Long-Term Inhibition of Rho-Kinase Suppresses Angiotensin II–Induced Cardiovascular Hypertrophy in Rats In Vivo

Effect on Endothelial NAD(P)H Oxidase System

Midoriko Higashi, Hiroaki Shimokawa, Tsuyoshi Hattori, Junko Hiroki, Yasushi Mukai, Keiko Morikawa, Toshihiro Ichiki, Shosuke Takahashi, Akira Takeshita

Abstract—Intracellular signaling pathway mediated by small GTPase Rho and its effector Rho-kinase plays an important role in regulation of vascular smooth muscle contraction and other cellular functions. We have recently demonstrated that Rho-kinase is substantially involved in angiotensin II–induced gene expressions and various cellular responses in vitro. However, it remains to be examined whether Rho-kinase is involved in the angiotensin II–induced cardiovascular hypertrophy in vivo and, if so, what mechanisms are involved. Long-term infusion of angiotensin II for 4 weeks caused hypertrophic changes of vascular smooth muscle and cardiomyocytes in rats. Both changes were significantly suppressed by concomitant oral treatment with fasudil, which is metabolized to a specific Rho-kinase inhibitor, hydroxyfasudil, after oral administration. Angiotensin II caused a perivascular accumulation of macrophages and Rho-kinase activation, both of which were also significantly suppressed by fasudil. Vascular NAD(P)H oxidase expression (nox1, nox4, gp91phox, and p22phox) and endothelial production of superoxide anions were markedly increased by angiotensin II, both of which were also significantly suppressed by fasudil. Thus, fasudil ameliorated the impaired endothelium-dependent relaxations caused by angiotensin II without affecting vasodilator function of vascular smooth muscle. These results provide evidence that Rho-kinase is substantially involved in the angiotensin II–induced cardiovascular hypertrophy in rats in vivo. The suppression of endothelial NAD(P)H oxidase upregulation and resultant superoxide production and the amelioration of endothelial vasodilator function may be involved in this process. (Circ Res. 2003;93:767-775.)

Key Words: angiotensin II ■ Rho-kinase ■ arteriosclerosis ■ cardiovascular hypertrophy ■ superoxide anion

Angiotensin II is a vasoconstrictor agent that exerts numerous cardiovascular effects, including vascular inflammation and proliferation and subsequent development of arteriosclerosis.1–3 During agonist-induced contraction of vascular smooth muscle cells (VSMCs), phosphorylation of myosin light chain (MLC) is a crucial step for force development. The extent of MLC phosphorylation depends on the balance between the activity of Ca²⁺/calmodulin-dependent MLC kinase and that of myosin phosphatase.4 It has been demonstrated that Rho-kinase/ROKα/ROCK II (an isoform to p160ROCK/ROKβ/ROCK I),5 which is an effector of the small GTPase Rho, inhibits MLC phosphatase activity by phosphorylating its myosin-binding subunit6,7 and thus plays a central role in agonist-induced Ca²⁺ sensitization and hypercontraction of VSMCs.4,5,7 We have demonstrated that Rho-kinase plays an important role in angiotensin II–induced mRNA expression of monocyte chemoattractant protein-18 and of plasminogen activator inhibitor-19 in cultured rat aortic VSMCs. We have recently demonstrated that in cultured human coronary VSMCs, the expression of Rho-kinase is enhanced by inflammatory stimuli, such as angiotensin II and interleukin-1β (IL-1β).10 However, it remains to be determined whether Rho-kinase is involved in angiotensin II–induced cardiovascular hypertrophy in vivo and, if so, what mechanisms are involved. The present study was thus designed to examine these points in rats in vivo.

Materials and Methods

Animals
This experiment was reviewed and approved by the Committee on Ethics of Animal Experiments of the Kyushu University. A total of 111 adult male WKY rats (14 to 17 weeks old, weighing 300 to 350 g) obtained from the colony at the Kyushu University were used. Animals were anesthetized with intraperitoneal pentobarbital (50
mg/kg), and an osmotic minipump (Alzet model 2ML4 or 2ML1) was implanted subcutaneously. The pumps contained angiotensin II dissolved in 0.15 mol/L NaCl containing 10 mmol/L acetic acid. The infusion rate was 0.75 mg/kg per day (for 4 weeks for the protocol of cardiovascular hypertrophy) or 1 week (for the protocol of superoxide production and endothelial function). Sham-operated control rats underwent an identical surgical procedure, but with a pump containing vehicle alone. Both angiotensin II–infused and sham-operated animals received either a low dose (30 mg/kg per day) or a high dose (100 mg/kg per day) of fasudil or vehicle in their drinking water beginning at the day of pump implantation.11

