Cerebral vascular dysfunction in TallyHo mice: a new model of Type II diabetes

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Didion SP, Lynch CM, Faraci FM. Cerebral vascular dysfunction in TallyHo mice: a new model of Type II diabetes. Am J Physiol Heart Circ Physiol 292: H1579–H1583, 2007. First published November 22, 2006; doi:10.1152/ajpheart.00939.2006.—The purpose of this study was to characterize vascular responses and to examine mechanisms of vascular dysfunction in TallyHo mice, a new polygenic model of Type II diabetes. Responses of cerebral arterioles and carotid arteries were examined in vivo by using a cranial window and in vitro by using tissue baths, respectively. Dilatation of cerebral arterioles (baseline diameter = 33 ± 1 μm) in response to acetylcholine, but not to nitroprusside, was markedly reduced (P < 0.05) in TallyHo mice. Responses of cerebral arterioles to acetylcholine in TallyHo mice were restored to normal with polyethylene glycol-superoxide dismutase (100 U/ml; a superoxide scavenger). Responses to acetylcholine were also greatly impaired (P < 0.05) in the carotid arteries from TallyHo mice. Phenylephrine- and serotonin-, but not to KCl- or U46619, induced contraction was increased two- to fourfold (P < 0.05) in carotid arteries of TallyHo mice. Responses to phenylephrine and serotonin were reduced to similar levels in the presence of Y-27632 (an inhibitor of Rho kinase; 3 μmol/l). These findings provide the first evidence that vascular dysfunction is present in TallyHo mice and that oxidative stress and enhanced activity of Rho kinase may contribute to altered vascular function in this genetic model of Type II diabetes.

carotid artery; cerebral circulation; endothelium; microcirculation

EPIDEMIOLOGICAL STUDIES suggest that there is a marked increase in the number of vascular complications in patients with diabetes (21, 22, 51). For example, results from the Framingham study have found a two- to fourfold increased risk associated with diabetes and the development of other cardiovascular risk factors, including atherosclerosis, carotid artery disease, and stroke (21). Although Type II diabetes accounts for the majority (~90%) of diabetes in humans (3), relatively little is known regarding vascular responses and mechanisms that produce vascular dysfunction in Type II diabetes compared with Type I diabetes.

Whereas studies using ob/ob (leptin-deficient) and db/db (leptin receptor-deficient mice) have provided much insight into vascular alterations in the genetic models of Type II diabetes (8, 19, 25, 42, 43–46), such monogenic deficiencies in leptin and the leptin receptor are rare and are not always associated with the development of diabetes in humans (14). TallyHo mice represent a newly defined polygenic model of Type II diabetes and are characterized by hyperglycemia, hyperinsulinemia, hyperlipidemia, and moderate obesity (23, 24, 52). It has been suggested that TallyHo mice may be more representative of the polygenic nature of Type II diabetes in humans (23).

Because nothing is known regarding vascular responses in TallyHo mice and because Type II diabetes is a major risk factor for carotid artery disease and stroke (21, 22, 51), the first goal of the present study was to characterize responses in carotid artery and cerebral arterioles in TallyHo mice. As a part of these studies, we examined whether superoxide plays a role in alterations of vascular function in this model.

Although previous studies have examined mechanisms that contribute to altered vasoconstrictor responses in Type II diabetes (16, 19, 25, 40, 42, 43), the contribution of Rho kinase to contractile responses in diabetic blood vessels has not been extensively examined (40). Rho kinase has emerged as a major regulator of contractile responses in blood vessels (1, 5, 29). For example, increased Rho kinase activity has been implicated in cerebral vasospasm as well as in the enhanced cerebral artery tone associated with hypertension (5). Because Rho kinase activity can be influenced by both nitric oxide (NO) and superoxide (4, 18), we hypothesized that superoxide-mediated endothelial function would be present and would be associated with altered vasoconstrictor responses in TallyHo mice. Thus, a second goal was to examine whether contractile responses in the carotid artery, a large muscular artery, are altered in TallyHo mice and to determine whether such alterations can be accounted for by increased function of Rho kinase.

METHODS

Experimental animals. TallyHo mice (male; n = 50) and their non-diabetic controls (male C57Bl/6; n = 62) were obtained from Jackson Laboratories (Bar Harbor, ME). Animals had access to food and water ad libitum and were housed in the Animal Care Unit at The University of Iowa. All experimental protocols were reviewed and approved by the University of Iowa Animal Care and Use Committee before the start of all studies described herein and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Systolic blood pressure in conscious mice was measured using tail-cuff plethysmography (Visitech Systems, Apex, NC) as previously described (8, 49). Blood glucose was determined by using an Acu-Chek Advantage glucometer (Roche, Indianapolis, IN) (7, 49).

