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Enhanced role for RhoA-associated kinase in adrenergic-mediated vasoconstriction in gracilis arteries from obese Zucker rats

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Naik, Jay S., Lusha Xiang, and Robert L. Hester. Enhanced role for RhoA-associated kinase (ROK)-mediated increases in Ca2+ sensitivity in α1-adrenergic vasoconstriction in arteries from obese Zucker (OZ) rats. Am J Physiol Regul Integr Comp Physiol 290: R154–R161, 2006. First published September 1, 2005; doi:10.1152/ajpregu.00245.2005.—Obesity, insulin resistance, dyslipidemia, and hypertension are components of the pathophysiological state known as metabolic syndrome. Adrenergic vasoconstriction is mediated through increases in cytosolic Ca2+ and the myofilaments’ sensitivity to Ca2+. In many pathophysiological states, there is an enhanced role for Rho kinase (ROK)-mediated increases in Ca2+ sensitivity of the contractile apparatus. Thus we hypothesized that there is a greater role for ROK-mediated increases in Ca2+ sensitivity in α1-adrenergic vasoconstriction in arteries from obese Zucker (OZ) rats. Therefore, small gracilis muscle arteries from 11- to 12-wk-old and 16- to 18-wk-old lean and OZ rats were isolated, cannulated, and pressurized to 75 mmHg. For some experiments, vessels were loaded with fura 2-AM. Changes in luminal diameter and vessel wall Ca2+ concentration ([Ca2+]i) were measured in response to phenylephrine (PE), the thromboxane mimetic U-46619, and KCl. α1-Adrenergic vasoconstriction was similar between 11- to 12-wk-old lean and obese animals and greater in older obese animals compared with controls. PE-induced increases in vascular smooth muscle cell [Ca2+]i were blunted in OZ animals compared with lean controls in both age groups of animals. KCl and U-46619 elicited similar vasoconstriction and vascular smooth muscle cell [Ca2+]i in both groups. ROK inhibition attenuated PE vasoconstriction to a greater degree in arteries from 11- to 12-wk-old OZ rats compared with lean animals; ROK inhibition in arteries from older rats right shifted both concentration-response curves to the same point. Total RhoA and ROKα protein expressions were similar between groups. These results suggest an enhanced role for the ROK pathway in α1-adrenergic vasoconstriction in metabolic syndrome.

vascular smooth muscle; vascular reactivity; isolated vessel; fura 2; phenylephrine; Type 2 diabetes

METABOLIC SYNDROME IS A COHORT of pathophysiological conditions that include obesity, insulin resistance, dyslipidemia, and hypertension. Epidemiological reports suggest that ~24% of adults in the United States over 20 yr of age meet the criteria for metabolic syndrome established by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults III (15). On the basis of year 2000 census data, ~47 million Americans have metabolic syndrome (16). Metabolic syndrome has been shown to be associated with alterations in vascular reactivity. Indeed, blunted endothelial-dependent vasodilation and augmented vasoconstriction have been demonstrated in both obese humans (3, 14, 26, 43, 47) and animals (10, 17–20, 30, 34, 37, 51). However, whether this corresponds to an alteration in vascular smooth muscle (VSM) Ca2+ handling in metabolic syndrome is not known.

Increases in cytosolic Ca2+ concentration ([Ca2+]i) in VSM cells result in phosphorylation of the 20-kDa regulatory myosin light chain, resulting in shortening of VSM. The degree of shortening reflects the relative activities of the Ca2+/calmodulin-dependent myosin light chain kinase and the Ca2+-independent myosin light chain phosphatase (MLCP). Regulation of the activity of MLCP mediates the myofilaments’ sensitivity to Ca2+ (11, 24, 27, 36). MLCP consists of a 37- to 38-kDa catalytic subunit (PP1c), an associated 110- to 130-kDa targeting subunit (MYPT1), and a third 20-kDa subunit of unknown function. Phosphorylation of the MYPT1 subunit of MLCP inhibits its activity, increasing the Ca2+ sensitivity of the contractile apparatus for a given [Ca2+]i. It is well established that MLCP can be inhibited via activation of the small G protein RhoA. On activation, RhoA translocates to the membrane where it activates Rho kinase (ROK), which can inhibit MLCP by phosphorylation of MYPT1 (13). ROK has been shown to be a component of the vasoconstriction in response to phenylephrine (PE), serotonin, endothelin, prostanoglandins F2α, thromboxane A2, ANG II, and histamine (32, 38, 42, 44). Moreover, a critical role for ROK has been demonstrated in animal models of systemic (6, 23, 28, 40) and pulmonary hypertension (12, 33). For example, inhibition of ROK decreased forearm vascular resistance to a greater degree in hypertensive subjects compared with control individuals (31). Moreover, the specific ROK inhibitor Y-27632 markedly decreased blood pressure in various models of hypertension but not in normotensive animals (48). However, a direct assessment of alterations in VSM Ca2+ sensitivity using small resistance arteries in a model of metabolic syndrome has not been performed. Thus we hypothesized that there is an enhanced role for ROK-mediated increases in Ca2+ sensitivity in skeletal muscle resistance arteries in an animal model of metabolic syndrome, the obese Zucker (OZ) rat. The OZ rat possesses nonfunctional leptin receptors and thus exhibits increased food intake, rapidly developing obesity, insulin resistance, dyslipidemia, and hypertension (4, 17–20, 37, 51). Thus the OZ rat provides a useful tool for the study of vascular function during a cohort of pathophysiological conditions rapidly growing in prevalence.

