Endothelial dysfunction and hypercontractility of vascular myocytes are ameliorated by fluvastatin in obese Zucker rats

Hiroaki Nishimatsu,1 Etsu Suzuki,2 Hiroshi Satonaka,2 Ryo Takeda,2 Masao Omata,2 Toshiro Fujita,2 Ryozo Nagai,2 Tadaichi Kitamura,1 and Yasunobu Hirata2

1Departments of Urology and 2Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Tokyo, Japan

Submitted 27 July 2004; accepted in final form 13 November 2004

Nishimatsu, Hiroaki, Etsu Suzuki, Hiroshi Satonaka, Ryo Takeda, Masao Omata, Toshiro Fujita, Ryozo Nagai, Tadaichi Kitamura, and Yasunobu Hirata. Endothelial dysfunction and hypercontractility of vascular myocytes are ameliorated by fluvastatin in obese Zucker rats. Am J Physiol Heart Circ Physiol 288: H1770–H1776, 2005. First published November 18, 2004; doi:10.1152/ajpheart.00751.2004.—To study the mechanisms of vascular dysfunction in diabetes mellitus, we examined the responses of the aorta to adrenomedullin (AM) and ANG II in obese Zucker (OZ), lean Zucker (LZ), and OZ rats administered fluvastatin (OZ + Flu). AM-induced endothelium-dependent vasorelaxation was impaired in OZ rats compared with LZ rats, and fluvastatin restored AM-induced, endothelium-dependent vasorelaxation (%Δtension at 10−7 mol/l AM; OZ, −85.1 ± 3.1%; LZ, −50.7 ± 2.5%; OZ + Flu, −75.6 ± 2.7%). Expression of endothelial nitric oxide synthase (eNOS) and Akt phosphorylation in response to AM (10−7 mol/l) were also diminished in OZ rats. Fluvastatin restored the eNOS expression and Akt phosphorylation (relative intensity): LZ, 2.3 ± 0.4; OZ, 1.0 ± 0.2; OZ + Flu, 1.8 ± 0.3. Akt phosphorylation (relative intensity): LZ, 2.3 ± 0.2; OZ, 1.0 ± 0.3; OZ + Flu, 1.9 ± 0.2. ANG II-induced vasoconstriction was enhanced in the aortic rings of OZ rats compared with LZ rats, and this enhanced vasoconstriction was partially normalized by fluvastatin and was abolished when the aorta of OZ rats was preincubated with the Rho kinase inhibitor Y-27632. GTPyS-induced contraction of permeabilized aortic smooth muscle cells, which is an indicator of the Rho-dependent Ca2+ sensitization of contraction, was enhanced in OZ rats compared with LZ rats, and this enhanced contraction was suppressed in OZ + Flu rats. These results suggested that endothelium-dependent vasorelaxation was impaired, Ca2+ sensitization of contraction was augmented in blood vessels of OZ rats and that fluvastatin restored vascular function by activating the Akt-dependent pathway and inhibiting the Rho-dependent pathway.

nitric oxide; Akt; Rho

IT IS WELL KNOWN that diabetes mellitus (DM) is a major risk factor for vascular diseases such as atherosclerosis and restenosis after angioplasty. Most diabetic patients have type II DM, which is characterized by obesity and insulin resistance. It has been shown that DM is often associated with vascular dysfunction. Endothelium-dependent vasorelaxation in response to acetylcholine (ACh) was impaired in obese Zucker (OZ) rats, an animal model of insulin resistance (35). Neointimal formation after balloon injury of the carotid artery was enhanced in OZ rats compared with the control lean Zucker (LZ) rats (20, 21). Furthermore, it has been demonstrated that neointimal proliferation in coronary arteries after stent implantation was accelerated in diabetic patients (11). However, the mechanisms by which DM is often associated with vascular dysfunction are not clearly understood.