Cranial window studies. Mice were anesthetized with pentobarbital sodium (75–90 mg/kg ip). Anesthesia was supplemented regularly at ~10–20 mg·kg⁻¹·h⁻¹. The trachea was cannulated, and animals were ventilated mechanically with air and supplemental oxygen. A femoral artery was cannulated for measurement of systemic pressure.

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and to sample arterial blood for determination of glucose and insulin levels. Arterial pressure (under anesthesia) was similar ($P > 0.05$) in the two groups and averaged 75 ± 2 mmHg. Arterial blood gases were measured and were also similar ($P > 0.05$) in the groups ($P_{O_2}$, 159 ± 7; $P_{CO_2}$, 36 ± 1; pH, 7.30 ± 0.01).

A cranial window was made over the left parietal cortex, and a segment of a pial arteriole was exposed as described previously (2, 8, 9). Application of vehicle did not affect vessel diameter. Diameter of one arteriole per animal was measured under control conditions and during drug application. Changes in arteriolar diameter were measured in response to the endothelium-dependent dilator acetylcholine (ACh; 1 and 10 μmol/l) and to the endothelium-independent dilator nitroprusside (0.1 and 1 μmol/l). Studies from our laboratory have shown that the response to ACh in cerebral arterioles is mediated by NO (4, 11).

In a second group, we examined the role of superoxide in alterations in vascular responses. Thus responses to ACh repeated in the presence of polyethylene glycol-superoxide dismutase (PEG-SOD; 100 U/ml) or vehicle (control). We have shown previously that this concentration of PEG-SOD is efficacious in reducing superoxide levels in blood vessels and improving endothelium-dependent responses (8, 9). The cranial window was treated with PEG-SOD or vehicle for 30 min before and during the second application of ACh.

**Carotid artery studies.** Function of carotid arteries was examined as previously described (6, 9, 49). After a 45-min equilibration period, vessels were precontracted submaximally (50–60% of maximum) with the thromboxane analog 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F$_2$α (U-46619). After a stable plateau was reached, concentration-response curves were generated to ACh (0.01 to 100 μmol/l) and for the endothelium-independent, non-NO dilator papaverine (0.03–30 μmol/l). We have previously shown responses of the carotid artery in mice to ACh are mediated by endothelial NO synthase and NO (9, 14).

Contractile responses to phenylephrine (0.01–100 μmol/l), potassium chloride (1–10 mmol/l), serotonin (0.01–10 μmol/l), and U46619 (0.03–3 μg/ml) were examined in TallyHo and control mice. To examine the role of Rho kinase, responses to these agonists were also examined before and after 30-min incubation with Y-27632 (a selective Rho kinase inhibitor; 3 μmol/l) (8).

**Drugs.** ACh, nitroprusside, phenylephrine, papaverine, PEG-SOD, and serotonin were obtained from Sigma (St. Louis, MO), and all were dissolved in saline. U46619 was obtained from Cayman Chemical (Ann Arbor, MI) and dissolved in 100% ethanol with subsequent dilutions being made with saline. Y-27632 was obtained from CalBioChem (San Diego, CA) and dissolved in milli-Q water. All other reagents were of standard laboratory grade.

**Statistical analysis.** All data are expressed as means ± SE. Relaxation to ACh and papaverine in carotid artery is expressed as a percent relaxation to U46619-induced contraction. Comparisons of relaxation and contraction in response to these reagents were made using analysis of variance followed by Bonferroni’s multiple comparisons test. Arteriolar responses are presented as a percent change in diameter compared with baseline. Statistical analysis was performed using paired or unpaired t-tests or ANOVA were appropriate. Statistical significance was accepted at $P < 0.05$.

**RESULTS**

**Basal parameters.** TallyHo mice and control mice were of similar age (5 ± 1 mo). Body weight (38 ± 1 and 29 ± 1 g) and blood glucose levels (447 ± 31 and 130 ± 6 mg/dl) were greater ($P < 0.05$) in TallyHo compared with controls, respectively. Systolic blood pressure (conscious) was similar ($P > 0.05$) in both groups (117 ± 3 and 118 ± 3 mmHg in TallyHo and control mice, respectively).

**Cerebral vasodilator responses in TallyHo mice.** Arteriolar diameter was similar ($P > 0.05$) under baseline conditions in both groups of mice (control = 32 ± 1 μm and TallyHo = 34 ± 1 μm). ACh produced dilatation of cerebral arterioles in control mice (Fig. 1). In contrast, dilatation in response to ACh was markedly impaired in TallyHo mice (Fig. 1). For example, 10 μmol/l ACh produced 30 ± 3% and 7 ± 1% dilatation in control and TallyHo mice, respectively. Nitroprusside produced vasodilatation that was similar in control and TallyHo mice (Fig. 1). These results suggest that the impaired response in TallyHo mice was selective for endothelium.