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METHODS

Animals

All animal protocols employed in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Male lean and OZ rats (for age = 11–12 wk, body wt = 337 ± 9 and 500 ± 9 g for lean and obese animals, respectively; for age = 16–18 wk, body wt = 425 ± 6 and 633 ± 10 g for lean and obese animals, respectively; Harlan) were used for these experiments.

Isolated Gracilis Artery Preparation

Rats were anesthetized with pentobarbital sodium (150 mg/kg ip). The gracilis muscle was surgically dissected from the anesthetized rat and secured in a Silastic-coated petri dish containing dissection solution [composed of (in mM) 130 NaCl, 4 KCl, 1.2 MgSO4, 4 NaHCO3, 1.8 CaCl2, 10 HEPES, 1.18 KH2PO4, 6 glucose, and 0.03 EDTA]. Small gracilis arteries (passive diameter at 75 mmHg = 163 ± 6 μm) were dissected free from the muscle, placed in a vessel chamber (Living Systems) containing dissection solution, cannulated with glass micropipettes, and secured with ligatures. Vessels were superfused (5 ml/min) with warmed (37°C), aerated (21% O2-5% CO2-balance N2) physiological saline solution (PSS) composed of (in mM) 119 NaCl, 5.9 KCl, 25 NaHCO3, 1.18 NaH2PO4, 1.17 MgSO4, 2.5 CaCl2, 0.026 EDTA, and 5.5 glucose. Intraluminal pressure was slowly increased to 100 mmHg using a column of PSS, vessels were stretched to remove bends, and pressure was reduced to 75 mmHg for a 30-min equilibration period. Viability of the artery was assessed by a single dose of the α1-adrenergic agonist PE (10 μM) followed by 10 μM ACh. Arteries then underwent a 15-min washout period. Passive diameter was determined at the end of each experiment by superfusing arteries with Ca2+-free PSS [composed of (in mM) 119 NaCl, 5.9 KCl, 25 NaHCO3, 1.18 NaH2PO4, 1.17 MgSO4, 2.5 EGTA, and 5.5 glucose] for 30 min.

Vasoconstritor and VSM [Ca2+] Responses to PE, U-46619, and KCl

Pressurized arteries were loaded with the cell-permeant ratiometric Ca2+-sensitive fluorescent dye fura 2-AM (Molecular Probes). Fura 2-AM was dissolved in anhydrous DMSO at a concentration of 1 mM. Immediately before cells were loaded, fura 2-AM was mixed with 0.5 volumes of a 20% solution of pluronic acid in DMSO, and this mixture was diluted with dissecting solution to yield a final concentration of 2 μM fura 2-AM and 0.05% pluronic acid. Vessels were incubated in this solution for 45 min at room temperature in the dark. Immediately before cells were loaded, fura 2-AM was mixed with 0.5 volumes of a 20% solution of pluronic acid in DMSO, and this mixture was diluted with dissecting solution to yield a final concentration of 2 μM fura 2-AM and 0.05% pluronic acid. Vessels were incubated in this solution for 45 min at room temperature in the dark. Arterial lumen was determined at the end of each experiment by superfusing arteries with Ca2+-free PSS [composed of (in mM) 119 NaCl, 5.9 KCl, 25 NaHCO3, 1.18 NaH2PO4, 1.17 MgSO4, 2.5 EGTA, and 5.5 glucose] for 30 min.

