ORIGINAL ARTICLE

Increased Rho-Kinase Activity in Hypertensive Patients With Left Ventricular Hypertrophy

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BACKGROUND
There is experimental evidence on the role of Rho-kinase (ROCK) activation in cardiac hypertrophy but no information on its role in human hypertension and left ventricular hypertrophy (LVH). We hypothesized that ROCK activity is higher in hypertensive patients with LVH compared with hypertensive patients without LVH.

METHODS
We conducted a cross-sectional study comparing untreated hypertensive patients with (n = 41) and without LVH (n = 46) determined by echocardiography with a healthy normotensive control group (n = 51). Measurements included LV mass, dimensions, and function and ROCK activity determined in circulating leukocytes by measuring Western blot levels of phosphorylated to total myosin light chain phosphatase 1 (MYPT1-p/t).

RESULTS
Compared with normotensive subjects, MYPT1-p/t was significantly increased by 4.5-fold in the hypertensive patients without LVH and by 9-fold in the hypertensive patients with LVH. Compared with the hypertension without LVH group, MYPT1-p/t was significantly increased by 2-fold in the hypertension with LVH gorup. In patients with eccentric LVH, the mean MYPT1-p/t ratio was significantly higher by 4-fold compared with hypertensive patients without eccentric LVH. Patients with an E/e´ ratio ≥15 (n = 6) showed a higher MYPT1-p/t ratio (by 26%) compared with patients with a lower E/e´ ratio ($P \leq 0.01$).

CONCLUSIONS
ROCK activity is higher in hypertensive patients with LVH compared with hypertensive patients without LVH, and it is further increased when eccentric LVH is present. Thus, in hypertension, ROCK activation is related to pathological cardiac remodeling and might have a role as an LVH marker.

Keywords: blood pressure; hypertension; hypertrophy; remodeling; Rho-kinase.

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The small guanosine triphosphatase Rho and its target Rho-kinase (ROCK) play significant roles in both blood pressure regulation and vascular smooth muscle contraction. Rho is activated by agonists of receptors coupled to cell membrane G protein (such as angiotensin II, endothelin, or noradrenalin), by growth factors, or by cytokines.[1–4] Once Rho is activated, it translocates to the cell membrane where it activates ROCK. Then ROCK phosphorylates myosin light chain phosphatase, which is then inhibited. This sequence stimulates vascular smooth muscle contraction, stress fiber formation, and cell migration. In this way, Rho and ROCK activation have important effects on several cardiovascular diseases.[2–6]

Additionally, there is experimental evidence on the significant role of ROCK activation in the development of cardiac hypertrophy, remodeling, and ventricular dysfunction. In Dahl salt-sensitive hypertensive rats, increased left ventricular weight was significantly ameliorated by using the ROCK inhibitor Y-276327.[7] Upregulated RhoA protein, ROCK gene expression, and myosin light-chain phosphorylation in the hypertrophy stage were also suppressed by the ROCK inhibitor.[7] In hypertensive rats, the ROCK inhibitor fasudil attenuated myocardial fibrosis possibly through suppression of monocyte/macrophage infiltration of the heart.[8] Moreover, ROCK is significantly activated in the aortic wall in normotensive rats with genetically high angiotensin I–converting enzyme and angiotensin II, and it causes activation of genes that promote vascular remodeling, such as monocyte chemoattractant protein 1, transforming growth factor β1, and plasminogen activator inhibitor 1, and also increases vascular oxidative stress.[9] In Dahl salt-sensitive hypertensive

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rats, long-term ROCK inhibition with fasudil ameliorated diastolic heart failure,[10] and in rats with pressure overload hypertrophy, selective ROCK inhibition with GSK-576371 improved left ventricular (LV) geometry, collagen deposition, and diastolic function.[11] In mice overexpressing Gαq, ROCK1 gene deletion prevented LV dilatation and systolic dysfunction.[12]

In humans, Hata *et al.* recently observed higher ROCK activity by 37% in peripheral blood leukocytes in untreated patients with hypertension compared with healthy individuals. Additionally, in patients with hypertension treated with antihypertensive agents, ROCK activity was lower in patients using calcium channel blockers compared with patients treated with renin-angiotensin system inhibitors, diuretics, or β-blockers.[13]

Even though there is evidence on the role of ROCK activation in the development of hypertension and cardiovascular remodeling, there is no information on the role of ROCK activation in human hypertension and LV hypertrophy (LVH). We hypothesized that ROCK activity in circulating leukocytes is higher in hypertensive patients with LVH than in hypertensive patients without LVH.

