ELSEVIER

Endothelial-dependent vasodilation is reduced in mesenteric arteries from superoxide dismutase knockout mice

Christy-Lynn M. Cooke, Sandra T. Davidge*

Perinatal Research Centre, Departments of Obstetrics/Gynecology and Physiology, 232 HMRC, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Abstract

Objective: Oxidative stress has increasingly been implicated in the development and progression of many vascular diseases. Previous work from our laboratory indicated that peroxynitrite alters vasoactive pathways in endothelial cells, which could potentially reduce vascular relaxation. To test this hypothesis in vivo, we utilized an animal model of endogenous oxidative stress, the CuZn superoxide dismutase (SOD) knockout mouse, to assess vascular function. *Methods:* Vascular reactivity of mouse mesenteric arteries was assessed in the presence or absence of inhibitors to nitric oxide synthase (NOS) and/or prostaglandin H synthase (PGHS). Endothelial-dependent function was also measured after the addition of exogenous SOD. Peroxynitrite formation was detected by nitrotyrosine immunofluorescence in mesenteric arteries. *Results:* Our data indicate that endothelial-dependent relaxation responses to methacholine are highly reduced in SOD−/− mice ($P<0.01$, ANOVA). In only the wild-type mice, NOS or PGHS inhibition significantly blunted relaxation, suggesting that vasodilators from these pathways are present only in the controls and not in SOD−/− mice. A combination of NOS and PGHS inhibitors reduced methacholine relaxation in both wild-type and SOD−/− mice. This residual EDHF-like relaxation was not different between groups. After incubation with exogenous SOD, endothelial-dependent relaxation could be partially restored in SOD−/− mice, due to increased NOS-mediated vasodilation. In addition, peroxynitrite formation was significantly elevated in mesenteric arteries from SOD−/− mice. *Conclusion:* Our data suggest that in a novel animal model of oxidative stress, vessel function is compromised due to alterations in NOS and PGHS-dependent relaxation responses.
© 2003 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Endothelium; Mesenteric artery; Superoxide dismut; SE knockout mouse; Vascular function; NOS; PGHS

1. Introduction

Oxidative stress occurs when biological systems encounter either an excess of pro-oxidants and/or have reduced anti-oxidant capacity, and has been hypothesized to play a role in the pathophysiology of cardiovascular disease [1]. Superoxide anion is a key mediator in many of the deleterious actions of reactive oxygen species. The superoxide radical itself has been implicated in mediating vascular dysfunction [2], but superoxide anion also initiates the formation of many reactive oxygen species. For example,

superoxide anion is the precursor to hydrogen peroxide, which can be hydrolyzed into the potent hydroxyl radical, an initiator of lipid peroxidation. Further, superoxide anion scavenges nitric oxide, forming peroxynitrite, which occurs at a rate significantly more rapid than the reaction of superoxide with the enzyme superoxide dismutase (SOD) [3,4].

The reaction of superoxide with nitric oxide reduces the bioavailability of nitric oxide as a vasodilator. Additionally, peroxynitrite itself substantially impacts the vascular system. We previously found that peroxynitrite affects endothelial cells in culture by reducing protein levels of prostacyclin synthase, as well as by inducing nuclear translocation of the oxidant-sensitive transcription factor, NFκ-B [5]. Other laboratories have found that peroxynitrite activates the enzyme upstream of prostacyclin synthase, prostaglandin H synthase (PGHS) which also produces the vasoconstrictor thrombox-

ane, in addition to prostacyclin [6]. Overall, these alterations may shift the balance between dilators and constrictors, leading to a reduced capacity for vasorelaxation.

Pro-oxidants are typically maintained at low intracellular levels by both enzymatic and nonenzymatic mechanisms. Pertinent to our study, superoxide dismutase (SOD) is the enzyme responsible for converting the free radical superoxide anion into hydrogen peroxide, which is then neutralized by catalase into water [7]. SOD exists as three isoforms; SOD-1 is copper–zinc dependent and localized to the cytosol (CuZn SOD), SOD-2 is manganese dependent and found in the mitochondria (Mn SOD) and there is also an extracellular SOD (EC-SOD) [1]. The Mn SOD isoform plays a critical role in the protection from oxidants inside the mitochondria, which produces copious amounts during cellular respiration. Although homozygous knockout Mn SOD mice die within the first 10 days of life, [8], the phenotype of the heterozygotes includes increased susceptibility to oxygen toxicity [9] as well as altered cardiac mitochondrial function and increased oxidative injury [10]. On the other hand, the cytosolic isoform, CuZn SOD scavenges superoxide that has been produced from enzymatic processes, such as NADPH oxidase [11]. Thus, the CuZn SOD $-/-$ mouse constitutes a proficient model to assess the influence of elevated superoxide anion, in vivo, on vascular function.

Based on the importance of oxidative stress in the pathogenesis of vascular dysfunction, we sought to determine the effect of increased cytosolic superoxide radicals on blood vessel reactivity, focussing on endothelial cell vasoactive pathways that are altered by pro-oxidant molecules. We hypothesized that resistance-sized blood vessels from SOD $-/-$ mice will have reduced endothelial-mediated relaxation and enhanced vasoconstriction, due to impaired PGHS and nitric oxide-dependent mechanisms.