Effect of ROK Inhibition of PE- and U-46619-Induced Constriction

Pressurized resistance arteries were isolated and cannulated as described above. Decreases in luminal diameter were determined in response to serial administration of PE (10 nM to 10 μM) or U-46619 (1 pM to 0.1 μM). Arteries were washed with PSS for 15 min and then pretreated with the ROK inhibitor Y-27632 for 30 min (25). The PE dose-response curve was then repeated. Luminal diameter was measured from a bright-field image taken just before the administration of the next concentration of PE using MetaMorph 4.5 software (Universal Imaging). Preliminary experiments in arteries from lean and OZ rats demonstrated that successive PE and U-46619 dose-response curves could be performed without tachyphlaxis (data not shown).

Total RhoA and ROKα Protein Expression

To examine levels of total RhoA and ROKα, femoral arteries from pentobarbital sodium-anesthetized 16- to 18-wk-old lean (n = 5) and obese (n = 5) rats were dissected free and snap-frozen in liquid N2. Femoral arteries from two animals were pooled and treated as a single sample. Each sample was homogenized in 10 mM Tris-HCl homogenization buffer containing 255 mM sucrose, 2 mM EDTA, protease inhibitor cocktail (Pierce), and 1 mM PMSF. Samples were centrifuged at 14,000 g for 10 min at 4°C to remove insoluble debris. The supernatant was collected, and sample protein concentrations were determined by the Lowry method. A molecular weight standard (Bio-Rad) was added to each gel, and proteins were separated by SDS-PAGE (15%/7.5% Tris-HCl gels; Bio-Rad) and transferred to polyvinylidene difluoride membranes. Blots were blocked for 1 h at room temperature with blocking buffer (LI-COR Biosciences) containing 0.01% Tween 20. Blots were then incubated overnight at 4°C with a mouse monoclonal antibody for RhoA (1:500; BD Biosciences) or ROKα (1:750; BD Biosciences). For immunoochemical labeling, all blots were incubated for 1 h at room temperature with donkey anti-mouse IgG (1:5,000; IRDye 700DX, LI-COR Biosciences). RhoA (~21 kDa) and ROKα (~180 kDa) bands were detected with an Odyssey infrared imaging system (LI-COR Biosciences). Subsequent to RhoA and ROKα detection, blots were washed in PBS containing 0.1% Tween 20 and incubated for 1 h with mouse monoclonal antibody for α-actin (1:1,000; Sigma). Immunoochemical labeling of α-actin was achieved by incubating blots for 1 h at room temperature with donkey anti-mouse IgG (1:5,000; IRDye 700DX, LI-COR Biosciences), and blots were then reexamined with an Odyssey infrared imaging system (LI-COR Biosciences). Quantification of the bands was accomplished by densitometric analysis of scanned images using MetaMorph 4.5 software (Universal Imaging). Bands for RhoA and ROKα were normalized to those of α-actin.

Statistics and Data Analysis

Changes in vessel luminal diameter in response to PE and KCl were expressed as a percentage of baseline diameter. Because arteries treated with Y-27632 had less vascular tone than under control conditions, for this experiment, changes in luminal diameter were expressed as a percent of passive diameter. Data were analyzed using two-way repeated-measures ANOVA or t-test as appropriate. Individual groups were compared using the Student-Newman-Keuls post hoc test. A probability of P ≤ 0.05 was accepted as statistically significant for all comparisons.

RESULTS

Vascular Reactivity in 11- to 12-wk-Old Lean and OZ Rats

PE-induced constrictor and VSM [Ca2+] responses. Changes in luminal diameter in response to PE in gracilis arteries from 11- to 12-wk-old lean and obese animals are
A. PE administration produced a concentration-dependent decrease in luminal diameter in gracilis arteries from both lean and OZ rats. There were no differences in reactivity to PE between groups. Changes in VSM cell [Ca$^{2+}$] in response to PE in gracilis arteries from lean and OZ rats are presented in Fig. 1B. PE produced a concentration-dependent increase in VSM cell [Ca$^{2+}$] in arteries from lean animals. In contrast, PE-induced vasoconstriction was largely independent of increases in VSM cell [Ca$^{2+}$] in arteries from obese animals. The Ca$^{2+}$ independence of PE-mediated vasoconstriction in arteries from obese animals is graphically represented in Fig. 1C. Note the roughly linear relationship between VSM cell [Ca$^{2+}$] and luminal diameter in arteries from lean rats and that this relationship is absent in arteries from obese animals (Fig. 1C).

