Selective cerebral vascular dysfunction in Mn-SOD-deficient mice


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Faraci, F. M., M. L. Modrick, C. M. Lynch, L. A. Didion, P. E. Fegan, and S. P. Didion. Selective cerebral vascular dysfunction in Mn-SOD-deficient mice. J Appl Physiol 100: 2089–2093, 2006. First published March 2, 2006; doi:10.1152/japplphysiol.00939.2005.—We tested the hypothesis that the mitochondrial form of superoxide dismutase [manganese superoxide dismutase (Mn-SOD)] protects the cerebral vasculature. Basilar arteries (baseline diameter ∼140 μm) from mice were isolated, cannulated, and pressurized to measure vessel diameter. In arteries from C57BL/6 mice preconstricted with U-46619, acetylcholine (ACh; an endothelium-dependent vasodilator) produced nitric oxide-mediated dilation that was similar in male and female mice and abolished by an inhibitor of nitric oxide synthase. Vasodilation to ACh was not altered in heterozygous male or female Mn-SOD-deficient (Mn-SOD+/−) mice compared with wild-type littermate controls (Mn-SOD+/+). Constriction of the basilar artery to arginine vasopressin, but not KCl or U-46619, was increased in Mn-SOD+/− mice (P < 0.05), and this effect was prevented by tempol, a scavenger of superoxide. We also examined responses of cerebral (pial) arterioles (branches of the middle cerebral artery, control diameter ∼30 μm) to ACh in anesthetized mice using a cranial window. Responses to ACh, but not nitroprusside (an endothelium-independent agonist), were reduced (P < 0.05) in cerebral arterioles in Mn-SOD+/− mice, and this effect was prevented by tempol. Thus these are the first data on the role of Mn-SOD in cerebral circulation. In the basilar artery, ACh produced nitric oxide-mediated dilation that was similar in male and female mice. Under normal conditions in cerebral arteries, responses to ACh were not altered but constrictor responses were selectively enhanced in Mn-SOD+/− mice. In the cerebral microcirculation, there was superoxide-mediated impairment of responses to ACh.

dothelial function, and limits vasoconstrictor responses in the carotid artery and cerebral circulation (4–6).

The mitochondrial content of cerebral endothelium is greater than that in other cells (20), and endothelium appears to express higher levels of Mn-SOD than other portions of the vessel wall (1, 9). Astrocytes can induce expression of Mn-SOD in cerebral endothelium (22, 23). Thus the functional importance of Mn-SOD may differ along the vascular tree and could potentially be more important in cerebral microvessels and in the metabolically active cerebral endothelium (the blood-brain barrier) (18).

Using genetically altered mice deficient in expression of Mn-SOD, the present study had several goals. First, we determined whether deficiency in Mn-SOD produces endothelial dysfunction in cerebral arteries and brain microvessels. Second, we determined whether vasoconstrictor responses of cerebral arteries were enhanced in Mn-SOD-deficient (Mn-SOD+/−) mice. Lastly, because vascular responses may vary with gender (12), we examined responses in both male and female wild-type (Mn-SOD+/+) and Mn-SOD+/− mice. In these experiments we used Mn-SOD+/− mice because mice that are completely deficient in Mn-SOD (Mn-SOD−/−) die shortly after birth (21). In addition, studies of Mn-SOD−/− mice may provide a better model for disease states or genetic polymorphisms in which activity of Mn-SOD is reduced but not eliminated.

METHODS

Experimental animals. The animal protocol used in these experiments was reviewed and approved by the University of Iowa Animal Care and Use Committee. Mice for this study (8 ± 1 mo of age) were derived from the breeding of Mn-SOD+/+ and Mn-SOD−/− mice to generate the same genotypes within the same litter, thus providing littermate controls. Breeding pairs of mice used for these experiments were obtained from the Jackson Laboratories and have been backcrossed more than 10 generations to the C57BL/6 background. Additional C57BL/6 mice (2–3 mo old) were used in some studies (see below). Mice were fed regular chow and water was available ad libitum. Genotyping of mice was performed by PCR of DNA from tail biopsies as described previously (1). Mn-SOD protein expression in aorta was performed using Western blotting as described previously in detail (1, 4, 5).

