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Nitric Oxide and Blood Pressure in Mice Lacking Extracellular-Superoxide Dismutase

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Nitric oxide is a major vasorelaxant and regulator of the blood pressure. The blood vessels contain several active sources of the superoxide radical, which reacts avidly with nitric oxide to form noxious peroxynitrite. There are large amounts of extracellular-superoxide dismutase (EC-SOD) in the vascular wall. To evaluate the importance of EC-SOD for the physiology of nitric oxide, here we studied the blood pressure in mice lacking the enzyme. In chronically instrumented non-anaesthetized mice there was no difference in mean arterial blood pressure between wild-type controls and EC-SOD mutants. Extensive inhibition of nitric oxide synthases with *N*-monomethyl-L-arginine however resulted in a larger increase in blood pressure, and infusion of the nitric oxide donor nitrosoglutathione caused less reduction in blood pressure in the EC-SOD null mice. We interpret the alterations to be caused by a moderately increased consumption of nitric oxide by the superoxide radical in the EC-SOD null mice. One role of EC-SOD may be to preserve nitric oxide, a function that should be particularly important in vascular pathologies, in which large increases in superoxide formation have been documented.

Keywords: Nitric oxide; Superoxide radical; Blood pressure; Oxidative stress; Blood vessels

INTRODUCTION

The free radical nitric oxide (NO) exerts a variety of physiological actions in the vasculature. NO is primarily formed by the endothelial nitric oxide synthase (NOS) and regulates the blood pressure and flow by causing relaxation of smooth muscle cells.[1,2] Other effects of the NO are reduction of adhesion and activation of both leukocytes[3] and platelets.[4] Under inflammatory conditions cytokines stimulate synthesis of the inducible NOS in a variety of cells in the vasculature. The large amounts of NO formed under such conditions may cause both cytotoxic effects and marked vasodilation.

The superoxide anion radical is formed by several mechanisms in the vascular wall, but its physiological effects are less well understood. The radical may be formed by membrane-bound NAD(P)H oxidases in smooth muscle cells,[5] the endothelium,[6] and adventitial fibroblasts,[7] NOS[8] and by xanthine oxidase.[9] Under pathological conditions there are large increases in superoxide formation from a variety of sources.[10–14] The superoxide radical and NO react extremely rapidly to form the noxious peroxynitrite.[15] Peroxynitrite is a strongly oxidizing and nitrating compound.[16]

Superoxide dismutases (SOD) disproportionate the superoxide radical to form hydrogen peroxide and oxygen. There are three isoenzymes, the cytosolic CuZn-SOD, the mitochondrial matrix Mn-SOD, and the secreted extracellular-SOD (EC-SOD).[17] Owing to limited membrane penetration of the superoxide anion radical, the SOD isoenzymes primarily exert their protective actions in their respective compartments.[18] EC-SOD exists anchored to heparan sulfate proteoglycans and is particularly abundant in the vascular wall and accounts for about half the total SOD activity in both murine and human aorta.[19] EC-SOD also occurs in plasma and forms an equilibrium between the

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plasma phase and the endothelial cell surfaces.[20] An important function of EC-SOD could be to preserve NO against reaction with the superoxide radical in the vascular wall interstitium and in the blood. We have previously shown that EC-SOD bound to aorta *in vitro* augments NO-induced relaxation in the presence of the superoxide radical generator pyrogallol.[21]

Mice lacking EC-SOD have been produced by homologous recombination in embryonic stem cells.[22] In the present study we analyzed some aspects of nitric oxide physiology in EC-SOD null mice and find evidence for increased NO consumption in the vasculature of the mice, but no alteration in the basal blood pressure.

MATERIALS AND METHODS

Generation of EC-SOD Mutant Mice

The EC-SOD null mutant mice and corresponding wild type mice (background genotypes C57Bl/6 × 129/SV) were obtained from a breeding colony established at Umeå University using animals previously generated by homologous recombination.[22] The mice were 6 months old and all male. The study was approved by the animal ethics committee at Umeå University.

Chronic Conscious Instrumentation, Blood Pressure Measurements and Drug Administration

The mice (EC-SOD mutant and wild type) were anaesthetized briefly with 2% isofluorane. A cannula line was implanted in the femoral artery and femoral vein, tunnelled subcutaneously to exit at the upper back and connected to a swivel tether system for continuous registration of blood pressure and drug administration, respectively.[23] Physiological saline containing 25 U/ml heparin was administrated as a continuous infusion via the femoral artery (50 μl/h) and femoral vein (50 μl/h) to maintain patency of cannulae lines. Following recovery from surgery, blood pressure was measured over the following 24 h period. Only animals showing a stable mean arterial blood pressure were entered into the study.