METHODS

Study design

This was a cross-sectional study comparing untreated hypertensive patients with and without LVH with a healthy, normotensive control group. The 3 groups were similar in age and sex.

The study was approved by the Research Committee of the Medical School, Pontificia Catholic University of Chile, and was funded by Fondecyt 1085208 and 1121060 (Chile). Patients with a recent diagnosis of hypertension (<3 months) from our ambulatory center, without antihypertensive treatment, with serum creatinine <1.5 mg/dl, without diabetes, who were nonobese (body mass index <30 kg/m^2), and who were in sinus rhythm were invited to participate and consecutively recruited (n = 87). Control subjects (n = 41) were healthy, normotensive subjects with similar age and sex invited to participate and recruited among hospital staff and patients' relatives with a strictly normal medical record. In the control group, diagnosis of hypertension was excluded with the medical record and also with 2 blood pressure measurements <140/90 mm Hg obtained on 2 different days (3 measurements each day) without use of any antihypertensive drug.

Exclusion criteria were aged <18 years; clinical history of heart failure or any heart disease; neoplastic disease in the last 4 years; active infection in the last 8 weeks; use of statins (because in addition to inhibiting cholesterol biosynthesis, statins also inhibit the formation of isoprenoid intermediates, which are required for the activation of the RhoA/ROCK pathway and inhibit circulating ROCK activity in humans);[14] and chronic lung, liver, or kidney disease. The study complied with the Declaration of Helsinski and was approved by the Ethics Committee of our institution; an informed consent was obtained from all individuals.

Blood pressure measurements

In all subjects, both systolic and diastolic blood pressure were measured in seated position using a standard mercury sphyngomanometer. Phase I and V of Korotkoff sounds were used for this purpose. Three measurements on 2 separate days were performed after 5 minutes of rest in a seated position. The diagnosis of hypertension was made with a blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic.

Ecocardiographic measurements and noninvasive arterial stiffness evaluation

Echocardiograms were obtained with a 2.5-MHz transducer (Philips S5-1) at the time of blood pressure determinations, sampling with a Phillips IE-33 instrument (Phillips, Andover, MA) to evaluate LV function, geometry, and mass. All measurements were performed blindly by an expert operator according to the recommendations of the American Society of Echocardiography.[15] The following variables in the parasternal short axis were measured: end-diastolic interventricular septal thickness and posterior wall thickness and end-diastolic and end-systolic LV dimensions. With these variables, LV mass and LV mass index were calculated according to the formula developed by Devereux *et al.*[16] and modified by the American Society of Echocardiography. As assessed by Bland–Altman analysis, intraobserver variability for LV mass index was −0.2 g/m^2 (range = −14.3 to 13.9 g/m^2; SD = 1.96) and interobserver variability for LV mass index was −1.3 g/m^2 (range =−16.9 to 14.3 g/m^2; SD = 1.96).

Ejection fraction was measured by the biplane Simpsons method. Left atrium area, transmitral pulsed wave analysis, and mitral annulus tissue Doppler (e´) using the mean value (medial and medial) were measured in the apical 4-chamber view. Diastolic dysfunction was considered with a E/e´ ratio ≥15.[17] LVH was considered when LV mass index was >95 g/m^2 for women and >115 g/m^2 for men. Eccentric LVH was defined with a posterior wall/diastolic diameter ratio <0.42.[15,16]

Carotid-to-femoral pulse wave velocity (PWV), a noninvasive and indirect index of arterial stiffness, was determined in all subjects with Complior 1 equipment (Createch Industrie, Massy, France).[18]