Studies of cerebral arteries in vitro and cerebral arterioles in vivo. After an overdose of anesthesia (pentobarbital sodium, 150–200 mg/kg ip), the brain was rapidly removed and placed in ice-cold Krebs buffer. As described previously (12, 31), the basilar artery was isolated, cannulated, and pressurized to measure vessel diameter. In arteries from C57BL/6 mice preconstricted with U-46619, acetylcholine (ACh; an endothelium-dependent vasodilator) produced nitric oxide-mediated dilation that was similar in male and female mice and abolished by an inhibitor of nitric oxide synthase. Vasodilation to ACh was not altered in heterozygous male or female Mn-SOD-deficient (Mn-SOD+/−) mice compared with wild-type littermate controls (Mn-SOD+/+). Constriction of the basilar artery to arginine vasopressin, but not KCl or U-46619, was increased in Mn-SOD+/− mice (P < 0.05), and this effect was prevented by tempol, a scavenger of superoxide. We also examined responses of cerebral (pial) arterioles (branches of the middle cerebral artery, control diameter ∼30 μm) to ACh in anesthetized mice using a cranial window. Responses to ACh, but not nitroprusside (an endothelium-independent agonist), were reduced (P < 0.05) in cerebral arterioles in Mn-SOD+/− mice, and this effect was prevented by tempol. Thus these are the first data on the role of Mn-SOD in cerebral circulation. In the basilar artery, ACh produced nitric oxide-mediated dilation that was similar in male and female mice. Under normal conditions in cerebral arteries, responses to ACh were not altered but constrictor responses were selectively enhanced in Mn-SOD+/− mice. In the cerebral microcirculation, there was superoxide-mediated impairment of responses to ACh.

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isolated using a dissecting microscope, cannulated onto glass micropipettes filled with Krebs buffer in an organ chamber, and secured with nylon monofilament. Arteries were pressurized to 60 mmHg. Using a microscope and a video camera, vessel images were projected on a video monitor. An electronic dimension analyzer was then used to measure lumen diameter.

Once prepared, basilar arteries were allowed to equilibrate for at least 30 min at a distending pressure of 60 mmHg before protocols were initiated. We examined changes in diameter of the basilar artery in response to KCl (50 mM) (n = 22 Mn-SOD+/−; 16 Mn-SOD−/− mice) and cumulative doses of the thromboxane A2 mimetic U-46619 (n = 16 Mn-SOD+/−; 11 Mn-SOD−/− mice) and arginine vasopressin (n = 13 Mn-SOD+/−; 15 Mn-SOD−/− mice). In a subgroup of Mn-SOD−/− mice (n = 5 Mn-SOD), the effects of tempol (1 mM, a scavenger of superoxide) were used to examine a mechanism that may contribute to enhanced vasoconstrictor responses.

For studies in which acetylcholine or other vasodilators were used, basilar arteries were constricted by 30% (~60% of the response to 50 mM KCl) with U-46619. Acetylcholine is an endothelium-dependent agonist (10) and thus was used to examine endothelial function. After development of a stable baseline diameter, cumulative dose-response curves were obtained.

Nitric oxide (NO) is known to mediate dilation of cerebral arteries in response to acetylcholine in several species, including humans (10). TO determine whether responses to acetylcholine are also NO-mediated in the basilar artery of mice, we performed studies using acetylcholine in several species, including humans (10). Acetylcholine produces maximal vasodilation.

For studies in vivo, mice were anesthetized with pentobarbital sodium (75–90 mg/kg ip), supplemented regularly at ~20 mg·kg−1·h−1. Animals were ventilated mechanically with supplemental oxygen, and arterial blood pressure and blood gases were monitored as described previously (7, 11, 24, 31). A cranial window was made over the left parietal cortex, the window was constantly suffused with artificial CSF, and a pial arteriole (branches of the middle cerebral artery) was exposed. Arteriolar diameter was measured using a microscope equipped with a television camera coupled to a video monitor and an image-shearing device. The diameter of one arteriole per animal was measured under control conditions during topical application of drugs. Arterial pressure was similar in the two groups of mice and averaged 75 ± 2 and 79 ± 2 mmHg (mean ± SE) in Mn-SOD+/− and Mn-SOD−/− mice, respectively. Arterial blood gases were also similar (Pco2 = 35 ± 1 Torr, Po2 = 163 ± 9 Torr, and pH = 7.32 ± 0.017). The gases and pH of the artificial CSF were Pco2 = 41 ± 1 Torr, Po2 = 87 ± 5 Torr, and pH = 7.30 ± 0.01. In these studies, responses of arterioles to topical application of acetylcholine (1 and 10 μM) and nitroprusside (an endothelium-independent vasodilator, 0.1 and 1 μM) were examined. In a subgroup of the mice (n = 7 of each genotype), tempol was used to examine a mechanism that may contribute to impaired endothelial responses.

Statistical analysis. Constriction in response to U-46619, KCl, and arginine vasopressin was determined by calculating the percent reduction in vessel diameter from the baseline control level. For vasodilator responses, results are expressed as percent dilation (% of induced tone), with 100% representing the difference between the resting value under basal conditions and the constricted value with U-46619. As appropriate, comparisons were made using paired or unpaired t-tests or ANOVA with repeated measures followed by Student-Newman-Keuls test to detect individual differences. A P < 0.05 was defined as significant.