Accumulated evidence suggests that the renin-angiotensin system (RAS) is implicated not only in the control of blood pressure but also in the pathogenesis of atherosclerosis (1, 30). It has also been reported that ANG II inhibits insulin signaling and induces insulin resistance (3, 33). Furthermore, blockade of RAS reportedly improved vascular function and insulin resistance in diabetic animals (8, 9), suggesting pivotal roles of RAS in the development of vascular dysfunction and insulin resistance in a diabetic state. Adrenomedullin (AM) is a novel peptide that has a potent vasorelaxant activity (10). Recently, with the use of AM gene knockout mice, it has been clarified that endogenous AM is involved in blood pressure control, because AM+− mice show significantly higher blood pressure than wild-type mice (25). We have shown that AM induces vasorelaxation, at least partly, in an endothelium-dependent manner and that AM-induced endothelium-dependent vasorelaxation is mediated by the phosphatidylinositol-3-kinase (PI3K)/Akt-dependent pathway (18). However, little is known as to what kinds of alterations in response to ANG II and AM occur in blood vessels of diabetic animals.

Recently, it has been demonstrated that 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) have a potent antiatherogenic effect, which appears to be, at least in part, independent of their effects on serum lipids (32). Two intracellular pathways through which statins exert beneficial effects on blood vessels have been identified. One is the activation of the protein kinase Akt. Akt is located downstream of PI3K; it phosphorylates and activates endothelial nitric oxide (NO) synthase (eNOS), which in turn induces NO production and vasorelaxation (2, 5). Statins reportedly activate Akt and eNOS (12). The other is the inhibition of the small GTP-binding protein Rho. Statins suppress Rho activation by inhibiting the attachment of geranylgeranylated Ras to Rho, which is an important posttranslational modification and is required for the membrane translocation and activation of Rho (13, 14). Rho has been shown to be implicated in the proliferation of vascular smooth muscle cells (VSMCs) (23, 24) and the suppression of eNOS expression in vascular endothelial cells (13). Furthermore, Rho activates Rho kinase, which in turn phosphorylates and inactivates myosin light chain phosphatase (MLCP) (6). Because MLCP dephosphorylates myosin light chain and induces relaxation of VSMCs, inactivation of MLCP results in vasoconstriction.

Address for reprint requests and other correspondence: E. Suzuki, Division of Nephrology and Endocrinology, #202, The Dept. of Internal Medicine, Faculty of Medicine, Univ. of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 Japan (E-mail: suzuki-2im@h.u-tokyo.ac.jp)
Thus activation of Rho increases the sensitivity of VSMCs contraction to a given intracellular Ca\(^{2+}\) concentration. This phenomenon is called Ca\(^{2+}\) sensitization of contraction. It is therefore expected that statins ameliorate vascular dysfunction observed in the state of DM via the activation of Akt and suppression of Rho.

This study was undertaken to examine whether AM-induced endothelium-dependent vasorelaxation and ANG II-induced vasosconstriction are altered in OZ rats, an animal model of DM; and if so, what are the mechanisms by which the alterations of the responses to these vasoactive substances occur in blood vessels of OZ rats. We especially examined the role of Akt and Rho in the alterations of vascular responses to these vasoactive substances. We also examined whether the HMG-CoA reductase inhibitor fluvastatin would improve vascular function in OZ rats.

**MATERIALS AND METHODS**

Reagents. Phospho-specific anti-Akt antibody that recognizes catalytically active Akt was obtained from New England BioLabs (Beverly, MA). Anti-Akt and anti-eNOS antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). GTP\_\_S, A-23187, \(\beta\)-escin, LY-294002, and calmodulin were purchased from Sigma (St. Louis, MO), and Y-27632 was purchased from Calbiochem-Novabiochem (San Diego, CA). Fluvastatin was kindly supplied by Tanabe Seiyaku (Tokyo, Japan).