B. KCl elicited a concentration-dependent decrease in luminal diameter in arteries from lean and OZ rats (Fig. 2A). In contrast to PE, KCl produced a concentration-dependent increase in VSM cell [Ca$^{2+}$] in arteries from both lean and OZ rats (Fig. 2A). In contrast to PE, KCl produced a concentration-dependent increase in VSM cell [Ca$^{2+}$] in arteries from both lean and OZ rats (Fig. 2A).
There were no significant differences between groups.

**Effect of ROK inhibition on PE-induced constriction.** Administration of Y-27632 increased basal diameter from 121 ± 9 to 141 ± 9 μm and from 125 ± 15 to 142 ± 12 μm in lean and OZ rats, respectively. Passive diameters were 148 ± 13 and 148 ± 14 μm for arteries from lean and obese animals, respectively. Decreases in luminal diameter in response to PE in gracilis arteries from lean and OZ rats under control conditions or after treatment with the ROK inhibitor Y-27632 are presented in Fig. 3. Under control conditions, PE induced a rightward shift in the PE concentration-response curve in arteries from lean and obese animals. The responses from the Y-27632-treated OZ rats were significantly different from the Y-27632-treated lean animals.

**Vascular Reactivity in 16- to 18-wk-Old Lean and OZ Rats**

**PE-induced constrictor and VSM [Ca²⁺] responses.** Changes in luminal diameter in response to PE in gracilis arteries from 16- to 18-wk-old lean and obese animals are presented in Fig. 4A. PE administration produced a concentration-dependent decrease in luminal diameter in gracilis arteries from both lean and OZ rats. In contrast to what was observed in younger animals, vasoconstrictor reactivity was significantly greater in arteries from obese animals compared with lean controls. Changes in VSM cell [Ca²⁺] in response to PE in gracilis arteries from lean and OZ rats are presented in Fig. 4B. PE produced a concentration-dependent increase in VSM cell [Ca²⁺] in arteries from both groups. However, the increase in VSM cell [Ca²⁺] was significantly blunted in arteries from obese animals compared with control. Surprisingly, in response to 10 μM PE, VSM Ca²⁺ fell below baseline in arteries from obese animals. The Ca²⁺ independence of PE-mediated vasoconstriction in arteries from obese animals is graphically represented in Fig. 4C. Note the roughly linear relationship between VSM cell [Ca²⁺] and luminal diameter in arteries from lean rats and that this relationship is absent in arteries from obese animals.

**Effect of Rho-kinase inhibition on PE-induced constriction.** Decreases in luminal diameter in response to PE in gracilis arteries from lean and OZ rats under control conditions or after treatment with Y-27632 are presented in Fig. 5. Administration of Y-27632 increased basal diameter from 126 ± 21 to 139 ± 20 μm and from 129 ± 3 to 160 ± 11 μm in lean and OZ rats, respectively. Passive diameters were 143 ± 20 and 164 ± 9 μm for arteries from lean and obese animals, respectively. Under control conditions, PE-induced constriction was signif-
显著地在动脉从OZ rats与lean animals。 Pretreatment with Y-27632 produced a rightward shift in the PE concentration-response curves in arteries from both lean and OZ rats. In the presence of the ROK inhibitor, vasoconstriction to PE was similar between groups.

U-46619-induced vasoconstrictor and VSM [Ca\(^{2+}\)] responses. Changes in luminal diameter in response to U-46619 are presented in Fig. 6A. U-46619 elicited a concentration-dependent decrease in luminal diameter in arteries from both groups. There were no differences in reactivity to U-46619 between groups. Pretreatment with Y-27632 abolished the vasoconstriction to U-46619 in arteries from both groups (Fig. 6A). VSM cell Ca\(^{2+}\) did not increase until the highest concentration of U-46619 (Fig. 6B).

Total RhoA and ROK\(\alpha\) protein expression. Total RhoA and ROK\(\alpha\) protein expression in femoral arteries from lean and OZ rats are presented in Fig. 7. There were no differences in total RhoA or ROK\(\alpha\) expression between groups.

