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Diesel exhaust exposure enhances venoconstriction via uncoupling of eNOS

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ABSTRACT

Environmental air pollution is associated with adverse cardiovascular events, including increased hospital admissions due to heart failure and myocardial infarction. The exact mechanism(s) by which air pollution affects the heart and vasculature is currently unknown. Recent studies have found that exposure to air pollution enhances arterial vasoconstriction in humans and animal models. Work in our laboratory has shown that diesel emissions (DE) enhance vasoconstriction of mouse coronary arteries. Thus, we hypothesized that DE could enhance vasoconstriction in arteries and veins through uncoupling of endothelial nitric oxide synthase (eNOS). To test this hypothesis, we first bubbled DE through a physiological saline solution and exposed isolated mesenteric veins. Second, we exposed animals, whole body, to DE at 350 μg/m^3 for 4 h, after which mesenteric arteries and veins were isolated. Results from these experiments show that saline bubbled with DE as well as inhaled DE enhances vasoconstriction in veins but not arteries. Exposure to several representative volatile organic compounds found in the DE-exposed saline did not enhance arterial constriction. L-nitro-arginine-methyl-ester (L-NAME), an eNOS inhibitor, normalized the control vessels to the DE-exposed vessels implicating an uncoupling of eNOS as a mechanism for enhanced vasoconstriction. The principal conclusions of this research are 1) veins exhibit endothelial dysfunction following in vivo and ex vivo exposures to DE, 2) veins appear to be more sensitive to DE effects than arteries, and 3) DE components most likely induce endothelial dysfunction through the uncoupling of eNOS.

Introduction

Current epidemiological research has associated environmental air pollution with adverse cardiovascular events. Furthermore, environmental air pollution has been associated with increased incidences of myocardial infarction (Peters et al., 2001), stroke (Villeneuve et al., 2006; Wellenius et al., 2005), and ischemic heart disease (Pope et al., 2004). It is believed that many of the cardiovascular effects of environmental air pollution are related to increased vasoconstriction following exposure. Currently, there are several studies in both animals and humans that associate exposure to air pollution with endothelial cell dysfunction, leading to a reduction in vasodilatory capacity and/or an enhancement of vasoconstriction.

In human clinical and panel studies, environmental air pollution has been shown to decrease brachial artery blood flow through a reduction in flow mediated dilation (Briet et al., 2007; Rundell et al., 2007; Brook et al., 2002). Other studies have indicated that forearm blood flow, a technique for measuring overall vascular perfusion, is reduced following inhalation of environmental air pollutants (Mills et al., 2007, 2005; Tornqvist et al., 2007, Dales et al., 2007). Mechanistically, it appears that the reduction in blood flow following exposure to environmental air pollutants is due to endothelial cell dysfunction (Briet et al., 2007; Mills et al., 2005). In animal models of vascular function, various air pollutants have been shown to induce enhanced arterial vasoconstriction (Campen et al., 2005; Proctor et al., 2006; Li et al., 2005).

Endothelial nitric oxide synthase (eNOS) is responsible for the majority of endogenous nitric oxide found in the vascular endothelial cells (Forstermann and Munzel, 2006). eNOS can be activated through a variety of pathways most notably acetylcholine/muscarinic receptor binding and endothelin-1 (ET-1)/ET_B secondary receptor binding. Reduced production and efficacy of NO in the vasculature is a hallmark of endothelial cell dysfunction. Central to endothelial cell dysfunction is physical and/or functional uncoupling of eNOS, after which eNOS ostensibly produces only superoxide (O_2^-) and no NO, dramatically reducing the vasculature's ability to vasodilate through this major pathway, and potentially affecting prostaglandin and endothelial-derived hyperpolarizing factor pathways as well (Liu et al., 2006). Sequestration of free NO through the reaction between NO and O_2^- to form peroxynitrite (ONOO−) also occurs (Forstermann and Munzel, 2006).