**Ex vivo experiments.** All experiments conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). OZ and LZ rats were fed on standard chow. Some OZ rats were fed on standard chow supplemented with fluvastatin (5 mg/kg) for 4 wk before the experiments. Sixteen-week-old OZ and LZ rats were used for the experiments. The effects of AM on the tension of rat aortic rings were examined as previously described (18). The effects of ANG II on the contraction of aortic rings were examined basically in OZ rats. The effects of AM on the tension of rat aortic rings before protein extraction. Serum total cholesterol, triglycerides, and sugar were measured by the tail-cuff method. The body weight was significantly higher in 16-wk-old OZ rats than that in age-matched LZ rats. Although systolic blood pressure measured by the tail-cuff method tended to increase in OZ rats, no significant difference was observed between OZ and LZ rats. Serum total cholesterol, triglycerides, and sugar were significantly higher in OZ rats than those in LZ rats. Treatment with fluvastatin did not significantly affect these parameters except for blood sugar, which was significantly lower in OZ rats administered fluvastatin (OZ + Flut) than in OZ rats (Table 1).

**AM-induced, endothelium-dependent vasorelaxation is impaired in OZ rats.** To assess endothelial function in OZ rats, we examined endothelium-dependent vasorelaxation in response to ACh and AM in OZ rats and LZ rats. ACh-induced endothelium-dependent vasorelaxation was significantly diminished in aortic rings with intact endothelium (E+) prepared from OZ rats compared with that observed in E+ aortic rings prepared from LZ rats (Fig. 1A, left). Fluvastatin did not affect ACh-induced, endothelium-dependent vasorelaxation in LZ rats (data not shown). However, when fluvastatin was administered to OZ rats, ACh-induced endothelium-dependent vasorelaxation of E+ aortic rings was restored to almost the same level as that observed in E+ aortic rings prepared from LZ rats. When endothelium was denuded (E−), ACh did not induce vasorelaxation of aortic rings prepared from these three groups.

**Preparation of protein extracts and Western blot analysis.** Protein extracts were prepared from rat aortas as previously described (18). Western blot analysis was performed as previously described (28). Fifty micrograms of each protein extract were subjected to Western blot analysis. Primary antibodies were used at a dilution of 1:100 except for anti-phospho-Akt antibody, which was used at a dilution of 1:500.

**Northern blot analysis.** Northern blot analysis was performed as previously described (17). The cDNA probe for the detection of ANG II type I receptor was prepared by reverse transcription (RT)-polymerase chain reaction (PCR). Total RNA extracted from Wistar rat aorta was subjected to RT using a random primer. The cDNAs were used for subsequent PCR. The primers used for PCR were as follows: sense primer, 5'-GGATCCATCCTTTAATCATGTAGAAGA-3'; antisense primer, 5'-CTCGAGGTTAGATGACGGGTGGGCAAA-3'.

**Measurement of cGMP and cAMP production.** Measurement of cGMP and cAMP production in the rat aortas was performed as previously described (18).

**Statistical analyses.** Values are means ± SE. The statistical analyses were performed using analysis of variance followed by the Student-Newman-Keuls test. Differences with a P value of <0.05 were considered statistically significant.

**RESULTS**

**Physical and metabolic features of OZ and LZ rats.** Body weight was significantly higher in 16-wk-old OZ rats than that in age-matched LZ rats. Although systolic blood pressure measured by the tail-cuff method tended to increase in OZ rats, no significant difference was observed between OZ and LZ rats. Serum total cholesterol, triglycerides, and sugar were significantly higher in OZ rats than those in LZ rats. Treatment with fluvastatin did not significantly affect these parameters except for blood sugar, which was significantly lower in OZ rats administered fluvastatin (OZ + Flut) than in OZ rats (Table 1).