We have previously confirmed that hydroxyfasudil is a specific Rho-kinase inhibitor because its specificity for Rho-kinase is 100 times higher than for protein kinase C and 1000 times higher than for MLC kinase.12 Further, the inhibitory effect of hydroxyfasudil on 16 kinases, including Rho-kinase, has recently been examined. Among the kinases tested, hydroxyfasudil at 10 μmol/L showed more than 50% inhibition only for Rho-kinase (97.6%) (Dr Seto, Asahi Kasei Co, personal communication, 2003). In a preliminary study, the plasma concentration of hydroxyfasudil in rats that was reduced to be 100 μmol/L per kg per day for 1 week was 435±102 and 502±1.36 nmol/L in the control and the angiotensin II–infused rats, respectively (n=4 each). Thus, we consider that hydroxyfasudil is a reasonably selective inhibitor for Rho-kinase in the present study. Systolic blood pressure was measured immediately before surgery and at the end of the experiment by tail cuff plethysmography.11

Histological Analysis
After 4 weeks of angiotensin II infusion, the animals were again anesthetized with intraperitoneal pentobarbital (50 mg/kg) and their thoracic aortas were cannulated. The coronary vasculature was perfused with PBS containing sodium nitroprusside (10 μmol/L) and then fixed with methacarn solution and subsequently prepared for histological analysis as previously described.11 Left ventricular (LV) weight was measured, and the ratio of LV weight to body weight was calculated to determine an index of LV hypertrophy. Four sections were obtained from each heart and mounted on slides and stained with Masson’s trichrome or H&E. To evaluate the coronary arterial wall thickness and perivascular fibrosis, short-axis images of small coronary arteries (with an internal diameter of 50 to 200 μm) were scanned at ×200 magnification. The wall to lumen ratio (the ratio of medial thickness to internal diameter) and the area of perivascular fibrosis (the ratio of the fibrosis area surrounding the vessel to the total vessel area) were calculated. In each heart, ~40 small arteries were examined. To evaluate the extent of cardiomyocyte hypertrophy, cross-sectional images of cardiomyocytes were scanned at ×400 magnification. Approximately 10 cross-sections of cardiomyocytes were analyzed in each heart. Average values for each heart were used for analysis.

Immunostaining for Macrophages
To evaluate the effect of fasudil on macrophage accumulation, the specimens obtained from each treatment group were also immunostained with anti-rat ED-1 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif). After incubation with the biotinylated, affinity-purified secondary antibody and avidin-biotin amplification, the immunostained regions containing phosphorylated ERM family proteins were visualized by ECL Western blotting (Santa Cruz Biotechnology).11

Organ Chamber Experiments
Animals were anesthetized with pentobarbital and exsanguinated. The thoracic aortas were carefully dissected and cleaned off any perivascular tissue under microscope in physiological salt solution (PSS) of the following composition (in mmol/L): NaCl 121, KCl 4.7, NaHCO3 24.7, MgSO4 12.2, CaCl2 2.5, KH2PO4 1.2, and glucose 5.8, aerated with 95% O2 and 5% CO2. In some experiments, the endothelium was denuded using gentle rubbing of the luminal surface with an appropriate silk. The rings from each thoracic aorta (~5 mm in length) were mounted vertically between two hooks in organ chamber myographs (Medical Supply Co), which were filled with PSS and kept at 37°C. Isometric tension was measured with force transducers (Nihon Kohden Co).15 Each preparation was stretched in a stepwise manner to an optimal length, at which point the force induced by 118 mmol/L KCl became maximal and constant. After equilibration for at least 30 minutes, the rings were then precontracted with prostaglandin F2α (3 to 10 μmol/L). After a stable contraction was achieved, the rings were exposed to acetycholine (ACH, 10−10 to 10−3 mol/L) with or without L-NNA (100 μmol/L) to evaluate endothelial vasodilator function. Acute effect of indomethacin (10 μmol/L) was also examined to evaluate the possible contribution of cyclooxygenase-derived endothelium-derived contracting factors. Endothelium-independent relaxations to sodium nitroprusside (SNP, 10−11 to 10−8 mol/L) were examined in rings without endothelium.