Endothelial cell dysfunction is a hallmark of many diseases, including diabetes and atherosclerosis, which are associated with an increased risk of adverse outcome due to exposure to air pollution (Pope et al., 2004). Furthermore, animal models of diabetes and atherosclerosis appear to be sensitive to the vascular effects of environmental air pollutants (Lund et al., 2007; Proctor et al., 2006; Campen et al., 2005). However, to date no studies, human or animal,

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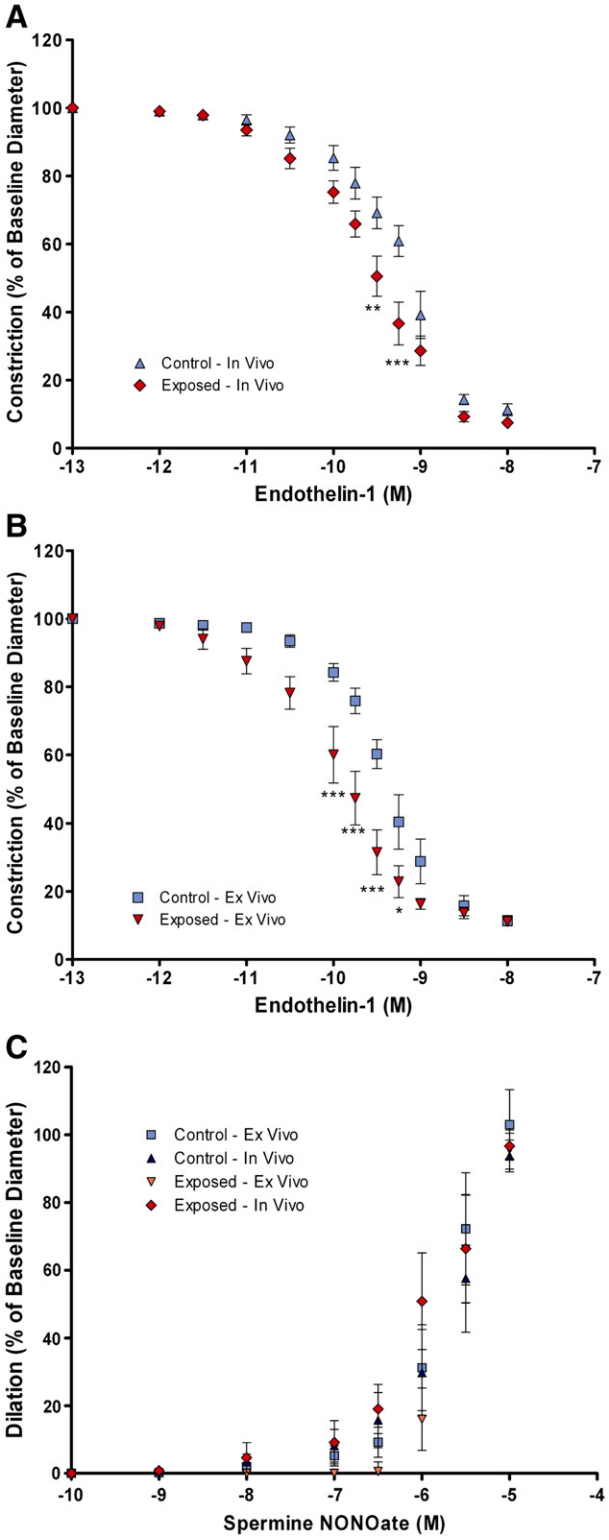

Fig. 1. DE enhances ET-1 induced vasoconstriction in mouse mesenteric veins. Dose-response curves for ET-1 (panels A and B) and spermine NONOate (panel C) for control, and DE in vivo or ex vivo. C57BL/6 mice were exposed to either filtered air or DE (350 μg/m^3) for 4 h and then terminated. The mesenteric veins were isolated in naive saline, cannulated, and ET-1 or spermine NONOate curves were performed (panels A and C) as described in the Materials and methods section. In panel B DE was bubbled through 1 L of PSS for 1 h and then mixed 1:4 with naive PSS, and filtered prior to use as the superfusate. In both inhalation and ex vivo exposures, DE enhanced ET-1-induced constriction (panels A and B) but did not alter vasodilatation induced by spermine NONOate (panel C) ($n>6$/group, ***$p<0.001$,**$p<0.01$, *$p<0.05$).

have shown an enhancement of venous tone following exposure to environmental air pollution, nor have they shown evidence of eNOS uncoupling following inhalation exposure. Seminal observations of air pollution associated hospital admissions for dyspnea may have indirectly implicated enhanced vasoconstriction as a possible mechanism (Higgins et al., 1995). Venous tone is a clear contributor to the pulmonary symptoms of heart failure exacerbation and Nesiritide, a commercially-available B-type natriuretic peptide, significantly reduces venous tone and improves shortness of breath in acutely symptomatic patients (Gehlbach and Geppert, 2004). In the present study we show that a common environmental air pollutant, DE, can enhance venous constriction in an isolated vessel model and that the enhancement of vasoconstriction is related to uncoupling of eNOS.