Table 1. Physical and metabolic characteristics of the three groups of rats

<table>
<thead>
<tr>
<th></th>
<th>LZ</th>
<th>OZ</th>
<th>OZ + Flut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>292.7±2.5</td>
<td>416.6±6.1†</td>
<td>402.9±4.2†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>141.5±4.0</td>
<td>152.1±3.5</td>
<td>150.3±2.8</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>54.4±2.0</td>
<td>104.8±6.1†</td>
<td>103.5±2.4†</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>30.1±2.8</td>
<td>190.6±18.2‡</td>
<td>185.3±19.2‡</td>
</tr>
<tr>
<td>Blood sugar, mg/dl</td>
<td>106.7±3.2</td>
<td>220.1±5.8†</td>
<td>189.2±4.1†</td>
</tr>
</tbody>
</table>

The parameters of 16-wk-old rats were compared. Values are means ± SE. LZ, lean Zucker; OZ, obese Zucker; OZ + Flut, obese Zucker + fluvastatin. *P < 0.05 vs. OZ rats (n = 10); †P < 0.0001 vs. LZ rats.
We next examined AM-induced, endothelium-dependent vasorelaxation (Fig. 1B). AM-induced vasorelaxation of E– aortic rings did not differ significantly among LZ, OZ, and OZ + Flu rats. Furthermore, LY did not affect AM-induced vasorelaxation of E– aortic rings in the three groups. These results suggested that fluvastatin improved AM-induced endothelium-dependent vasorelaxation via the PI3K-dependent pathway.

**AM-induced Akt phosphorylation and eNOS expression are impaired in OZ rats.** We have previously shown that AM induces endothelium-dependent vasorelaxation via the PI3K/Akt-dependent and NO/cGMP-dependent pathways in rat aortas (18). Thus we examined Akt phosphorylation in the aortas of OZ and LZ rats, which were stimulated with AM. Because AM-induced Akt phosphorylation peaked around 15 min post-stimulation (data not shown), the aortas were stimulated with 10⁻⁷ M AM for 15 min in this experiment. AM-induced Akt phosphorylation was significantly reduced in the aortas from OZ rats (Fig. 2A). Fluvastatin restored AM-induced Akt phosphorylation in OZ rats.

**AM-induced eNOS expression was also diminished in OZ rats.** We have previously shown that AM induces endothelium-dependent vasorelaxation via the PI3K/Akt-dependent and NO/cGMP-dependent pathways in rat aortas (18). Hence we examined eNOS expression in the aortas of OZ and LZ rats, which were stimulated with AM. Because eNOS protein expression peaked around 15 min post-stimulation (data not shown), the aortas were stimulated with 10⁻⁷ M AM for 15 min in this experiment. AM-induced eNOS expression was significantly reduced in the aortas from OZ rats (Fig. 2B). Fluvastatin restored AM-induced eNOS expression in OZ rats.

**Statins improve vascular function in Zucker rats.** By increasing Akt phosphorylation and eNOS expression, fluvastatin improves endothelium-dependent vasorelaxation in OZ rats.

---

**Fig. 1.** A: impairment of ACh-induced, endothelium-dependent vasorelaxation and its amelioration by fluvastatin. Aortic rings were prepared from lean Zucker (LZ) rats, obese Zucker (OZ) rats, and OZ rats administered fluvastatin (OZ + Flu). Aortic rings with (E+) or without (E–) endothelium were precontracted with norepinephrine and incubated with the indicated concentrations of ACh to measure endothelium-dependent vasorelaxation. *P < 0.05 vs. LZ rats (n = 7). B: impairment of adrenomedullin (AM)-induced, endothelium-dependent vasorelaxation and its amelioration by fluvastatin. Experiments were performed in the same way as in A except that AM was used instead of ACh to induce endothelium-dependent vasorelaxation. In some experiments, aortic rings were pretreated with 20 μmol/l LY-294002 (LY). *P < 0.05 vs. LZ rats without LY pretreatment (n = 7).