N-monomethyl-L-arginine (L-NMMA) (1–1000 mg/kg body weight intravenously) was administrated as a bolus over 30 s to the mice. The maximum increase in blood pressure was measured over the 20 min period between successive doses of L-NMMA.

Nitrosoglutathione (GSNO) (37.5–600 μg/kg intravenously) was administered as a 3 min infusion. The decrease in blood pressure was recorded over a 30 min period between successive increasing doses of GSNO. The area of blood pressure depression below the basal level was determined by computerized planimetry and was expressed as arbitrary units.

RESULTS

The present study was carried out with conscious non-anaesthetized mice to avoid all the various influences on blood pressure caused by anaesthetics. We started with recording the mean arterial blood pressure (MABP) over 12 h, but found no differences between the wild type and EC-SOD null mice (Fig. 1). Using hourly values over 12 h, the MABP's were 109 ± 3 (SEM) mmHg for wild type mice ($n = 9$) and 107 ± 3 (SEM) mmHg for the null mutant mice ($n = 12$).

Administration of the nitric oxide synthase inhibitor L-NMMA as an intravenous bolus elevated the MABP in a dose-dependent manner in both mouse groups (Fig. 2). At the highest dose, 1000 mg/kg, the response of the EC-SOD null mutant mice was significantly greater than in the wild type mice, $p = 0.028$.

Conversely, the nitric oxide donor GSNO caused with both groups of mice a dose-dependent depression of the blood pressure (Fig. 3). The depression area was numerically smaller in the null mutant mice at all doses and reached statistical significance in some.

DISCUSSION

Reductions in the concentration of NO causes marked increases in blood pressure in many species, as shown both by the effects of NOS inhibitors[24] and

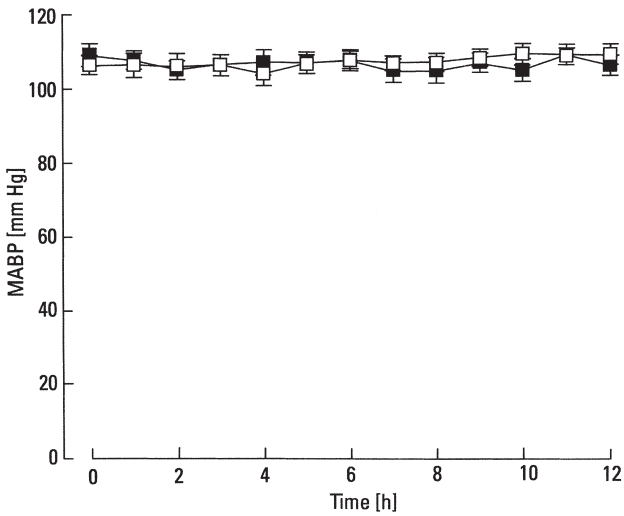

FIGURE 1 Mean arterial blood pressure (MABP) in the conscious mouse. The blood pressure was monitored in chronically instrumented mice as described under "Materials and methods section". ■, indicates results for 9 wild type mice and □, for 12 EC-SOD null mutants. The bars indicate SEM.

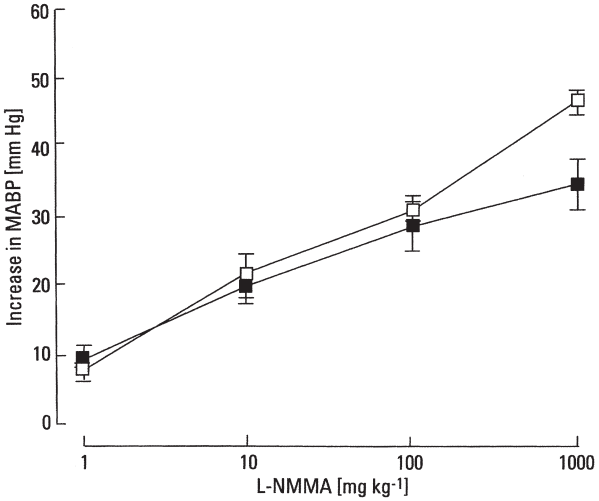

FIGURE 2 Effect on L-NMMA on mean arterial blood pressure. L-NMMA was infused as a bolus, and the maximal increase in MABP for each successive increasing dose recorded in 5 wild type mice (■) and 4 EC-SOD null mutant mice (□). The bars indicate SEM. The difference at the highest dose was statistically significant, $p = 0.028$, Students *T*-test.

