effects of damage[121]. This lab has also shown significant findings of the role of HO-1 and the attenuating effects it can have with hypertension[1,122,123]. We have also looked from the other perspective, namely ROS generation. We have recently observed that attenuation of ROS generation with pNaKtide[124,125], a peptide designed to ameliorate the Na/K-ATPase mediated feed forward amplification of ROS[50], prevents phenotypical changes within adipocytes as well as ameliorates diet induced obesity in mice[35].

CONCLUSION

There is clearly a very strong relationship between obesity and hypertension. While a plethora of mechanisms potentially link obesity to hypertension, we are left with the provocative possibility that adipocyte biology may play an important role in blood pressure regulation, a topic which to date has not been systematically explored.

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