**Fig. 2.** A: impairment of AM-induced Akt phosphorylation and its restoration by fluvastatin in aortas of OZ rats. Rat thoracic aortas from LZ, OZ, and OZ + Flu rats were incubated with 10⁻⁷ mol/l AM for 15 min. Fifty micrograms of each protein extract was immunoblotted with a phospho-specific anti-Akt antibody (*pAkt), which recognizes catalytically active Akt, or anti-Akt antibody (Total Akt), which recognizes total Akt1/2, regardless of whether Akt is phosphorylated or not. The relative intensity of each band is shown in the lower histogram. *P < 0.05 vs. LZ rats (n = 5). B: suppression of endothelial nitric oxide synthase (eNOS) expression and its restoration by fluvastatin in aortas of OZ rats. Protein extracts from the rat thoracic aortas were subjected to Western blot analysis. The relative intensity of each band is shown in the lower histogram. *P < 0.05 vs. LZ rats (n = 5).
OZ rats compared with that in aortas from LZ rats (Fig. 2A). AM-induced Akt phosphorylation was restored when OZ rats were treated with fluvastatin. We next measured eNOS expression in the aortas of LZ, OZ, and OZ + Flu rats. Its expression was significantly reduced in OZ rats compared with LZ rats, and fluvastatin restored its expression (Fig. 2B).

We also measured cGMP production in the aortas. cGMP production in both the nonstimulated basal condition and AM-stimulated condition was significantly reduced in the aortas of OZ rats compared with that in the aortas of LZ rats (Fig. 3A). Fluvastatin significantly restored cGMP production under both basal and AM-stimulated conditions. In contrast cAMP production did not significantly differ among the three groups in both the basal and AM-stimulated states (Fig. 3B).

**ANG II-induced vasoconstriction is enhanced in aortas of OZ rats.** We next examined whether the contraction of VSMCs was changed in the aortas of OZ rats. We used ANG II as an agonist to induce vasoconstriction. ANG II-induced vasoconstriction was significantly augmented in E− aortic rings prepared from OZ rats compared with that in E− aortic rings from LZ rats (Fig. 4A). In OZ rats administered fluvastatin, the enhanced vasoconstriction was partially but significantly reduced. When the aortic rings were pretreated with the Rho kinase inhibitor Y-27632, ANG II-induced vasoconstriction was partially but significantly reduced. When the aortic rings were permeabilized with β-escin, ANG II-induced vasoconstriction was significantly reduced, and the difference in the extent of ANG II-induced vasoconstriction observed among the three groups disappeared. To study whether the expression level of the type I receptor for ANG II differs between LZ and OZ rats, we examined its expression by Northern blot analysis. As shown in Fig. 4B, the expression of ANG II type I receptor did not differ remarkably between OZ and LZ rats regardless of the age of the rats examined, suggesting that the hypercontractility of vascular myocytes in response to ANG II observed in OZ rats might be mediated by pathways located downstream of ANG II receptors and that the Rho-dependent pathway might be implicated in the hypercontractility of vascular myocytes in OZ rats.