2. Materials and methods

2.1. Animal housing and protocols

Heterozygous pair of B6, 129S-SOD1 mice were purchased from Jackson Laboratories (Bar Harbour, ME), and were subsequently bred at our facility. Mice were housed in a virus-antigen free environment, kept at 20% humidity, under 12 h light/dark cycles and fed standard laboratory chow and water ad libitum. Genotype was determined at the University of Alberta Transgenic Facility using specific primers for the mutated gene. Experimental groups consisted of wild type (control; females $n = 6$ and males $n = 8$) vs. homozygous knockout littermates (SOD $-/-$; females $n = 5$ and males $n = 7$). Initial experiments considered males and females as separate groups, but after data analysis, we found that there was not a statistical difference, thus data from both groups were pooled. Mice were used between 5 and 7 months of age. All protocols used

for this study were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and were in accordance with the Canadian Council on Animal Care.

2.2. Vessel function

This study focuses on the characterization of vascular reactivity in mesenteric arteries, since they are small resistance sized arteries that contribute substantially to overall peripheral vascular resistance [12]. Mesenteric arcades were removed from the animal after cervical dislocation and placed immediately into ice-cold Dulbecco Modified Essential Medium (DMEM) buffer (1 mmol/l sodium pyruvate, 25 mmol/l sodium bicarbonate, 5 mmol/l HEPES, 5 mmol/l D + glucose; pH 7.4) which was used to maintain vessel viability [13]. Second-order mesenteric arteries (~ 150 μm) were cleaned free of fat and connective tissue under a light microscope. After threading with two smooth 20 μm tungsten wires, vessels were mounted in an isometric wire myograph system (Kent Scientific, Litchfield, CT), warmed to 37 °C (bathed in 5 ml of DMEM buffer) and equilibrated for 30 min.

2.3. Myograph protocols

At the start of each experiment, vessel length was measured using a micrometer and a passive circumference–tension curve was performed for each vessel to determine the optimum resting tension. Cumulative dose response curves were initially performed for phenylephrine (1–50 μmol/l). Vessels were used for subsequent relaxation curves if they attained a minimum of 1.0 mN/mm of tension in response to phenylephrine. Phenylephrine dose response curves were repeated after a 20 min pre-incubation with either N^G-nitro-L-arginine methyl ester (L-NAME; 100 μmol/l; nitric oxide synthase (NOS) inhibitor) or meclofenamate (1.0 μmol/l, PGHS inhibitor). The concentration of phenylephrine required to produce an 80% response (EC_{80}) was used to pre-constrict the vessels for subsequent vasodilation curves because this dose provides a greater range over which relaxation could be assessed. Methacholine (muscarinic agonist; endothelial-dependent vasodilator) dose response curves (10 nmol/l–10 μmol/l) were performed alone or after a 20-min incubation with L-NAME alone, meclofenamate (MECLO) alone, L-NAME and MECLO together, or L-NAME, MECLO plus inhibitors of calcium-sensitive potassium channels (apamine, 10 μmol/l and charybdotoxin, 0.1 μmol/l).

In a separate set of experiments, methacholine curves were also assessed after a 1-h pre-incubation with the soluble polyethylene glycol–SOD (PEG–SOD; 50 units/ml) either alone or in combination L-NAME (20 min, 100 μM). Relaxation responses were also measured over a range of doses (1.0 nmol/l–10 μmol/l) to an exogenous nitric oxide donor, sodium nitroprusside (SNP), as well as a stable prostacyclin

analogue, epoprostenol. All drugs, unless otherwise stated, were purchased from Sigma (St. Louis, MO).

2.4. Immunohistochemistry for peroxynitrite formation and vasoactive enzymes

At the time of dissection, mesenteric arteries that were not used for myograph experiments were fixed with 4% formaldehyde, and dehydrated overnight in 30% sucrose at 4 °C. Arteries were embedded in tissue freezing compound (O.C.T. Tissue Tek™, Fisher Scientific) and sectioned using a cryostat (−25 °C) at a thickness of 10 μm. The immunostaining protocol is described in brief. Slides were fixed with acetone, and blocked for 30 min at room temperature with 2% bovine serum albumin (BSA) in PBS (pH 7.4). Sections were then incubated with rabbit polyclonal anti-nitrotyrosine (1:100; Transduction Laboratories, San Diego, CA), prostacyclin synthase, PGHS-1 (1:200; Cayman Chemicals, Ann Arbor, MI) or eNOS (1:200; Santa Cruz, CA) overnight at 4 °C. The anti-rabbit secondary antibody, containing a rhodamine-TRITC fluorescent tag, was incubated for 40 min at room temperature. Sections were mounted with Vectashield H-1200 solution (containing DAPI, Burlingame, CA) and slides were sealed. For detection of nitrotyrosine fluorescence in mesenteric arteries, images were captured using an Olympus Microscope System (Model BX40) with Reflected Light Fluorescence Attachment (Model BX-FLA) and analyzed with Image Pro-Plus software (Media Cybernetics, Silver Spring, MD). Negative controls were performed by incubating slides either without primary or secondary antibody, or incubating with excess nitrotyrosine (10 μM in PBS, Sigma) before the addition of primary antibody [14]. All negative controls showed minimal immunofluorescence.