Measurement of Vascular Superoxide Anion Production
Superoxide anion production was measured using lucigenin chemiluminescence as previously described.1 Briefly, the thoracic aortas were carefully dissected and cleaned off any perivascular tissue and blood contaminants under microscope in PSS and then placed in HEPES-buffered PSS. In a preliminary study, we confirmed no adhesion of inflammatory cells to the endothelium (data not shown). Scintillation vials containing 1 mL HEPES-buffered PSS with 5 μmol/L lucigenin (bis-N-methylacridinium nitrate) were placed into a scintillation counter (Lucinescence Reader BRL 301, ALOKA). We used Tiron (10 μmol/L), a superoxide scavenger, in all experiments to confirm the validity of our technique with lucigenin.1 After dark adaptation, background counts were recorded for 3 minutes and then three vascular segments (~5 mm in length) from each thoracic aorta were added to the vial. Scintillation counts were then recorded every 1 minute for 10 minutes and the respective background counts were subtracted. The vessels were then dried for determination of dry weight. Lucigenin counts were expressed as counts per minute per milligram of dry weight. The measurement was also performed in the presence of the NAD(P)H oxidase inhibitor, apocynin (100 μmol/L), which inhibits the activity of assembly of the components of NAD(P)H oxidase.16,17

Real-Time Polymerase Chain Reaction
Total RNA was isolated from rapidly frozen thoracic aortas and reverse transcribed. Quantification of NAD(P)H oxidase components, including nox1, nox4, gp91phox, and p22phox, was performed by amplification of cDNA using an ABI Prism 7000 real-time thermocycler. Message copy numbers were obtained from standard curves generated from genuine rat nox1, nox4, gp91phox, and p22phox template.18

Drugs
Angiotensin II, prostaglandin F2α, ACh, L-NNA, indomethacin, HEPES, lucigenin, Tiron, and apocynin were purchased from Sigma Chemical. Fasudil was provided by Asahi Kasei Co (Tokyo, Japan).
Statistical Analysis
Results are expressed as mean±SEM. Throughout the text, n represents the number of animals tested. A 2-way ANOVA was performed to evaluate the global statistical significance, and, if a significant F value was found, Bonferroni’s post hoc test was performed to identify the difference among the groups. Differences in a single parameter among groups were evaluated using one-way ANOVA followed by Fisher’s test for multiple comparisons. P<0.05 was considered to be statistically significant.

Results
Angiotensin II–Induced Hypertension
Angiotensin II infusion for 4 weeks significantly increased systolic blood pressure from 141±6 to 188±10 mm Hg in control rats (n=9, P<0.01). The long-term oral treatment with fasudil did not significantly suppress the angiotensin II–induced hypertension either at the low dose (195±8 mm Hg, n=11) or the high dose (195±10 mm Hg, n=11).

Angiotensin II–Induced Coronary Vascular Hypertrophy
The infusion of angiotensin II caused a significant increase in medial thickness (as evaluated by wall to lumen ratio) and perivascular fibrosis in the coronary arteries compared with sham-operated control animals (Figure 1). The concomitant treatment with fasudil significantly suppressed the angiotensin II–induced coronary vascular hypertrophy and perivascular fibrosis at both doses (Figure 1).

Angiotensin II–Induced Cardiac Hypertrophy
Angiotensin II caused a significant increase in LV weight and cardiomyocyte hypertrophy compared with control
rats (Figure 2). The simultaneous treatment with fasudil significantly suppressed the angiotensin II–induced LV hypertrophy at the high dose and also tended to do so at the low dose (Figure 2). The angiotensin II–induced cardiomyocyte hypertrophy also was significantly suppressed by fasudil at both doses (Figure 2).

**Angiotensin II–Induced Accumulation of Macrophages**

Angiotensin II caused a significant increase in perivascular accumulation of ED-1–positive macrophages (Figure 3). Fasudil dose-dependently and significantly suppressed the angiotensin II–induced accumulation of macrophages (Figure 3).

**Rho-Kinase Activation by Angiotensin II**

Western blot analysis demonstrated that the extent of phosphorylation of the ERM family proteins was significantly increased in angiotensin II–infused rats compared with control rats (Figure 4). The angiotensin II–induced activation of Rho-kinase was significantly inhibited to the basal levels by fasudil (Figure 4). By contrast, treatment with fasudil alone had no effect on Rho-kinase activity in control rats (Figure 4).
Angiotensin II–Induced Endothelial Dysfunction