ROCK activity in circulating leukocytes

ROCK activity was assessed by measuring the levels of phosphorylated to total myosin light chain phosphatase 1 (MYPT1-p/t), a direct downstream target of ROCK.[19,20] Venous blood containing EDTA was poured over Histopaque (Histopaque-1077; Sigma Chemical, St. Louis, MO) and centrifuged. The white cells were resuspended in phosphate buffered saline (PBS). After determining cell yield and viability by using the trypan blue exclusion test (4–80 × 106 viable cells; 95% viability), the cells were resuspended in lysis buffer. Protein content of supernatants was determined by the Bradford assay. Soluble fractions were heated at 95 °C

with sodium dodecylsulfate–polyacrylamide gel electrophoresis sample buffer for myosin light chain phosphatase 1 (MYPT1), Rho-associated kinase 1 (ROCK1), and Rho-associated kinase 2 (ROCK2) Western blot analysis. The leukocyte protein extracts were matched for protein, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 6% polyacrylamide gels, and electrotransferred to nitrocellulose. Membranes were blocked with 7% nonfat milk in PBS containing 0.05% Tween 20 at room temperature. Antiphospho-Thr853-MYPT1 (Phospho-myosin-binding subunit/MYPT1-P-Thr853; Cyclex, Woburn, MA) or anti-MYPT1 (BD Transduction Laboratories, Becton, Dickinson and Co, Franklin Lakes, NJ), anti-ROCK1 monoclonal antibody, and anti-ROCK2 monoclonal antibody (BD Biosciences, San Jose, CA) primary antibodies were diluted in blocking solution (1:700, 1:1,000, 1:500, and 1:2,000, respectively). Nitrocellulose membranes were incubated with primary antibody overnight at 4 °C. After washing in PBS containing 0.05% Tween 20, blots were incubated with horseradish peroxidase–linked secondary antibody, and specific binding was detected using enhanced chemiluminescence with exposure to Kodak film. Each blot was quantified by scanning densitometry with the Un-Scan-It software (Silk Scientific, Orem, UT).[19,20]

Statistical analysis

Results are presented as mean ± SEM or as a percentage. Normal distribution of MYPT1 ratio was tested with the Kolmogorov–Smirnov test. A significant P value (<0.05) was obtained for the MYPT1 ratio, and nonparametric tests were used. A χ^2 test (for categorical variables) and Kruskall–Wallis and Mann–Whitney U tests (for continuous variables) were used. To assess correlations, Pearson or Spearman methods were used.

Considering our previous data,[19] with an alpha error ≤0.05, power 80%, and an expected difference ≥30% in MYPT1-p/t values, the sample size should be at least 96 subjects.

RESULTS

Clinical characteristics and blood pressure

Eighty-seven hypertensive patients and 51 control subjects were consecutively included in this study. Among hypertensive patients, 41 had LVH, which was defined as an LV mass index >95 g/m^2 for women and >115g/m^2 for men.[15] In all patients, the etiology of hypertension was essential, and nobody was receiving antihypertensive drugs, statins, steroids, or anti-inflammatory drugs. Most clinical characteristics, including age, sex, and body mass index, as well as general laboratory blood tests, were similar among the 3 groups (Tables 1 and 2).

Compared with the normotensive control group, blood pressure (systolic/diastolic) was higher by 43/25.5 and 49.9/25.1 mm Hg in the hypertensive patients without LVH and in the hypertensive patients with LVH, respectively, and it was similar between both hypertensive groups (Table 1).

Echocardiographic characteristics

Compared with both the control group and the hypertension without LVH group, in patients with hypertension and LVH, the end-diastolic diameter, interventricular septum, posterior wall thickness, and LV mass index were significantly higher (Table 3), whereas ejection fraction was similar among the 3 groups (62.9 ± 0.3% vs. 61.2 ± 0.5%, respectively; not significant). Both left atrium area and diameter were significantly higher in hypertensive patients with LVH. The E/A ratio was higher in the normotensive group compared with both the hypertensive group with LVH and the hypertensive group without LVH (1.2 ± 0.04 vs. 0.9 ± 0.03 vs. 0.9 ± 0.04, respectively; $P < 0.01$).

