Possible Involvement of Rho-Kinase in the Pathogenesis of Hypertension in Humans

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Abstract—Rho-kinase plays an important role in modulating Ca$^{2+}$ sensitivity of vascular smooth muscle and has been suggested to be involved in the increased systemic vascular resistance in hypertensive animals. However, it remains to be examined whether this is also the case in patients with essential hypertension. Recently, it has been shown that fasudil is a specific Rho-kinase inhibitor. The aim of this study was to examine whether Rho-kinase is involved in the pathogenesis of hypertension in humans by using this Rho-kinase inhibitor. Studies were performed in hypertensive patients (HT group, n=14) and age-matched normotensive subjects (NT group, n=12). Forearm blood flow was measured by a strain-gauge plethysmograph during intra-arterial infusion of graded doses of fasudil (3.2, 6.4, 12.8, and 25.6 µg/min) or sodium nitroprusside (0.4, 0.8, 1.6, and 3.2 µg/min). Resting forearm vascular resistance was significantly higher in the HT group than in the NT group (22±4 versus 17±5 U, respectively; P<0.05). The extent of the increase in forearm blood flow evoked by fasudil was significantly greater in the HT group than in the NT group (12.3±1.4 versus 6.0±0.6 mL · min$^{-1}$ · 100 mL$^{-1}$, respectively; P<0.01). The percent decrease in forearm vascular resistance was significantly greater in the HT group than in the NT group (63.6±4.7% versus 29.6±3.9%, respectively; P<0.01). By contrast, forearm vasodilator response evoked by sodium nitroprusside was comparable between the 2 groups. These results provide the first evidence that Rho-kinase may be involved in the pathogenesis of the increased peripheral vascular resistance in hypertension in humans. (Hypertension. 2001;38:1307-1310.)

Key Words: hypertension, essential □ muscle, smooth, vascular □ blood flow □ vascular resistance

Increased systemic vascular resistance plays an important role in the pathogenesis of hypertension; however, the molecular mechanism remains to be elucidated. Phosphorylation of myosin light chain (MLC) is a crucial step for vascular smooth muscle (VSMC) contraction, the extent of which is regulated in a dual manner by MLC kinase and MLC phosphatase (MLCPh). Inhibition of MLCPh results in an increase in MLC phosphorylation and subsequent VSMC hypercontraction (Ca$^{2+}$ sensitization). Indeed, this Ca$^{2+}$ sensitization mechanism of VSMCs has been reported to be enhanced in hypertensive animals.

Rho-kinase/ROKα/ROCKII, which is activated by the small GTPase Rho, inhibits MLCPh activity and thus plays a key role in Ca$^{2+}$ sensitization and hypercontraction of VSMCs. It has been previously reported that Y-27632, a Rho-kinase inhibitor, preferentially lowers arterial pressure in rat models of hypertension in vivo, which indirectly suggests an involvement of Rho-kinase in hypertension. We have recently demonstrated that Rho-kinase is upregulated and plays a key role in VSMC contraction in a porcine model of coronary artery spasm and in spontaneously hypertensive rats (SHR). However, it remains to be examined if this is also the case in humans.

Fasudil, a protein kinase inhibitor, is currently used in Japan for the treatment of cerebral vasospasm after subarachnoid hemorrhage. It has been recently demonstrated that fasudil is a specific Rho-kinase inhibitor, as is Y-27632, although the latter has not been approved for human use. Thus, fasudil is regarded as a specific Rho-kinase inhibitor that can be used in humans.

The present study was thus designed to determine whether Rho-kinase is involved in the increased peripheral vascular resistance in patients with essential hypertension by examining the vasodilator effect of fasudil.

Methods

Subjects
Fourteen patients with essential hypertension (8 men and 6 women) and 12 normal subjects (7 men and 5 women) were enrolled in the present study. The mean age of the hypertensive patients was 56±9 years; that of the normotensive subjects, 55±10 years. The hypertensive patients had no other coronary risk factors. Normal subjects did not have systemic hypertension, diabetes mellitus, or hyperlipidemia. Nine of the hypertensive patients had not received any antihypertensive therapy previously. Although the remaining 5 patients had previously received antihypertensive therapy, all vasodilator agents were discontinued >24 hours before the study. Written

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informed consent was obtained from all subjects. The present study was approved by the ethics committee for human research in our institute.

General Procedures
The present study was performed with subjects in a supine position and in a postabsorptive state with room temperature at 25°C to 26°C. With the subjects under local anesthesia, the left brachial artery was cannulated with a 20-gauge intravascular cannula for drug infusion, and the cannula was connected to a pressure transducer for direct measurement of arterial pressure.

Measurement of FBF
Forearm blood flow (FBF) was measured by using a strain-gauge plethysmograph with a venous-occlusion technique, as previously reported.¹⁴ FBF (mL · min⁻¹ · 100 mL of forearm volume⁻¹) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff on the upper arm. The pressure in the venous-occlusion or congesting cuff was 40 mm Hg. Circulation to the hand was arrested by a cuff inflated around the wrist. An average of 4 measurements made at 15-second intervals was used for later analysis. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure (diastolic pressure plus one third of pulse pressure in mm Hg) by FBF. These values are expressed as units throughout the study.