In a separate group of TallyHo mice, dilatation in response to ACh was restored toward that observed in control mice by PEG-SOD (Fig. 1). These data suggest an important role for superoxide in the impaired endothelium-dependent response in TallyHo mice.

**Responses of carotid arteries in TallyHo mice.** ACh produced relaxation of the carotid artery in control mice (Fig. 2). In contrast, responses to ACh were markedly impaired in TallyHo mice (Fig. 2). For example, 100 μmol/l ACh produced 81 ± 6% and 35 ± 8% relaxation in control and TallyHo mice, respectively. In contrast, papaverine produced relaxation that was similar ($P > 0.05$) in control and TallyHo mice (Fig. 2), indicating that the impaired response to ACh was selective.

Serotonin and phenylephrine produced concentration-dependent contraction in the carotid artery from control mice (Fig. 3). However, both agonists produced significantly greater contraction in arteries from TallyHo mice compared with controls (Fig. 3). Augmented responses to serotonin and phenylephrine were selective, because contraction of the artery to potassium chloride (data not shown) and U46619 (Fig. 4) were similar ($P > 0.05$) in control and TallyHo mice.

In controls, contraction to serotonin and phenylephrine was markedly reduced by Y-27632 (3 μmol/l) (Fig. 3). Y-27632 also inhibited vasoconstrictor responses to serotonin and phenylephrine in TallyHo mice, such that responses were now similar to those observed in control vessels treated with...
Y-27632 (Fig. 3). These findings suggest that Rho kinase mediates a large proportion of the response to serotonin and phenylephrine in control mice and that the enhanced constrictor response to serotonin and phenylephrine in TallyHo mice can be accounted for by activity of Rho kinase.

Y-27632 had little effect on contractile responses to U44619 in control mice (Fig. 4), suggesting that Rho kinase is not a key mediator of contractile responses to U46619. However, Y-27632 partially inhibited contractile responsiveness to U46619 in TallyHo mice (Fig. 4). These results are consistent with the concept that Rho kinase activity is increased in TallyHo mice and that this increase may maintain contractile responses to U46619, perhaps as a compensatory mechanism.

**DISCUSSION**

There are several major new findings of the present study. First, responses to the endothelium-dependent agonist ACh were selectively impaired in carotid arteries as well as cerebral arterioles in TallyHo mice. Second, responses of cerebral arterioles in TallyHo mice to ACh could be restored to normal with PEG-SOD, suggesting a major role for superoxide in mechanisms of endothelial dysfunction. Third, contractile responses to serotonin and phenylephrine were greater in the carotid artery from TallyHo mice. Fourth, Y-27632 markedly inhibited contraction to serotonin and phenylephrine in control and TallyHo mice; however, the relative degree of inhibition was much greater in TallyHo mice compared with controls. These data represent the first study of vascular responses in TallyHo mice and suggest that activity of Rho kinase is increased and contributes to the enhanced contractile responses associated with Type II diabetes.

**Impaired endothelium-dependent responses in TallyHo mice: role of superoxide.** Peripheral vascular responses to endothelium-dependent agonists are impaired in experimental models and patients with Type II diabetes (16, 19, 25, 41, 42, 46). For example, aortic responses to ACh are impaired in ob/ob as well as db/db mice, two distinct models of Type II diabetes (19, 42, 46). In the present study, we found that responses to ACh were selectively impaired in the carotid artery in TallyHo mice. Consistent with our findings in the carotid arteries, we found that endothelium-dependent responses were also markedly impaired in cerebral microvessels in TallyHo mice. Collectively, these findings provide the first evidence that vascular responses are impaired in TallyHo mice. In addition, the present findings provide further evidence that impairment of endothelium-dependent response in Type II diabetes is not limited to large peripheral vessels but also extends to resistance vessels within the cerebral circulation (8, 50).

Increases in superoxide are associated with endothelial dysfunction in many diseases, including atherosclerosis, hypertension, and Type I diabetes (2, 6, 8, 9, 30). However, very few studies have examined responses in the cerebral circulation in Type II diabetes (4, 36, 50) and even fewer have examined potential mechanisms of vascular dysfunction in Type II diabetes (4, 27). Therefore, we determined whether responses of cerebral arterioles in TallyHo mice were superoxide mediated. We found that responses to ACh could be restored by using PEG-SOD in TallyHo mice toward those observed in control mice. These findings suggest that under normal conditions, superoxide does not impair responses to
endothelium-dependent agonists, consistent with findings in other blood vessels in mice and with other superoxide scavengers (6, 7, 12). Our results suggest that endothelium-dependent responses are impaired in the carotid artery and in cerebral arterioles in TallyHo mice and begin to implicate an important role for superoxide in this impairment.