Endothelium-dependent relaxation to ACh of the isolated aortas from angiotensin II–infused rats was significantly impaired compared with that from controls (Figure 5A). The impaired endothelium-dependent relaxation was not improved by acute administration of indomethacin (10 μmol/L), ruling out an involvement of cyclooxygenase-derived endothelium-derived contracting factors. The concomitant treatment with fasudil significantly ameliorated the angiotensin II–induced endothelial dysfunction (Figure 5A). The fasudil-mediated improvement of endothelial vasodilator function was abolished by L-NNA (100 μmol/L), indicating that the beneficial effect of fasudil on endothelial function is totally mediated by NO-dependent mechanism (Figure 5A). By contrast, endothelium-independent relaxation to SNP was comparable among the groups (Figure 5B). Treatment with fasudil alone had no effect on either endothelium-dependent or -independent relaxation in control rats (Figure 5).

Angiotensin II–Induced Superoxide Production

A mild amount of superoxide production was noted in control animals (Figure 6). Angiotensin II infusion for 1 week markedly increased vascular superoxide production (Figure 6). Endothelial denudation normalized superoxide production in angiotensin II–infused rats with a remaining production of superoxide anions in VSMCs (Figure 6). The amount of superoxide production in VSMCs tended to be higher in angiotensin II–infused rats than in control rats although statistically not significant (Figure 6). An NAD(P)H oxidase inhibitor, apocynin, markedly inhibited endothelial superoxide production in angiotensin II–infused animals (Figure 6). Importantly, the concomitant oral treatment with fasudil significantly suppressed the angiotensin II–induced endothelial production of superoxide anions (Figure 6). Treatment with fasudil alone had no effect on vascular superoxide production in control rats (Figure 6).

Angiotensin II–Induced Upregulation of Vascular NAD(P)H Oxidase

In the thoracic aortas of angiotensin II–infused rats, mRNA expressions of nox1, nox4, gp91phox, and p22phox were all significantly increased compared with control animals (Figure 7). The concomitant treatment with fasudil significantly suppressed all of these upregulations (Figure 7). Treatment with fasudil alone had no effect on NAD(P)H oxidase subunit mRNA expressions in control animals (Figure 7).

Discussion

The novel findings of this study are that Rho-kinase is substantially involved in angiotensin II–induced cardiovascular hypertrophy in vivo and that the mechanisms include enhanced oxidative stress associated with upregulation of endothelial NAD(P)H oxidase and resultant endothelial dysfunction, in which Rho-kinase plays a key role. To the best of our knowledge, this is the first study that demonstrates the involvement of Rho-kinase in the pathogenesis of angiotensin II–induced cardiovascular hypertrophy in vivo.

Cardiovascular Hypertrophy in a Rat Model With Angiotensin II Infusion

In the present study, to examine the involvement of Rho-kinase in the in vivo effect of angiotensin II, we used a well-established rat model with angiotensin II infusion that is characterized by cardiovascular hypertrophy.\textsuperscript{2,19,20} The mechanisms that have been proposed for the in vivo effects of angiotensin II include NAD(P)H oxidase activation and resultant endothelial dysfunction, in which Rho-kinase plays a key role. To the best of our knowledge, this is the first study that demonstrates the involvement of Rho-kinase in the pathogenesis of angiotensin II–induced cardiovascular hypertrophy in vivo.

Figure 3. Inhibitory effect of long-term treatment with fasudil on angiotensin II–induced perivascular accumulation of macrophages in rats. Results are expressed as mean±SEM.

Figure 4. Inhibitory effect of long-term treatment with fasudil on angiotensin II–induced Rho-kinase activation in the left ventricle. Results are expressed as mean±SEM.
Involvement of Rho-Kinase in the Signal Transduction of Angiotensin II

Accumulating evidence has indicated that Rho-kinase is substantially involved in the signal transduction initiated by various vasoactive factors, including angiotensin II, serotonin, thrombin, endothelin-1, norepinephrine, and platelet-derived growth factor. We have demonstrated that Rho-kinase plays an important role in angiotensin II-induced mRNA expression of monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 in cultured rat aortic VSMCs. We also have recently demonstrated that the expression of Rho-kinase itself is enhanced by inflammatory stimuli, such as angiotensin II and interleukin-1β. Protein kinase C and nuclear factor-κB seem to be involved in the expression of Rho-kinase.