Protocol
FBF, arterial pressure, and heart rate were measured at rest and during the administration of graded doses of fasudil (3.2, 6.4, 12.8, and 25.6 µg/min) or sodium nitroprusside (SNP; 0.4, 0.8, 1.6, and 3.2 µg/min). Each dose of fasudil was infused for 15 minutes, and the FBF was measured in the last minute of each infusion. Each dose of SNP was infused for 5 minutes, and FBF was measured after each infusion. Venous blood samples were drawn from the antecubital vein before and immediately after infusion of the peak dose of fasudil (25.6 µg/min) and at the end of the study for determination of the concentration of fasudil.

Drugs
The following drugs were used: fasudil (Asahi Chemical Industries) and SNP. All drugs were dissolved in physiological saline immediately before use.

Statistical Analysis
All results are expressed as mean±SEM. Values at rest were compared by unpaired t test. Responses to graded doses of drugs in each group were examined by ANOVA for repeated measures. Two-way ANOVA was used to compare responses of FBF and FVR in the 2 groups. Values of P<0.05 were considered to be statistically significant.

Results
Baseline Characteristics
Mean blood pressure at rest was significantly higher in hypertensive patients than in normotensive subjects (103±7 versus 73±6 mm Hg, respectively; P<0.01). Heart rate at rest did not significantly differ between the hypertensive patients and normotensive subjects (70±5 versus 69±6/min, respectively). Basal FBF did not differ between the hypertensive patients and normotensive subjects (3.2±1 versus 3.8±2 mL · 100 mL⁻¹ · min⁻¹). Basal FVR was significantly higher in the hypertensive patients than in the normotensive subjects. (22±4 versus 17±5 U, respectively; P<0.05). The 2 groups were comparable in age, gender difference, body mass index, plasma total cholesterol, triglycerides, LDL and HDL cholesterol, hemoglobin A₁c, and current smoking habits (data not shown).

Plasma Concentrations of Fasudil
Just after the peak dose of fasudil, its plasma concentrations significantly increased in both groups; however, the levels were comparable between the hypertensive patients and normotensive subjects (71±22 versus 72±23 μmol/L, respectively).

Forearm Vascular Responses to Fasudil and SNP
Fasudil significantly evoked dose-dependent increases in FBF in the hypertensive patients but not in the normotensive subjects (Figure 1). By contrast, SNP caused comparable increases in FBF in the 2 groups (Figure 1). Fasudil evoked significantly greater decreases in FVR in the hypertensive patients compared with the normotensive subjects, thus normalizing FVR in those patients (Figure 2). In contrast, SNP caused comparable decreases in FVR in the 2 groups (Figure 2). Systemic arterial blood pressure and heart rate did not change significantly during the intra-arterial infusion of fasudil or SNP in either group (data not shown).

Discussion
The present study was conducted on the assumption that fasudil is a specific Rho-kinase inhibitor.⁷,¹¹,¹² Davies et al¹¹ recently examined the specificity of 16 commonly used protein kinase inhibitors against 24 protein kinases. They found that the inhibitory effect of fasudil on Rho-kinase is as specific as that of Y-27632.¹¹ Because Y-27632 has not been
approved for human use, we chose fasudil as a specific Rho-kinase inhibitor for the present study.

The major finding of the present study was that the forearm vasodilator response to fasudil was significantly greater in hypertensive patients than in normal subjects, whereas SNP caused a similar forearm vasodilator response in the 2 groups. The extent of the increase in plasma concentration of fasudil was comparable between the 2 groups, and the administration of fasudil into the forearm circulation did not significantly change systemic blood pressure or heart rate in either group. The preferential vasodilator effect of fasudil in hypertensive patients was not due to the structural change of the arterial wall, because the response to SNP was comparable between the 2 groups. These results provide the first evidence that Rho-kinase may be involved in the pathogenesis of the increased peripheral vascular resistance in hypertensive patients.

The involvement of Rho-kinase in hypertension was first indirectly suggested by Uehata et al. inasmuch as they observed that Y-27632 preferentially lowered blood pressure in various rat models of hypertension in vivo. Recently, we were able to directly demonstrate that in hypertensive blood vessels of SHR, both the expression and the activity of Rho-kinase are enhanced, causing VSMC hyperreactivity through suppression of MLCPh. The same molecular mechanism appears to be involved in the pathogenesis of coronary artery spasm in our porcine model. Thus, it is conceivable that Rho-kinase is involved in a wide variety of disorders with VSMC hyperreactivity.

Rho-kinase plays an important role in various cellular functions, not only in VSMC contraction but also in actin cytoskeleton organization, cell adhesion and motility, cytoskeleton, and gene expression. Indeed, we and others have recently demonstrated that Rho-kinase is involved in the pathogenesis of arteriosclerosis in general. We also have recently demonstrated that long-term oral treatment with fasudil inhibits the development of coronary vascular lesions (medial thickening and perivascular fibrosis) in SHR in vivo. The present study suggests that Rho-kinase is also substantially involved in the pathogenesis of hypertension in humans. Taken together, it is suggested that Rho-kinase could be regarded as a novel therapeutic target for the treatment of hypertension.

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References


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Correction

The authors of an article in the December issue of *Hypertension* by Masumoto et al (Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. *Hypertension*. 2001;38:1307–1310) would like to make the following correction:

In the Results section, “Plasma Concentrations of Fasudil” (p 1308), the levels were comparable between the hypertensive patients and the normotensive subjects: 710±94 versus 722±84 nmol/L, not 71±22 versus 72±23 μmol/L.