RESULTS

Dilator responses of the basilar artery. Baseline diameter of the basilar artery in C57BL/6 mice was 132 ± 4 μm (n = 15). Responses of the basilar artery to acetylcholine (0.1–100 μM) were reproducible (data not shown). For example, 10 μM acetylcholine dilated the basilar artery by 73 ± 5 and 69 ± 2% during the first and second application, respectively (n = 6). Treatment with L-NNA completely prevented responses to acetylcholine (n = 9). In response to 0.1 and 10 μM acetylcholine, for example, the basilar artery dilated by 28 ± 4 and 66 ± 3% in the absence and 0 ± 1 and 0 ± 1% in the presence of L-NNA. This inhibitory effect was selective, as the basilar artery dilated by 104 ± 1% in response to papaverine in the presence of L-NNA (n = 5).

Responses to acetylcholine were similar in male and female mice and thus were combined. Baseline diameters of the basilar arteries in Mn-SOD+/− and Mn-SOD−/− mice were 144 ± 3 and 148 ± 3 μm, respectively (n = 24 and n = 16). Dilation of the basilar artery in response to acetylcholine was similar in Mn-SOD+/− and Mn-SOD−/− mice (Fig. 1). Papaverine also produced similar vasodilation in the two groups of mice (Fig. 1).

Our laboratory has shown previously that levels of Mn-SOD protein in the vasculature are ~50% of normal in Mn-SOD−/− mice on a CD-1 genetic background (1). In the present study, we found a similar reduction in levels of Mn-SOD protein using mice on the C57BL/6 background (Fig. 1).

Constrictor responses of the basilar artery in Mn-SOD+/− mice. Arginine vasopressin produced constriction of the basilar artery, and the response was similar in male and female mice with reductions in diameter starting at a concentration of 1 nM (Fig. 2). Vasoconstrictor responses to vasopressin were enhanced in Mn-SOD−/− mice and prevented by treatment with tempol (Fig. 2). This difference in responsiveness to vasopressin was selective, because constriction of the basilar artery in response to KCl and U-46619 was similar in the two genotypes (Fig. 2).

Responses of cerebral arterioles in vivo. Baseline diameters were similar in cerebral arterioles from Mn-SOD+/− and Mn-SOD−/− mice [29 ± 1 and 28 ± 1 μm in wild-type (n = 18)
and Mn-SOD\textsuperscript{+/−} mice (n = 20), respectively. Dilation of cerebral arterioles in response to acetylcholine was impaired in Mn-SOD\textsuperscript{+/−} mice compared with controls, and this impairment was prevented by tempol (Fig. 3). Tempol had no effect on responses to acetylcholine in control mice (Fig. 3). In contrast to responses to acetylcholine, vasodilator responses to nitroprusside were similar in Mn-SOD\textsuperscript{+/+} and Mn-SOD\textsuperscript{+/−} mice (Fig. 3). The finding that responses to acetylcholine (an NO-mediated response), but not nitroprusside (an NO donor), are impaired by superoxide is consistent with many previous studies related to oxidative stress. This difference may reflect the susceptibility of endogenously produced NO (which must diffuse from endothelium to vascular muscle) vs. NO released by nitroprusside, which may be released intracellularly near its site of action.

**DISCUSSION**

There are several new findings in this study. First, dilation of the basilar artery in the mouse to acetylcholine was completely inhibited by l-NNA, indicating that this response is mediated by NO. Second, acetylcholine-induced dilation of the basilar artery was similar in wild-type and Mn-SOD\textsuperscript{+/−} mice, but it was reduced in small cerebral arterioles in Mn-SOD\textsuperscript{+/−} mice. These findings suggest that under otherwise normal conditions, deficiency in Mn-SOD impairs microvascular endothelial function in brain. The studies with tempol suggest that the mechanism that produces this impairment involves superoxide. Third, vasopressin was a potent constrictor of the basilar artery, and this response was selectively increased in Mn-SOD\textsuperscript{+/−} mice. Augmented responses to vasopressin involved superoxide as enhanced effects in Mn-SOD\textsuperscript{+/−} mice were prevented by tempol. Vascular responses to all the stimuli tested were not affected by gender.

Under normal conditions, the mitochondrial electron transport chain is a major source of superoxide, converting up to perhaps 5% of molecular oxygen to superoxide (21). Because of its subcellular localization, Mn-SOD represents a major defense mechanism against oxidative stress (21, 27). Using genetically altered mice, recent studies have demonstrated that Mn-SOD\textsuperscript{+/−} mice exhibit increased cell dysfunction and cell death in models of ischemia and brain injury (27).