2.5. Data analysis and statistics

Dose response curves are graphically depicted as either percent relaxation or constriction and each point represents mean ± standard error of the mean. EC_{50} concentrations were calculated and compared between groups for the phenylephrine, SNP and epoprostenol responses using either a Student's t-test or a One Way Analysis of Variance (ANOVA), where appropriate. However, since methacholine-induced responses were non-sigmoidal in shape and because of the variability in the slopes of the curves, we were not able to use EC_{50} doses for comparison. Thus, statistical significance between wild type and SOD−/− mice, as well as the effect of inhibitors for methacholine relaxation was determined using a Two-way ANOVA for repeated measures, followed by a Tukey post hoc test. Statistical significance was considered at $P < 0.05$. Immunofluorescence for nitrotyrosine was quantified using Sigma Scan 5.0 software, which measured mean optical intensity of the red fluorescence in mesen-
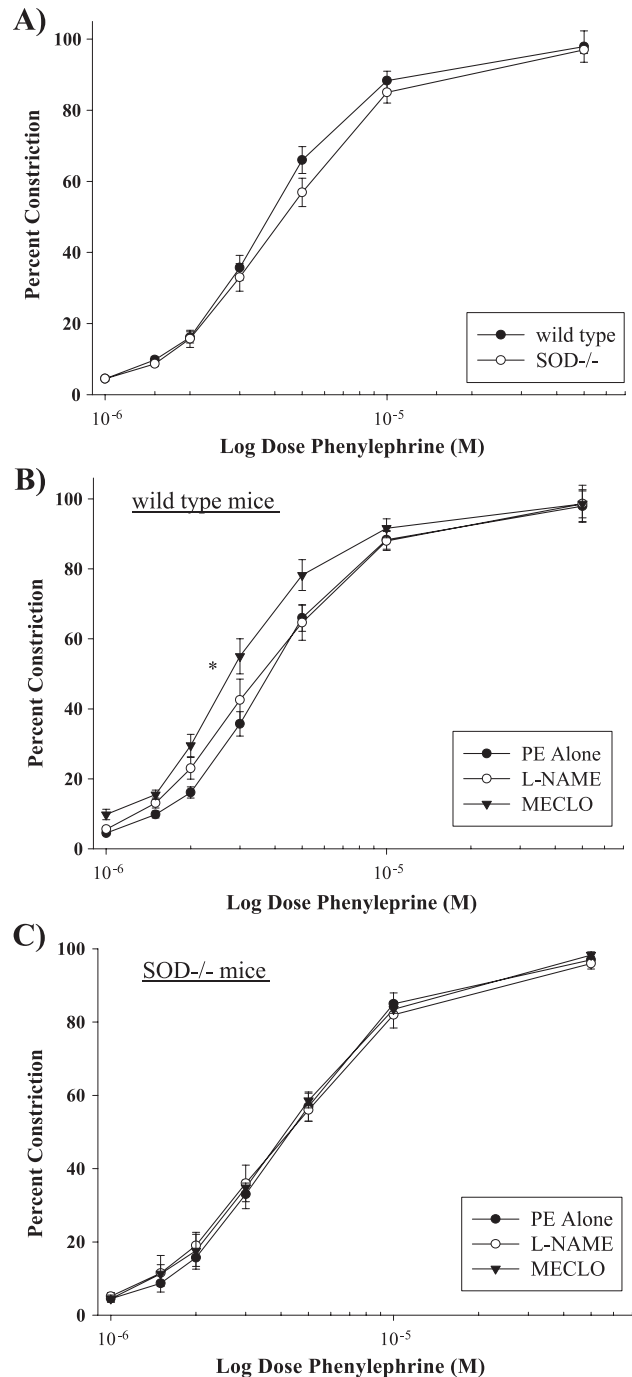

Fig. 1. (Panel A) Concentration response curves to phenylephrine in wild type (●; $n = 14$) and SOD−/− (○; $n = 12$) mice. Phenylephrine-induced vasoconstriction in wild type mice (Panel B) and SOD−/− mice (Panel C) in the presence or absence of NAME (○; 100 μmol/l) or meclofenamate (MEC; ▼; 1.0 μmol/l). Responses are expressed as percent constriction and each value represents the mean ± S.E.M. *$P < 0.05$ for PE alone vs. MECLO in wild type mice.

teric vessels. These data were normalized for vessel size by dividing the optical intensity by area and data are expressed using this ratio. Statistical analysis was performed using a Student's t-test, $P < 0.05$.

3. Results

3.1. Vascular reactivity to phenylephrine in SOD−/− and wild type mesenteric arteries

There were no significant differences in overall sensitivity to phenylephrine between wild type and SOD − / − mice
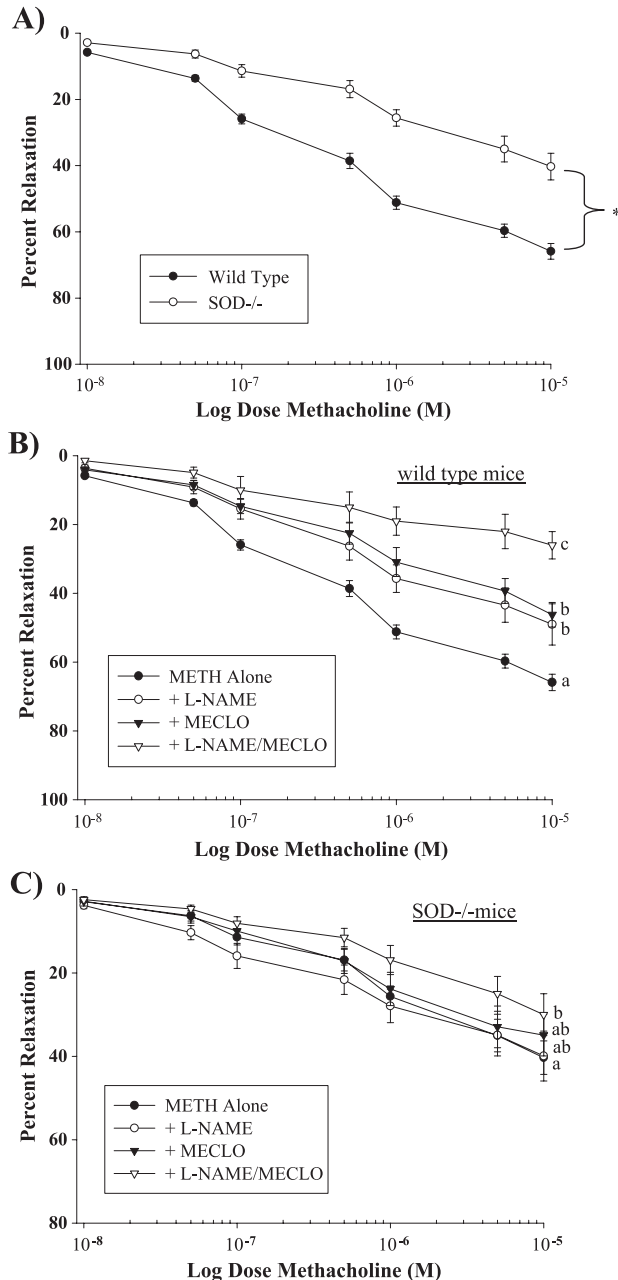