Materials and methods

Animals. Male C57BL/6 mice were obtained from Taconic at 8–10 weeks of age and housed in an AAALAC approved facility. Animals were provided with food and water *ad libitum*. All animal procedures were reviewed and approved by institutional animal care and use committee of Lovelace Respiratory Research Institute. Animals were used in accordance with National Institutes of Health guidelines for laboratory animals.

Whole body and ex vivo exposures. Mice were exposed whole body to either filtered air or 350 μg/m^3 of whole DE for a single 4 h period. DE was freshly derived from a single cylinder Yanmar diesel generator burning #2 certified diesel fuel (Chevron-Phillips, Borger, TX) under 100% load (Campen et al., 2005). For ex vivo assays, 1 L of the physiological saline solution (PSS) that acted as the superfusate, a solution to preserve metabolic and physiological function of the vessels, was bubbled with DE ($PM_{2.5}$ concentration of 2–3 mg/m^3 with a flow rate of 500 mL/min) via an impinger as previously described (Campen et al., 2005). Arteries were also exposed to concentrations of single compounds at concentrations similar to those found in impinged PSS ($\sim 10^{-7}$ M). Namely, we examined 3 carbonyls (formaldehyde, acetaldehyde, and acetone) and 2 alkanes (hexadecane and pristane). The carbonyls readily dissolved into the PSS, while the alkanes required dissolution into DMSO, necessitating an additional group of controls to ascertain DMSO effects. LCMS quantification of the compounds in PSS after warming and passage through the isolated vessel apparatus confirmed that the formaldehyde (1.5×10^{-7} M) and acetaldehyde (0.8×10^{-7} M) were still in solution, however acetone was entirely dissipated. Hexadecane and pristane both showed higher stability in solution with DMSO.

Additional details pertaining to the exposure system and characterization of exposure atmospheres have been described elsewhere (McDonald et al., 2004). Immediately following whole body exposures animals were terminated for use in the isolated vessel preparation.

Isolated vessel preparation. Mice were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and the intestinal arcade was removed.

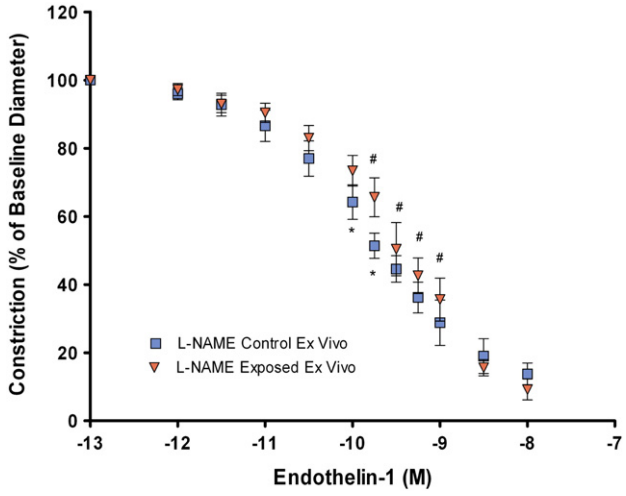

Fig. 2. L-NAME normalizes ET-1 vasoconstriction in DE ex vivo vessels to control levels. Control and DE-exposed veins were bathed in 1 μmol/L L-NAME in the superfusate during the entire procedure (see Materials and methods). L-NAME returned exposed vessel constriction to control levels such that there was a significant difference between L-NAME diesel and ex vivo DE veins. Furthermore, L-NAME enhanced ET-1 induced constriction to that of DE-exposed veins such that constriction at 10^{-10} and $10^{-9.75}$ M ET-1 was significantly elevated compared to control vessels ($n\geq5$/group, #=$p<0.05$ L-NAME diesel vs. ex vivo diesel, *=$p<0.05$ L-NAME control vs. control ex vivo).