It has been shown that levels of Mn-SOD protein are ~50% of normal in the vasculature (aorta and mesenteric arteries) of Mn-SOD\textsuperscript{+/−} mice (1, 32). These findings are expected based on the fact that levels of mRNA are almost always at 50% of normal in genetically altered mice with heterozygous gene deficiency (28). Previous studies have found that this level of Mn-SOD deficiency does not alter relaxation of the aorta to acetylcholine (1, 3). In the present study, we found that responses to acetylcholine are normal in the basilar artery in Mn-SOD\textsuperscript{+/−} mice. Although these findings suggest that endothelial function is normal in cerebral arteries in Mn-SOD\textsuperscript{+/−} mice under control conditions, we speculate that deficiency in Mn-SOD may predispose the vessels to endothelial dysfunction under conditions that promote oxidative stress. This hypothesis would be consistent with finding that mice with combined hypercholesterolemia and Mn-SOD deficiency exhibit enhanced vascular mitochondrial injury and atherosclerosis (2).

In the present experiments, Mn-SOD\textsuperscript{+/−} mice were used because mice that are completely deficient in Mn-SOD (Mn-SOD\textsuperscript{−/−}) die shortly after birth (21). We recognize that gene-targeted models may potentially express compensatory mechanisms that could affect their overall phenotype (13). In this regard, it is noteworthy that deficiency in Mn-SOD does not result in compensatory vascular expression by the remaining SOD isoforms (9, 32).

In contrast to the basilar artery, we obtained evidence for superoxide-mediated endothelial dysfunction in cerebral arterioles of Mn-SOD\textsuperscript{+/−} mice. It is not clear why dysfunction was detected in cerebral arterioles but not in cerebral arteries in these mice. Based on current concepts regarding mRNA levels in mice lacking one copy of a gene (28) and data that expression of Mn-SOD is reduced in aorta and mesenteric arteries of Mn-SOD\textsuperscript{+/−} mice (1, 32), it seems likely that levels of Mn-
SOD would be reduced to a similar extent in both types of blood vessels. We have considered the possibility that methodological differences might account for these observations but believe that such an explanation is unlikely. In previous studies using the same methods but with mice expressing other genetic manipulations, our laboratory has observed a similar phenotype in cerebral arterioles and the basilar artery (31). In contrast to the present findings with Mn-SOD, endothelial dysfunction was observed in both cerebral arterioles (4) and the basilar artery (M. L. Modrick, S. P. Didion, and F. M. Faraci, unpublished observations) in CuZn-SOD-deficient mice. Our data do not exclude possible regional or segmental differences in effects of Mn-SOD deficiency on cerebral blood vessels. If the density and activity of mitochondria as well as expression of Mn-SOD are relatively higher in cerebral arterioles than in cerebral arteries, deficiency in Mn-SOD might have a greater impact on function in brain microvessels. Mn-SOD expression is induced in cerebral endothelium by astrocytes (22, 23). Thus it seems conceivable that Mn-SOD may be functionally more important in cerebral microvessels. Regardless of the mechanism that accounts for this difference, our data on cerebral arterioles are consistent with very recent findings in skeletal muscle arterioles of Mn-SOD−/− mice, which exhibit impaired responses to acetylcholine (32).

Vasoconstrictor responses to vasopressin were selectively enhanced in the basilar artery in Mn-SOD−/− mice. In contrast, we found that responses to U-46619 and KCl were normal in these vessels. The latter findings are consistent with previous results obtained in aorta in which contraction to prostaglandin F2α and serotonin were normal in Mn-SOD−/− mice (1). Our findings with tempol suggest that the mechanism(s) that accounts for increased responses to vasopressin in cerebral arterioles in Mn-SOD−/− mice involves superoxide. The conclusion that superoxide could produce constriction of cerebral vessels or enhance responses of cerebral vessels to vasoconstrictors is consistent with previous studies on effects of β-amyloid peptide on cerebral circulation (19). Regardless of the mechanism, increased vasoconstrictor response to vasopressin may have functional consequences during activation of vasopressinergic nerves that innervate cerebral arteries (14) or in response to increases in circulating and/or cerebrospinal fluid concentrations of vasopressin that occur in disease states including hypoxia and intracranial hypertension (25, 30).

Our findings of vascular phenotypes in Mn-SOD−/− mice may have implications for genetic conditions (genetic polymorphisms) or disease states in which expression or activity of Mn-SOD is reduced. Vascular expression of Mn-SOD may be reduced under some pathophysiological conditions (16, 26, 29). Even in the absence of reductions in expression, activity of Mn-SOD can be reduced during oxidative and nitrosative stress as a result of selective nitrination and inactivation of the enzyme (8, 9). Under these conditions, deficiency in Mn-SOD activity may be associated with cerebral microvascular endothelial dysfunction and preservation or selective enhancement of vasoconstrictor responses. Such changes could contribute to chronic cerebrovascular disease or stroke.

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