Involvement of Rho-Kinase in Angiotensin II–Induced Cardiovascular Hypertrophy In Vivo

In the present study, the long-term oral treatment with fasudil markedly suppressed the development of angiotensin II–induced cardiovascular hypertrophy, indicating an involvement of Rho-kinase in the process in vivo. The cardiovascular hypertrophy caused by angiotensin II infusion may not be the consequence of elevated blood pressure because the process was significantly inhibited by fasudil at its nonhypotensive doses. Because Rho-kinase is involved in various cellular functions, the inhibitory effect of long-term blockade of Rho-kinase on the cardiovascular hypertrophy seems to be mediated by multiple mechanisms. It is highly possible that long-term treatment with fasudil inhibits some crucial steps of angiotensin II–induced cardiovascular hypertrophy, such as proliferation and migration of VSMCs and cardiomyocytes and recruitment of inflammatory cells into cardiovascular tissues. We consider that endothelial dysfunction caused by NAD(P)H oxidase activation is substantially involved in the pathogenesis of cardiovascular hypertrophy and that the amelioration of NAD(P)H oxidase activity by fasudil mainly accounts for its inhibitory effect on cardiovascular hypertrophy in the present in vivo study.

Involvement of Rho-Kinase in Angiotensin II–Induced NAD(P)H Oxidase Upregulation In Vivo

In the present study, endothelial denudation almost normalized the increased superoxide production. Because we cleaned off perivascular tissue, another possible source of superoxide anions, and because we confirmed no adhesion of inflammatory cells to the endothelium, we consider that the main source of superoxide production is the endothelium with a small contribution of VSMCs.

Figure 5. Long-term treatment with fasudil ameliorates angiotensin II–induced endothelial dysfunction. A, Endothelium-dependent relaxation to ACh was significantly impaired in angiotensin II–infused rats, normalized by the fasudil treatment but not restored in the presence of indomethacin (10 μmol/L). The beneficial effect of fasudil was abolished by L-NNA. The fasudil treatment alone had no effect on the relaxation to ACh in control rats. B, Endothelium-independent relaxation to SNP was comparable in control and angiotensin II–infused rats with or without the fasudil treatment. The fasudil treatment alone had no effect on the relaxation to SNP in control rats. Results are expressed as mean ± SEM.

Figure 6. Long-term treatment with fasudil suppresses angiotensin II–induced endothelial production of superoxide anions. Angiotensin II–induced increase in the superoxide production was acutely and significantly attenuated in the presence of apocynin (100 μmol/L). The fasudil treatment also normalized the superoxide production, whereas the treatment alone had no effect on vascular superoxide production. Results are expressed as mean ± SEM.
In the vasculature, the most important enzyme responsible for superoxide production is NAD(P)H oxidase. Angiotensin II induces complex formation and translocation of cytosolic NAD(P)H oxidase subunits (p47phox, p67phox, and p40phox), which associate with membrane-bound subunits (p22phox and gp91phox), resulting in activation of NAD(P)H oxidase and generation of superoxide anions. Increased endothelial production of superoxide anions in angiotensin II-infused rats was normalized by apocynin, indicating an increased activity of endothelial NAD(P)H oxidase in angiotensin II-infused animals. We consider that the increased endothelial production of superoxide anions is caused primarily by angiotensin II-induced upregulation of NAD(P)H oxidase, because it has been demonstrated that angiotensin II-induced NAD(P)H oxidase activation is closely coupled to the increased expression of the enzyme in rats.

NAD(P)H oxidase was originally found in neutrophils and is composed of five subunits, p40phox, p47phox, p67phox, p22phox, and gp91phox (nox1 and nox4). Although all subunits of neutrophil NAD(P)H oxidase are found in endothelial cells and adventitial fibroblasts, gp91phox is barely detectable in rat aortic VSMCs whereas homologues of gp91phox (nox1 and nox4) are expressed in those cells. It was shown in VSMCs in vitro that nox4 is expressed in higher amounts than nox1 and that expressions of both of them are elevated by angiotensin II. This suggests that nox4 may be the predominant vascular nox isoform, whereas nox1 may also produce superoxide anions when stimulated by angiotensin II. In this study, we also observed that nox4 was expressed in higher amounts than nox1 and that the expression of both nox isoforms was increased by angiotensin II infusion for 1 week in the rat aorta in vivo. This implies that there are distinct nox isoforms in VSMCs with different substrate specificities and different modes of regulation.

In the present study, long-term concomitant treatment with fasudil markedly suppressed angiotensin II-induced upregulation of all NAD(P)H oxidase subunits. The inhibitory effect of fasudil indicates that Rho-kinase is substantially involved in angiotensin II-induced upregulation of endothelial NAD(P)H oxidase in vivo.