Enhanced Ca²⁺ sensitization of contraction in aortas of OZ rats is ameliorated by fluvastatin. Because it is known that statins inhibit the activation of Rho and that Rho is implicated in Ca²⁺ sensitization of contraction (6, 13, 14), we hypothesized that Ca²⁺ sensitization of contraction would be enhanced in blood vessels of OZ rats. To test this hypothesis, we prepared skinned fibers of aortic smooth muscle cells and examined the responses of the skinned fibers to Ca²⁺ and GTPyS, which stimulates Rho-dependent Ca²⁺ sensitization of contraction (7). Increases in Ca²⁺ concentration induced contraction in a dose-dependent manner (Fig. 5A). Ca²⁺-dependent contraction reached a maximal level when pCa (−log [Ca²⁺]) was adjusted to 4.5. Ca²⁺-dependent contraction was not remarkably inhibited by Y-27632 (data not shown), suggesting that the Rho kinase-dependent Ca²⁺ sensitization of contraction did not contribute to the Ca²⁺-dependent contraction when β-escin was used to permeabilize VSMCs. Similar results have
been previously reported (22). Responses to Ca\textsuperscript{2+}/H11001 did not significantly differ among skinned fibers prepared from LZ, OZ, and OZ/H11001 Flu rats. GTP\textsuperscript{S} also induced contraction at pCa 6.0 in a dose-dependent fashion (Fig. 5B). GTP\textsuperscript{S}-induced contraction was inhibited approximately to 20% when 10 M Y-27632 was added (data not shown), suggesting that GTP\textsuperscript{S} promoted vasoconstriction in the skinned fibers via the Rho kinase-dependent pathway. GTP\textsuperscript{S}-induced contraction was significantly more potent in skinned fibers prepared from OZ rats than in those prepared from LZ rats. Fluvastatin significantly suppressed GTP\textsuperscript{S}-induced contraction. These results suggested that Ca\textsuperscript{2+} sensitization of contraction was enhanced in aortas of OZ rats and that fluvastatin suppressed the enhanced Ca\textsuperscript{2+} sensitization of contraction.

**DISCUSSION**

It is well known that endothelium-dependent vasorelaxation induced by ACh is impaired in animal models of type II DM (35). Because we have found that AM induces endothelium-dependent vasorelaxation (18), we examined whether AM-induced, endothelium-dependent vasorelaxation was impaired in OZ rats. We found that AM-induced, endothelium-dependent vasorelaxation, but not endothelium-independent vasorelaxation, was impaired in OZ rats compared with LZ rats. This impairment of endothelium-dependent vasorelaxation was associated with diminished AM-induced Akt phosphorylation and cGMP production. Interestingly, fluvastatin restored AM-induced endothelium-dependent vasorelaxation but not endothelium-independent vasorelaxation, and this improvement
was associated with the recovery of AM-induced Akt phosphorylation and cGMP production. Furthermore, pretreatment with LY abolished the difference in the extent of AM-induced endothelium-dependent vasorelaxation among LZ, OZ, and OZ + Flu rats. LY did not affect AM-induced endothelium-independent vasorelaxation. It has been reported that Akt-dependent phosphorylation of eNOS is necessary for a full activation of eNOS and endothelium-dependent vasorelaxation (2, 5). We have recently demonstrated that AM induces endothelium-dependent vasorelaxation via the PI3K/Akt-dependent pathway (18). Thus impairment of PI3K/Akt activation in response to AM may be implicated in the reduction of AM-induced, endothelium-dependent vasorelaxation in OZ rats. It has been reported that statins induce angiogenesis via activation of Akt and eNOS in vascular endothelial cells (12). Statins appear to stimulate membrane translocation of Akt and its activation (26). On the other hand, several reports showed that statins inhibited PI3K activation or Akt phosphorylation in response to growth factors such as insulin, platelet-derived growth factor, and ANG II, especially in cell types other than vascular endothelial cells (4, 15, 16, 36). Although the reason for this discrepancy remains unclear, it was also reported that statins did not activate Akt in vascular smooth muscle cells or cardiac myocytes (12). Furthermore, atorvastatin reportedly stimulated angiogenesis at low concentrations but inhibited angiogenesis at high concentrations (31). It seems that at low concentrations statins activate Akt and eNOS, at least in vascular endothelial cells. Thus our results suggested that fluvastatin improved AM-induced, endothelium-dependent vasorelaxation in OZ rats, at least in part, via its effect on Akt and eNOS. We also found that eNOS expression was diminished in the aortas of OZ rats compared with LZ rats, and fluvastatin restored eNOS expression. It has been reported that statins inhibit the Rho-dependent pathway via suppression of Rho geranylgeranylation, and stimulate eNOS expression (13). Our results were compatible with those findings. Thus it appears that both impairment of PI3K/Akt activation and diminished eNOS expression are implicated in the decreased endothelium-dependent vasorelaxation in OZ rats.

