Increased Oxidant Activity Mediates Vascular Dysfunction in Vibration Injury

Jennifer M. Hughes, Oliver Wirth, Kristine Krajnak, Roger Miller, Sheila Flavahan, Dan E. Berkowitz, Dan Welcome, and Nicholas A. Flavahan

Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland (J.M.H., S.F., D.E.B., N.A.F.); and Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia (O.W., K.K., R.M., D.W.)

Received August 8, 2008; accepted October 23, 2008

ABSTRACT

Occupational exposure to hand-operated vibrating tools causes a spectrum of pathological changes in the vascular, neurological, and musculoskeletal systems described as the hand-arm vibration syndrome (HAVS). Experiments were performed to determine the effects of acute vibration on the function of digital arteries. Rats paws were exposed to a vibrating platform (4 h, 125 Hz, constant acceleration of 49 m/s² root mean squared), and digital artery function was assessed subsequently in vitro using a pressure myograph system. Constriction to phenylephrine or 5-hydroxytryptamine was reduced in digital arteries from vibrated paws. However, after endothelium denudation, constriction to the agonists was no longer impaired in vibrated arteries. Inhibition of nitric-oxide synthase (NOS) with N°-nitro-L-arginine methyl ester (L-NAME) increased constriction to phenylephrine or 5-hydroxytryptamine in vibrated but not control arteries and abolished the vibration-induced depression in constrictor responses. However, nitric oxide (NO) activity, determined using the NO-sensitive probe 4-amino-5-methylamino-2’, 7’-difluorofluorescein, was reduced in vibrated compared with control arteries. Endogenous levels of reactive oxygen species (ROS), determined using the ROS-sensitive probe 5-(and 6)-chloromethyl-2’, 7’-dichlorodihydrofluorescein, were increased in vibrated compared with control arteries. The increased ROS levels were abolished by L-NAME or by catalase, which degrades extracellular hydrogen peroxide. Catalase also increased constriction to phenylephrine or 5-hydroxytryptamine in vibrated but not control arteries and abolished the vibration-induced depression in constrictor responses. The results suggest that acute vibration causes vascular dysfunction in digital arteries by increasing ROS levels, which is probably mediated by uncoupling of endothelial NOS. Therefore, therapeutic strategies to inhibit ROS or augment NO activity may be beneficial in HAVS.

Hand-arm vibration syndrome (HAVS) is a major occupational disease that causes considerable morbidity among workers exposed to vibration and is characterized by disorders of the vascular, sensorineural, and musculoskeletal systems (Bernard et al., 1998; Fridén, 2001; Weir and Lander, 2005). Approximately 1.45 million workers in the United States are exposed to hand-transmitted vibration, and based on data from epidemiological studies, approximately 50% of those workers will develop HAVS (Bernard et al., 1998). The predominant vascular disorder is heightened constriction of digital arteries resulting in reduced cutaneous blood flow (Fridén, 2001; Weir and Lander, 2005). The onset and progression of the disease are poorly understood at the cellular and molecular level. Although heightened activity of the sympathetic nervous system and endothelial injury have been proposed as important pathogenic mechanisms, clinical studies have demonstrated reduced vasoconstriction to α-adrenergic activation and normal dilation to the endothelium-dependent agonist acetylcholine in individuals who use vibrating tools (Ekenvall and Lindblad, 1986; Chen et al., 1994; Kennedy et al., 1999; Stoyneva et al., 2003). HAVS may also be associated with medial thickening and smooth muscle hypertrophy, resulting in increased wall/lumen ratios and...
reduced internal diameters in small arteries and arterioles (Takeuchi et al., 1986; Kent et al., 1991; Greenstein et al., 1994; Hashiguchi et al., 1994; Littleford et al., 1997).