Fig. 2. (Panel A) Methacholine concentration response curves in wild type mice (●; $n = 14$) and SOD − / − (○; $n = 12$) mice. Mesenteric artery relaxation in wild type mice (Panel B) and SOD − / − mice (Panel C) in the presence or absence of L-NAME (○; 100 μmol/l) or meclofenamate (MECLO; ▼; 1.0 μmol/l) or both L-NAME and MECLO (▽). Responses are expressed as percent relaxation and each value represents the mean ± S.E.M. *$P < 0.05$ vs. wild type. Different letters indicate statistically different dose response curves at $P < 0.05$.
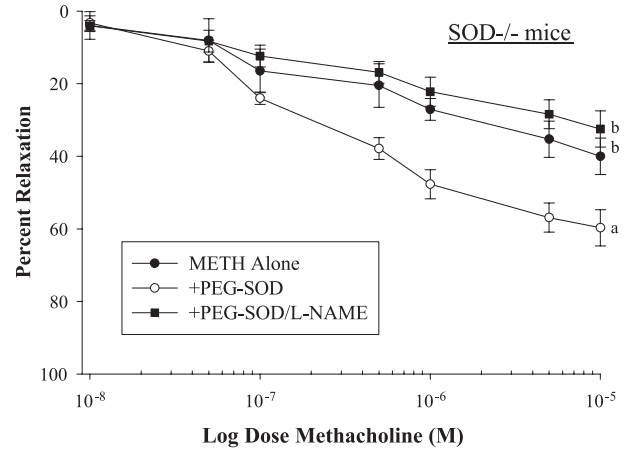

Fig. 3. Endothelial-dependent relaxation in mesenteric arteries from SOD − / − mice after 1-h incubation with PEG–SOD. Methacholine-induced relaxation in vessels from SOD − / − mice *before* PEG–SOD (●; $n = 6$), *after* PEG–SOD alone (○; $n = 6$) and after both PEG–SOD and L-NAME (■; 20 min, 100 μM; $n = 5$). Responses are expressed as percent relaxation and each value represents the mean ± S.E.M. Different letters indicate statistically significant dose response curves at $P < 0.05$.

(EC$_{50}$ values: 4.53 ± 0.45 μmol/l vs. 5.03 ± 0.51 μmol/l; Fig. 1A). Interestingly, the effect of PGHS inhibition on vasoconstriction was different between groups. In wild type mice, pre-incubation with MECLO (PGHS inhibitor) significantly enhanced the mesenteric sensitivity to phenylephrine, as indicated by a lower EC$_{50}$ concentration (EC$_{50}$: 3.18 ± 0.20 μmol/l vs. 4.53 ± 0.45 μmol/l; $P < 0.05$; Fig. 1B) suggesting modulation by a PGHS-dependent vasodilator. In contrast, MECLO had no effect on the phenylephrine dose response curve in SOD − / − mice (EC$_{50}$: 4.48 ± 0.41 μmol/l vs. 5.03 ± 0.51 μmol/l; Fig. 1C). There was a trend towards enhanced sensitivity to phenylephrine in wild type mice after L-NAME treatment; however, this effect did not reach statistical significance (EC$_{50}$: 3.55 ± 0.32 μmol/l vs. 4.53 ± 0.45 μmol/l; $P = 0.09$; Fig. 1B). Pre-incubation with the NOS inhibitor did not shift the constrictor response in SOD − / − mice (EC$_{50}$: 4.85 ± 0.32 μmol/l vs. 5.03 ± 0.51 μmol/l; Fig. 1C).

3.2. Endothelial-dependent relaxation in SOD−/− and wild type mice; role of NOS and PGHS

Methacholine-induced relaxation was significantly impaired in SOD − / − mice compared to wild type mice (Fig. 2A). In wild type mice, methacholine relaxation was significantly blunted after incubation with either L-NAME or MECLO (Fig. 2B). Conversely, in mesenteric arteries from SOD − / − mice, neither L-NAME nor MECLO had a significant effect on methacholine induced relaxation (Fig. 2C). These data suggest that NOS and PGHS-mediated vasorelaxation in SOD − / − mice is compromised.

By incubating vessels with a combination of both L-NAME and MECLO, we assessed the remaining component of endothelial-dependent relaxation. In mesenteric

arteries from both wild type and SOD $-/-$ mice, blocking NOS and PGHS significantly reduced methacholine-induced relaxation (Fig. 2B and C). However, this residual relaxation was not statistically different between groups (maximum relaxation: $26 \pm 4.3\%$ vs. $29 \pm 5.0\%$, $P = 0.6$). We also incubated mesenteric vessels from both wild type and SOD $-/-$ mice with a combination of apamine and charybdotoxin (in the presence of L-NAME and MECLO) and these inhibitors completely blocked relaxation (data not shown), indicating that the remaining relaxation was indeed

mediated by a factor(s) that acts on potassium channels (EDHF-like).