Although no changes were observed in the expression of the type I receptor for ANG II, aortas of OZ rats contracted in response to ANG II more potently than those of LZ rats, and this hypercontractility was partially reduced by fluvastatin. Furthermore, pretreatment of aortic rings with Y-27632 abolished the difference in the extent of contraction in response to ANG II. These results suggested that the Rho-dependent pathway was implicated in the hypercontractility of aortas from OZ rats. Because Rho is reportedly implicated in Ca\(^{2+}\) sensitization of contraction (7), we examined whether Ca\(^{2+}\) sensitization of contraction was changed in the aortas of OZ rats with the use of skinned fibers. GTP\(\gamma\)S induced vasoconstriction more potently in aortas of OZ rats compared with those of LZ rats, and this GTP\(\gamma\)S-induced vasoconstriction was ameliorated in OZ rats administered fluvastatin. Because statins reportedly inhibit geranylgeranylpyrophosphate anchoring on Rho (13), it is possible that fluvastatin inhibited Rho-induced Ca\(^{2+}\) sensitization by suppressing Rho geranylgeranylation.

In contrast, Ca\(^{2+}\)-dependent vasoconstriction did not significantly differ between OZ and LZ rats. Rho-dependent Ca\(^{2+}\) sensitization of contraction did not appear to contribute to Ca\(^{2+}\)-dependent vasoconstriction in our system because Ca\(^{2+}\)-dependent vasoconstriction was not inhibited by Y-27632. Thus it was suggested that the relaxant capacity rather than contractile capacity was changed in the aortas of OZ rats and that hypercontractility in response to ANG II might be partly due to the reduced relaxant capacity. However, it was reported that intracellular Ca\(^{2+}\) mobilization in response to phenylephrine via the voltage-dependent Ca\(^{2+}\) channel was increased in the aortas of OZ rats (19). It is, therefore, possible that the hypercontractility in response to ANG II might be due not only to an increased Ca\(^{2+}\) sensitization of contraction but also to an enhanced intracellular Ca\(^{2+}\) mobilization.

Systolic blood pressure of OZ rats used in this study was not significantly higher than that of LZ rats. Although there is a discrepancy in the previous reports about the blood pressure of OZ rats, relatively young OZ rats (<25 wk old) did not show higher blood pressure than their age-matched LZ rats in most studies (27, 34). Thus our results were compatible with previous reports.

Although we did not use a model of atherosclerosis in this study, downregulation of PI3K/Akt and activation of Rho potentially promote the development of atherosclerosis, because decrease of NO production induces the expression of monocyte chemoattractant protein-1 (29), and activation of Rho stimulates VSMCs proliferation (23, 24). Future studies will be required to examine the role of these pathways in the development of atherosclerosis.

In summary, AM-induced endothelium-dependent vasorelaxation was impaired and ANG II-induced vasoconstriction was increased in aortas of OZ rats. These changes seemed to be mediated by downregulation of PI3K/Akt and activation of the Rho-dependent pathway. Fluvastatin appeared to restore vascular function via the activation of Akt and suppression of the Rho-dependent pathway. It may be useful to modulate these pathways to treat diabetic patients.

**ACKNOWLEDGMENTS**

We thank Tanabe Seiyaku Co., LTD for supplying fluvastatin.

**GRANTS**

This study was supported in part by Grants-in-Aid 13670695 (to E. Suzuki), 15590725 (to E. Suzuki), 13470141 (to Y. Hirata), and 10218202 (to Y. Hirata), and by the Advanced and Innovative Research program in Life Sciences (to Y. Hirata) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**REFERENCES**

Disease-potential binding proteins—rاث-family or trimeric proteins or both—in Ca\(^{2+}\) sensitization of smooth muscle. \textit{Proc Natl Acad Sci USA} 93: 1340–1345, 1996.