The tissue Doppler study revealed that hypertensive patients with LVH had significantly higher E/e′ ratio than the control subjects and the HT patients without LVH (8.7 ± 0.2 vs. 8.8 ± 0.3 vs. 11 ± 0.5, respectively; $P < 0.01$).

Table 1. Demographic characteristics and blood pressure in normotensive control subjects and in hypertensive patients with and without left ventricular hypertrophy

Characteristic	Controls (n = 51)	HT without LVH (n = 46)	HT with LVH (n = 41)	*P* value
Age, y	50.4 ± 3.1	50.2 ± 2.9	53.4 ± 3.5	NS
Men, %	49	53	51	NS
HR, bpm	75.4 ± 3.2	75.7 ± 3	75.5 ± 3.1	NS
SBP, mm Hg	113.5 ± 4.4	156.5 ± 5.1*	163.4 ± 5.9*	<0.01
DBP, mm Hg	75.2 ± 3.9	100.7 ± 4.9*	100.3 ± 4.5*	<0.01
MAP, mm Hg	87.2 ± 3.5	120.1 ± 4.9*	119.8 ± 4.6*	<0.01
Weight, kg	69.3 ± 9.1	73.4 ± 10.9	73.4 ± 11.2	NS
Height, cm	163 ± 8	165 ± 9	162 ± 9	NS
BMI	25.8 ± 1	26.1 ± 2	26.2 ± 2	NS

Data are shown as mean ± SEM unless otherwise indicated. P values were determined by Kruskal–Wallis or χ^2 test.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; HT, hypertensive patients; LVH, left ventricular hypertrophy; MAP, mean arterial pressure; NS, not significant; SBP, systolic blood pressure.

*$P < 0.05$ vs. controls.

Table 2. Laboratory tests

Test	Controls (n = 51)	HT without LVH (n = 46)	HT with LVH (n = 41)	*P* value
Total serum cholesterol, mg/dl	194 ± 5	203 ± 6	202 ± 5	NS
Serum HDL cholesterol, mg/dl	51 ± 2	48 ± 3	46 ± 2	NS
Serum LDL cholesterol, mg/dl	129 ± 4	131 ± 5	131 ± 5	NS
Serum triglycerides, mg/dl	126 ± 13	176 ± 35	220 ± 28	0.004
AST, U/L	23 ± 1	27 ± 2	25 ± 1	NS
Uric acid, mg/dl	4.6 ± 0.1	5.4 ± 0.3	4.9 ± 0.3	NS
Serum glucose, mg/dl	90 ± 1.8	93 ± 2	94 ± 3	NS
Serum creatinine, mg/dl	0.77 ± 0.08	0.86 ± 0.1	0.82 ± 0.03	NS
Serum potassium, meq/L	4.17 ± 0.06	4.24 ± 0.06	4.11 ± 0.07	NS
Serum sodium, meq/L	139 ± 1	131 ± 4	140 ± 1.5	NS
Hematocrit, %	41 ± 0.5	44 ± 0.5	42 ± 0.7	NS

Data are shown as mean ± SEM. *P* values were determined by Kruskall–Wallis test.

Abbreviations: AST, aspartate aminotransferase; HDL, high-density lipoprotein; HT, hypertensive patients; LDL, low-density lipoprotein; LVH, left ventricular hypertrophy; NS, not significant.