In order to determine whether the impaired endothelial-dependent relaxation in SOD $-/-$ mice was due to acute superoxide production leading to scavenging of nitric oxide, we treated mesenteric vessels with an exogenous cell permeable SOD (PEG–SOD) alone or in combination with L-NAME. Our data illustrate that pre-incubation with PEG–SOD was able to significantly enhance methacholine-induced relaxation in vessels from SOD $-/-$ mice.
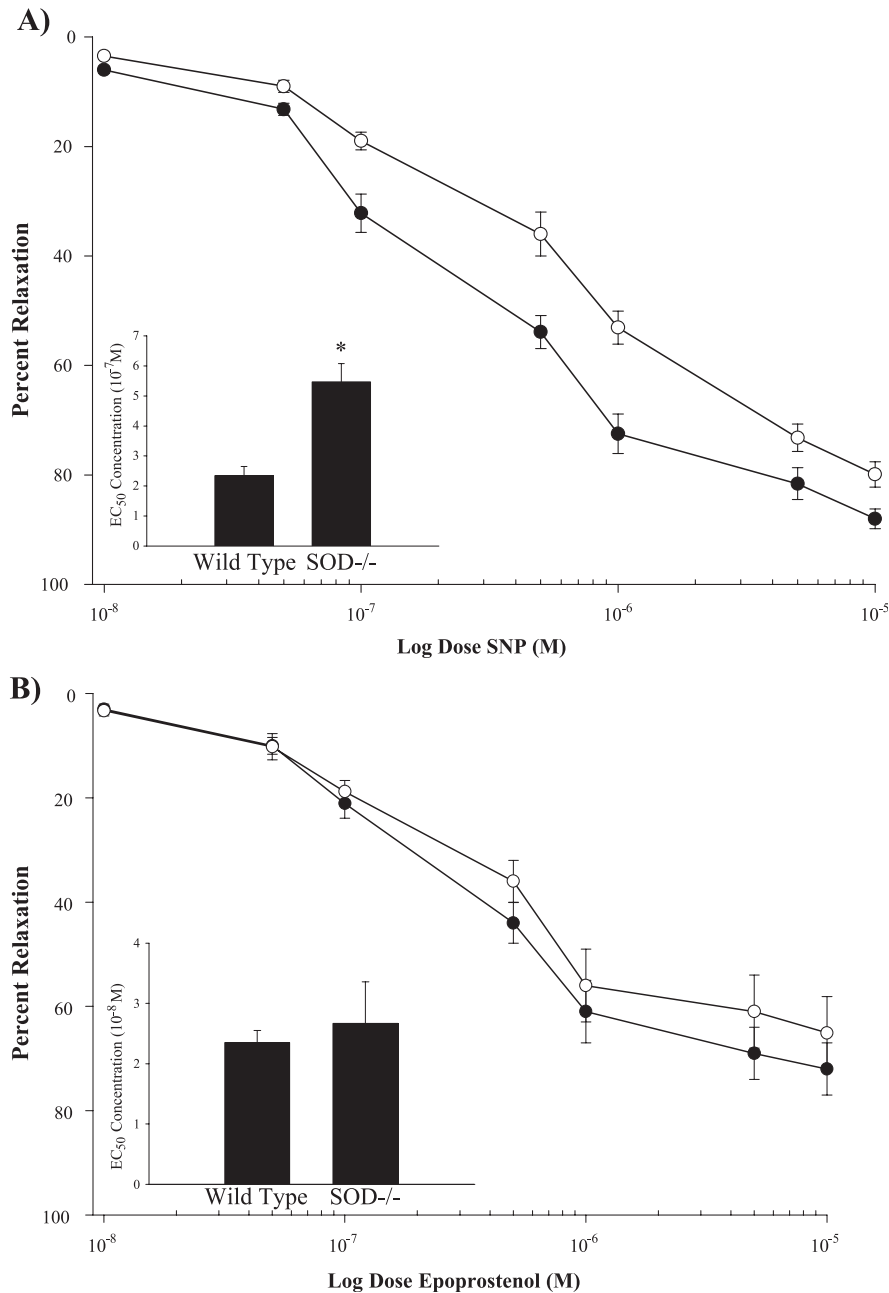
Fig. 4. Concentration response curves to endothelial-independent agonists. (Panel A) Sodium nitroprusside (SNP) induced relaxation in wild type (●; $n = 14$) and SOD $-/-$ (○; $n = 12$) mice. (Panel B) Epoprostenol-induced relaxation in wild type (●; $n = 6$) and SOD $-/-$ (○; $n = 6$) mice. Responses are expressed as percent relaxation and each value represents the mean $\pm$ S.E.M. Inset: bars indicate average EC$_{50}$ doses for wild type and SOD $-/-$ mice. *$P < 0.05$ vs. wild type.
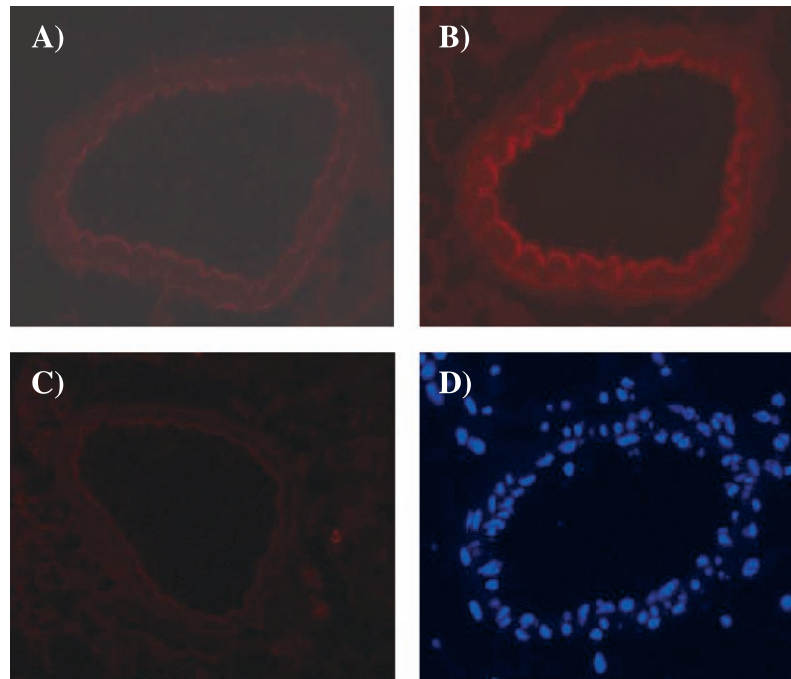
Fig. 5. Immunohistochemistry for nitrotyrosine, a marker of peroxynitrite formation. Representative sections of mesenteric arteries from (A) wild type mice ($n = 5$) and (B) SOD $-/-$ mice ($n = 5$) incubated overnight with rabbit polyclonal anti-nitrotyrosine antibody. Panel C is a negative control, which is a section incubated without primary antibody and panel D is the corresponding DAPI stained nuclei, in order to illustrate presence of the blood vessel. All images were taken at $100 \times$ magnification.