It is interesting to note that TallyHo mice have similar levels of systolic blood pressure (as measured using tail-cuff plethysmography) compared with controls. In contrast, db/db mice appear to be moderately hypertensive (28). Because hypertension per se has been shown to be associated with endothelial dysfunction (30), the present findings suggest that endothelial dysfunction in TallyHo mice is not related to a hypertensive phenotype. This is the first time, to our knowledge, that blood pressure has been evaluated in TallyHo mice. Such findings are important because they provide preliminary evidence that the vascular effects of Type II diabetes in TallyHo mice appear to occur independent of the confounding influence of differences in blood pressure.

**Enhanced constrictor responses in TallyHo mice: role of Rho kinase.** Whereas previous studies have shown that contractile responses are altered in Type I and Type II diabetes (15, 19, 25, 32, 33, 35, 37, 38, 42, 43, 45, 48), relatively few studies have examined mechanisms that produce these changes (15, 43). Activity of Rho kinase appears to be enhanced under pathophysiological conditions, including subarachnoid hemorrhage and hypertension (5, 30). Rho kinase increases calcium sensitivity and may represent a major mechanism in regard to enhanced contractile responses (55). We have shown previously that the resting tone of cerebral arterioles is enhanced in db/db mice and that this enhancement may occur as a result of a reduction in bioavailable NO (8). In the present study, we examined the role of Rho kinase in Type II diabetes and whether increased Rho kinase activity contributes to the increased contractile responses of carotid artery in TallyHo mice.

We found that phenylephrine- (i.e., α-adrenergic mediated) as well as serotonin-induced contraction was greater in carotid arterioles from TallyHo mice. These responses appear to be selective because KCl- and U46619-induced contraction was similar in TallyHo and control mice. We found that the very commonly used Rho kinase inhibitor Y-27632 reduced vasoconstrictor responses to phenylephrine and serotonin in both TallyHo and control mice. Y-27632 binds the ATP-binding site on Rho kinase and has been shown to be highly selective for Rho kinase (55). Our data with Y-27632 suggests the majority of the contractile response to phenylephrine and serotonin in blood vessels under normal conditions is mediated by Rho kinase, consistent with previous studies (49). Y-27632 reduced contractile responses to a similar level in arteries from both types of mice; however, the relative degree of inhibition was greater in TallyHo mice. This finding is consistent with an enhanced role of Rho kinase in phenylephrine-induced contraction in mesenteric arteries from Type II diabetic rats (40). The present data provide the first evidence that the functional importance of Rho kinase in arteries is increased in TallyHo mice.

There are two isoforms of Rho kinase, ROCK1 (ROKβ) and ROCK2 (ROKα), and Y-27632 inhibits both isoforms (1, 29). Thus we are not able distinguish whether the increase in Rho kinase reflects increases in activity of ROCK1 and/or ROCK2. In addition to increases in Rho kinase activity per se, there is some evidence to suggest that expression of Rho kinase may also increase under pathological conditions (39). Thus the enhanced contractile responses to serotonin and phenylephrine in TallyHo mice might be due to enhanced Rho kinase activity and/or enhanced Rho kinase expression.

Whereas the effects of Rho kinase are typically thought to reflect changes in expression/activity in smooth muscle, it should be noted that Rho kinase is also expressed in endothelium (10, 17). Rho kinase activity in endothelial cells has been shown to have multiple effects on NO and NO-related signaling (10, 17, 26, 27, 36, 47, 53). For example, upregulation of Rho kinase expression in endothelial cells in response to hypoxia is associated with reductions in endothelial NO synthase expression and NO levels (17, 53). Thus the reductions in phenylephrine- and serotonin-induced contraction produced by Y-27632 might reflect the effect of Rho kinase inhibition in both smooth muscle and endothelium.

In conclusion, in the present study, we found that endothelium-dependent relaxation is impaired in carotid arterioles and cerebral microvessels in TallyHo mice: a new model of Type II diabetes that mimics the polygenic nature of Type II diabetes in humans (23). These findings provide the first evidence that vascular dysfunction is present in any blood vessel in TallyHo mice. Contractile responses of carotid arterioles to both phenylephrine and serotonin were selectively enhanced in TallyHo mice through a mechanism that appears to involve Rho kinase. Because sensitivity to α-adrenergic stimulation and platelet activation are enhanced in patients with Type II diabetes (31, 44, 55), we speculate that enhanced vascular sensitivity to phenylephrine and serotonin combined with reductions in endothelium function may contribute to the increased risk of carotid artery disease and stroke associated with Type II diabetes (21, 22, 51).

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