**DISCUSSION**

The major findings of the present study are as follows: 1) \(\alpha_1\)-adrenergic mediated vasoconstriction was similar between 11- to 12wk-old lean and obese animals; however, PE-mediated vasoconstriction was augmented in 16- to 18wk-old OZ rats compared with lean controls; 2) PE-induced increases in VSM cell [Ca\(^{2+}\)] were blunted in obese animals from both age groups compared with their respective lean controls; 3) inhibition of ROK attenuated PE-mediated vasoconstriction to a greater degree in arteries from 11- to 12wk-old OZ rats compared with lean animals, whereas ROK inhibition in arteries from 16- to 18wk-old rats right-shifted both concentration-response curves to the same point; 4) U-46619 elicited a similar concentration-dependent vasoconstriction in arteries from lean and obese animals; 5) U-46619-mediated vasoconstriction was independent of changes in VSM cell [Ca\(^{2+}\)]; 6) inhibition of ROK abolished U-46619-induced vasoconstriction in arteries from both groups; and 7) total RhoA and ROK\(\alpha\) protein expressions were similar between groups. These results suggest that the augmented vasoconstrictor reactivity seen in older OZ rats is selective for \(\alpha_1\)-adrenergic receptors and may involve enhanced activation of the ROK pathway.

In the present study, vasoconstriction in response to PE was not augmented in gracilis arteries from 11- to 12wk-old OZ rats (Fig. 1A) but was augmented in older OZ rats compared with lean controls (Fig. 4A). This is consistent with previous studies in gracilis arteries and aortic strips that demonstrate increased adrenergic vasoconstriction in older OZ rats compared with lean animals (18, 19, 35, 37). For example, Frisbee and colleagues (18, 19, 37) demonstrated increased myogenic and agonist-induced vasoconstriction in 15wk-old OZ rats. In addition, in the mesenteric vasculature of ob/ob and db/db mice, vasoconstrictor reactivity was enhanced compared with control animals (30, 34). In the present study, PE elicited a concentration-dependent increase in VSM cell [Ca\(^{2+}\)] in arteries from lean rats, whereas PE-induced vasoconstriction was largely independent of changes in Ca\(^{2+}\) in arteries from OZ rats (Figs. 2B and 4B). These results are consistent with previous studies in animal models of hyperglycemia, which suggest an increased role for changes in VSM Ca\(^{2+}\) sensitivity in response to vasoconstrictor stimuli. For example, Abebe et al. (1) found that, in the absence of extracellular Ca\(^{2+}\), norepinephrine and methoxamine elicited greater increases in tension in aortic rings from type 1 diabetic animals compared with controls. Moreover, increasing extracellular Ca\(^{2+}\) in the presence of a fixed concentration of norepinephrine produced more tension in aortic rings from type 1 diabetic animals compared with control (2). Taken together, these results suggest that, in pathophysiological conditions that result in chronic elevations in blood glucose (i.e., metabolic syndrome), the mechanisms of \(\alpha_1\)-adrenergic vasoconstriction may be more dependent on changes in Ca\(^{2+}\) sensitivity.

It is unlikely that the failure of PE administration to produce increases in VSM cell [Ca\(^{2+}\)] is due to impaired voltage-gated
Ca$^2+$ channel function. Indeed, KCl elicited similar changes in VSM cell [Ca$^{2+}$] in arteries from lean and obese animals (Fig. 2). This is consistent with previous work in aortic rings from streptozotocin-treated rats (2). There are several potential mechanisms to explain the lack of rise in VSM cell [Ca$^{2+}$] in response to PE administration. For example, there may be alterations in the $\alpha_1$-adrenergic signaling cascade that results in diminished depolarization of the VSM cell membrane potential. Alternatively, there may be impaired store-operated Ca$^{2+}$ or receptor-operated Ca$^{2+}$ channel function that would result in diminished cytosolic Ca$^{2+}$. Indeed, Curtis et al. (9) demonstrated blunted capacitive Ca$^{2+}$ entry in retinal microvascular smooth muscle cells of type 1 diabetic rats. In addition, there may be enhanced Ca$^{2+}$ buffering in VSM cells from OZ rats. For example, elevated sarcoplasmic reticulum Ca$^{2+}$ pump expression and activity have been demonstrated in diabetic dyslipidemic pigs; these would increase Ca$^{2+}$ uptake into the sarcoplasmic reticulum, decreasing cytosolic Ca$^{2+}$ (22).