Furthermore, administering L-NAME to vessels treated with PEG–SOD blunted the enhanced relaxation, such that the relaxation was not different from control curves (Fig. 3). Interestingly, administering PEG–SOD to vessels from wild type mice caused a reduction in methacholine induced relaxation (maximum relaxation: $56.2 \pm 4.0\%$ vs. $22.9 \pm 3.2\%$; $P < 0.05$, data not shown). These data suggest that physiological, low levels of superoxide anion are important for mediating endothelial-dependent vascular functions [15–17].

3.3. Effect of exogenous nitric oxide and prostacyclin on mesenteric relaxation

Mesenteric arteries from wild type mice were significantly more sensitive to the nitric oxide donor, SNP, compared to arteries from SOD $-/-$ mice (EC$_{50}$: 0.24 ± 0.03 μmol/l vs. 0.65 ± 0.04 μmol/l; $P < 0.05$; Fig. 4A). In order to determine whether this effect was specific to nitric oxide, we also used the prostacyclin agonist, epoprostenol. Our data show that the responses to the prostacyclin analogue were not significantly different between groups (EC$_{50}$: 0.24 ± 0.02 nmol/l vs. 0.27 ± 0.06 nmol/l; Fig. 4B), suggesting that nitric oxide specifically is being interfered with in SOD $-/-$ mice.

3.4. Detection of peroxynitrite formation in SOD$-/-$ mice

Using fixed sections of mesenteric arteries, we performed immunohistochemistry for nitrotyrosine, which is a marker of

peroxynitrite formation [18]. Our data illustrate that peroxynitrite formation was elevated in mesenteric vessels of SOD $-/-$ mice (Fig. 5B) when compared to wild type mice (Fig. 5A). The mean intensity of immunofluorescence in wild type mice was 0.28 ± 0.03 compared with 0.78 ± 0.14 in vessels from SOD $-/-$ mice ($P < 0.05$; $n = 5$). In SOD $-/-$ mice, nitrotyrosine immunostaining was evident mainly in the endothelium, but was also present in vascular smooth muscle. Incubation of sections without primary antibody result in a very low signal (Fig. 5C) and was consistent for all other negative controls performed. Using immunohistochemistry, we also measured expression of the enzymes PGHS-1, prostacyclin synthase and eNOS in the mesenteric arteries from wild type and SOD $-/-$ mice and no differences between groups were detected (data not shown).

4. Discussion

This study utilized the CuZn SOD knockout mouse to highlight mechanisms behind oxidant-induced vascular dysfunction. We demonstrated that in this model, in which peroxynitrite levels are elevated, vascular relaxation is significantly impaired, due to reduced PGHS and nitric oxide-dependent vasodilation. Furthermore, although we did not observe an overall difference in vasoconstrictor responses between the two groups, PGHS inhibition increased phenylephrine sensitivity only in wild type mice, which corroborates our conclusion that SOD $-/-$

mice have an impaired PGHS modulation of vascular reactivity, in addition to the reduced nitric oxide-dependent vasorelaxation.

Our hypothesis that oxidative stress is mediating the vascular dysfunction in SOD − / − mice is supported by evidence that after pre-incubation with PEG–SOD, methacholine-induced relaxation in SOD − / − mice was enhanced. The use of PEG–SOD to restore oxidant-induced vascular dysfunction has been used in a mouse model of diabetes, where it was also effective at reversing the deleterious effects of superoxide anion [19]. In our study, we speculate that the acute removal of superoxide anions reduces scavenging of nitric oxide, thus increasing its bioactivity as a vasodilator. Supporting this theory, relaxation in PEG–SOD-treated vessels was significantly reduced after incubation with L-NAME, whereas in untreated vessels from SOD − / − mice, L-NAME did not affect methacholine-induced relaxation. Thus, exogenous SOD enhanced NOS-mediated relaxation in knockout mice. However, it is likely that chronic elevated levels of pro-oxidants also alter enzymes important to vascular function (such as prostacyclin synthase and/or eNOS), hence acute PEG–SOD could not fully compensate for these changes. Indeed, relaxation in vessels from SOD − / − mice was only partially restored after PEG–SOD treatment and did not vasodilate to the levels observed in wild type mice.