Table 3. Cardiac dimensions, left ventricular mass, and function

Echocardiographic parameter	Controls (n = 51)	HT without LVH (n = 46)	HT with LVH (n = 41)	*P* value
ESD, mm	27.9 ± 0.6	28.2 ± 0.6	29.9 ± 0.9	NS
EDD, mm	48.2 ± 0.5	48.7 ± 0.7	49.2 ± 1.0*	0.01
Septum thickness, mm	8.4 ± 0.2	8.9 ± 0.2	10.8 ± 0.3*	<0.01
PW thickness, mm	8.08 ± 0.1	8.5 ± 0.1	9.9 ± 1.2*	<0.01
LVMI, g/m^2	86.2 ± 2.0	86.9 ± 3.0	125.0 ± 4.0*	<0.01
EF, %	62.9 ± 0.3	61.6 ± 0.5	61.2 ± 0.5	NS
LA diameter, mm	36.6 ± 0.5	36.9 ± 0.5	39.6 ± 0.7*	<0.01
LA area, cm^2	17.8 ± 0.4	17.1 ± 0.4	20.5 ± 3.0*	<0.01
E wave velocity, cm/s	79.0 ± 2.0**	69.0 ± 2.0	73.0 ± 2.0	<0.01
A wave velocity, cm/s	66 ± 1.8	75 ± 2.3	81 ± 2.9*	<0.01
E/A	1.2 ± 0.04**	0.9 ± 0.03	0.9 ± 0.04	<0.01
e', cms/s	9.2 ± 0.2***	8.1 ± 0.3***	7.0 ± 0.3***	<0.01
E/e'	8.7 ± 0.2	8.8 ± 0.3	11.0 ± 0.5*	<0.01
DT, ms	201.0 ± 6.0**	227.0 ± 6.0	233.0 ± 12.0	0.02

Data are shown as mean ± SEM. *P* values were determined after Kruskall–Wallis test.

Abbreviations: DT, deacceleration time; e´, mean (lateral and medial) mitral annulus tissue doppler; E/A: transmitral filling waves ratio; E/e´, E wave (mitral pulse Doppler) and e´ (mitral annulus tissue Doppler) ratio; EDD, end-diastolic diameter; EF, ejection fraction; ESD, end-systolic diameter; HT, hypertensive patients; LA, left atrium; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; NS, not significant; PW, posterior wall.

*$P < 0.05$ vs. controls and hypertensive patients without left ventricular hypertrophy.

**$P < 0.05$ vs. hypertenstive patients with and without left ventricular hypertrophy, post hoc.

***$P < 0.05$ between groups, post hoc.

Arterial stiffness

In the normotensive control group, carotid-to-femoral PWV was 8.3 ± 0.9 m/sec, and it was significantly increased by 33% in both hypertensive groups. No differences were observed here between hypertensive patients with and without LVH (11.1 ± 1.8 vs. 11.5 ± 2.1, respectively; not significant) (Figure 1). A significant correlation between MYPT1-p/t and PWV was found ($r = 0.31$; $P = 0.01$).

ROCK activation in circulating leukocytes

The mean MYPT1-p/t ratio in circulating leukocytes, a downstream evidence for ROCK activation, was 2.1 ± 0.4 in the normotensive control group and it was 13.7 ± 2.7 in the hypertensive patients ($P < 0.01$). No differences were observed in the ROCK1 or ROCK2 isoforms measured in circulating leukocytes comparing the normotensive control group with the hypertensive patients.

Compared with the normotensive control group, the mean MYPT1-p/t ratio in circulating leukocytes was significantly increased by 4.5-fold (9.5 ± 2.7) in the HT without LVH group (n = 46) and by 9-fold (19 ± 4) in the HT with LVH group (n = 41) (Figure 2a, b). Mean MYPT1-p/t ratio in circulating leukocytes was significantly increased by 2-fold in the HT group with LVH compared to the HT group without LVH (Figure 2a, b).

Among hypertensive patients with LVH (n = 41), 18 (43%) had eccentric LVH (defined as posterior wall thickness to end diastolic diameter ratio <0.42), which means further LV remodeling. In these hypertensive patients with eccentric LVH hypertrophy, the mean MYPT1-p/t ratio was significantly higher by 4-fold compared with hypertensive patients with concentric LVH (33.5 ± 9.6 vs. 8.1 ± 2.1, respectively; $P < 0.001$) (Figure 3).