In the present study, inhibition of ROK produced a greater rightward shift in the PE concentration-response curve in arteries from 11- to 12-wk-old OZ rats compared with lean controls (Fig. 3). In addition, Y-27632 abolished the enhanced vasoconstriction observed in older OZ rats, suggesting a larger component of the PE-induced constriction is mediated through activation of ROK (Fig. 5). This finding is consistent with a study by Carter et al. (7) that demonstrated enhanced Y-27632-induced vasodilation in $\alpha_2$-adrenergic precontracted aortic rings from hypertensive rats compared with normotensive controls. In the present study, pretreating arteries with Y-27632 produced a similar basal vasodilatory response in arteries from 11- to 12-wk-old lean and obese animals, suggesting similar levels of basal ROK activation. However, gracilis arteries from older OZ rats exhibited slightly greater myogenic tone, which was abolished by Y-27632. Indeed, a recent finding of Didion et al. (10) demonstrated an increased vasodilatory response to Y-27632 in cerebral arteries in obese db/db mice compared with C57B1K mice, suggesting a greater role for ROK in determining resting vascular tone.

Nitric oxide has been shown to induce dilation partly through inhibition of the RhoA/ROK pathway (39). Chitaley and Webb (8) demonstrated that Y-27632 had a greater effect on PE-induced contraction in endothelium-denuded compared with intact aortic rings and that application of exogenous nitric oxide abolished the enhanced effect of Y-27632 observed in denuded rings. Because the OZ rat has been shown to exhibit endothelial dysfunction (17, 20) and increased oxidative stress (17, 19, 20, 37), it is possible that the greater inhibition of PE-mediated vasoconstriction in OZ rats in response to Y-27632 is due to decreased nitric oxide synthesis or bioavailability in these animals, resulting in higher basal ROK activity.

To investigate whether the enhanced reactivity in obese animals was selective for the $\alpha_1$-adrenergic receptor, vasoconstrictor responses to the thromboxane mimetic U-46619 were preformed. In the present study, unlike PE, U-46619-mediated vasoconstriction was similar between groups, suggesting that the enhanced reactivity is selective for the $\alpha_1$-adrenergic receptor (Fig. 6A). This is consistent with work by Stepp and Frisbee (18a) who demonstrated augmented vasoconstriction to NE but not to ANG II or endothelin. In addition, we demonstrated that U-46619 did not elicit increases in VSM cell Ca$^{2+}$ in arteries from either group (Fig. 6B). This is consistent with a study by Ungvari and Koller (49), who demonstrated that U-46619 caused vasoconstriction that was independent of changes in VSM cell Ca$^{2+}$ up to 0.01 $\mu$M in gracilis arteries from Wistar rats. Moreover, in the present study, pretreating arteries with Y-27632 completely abolished U-46619-mediated vasoconstriction in arteries from both groups (Fig. 6A). Taken together, these data suggest that the greater receptor-mediated vasoconstriction seen in OZ rats is selective for the $\alpha_1$-adrenergic receptor.

As mentioned above, we observed a ROK-dependent increase in PE-mediated vasoconstriction in arteries from 16- to 18-wk-old OZ rats. In contrast, U-46619 produced a similar degree of vasoconstriction between groups. This constriction was completely inhibited in both groups by pretreating arteries with Y-27632. These data suggest that there is not a generalized increase in the ROK pathway in OZ rats. Consistent with this postulate, total RhoA and ROK protein expressions in femoral arteries from 16- to 18-wk-old rats were similar between groups. These results may be due to an increase in $\alpha_1$-adrenergic receptor expression in VSM from OZ rats. However, the increase in VSM cell [Ca$^{2+}$] in response to PE was less in obese animals compared with lean animals, suggesting that the selective enhancement in PE-mediated vasoconstriction observed in these rats is unlikely because of a simple increase in receptor expression. Rather, one possibility that warrants further investigation is that there may be greater association between the $\alpha_1$-adrenergic receptor and components of the RhoA/ROK pathway within caveolae (45, 46).

Although the classic pathway for ROK activation is through RhoA, there is evidence to suggest that ROK can be activated by other signaling factors such as sphingosylphosphorylcholine (41) and arachidonic acid (5, 21). Indeed, PE administration has been shown to increase arachidonic acid in VSM cells (21). Furthermore, MLCP inhibition by ROK may be mediated by direct phosphorylation by the kinase itself (13) or indirectly.
through ROK-induced activation of protein kinase C-activated protein phosphatase inhibitor of 17 kDa (50), which acts on the catalytic subunit of MLCP. Whether these signaling components are involved in Ca\(_{2+}\) sensitization in response to PE in gracilis arteries from OZ rats should be investigated in future studies.

In summary, the results of the present study suggest that in this animal model of metabolic syndrome the mechanism of \(\alpha_1\)-adrenergic stimulation shifts to a greater reliance on ROK-mediated increases in Ca\(_{2+}\) sensitivity. Future studies should investigate the mechanisms responsible for this shift in signaling pathways.

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