Our data illustrates highly impaired endothelial-dependent relaxation, and is in agreement with a recent study that observed a significantly reduced response to a bolus administration of acetylcholine in the carotid artery of CuZn SOD − / − mice [20]. Further, in other animal models of oxidative stress (glutathione peroxidase gene (GPx-1) knockout mouse and apolipoprotein E knockout mouse) endothelial-dependent relaxation was found to be impaired, which also supports the findings of the current study [21,22]. However, the contribution of PGHS and nitric oxide in modulating the vascular reactivity was not demonstrated in these studies.

Our results illustrate that PGHS-mediated relaxation is compromised in mesenteric vessels from SOD − / − mice and thus, is partially responsible for the altered vascular reactivity. We speculate that the mechanism behind this reduced PGHS-mediated dilation is a decrease in prostacyclin (the predominant vasodilator produced by PGHS in mesenteric arteries) [23]. In the current study, we observed no difference in relaxation responses to exogenous prostacyclin, supporting the conclusion that production of a PGHS-dependent vasodilator is indeed reduced in SOD − / − mice, rather than the vessels being less sensitive to prostacyclin. In vessels from SOD − / − mice, we found increased evidence of nitrotyrosine, a marker of peroxynitrite. Evidence suggests that this reactive nitrogen species can alter the production of prostacyclin through a variety of mechanisms. For example, our laboratory found that peroxynitrite reduces protein levels of prostacyclin synthase in endothelial cells [5]. Additionally, Zou et al. [24,25] have extensively described how peroxynitrite inhibits the activity of prostacyclin synthase through a tyrosine nitrosylation-dependent reaction. In mesenteric arteries from SOD − / − mice, we did not detect reduced levels of prostacyclin synthase, suggesting that in this model, inhibition of prostacyclin synthase activity by peroxynitrite may be responsible for the reduced PGHS-dependent vasodilation.

Nitric oxide mediated vasodilation is also compromised in SOD − / − mice, as evidenced by reduced NOS-dependent endothelial-mediated relaxation and reduced sensitivity to the nitric oxide donor sodium nitroprusside. It is possible that nitric oxide is simply unavailable since it is being scavenged, forming peroxynitrite. However, reactive nitrogen species can also alter other aspects of the NOS pathway. For example, peroxynitrite can decrease the activity of eNOS via either directly uncoupling (oxidation) the zinc cluster of eNOS [26] or by the destabilization of tetrahydrobiopterin, an important co-factor for NOS activity [27]. These effects on eNOS activity may be involved in the impaired endothelial-dependent relaxation in mesenteric arteries from SOD − / − mice, since eNOS protein levels were not different between SOD − / − and wild type mice. Our conclusion is in agreement with other mouse models in which NOS-dependent relaxation was impaired [28]. Therefore, the mechanism(s) by which reactive nitrogen species impair vascular function are likely complex, affecting many different pathways involved in vascular relaxation.

EDHF-mediated relaxation has been described as endothelial-dependent relaxation that is not inhibited by NOS and PGHS antagonists, but is inhibited by potassium channel blockers [29]. In the SOD − / − mice, the magnitude of relaxation that is dependent on an EDHF-like factor was not different from arteries from wild type mice. This is in contrast to other models of vascular dysfunction, in which it is suggested that an EDHF-like factor is compensating, including in vessels from eNOS knockout mice [30], women with preeclampsia [31] and hypercholesterolemic rabbits [32].

In conclusion, our data illustrate that CuZn SOD knockout mice have reduced NOS and PGHS-dependent modulation of vascular reactivity. These abnormalities may be due to increased peroxynitrite-induced endothelial cell dysfunction, which is also indicative of a reduced capacity of nitric oxide to induced vasodilation. The findings of this study are relevant to clinical conditions of vascular dysfunction, because reduced SOD expression and activity (and increased peroxynitrite formation) are associated with preeclampsia [33,34], aging [35,36], atherosclerosis [37,38] and diabetes [39,40]. Using the CuZn SOD knockout mouse as a tool to better understand the mechanisms of oxidant-induced endothelial cell dysfunction provides new opportunities to investigate novel therapeutic targets for the treatment and prevention of vascular disease.

Acknowledgements

References

[1] Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. Arch Biochem Biophys 2001;389: 84–93.

[2] Fridovich I. Biological effects of the superoxide radical. Arch Biochem Biophys 1986;247:1–11.

[3] Saran M, Michel C, Bors W. Reaction of NO with O_2^-. Implications for the action of endothelium-derived relaxing factor (EDRF). Free Radic Res Commun 1990;10:221–6.

[4] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996;271:C1424–37.

[5] Cooke CL, Davidge ST. Peroxynitrite increases iNOS through NF-kappaB and decreases prostacyclin synthase in endothelial cells. Am J Physiol, Cell Physiol 2002;282:C395–402.

[6] Landino LM, Crews BC, Timmons MD, Morrow JD, Marnett LJ. Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis. Proc Natl Acad Sci U S A 1996;93:15069–74.

[7] Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 1999;31:261–72.

[8] Li Y, Huang TT, Carlson EJ, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 1995;11:376–81.