In the hypertensive patients without LVH (n = 46), the mean MYPT1 ratio in circulating leukocytes was 6.1 ± 3.3 in patients with normal LV geometry (n = 5) and 9.9 ± 3.9 in patients with concentric remodeling (n = 41; not significant).

Hypertensive patients with an E/e′ ratio ≥15 (n = 6) showed a significantly higher MYPT1-p/t ratio than patients with a lower E/e′ ratio (11.2 ± 0.9 vs. 8.9 ± 1.7; $P = 0.01$).

We did not observe a significant correlation between LV mass and other echocardiographic or clinical variables with MYPT1-p/t.

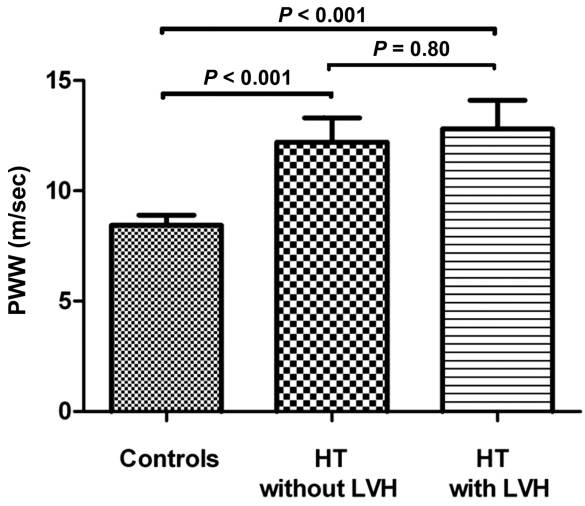

Figure 1. Carotid-to-femoral pulse wave velocity (PWV; mean ± SEM) in normotensive control subjects (n = 51) and in hypertensive (HT) patients with (n = 41) and without left ventricular hypertrophy (LVH) (n = 41). *P* values are for comparisons after significant Kruskall–Wallis test ($P < 0.001$).

DISCUSSION

The main finding of this study was that ROCK activity in circulating leukocytes was higher in hypertensive patients with echocardiographic evidence of LVH compared with hypertensive patients without LVH. Moreover, among hypertensive patients with LVH, those with an advanced remodeling process (eccentric LVH and those with a higher E/e′ ratio) showed increased ROCK activity compared with patients without those characteristics. Additionally, ROCK activity in circulating leukocytes was significantly higher in untreated hypertensive patients compared with normotensive control subjects, which is consistent with recently published data.[13]

Several animal studies have involved ROCK activation in the pathogenesis of hypertension. ROCK was upregulated in carotid and coronary arteries in spontaneous hypertensive rats compared with control rats, and the contractile response to phenylephrine and serotonin was significantly inhibited when hydroxyfasudil was added in the hypertensive group.[21] Uehata *et al.* observed that the administration of the specific ROCK inhibitor Y-27632 had a dose-dependent reduction