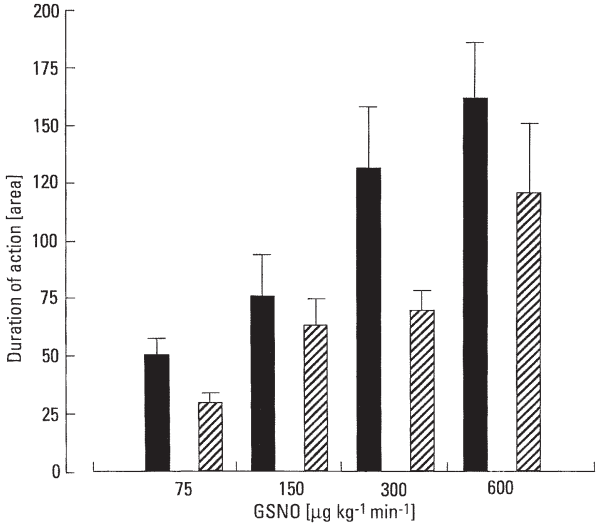

FIGURE 3 Effect of GSNO on mean arterial blood pressure. GSNO was infused over 3 min in 5 wild type mice (black bars) and 4 EC-SOD null mutants (striped bars). The decrease in MABP was recorded and calculated as described under "Materials and methods". The bars indicate SEM. The statistical significances for the differences were 0.04, 0.58, 0.07 and 0.32 (Students *T*-test) for 75, 150, 300 and 600 μg GSNO/kg/min, respectively.

by the increased MABP of mice lacking the endothelial NOS.[25] The effect of NO may be mediated both by relaxation of vascular smooth muscle cells and by transmittor effects in the CNS.[26] In the present study, we found no effect on the blood pressure of the absence of EC-SOD (Fig. 1). If there were an increased consumption of NO in the vasculature -and CNS- of the mice through formation of peroxynitrite, various compensatory mechanisms including possibly increased NO secretion normalized the MABP. To further investigate this issue, we tried to limit NO formation through NOS inhibition by L-NMMA, which caused increases in MABP (Fig. 2). At the highest dose, the increase was larger in the EC-SOD null mice, which might be explained by increased consumption of the little remaining NO. Complicating the interpretation, L-NMMA also increases formation of superoxide from NOS.[27] However that superoxide would probably not reach the extracellular space to any greater extent and be influenced by EC-SOD. Conversely the NO donor GSNO caused a dose-dependent reduction in blood pressure, which through autoregulation should limit endogenous NO formation (Fig. 3). In this situation, the blood pressure reductions were attenuated in the EC-SOD null mice. In the presence of reductants, CuZn-SOD decomposes nitrosothiols under formation of NO.[28] EC-SOD probably also catalyses that reaction since its active site is similar to that of CuZn-SOD.[29] However, the reaction is relatively slow and EC-SOD at the concentration prevalent in the vessel wall would probably not decompose much GSNO under the conditions of the present study. In aggregate, the studies suggest that there is a moderately increased consumption of NO in the EC-SOD null mice. It has been argued that owing to the very high reaction rate between superoxide and NO, the SOD isoenzymes in many situations can poorly compete for available superoxide. However, previous calculations based on the EC-SOD content in the human (and mouse) vascular wall interstitium[19] indicated that the enzyme should be able to compete with the NO levels prevalent under basal conditions, as in the present investigation.

The present study shows that healthy mice in the laboratory environment maintain a normal blood pressure in the absence of EC-SOD. Under a variety of pathological conditions, however, increased rates of superoxide radical formation have been indicated. Thus there is evidence for a markedly increased formation in hypercholesterolemia,[10] in atherosclerotic vessels,[11] in hypertension induced by angiotensin II,[12] in diabetes,[13] in nitroglycerin tolerance[14] and numerous other conditions. Under such circumstances much more NO should be consumed, and EC-SOD might have a more marked role in NO preservation. The vascular dysfunctions caused should mostly involve more local control of vessel tonus and leukocyte and platelet adhesion, than the overall blood pressure. It should also be noted that even if the blood pressure can be controlled by autoregulation including increased NO secretion, the peroxynitrite formed might exert a variety of toxic effects on the vasculature.[16] EC-SOD should under all circumstances reduce such adverse effects. One study on humans including blood pressure has been carried out. In a population

study involving 4500 individuals, plasma EC-SOD levels were lower in men than in women, and low levels were also seen in smokers and individuals with high fibrinogen and low tissue plasminogen activator activity.[30] There was, however, little relation with blood pressure, the only significant correlation was a positive association between diastolic blood pressure in women and the plasma EC-SOD level.

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