[9] Tsan MF, White JE, Caska B, Epstein CJ, Lee CY. Susceptibility of heterozygous MnSOD gene-knockout mice to oxygen toxicity. Am J Respir Cell Mol Biol 1998;19:114–20.

[10] Van Remmen H, Williams MD, Guo Z, et al. Knockout mice heterozygous for Sod2 show alterations in cardiac mitochondrial function and apoptosis. Am J Physiol, Heart Circ Physiol 2001;281: H1422–32.

[11] Wolin MS, Gupte SA, Oeckler RA. Superoxide in the vascular system. J Vasc Res 2002;39:191–207.

[12] Christensen KL, Mulvany MJ. Mesenteric arcade arteries contribute substantially to vascular resistance in conscious rats. J Vasc Res 1993;30:73–9.

[13] Loutzenhiser R, Bidani A, Chilton L. Renal myogenic response: kinetic attributes and physiological role. Circ Res 2002;90:1316–24.

[14] Thuraisingham RC, Nott CA, Dodd SM, Yaqoob MM. Increased nitrotyrosine staining in kidneys from patients with diabetic nephropathy. Kidney Int 2000;57:1968–72.

[15] Pomposiello S, Rhaleb NE, Alva M, Carretero OA. Reactive oxygen species: role in the relaxation induced by bradykinin or arachidonic acid via EDHF in isolated porcine coronary arteries. J Cardiovasc Pharmacol 1999;34:567–74.

[16] Sobey CG, Heistad DD, Faraci FM. Mechanisms of bradykinin-induced cerebral vasodilatation in rats. Evidence that reactive oxygen species activate K+ channels. Stroke 1997;28:2290–4 [discussion 2295].

[17] McIntyre M, Bohr DF, Dominiczak AF. Endothelial function in hypertension: the role of superoxide anion. Hypertension 1999;34:539–45.

[18] Tarpey MM, Fridovich I. Methods of detection of vascular reactive species: nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite. Circ Res 2001;89:224–36.

[19] Gunnett CA, Heistad DD, Faraci FM. Interleukin-10 protects nitric

[20] Didion SP, Ryan MJ, Didion LA, et al. Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. Circ Res 2002; 91:938–44.

[21] Forgione MA, Weiss N, Heydrick S, et al. Cellular glutathione peroxidase deficiency and endothelial dysfunction. Am J Physiol, Heart Circ Physiol 2002;282:H1255–61.

[22] Godecke A, Ziegler M, Ding Z, Schrader J. Endothelial dysfunction of coronary resistance vessels in apoE−/− mice involves NO but not prostacyclin-dependent mechanisms. Cardiovasc Res 2002;53: 253–62.

[23] Lennon EA, Poyser NL. Production of prostaglandins I2, E2 and F2 alpha by blood vessels of normotensive and hypertensive, male and female rats. Prostaglandins Leukot Med 1986;25:71–89.

[24] Zou M, Yesilkaya A, Ullrich V. Peroxynitrite inactivates prostacyclin synthase by heme-thiolate-catalyzed tyrosine nitration. Drug Metab Rev 1999;31:343–9.

[25] Zou M, Martin C, Ullrich V. Tyrosine nitration as a mechanism of selective inactivation of prostacyclin synthase by peroxynitrite. Biol Chem 1997;378:707–13.

[26] Zou MH, Shi C, Cohen RA. Oxidation of the zinc–thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest 2002;109:817–26.

[27] Kohnen SL, Mouithys-Mickalad AA, Deby-Dupont GP, et al. Oxidation of tetrahydrobiopterin by peroxynitrite or oxoferryl species occurs by a radical pathway. Free Radic Res 2001;35:709–21.

[28] d'Uscio LV, Baker TA, Mantilla CB, et al. Mechanism of endothelial dysfunction in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2001;21:1017–22.

[29] Campbell WB, Harder DR. Prologue: EDHF—what is it? Am J Physiol, Heart Circ Physiol 2001;280:H2413–6.

[30] Waldron GJ, Ding H, Lovren F, Kubes P, Triggle CR. Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. Br J Pharmacol 1999;128:653–8.

[31] Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR. Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and preeclampsia. Clin Sci (Lond) 2002;103:67–73.

[32] Najibi S, Cowan CL, Palacino JJ, Cohen RA. Enhanced role of potassium channels in relaxations to acetylcholine in hypercholesterolemic rabbit carotid artery. Am J Physiol 1994;266:H2061–7.

[33] Roggensack AM, Zhang Y, Davidge ST. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. Hypertension 1999;33:83–9.

[34] Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in normal and preeclamptic placentas. J Soc Gynecol Investig 1996;3:179–84.

[35] van der Loo B, Labugger R, Skepper JN, et al. Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med 2000;192: 1731–44.

[36] Jun T, Ke-yan F, Catalano M. Increased superoxide anion production in humans: a possible mechanism for the pathogenesis of hypertension. J Hum Hypertens 1996;10:305–9.

[37] Luoma JS, Stralin P, Marklund SL, et al. Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions: colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. Arterioscler Thromb Vasc Biol 1998;18:157–67.

[38] White CR, Brock TA, Chang LY, et al. Superoxide and peroxynitrite in atherosclerosis. Proc Natl Acad Sci U S A 1994;91:1044–8.

[39] Rosen P, Ballhausen T, Stockklauser K. Impairment of endothelium dependent relaxation in the diabetic rat heart: mechanisms and implications. Diabetes Res Clin Pract 1996;(Suppl. 31):S143–55.

[40] Hattori Y, Kawasaki H, Abe K, Kanno M. Superoxide dismutase recovers altered endothelium-dependent relaxation in diabetic rat aorta. Am J Physiol 1991;261:H1086–94.