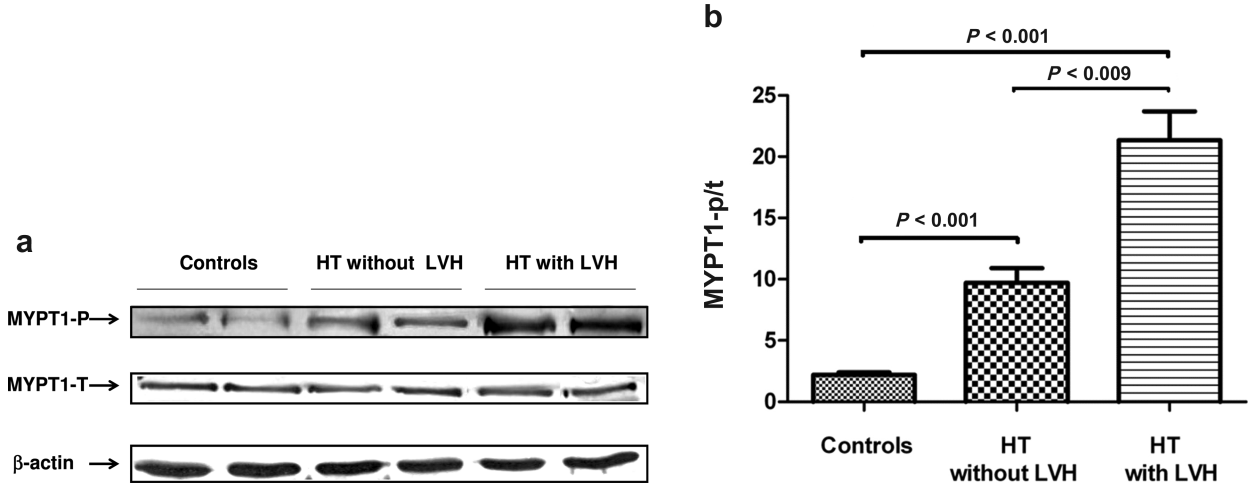

Figure 2. Rho-kinase activity in circulating leukocytes assessed by the Western blot levels of phosphorylated to total myosin light chain phosphatase 1 (MYPT1-p/t), a direct downstream target of Rho-kinase. (a) Representative Western Blots of MYPT1 (phosphorylated and total) in circulating leukocytes from normotensive controls (n = 2); hypertensive patients without LVH (n = 2) as well as from hypertensive patients with LVH (n = 2). (b) Bar graph showing Rho-kinase activity in circulating leukocytes assessed by the Western blot levels of phosphorylated to total myosin light chain phosphatase 1 (MYPT1-p/t) in normotensive controls (n = 51) and in hypertensive patients with (n = 41) and without LVH (n = 46) determined by echocardiography (mean ± SEM). *P* values are for comparisons after significant Kruskall–Wallis test ($P < 0.001$).

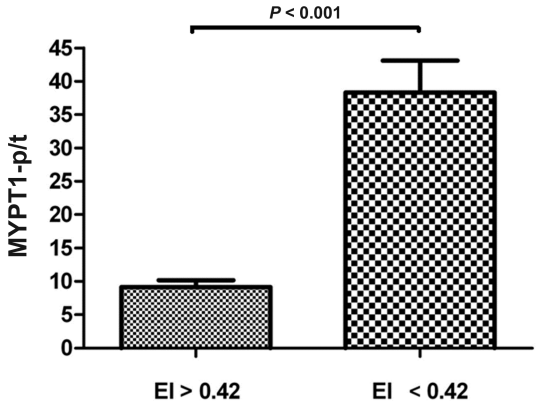

Figure 3. Rho-kinase activity in circulating leukocytes assessed by the Western blot levels of phosphorylated to total myosin light chain phosphatase 1 (MYPT1- p/t), a direct downstream target of Rho-kinase in hypertensive patients with and without eccentric left ventricular hypertrophy (LVH) determined by the echocardiographic eccentricity index (EI, mean ± SEM). An eccentricity index < 0.42 is indicative of eccentric LVH. *P* value determined by Mann–Whitney *U* test.

on blood pressure in spontaneous hypertensive, renal hypertensive, and DOCA salt rats.[22]

The first clinical evidence about the role of the RhoA/ROCK pathway in the pathogenesis of human hypertension was published by Masumoto *et al.*[23] Hypertensive patients received nitroprusside infusion with or without the ROCK inhibitor fasudil, which induced a larger vasodilator response in these patients than in control subjects, whereas the vasodilator response to nitroprusside was similar in both groups.[23] Similar results were observed in patients with heart failure with elevated arterial arm resistance and reduced flow-mediated vasodilatation, where fasudil did increase blood flow and reactive hyperemia compared with control subjects,[24] demonstrating the role of ROCK activation in increased vascular resistance and reduced forearm vasodilatation in these patients.[25] In our study, we observed that hypertensive patients had increased ROCK activity and PWV (with a weak but significant correlation between both parameters), showing that the RhoA/ROCK pathway might be involved, at least in part, in vascular remodeling.