Involvement of Rho-Kinase in Angiotensin II-Induced Endothelial Dysfunction

Clinical and experimental studies have demonstrated that endothelial dysfunction is an important early step in atherosclerosis. Endothelial dysfunction with long-term angiotensin II treatment is caused primarily by increased NAD(P)H oxidase–mediated vascular superoxide production. The present results suggest that Rho-kinase may play an important role in angiotensin II–induced endothelial dysfunction through upregulation of endothelial NAD(P)H oxidase and subsequent increase in endothelial superoxide production. It has previously been demonstrated that inhibition of Rho increases endothelial NO synthase expression. We also have recently demonstrated that in VSMCs, inhibition of Rho-kinase by hydroxyfasudil ameliorates hypoxia-induced down-regulation of endothelial NO synthase. Indeed, in the present study, the improved endothelium-dependent relaxation by the long-term treatment with fasudil was mediated by NO-dependent mechanism. Taken together, Rho-kinase seems to be substantially involved in the angiotensin II–induced endothelial dysfunction in vivo.

Limitations of the Study

Several limitations should be mentioned for the present study. First, a dose-dependent effect of fasudil was noted for LV hypertrophy (Figure 2B) and macrophage infiltration (Figure 3) but not for other parameters. The present doses of fasudil (30 and 100 mg/kg per day) were selected based on our
previous study. We have recently checked the serum concentrations of fasudil achieved by these two doses in rats. The area under the plasma-concentration curve of hydroxyfasudil (AUC$_{0-24}$, ng·h·mL$^{-1}$) in rats that received fasudil in drinking water was 627 and 1450 for the low-dose (30 mg/kg per day) and the high-dose (100 mg/kg per day) groups, respectively (n=4 each). Indeed, this AUC$_{0-24}$ was within the clinical therapeutic range of the specific Rho-kinase inhibitor in humans (unpublished data, 2003). Therefore, it is conceivable that a low dose (30 mg/kg per day) of fasudil was enough to suppress most of the angiotensin II–induced cardiovascular hypertrophic changes. Second, in the present study, fasudil had no effect on angiotensin II–induced hypertension. We previously demonstrated that acute oral administration of fasudil (30 mg/kg) selectively lowered blood pressure in SHR but not in WKY rats in vivo. In SHR, long-term treatment with fasudil did not lower blood pressure at the low dose (30 mg/kg per day), whereas the high dose of fasudil (100 mg/kg per day) significantly lowered blood pressure. In this study, rats received fasudil in drinking water. Because rats drink water mainly at the night, it is possible that blood pressure is decreased at night. This point remains to be examined in a future study by using telemetry monitoring system of blood pressure under conscious conditions. Third, we did not measure NAD(P)H oxidase activity in the present study. Finally, although we demonstrated the inhibitory effect of fasudil on the endothelial NAD(P)H oxidase expression and superoxide production by the endothelium, this is the correlative nature of the observation rather than the definitive cause and effect relationship between Rho-kinase function and NAD(P)H oxidase regulation in angiotensin II–induced cardiovascular hypertrophy. This point also remains to be clarified in a future study.

Clinical Implications

Accumulating evidence indicates that angiotensin II is substantially involved in the pathogenesis of a wide spectrum of cardiovascular diseases. The present study demonstrates that Rho-kinase plays an important role in the in vivo effects of angiotensin II. Additional studies are required to elucidate the role of Rho-kinase in the pathogenesis of cardiovascular diseases and the clinical usefulness of Rho-kinase inhibitors.

Acknowledgments

This study was supported in part by grants-in-aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan (Nos. 12032215, 12470158, 12877114, 13307024, and 13557068) and by the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan. The authors thank Professor S. Mohri at the Center of Biomedical Research, Kyusyu University Graduate School of Medical Sciences, for cooperation in this study, M. Sonoda and I. Kunihiro for excellent technical assistance, and Asahi Kasei Co (Tokyo, Japan) for providing fasudil.

References


Long-Term Inhibition of Rho-Kinase Suppresses Angiotensin II–Induced Cardiovascular Hypertrophy in Rats In Vivo: Effect on Endothelial NAD(P)H Oxidase System

Midoriko Higashi, Hiroaki Shimokawa, Tsuyoshi Hattori, Junko Hiroki, Yasushi Mukai, Keiko Morikawa, Toshihiro Ichiki, Shosuke Takahashi and Akira Takeshita

_Circ Res._ 2003;93:767-775; originally published online September 18, 2003;
doi: 10.1161/01.RES.0000096650.91688.28

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/93/8/767

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org//subscriptions/