There is experimental evidence on the role of the RhoA/ROCK pathway in the development of cardiac hypertrophy, remodeling, and ventricular dysfunction. Recent observations suggest that in heart failure, pathologic cardiac remodeling by itself could be a major determinant of ROCK activation. There are experimental studies evaluating ROCK inhibition and its effects on cardiac remodeling and LV dysfunction.[11,26] In rats with pressure overload hypertrophy (Dahl salt-sensitive rats), inhibition of this signaling pathway improved LV geometry, reduced collagen deposition, and improved diastolic function.[11,26]

In a recent study from our group, patients with stable systolic heart failure had increased levels of ROCK activity, determined by the MYPT1-p/t ratio in circulating leucocytes, as compared with healthy individuals and treated hypertensive patients.[19] In that study, ROCK activation was inversely correlated with the LV systolic function.[19]

In hypertensive patients treated with antihypertensive drugs, ROCK activity in circulating leukocytes was lower in patients using calcium channel blockers compared with patients treated with renin-angiotensin system inhibitors, diuretics, or β-blockers.[13] These findings suggest that the enhancement of ROCK activity by hypertension may cause leukocyte activation and enhanced leukocyte infiltration into the vascular wall,[13] leading to progression of vascular remodeling and atherosclerosis and suggesting a possible clinical relevance in estimating the degree of ROCK activity in these patients.[13] In a recent study in hypertensive patients treated for 48 weeks with antihypertensive drugs, ROCK activity in circulating leukocytes was lowered similarly by nifedipine and eplerenone from the 4th week, but endothelial function was only improved by eplerenone.[27] None of these studies assessed LVH parameters in these patients.

Regarding diastolic function, in our study, patients with an increased E/e´ ratio had increased ROCK activity, suggesting that this subgroup has a more advanced geometrical and functional remodeling process.

One limitation in this study is related to the biologic significance of ROCK activation in circulating leukocytes, whether it directly mediates myocardial remodeling or it implies similar activation in other cell types in the myocardium. No information is available in the literature on this specific aspect. However, circulating lymphocytes have been used to study β-adrenergic receptor signaling and to make extrapolations to the cardiac β-adrenergic receptor system, and they represent a valuable and reliable marker of the functional state of cardiac β-adrenergic receptor signaling.[28–30] Furthermore, the G protein–coupled receptor kinase 2 (GRK2 or β-ARK1) regulates β-adrenergic receptors in the heart, and its cardiac expression is elevated in human heart failure. A direct correlation between myocardial and circulating lymphocytes GRK2 activity has been found in patients with heart failure, implying that myocardial GRK2 expression and activity are mirrored by lymphocyte levels of this kinase in human heart failure,[30] which might be similar but needs to be proven with ROCK activation.

Additionally, LV geometry and function were assessed by volumetric parameters and not by deformation imaging or other surrogates of contractile dysfunction and fibrosis extension.

Another limitation was the absence of a group with medical treatment and with long-term follow-up to evaluate the relation between ROCK activity and LVH regression. However, this aspect was beyond the scope of this study, which aimed to characterize ROCK activation measured in circulating leukocytes in hypertensive patients with LVH.

We did not find a significant correlation between LV mass and other echocardiographic or clinical variables with MYPT1-p/t, probably because of the sample size and the dispersion of MYPT1-p/t values. It is also possible that 3-dimensional echocardiography could have improved LV mass quantification, maybe allowing a more precise differentiation among different levels of LV mass.

In conclusion, in hypertensive patients, ROCK activity is increased by 2-fold in circulating leukocytes in patients with LVH compared with hypertensive patients without LVH. Additionally, in hypertensive patients with eccentric LVH,

ROCK activity is increased by 4-fold in circulating leukocytes compared with hypertensive patients with concentric LVH and is higher in subjects with evidence of diastolic dysfunction as compared with subjects without diastolic dysfunction. These data suggest that ROCK activation in circulating human leukocytes is related, at least in part, to pathological geometrical and functional cardiac remodeling and might be used to identify hypertensive subjects with myocardial damage.

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DISCLOSURE

The authors declared no conflict of interest.

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