The goal of the present study was to examine the effects of acute in vivo vibration on the functional responses of isolated digital arteries and to determine mechanistic responses associated with vibration injury. No previous studies have attempted this type of analysis on digital arteries. Therefore, we developed a new model of vibration injury using the rat paw. Before performing physiological studies, the physical (i.e., biodynamic) response of the digits and paw to vibration was characterized. Responses of digital arteries from control and vibrated paws were then examined ex vivo to determine vascular responses associated with acute vibration injury and to identify potential underlying mechanisms.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (H1a:(SD)CVF rats; 6 weeks old) were obtained from Hilltop Laboratory Animals, Inc. (Scottsdale, PA) and housed in a temperature- and humidity-controlled facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Food (Teklad 2918 rodent diet; Harlan Teklad, Madison, WI) and water were provided ad libitum. Before each exposure session, rats were restrained using a modified cone-shaped plastic bag (72-6414; Harvard Apparatus Inc., Holliston, MA). The rat was placed inside the bag with the head facing the smaller opening of the bag (Fig. 1). The larger opening was closed off with tape. When the rat was secured, a small opening was cut into the right side of the bag so that the right paws and forelimbs of the rats could be exposed. Additional openings were cut into the bottom of the bag to allow urine to escape the bag. The rats quickly acclimated to the bag restraint and remained quite still throughout exposure sessions. All procedures were approved by the Institutional Animal Care and Use Committee.

**Vibration Apparatus.** The restrained rat rested in a support device that was secured to a pole stand and suspended over an electromagnetic shaker (V408; Ling Dynamic Systems, Herts, UK) so that its paw rested flat on a custom-designed vibrating platform affixed to the shaker (Fig. 1). The vibrating platform measured 9 cm square and 1.3 cm thick and was fabricated from a solid block of aluminum. Vibration measurements of the platform alone revealed that it was stable through a wide range of frequencies; the resonant frequency of the platform was well above 500 Hz, the highest frequency tested in the present study. Repeated vibration exposures occurred in a sound-attenuating cubicle (MED Associates, St. Albans, VT). To measure vibration transmissibility in the paw, a scanning laser vibrometer (PSV-300; Polytec, Germany) was positioned above the vibration platform to record the distribution of vibration at specified locations on the digits, palm, and wrist (Fig. 1). Software (version 2B; Polytec GmbH, Waldbronn, Germany) collected and recorded velocity measurements from several locations along the dorsal surface paw.

**Vibration Transmissibility.** Three rats were used to measure the vibration transmissibility in the rat paw to characterize the response of the paw to segmental vibration exposure. Before testing, these rats were lightly anesthetized with xylazine (5 mg/kg) and ketamine (40 mg/kg) to keep their paws completely still during operation of the scanning laser vibrometer. The anesthetized rat was placed in a cone-shaped plastic bag and placed over the shaker as described above. Velocity measurements were recorded from several locations along the dorsal surface of the paw, plus a reference point on the surface of the vibrating platform next to the paw. Six different vibration frequencies (32.5, 63, 125, 160, 250, and 500 Hz) were tested at three different unweighted accelerations (9.8, 49, and 100 m/s²). Measurement at each location lasted approximately 5 s, long enough for the software to record a reliable measurement.

**Vibration Exposure.** A separate set of nonanesthetized rats was used for in vitro analyses of vascular function. Each rat was randomly assigned to one of two groups: a vibration-exposed group or a restraint-control group. Rats in the vibration group were exposed to a 4-h period of vibration at 125 Hz and a constant acceleration of 49 m/s² root mean squared. The biodynamic response of the paw to this frequency and acceleration was fairly consistent at all measurement locations (Fig. 2). In addition, exposure to tools with a dominant vibration frequency near 125 Hz is associated with the development of HAVS in humans (Bovenzi et al., 1988). Rats in the restraint-control group were similarly restrained and placed in the same exposure chamber, except no vibration was applied to their paws or forelimbs. Exposure chambers were equipped with a video camera to monitor rats throughout the exposure and ensure that each rat’s paw stayed in contact with the vibrating platform at all times. After exposures, the rats were euthanatized using pentobarbital (100 mg/kg i.p.), and the right paw was immediately removed, immersed in ice-cold Dulbecco’s modified Eagle’s medium that was maintained at 4°C, and transported from the National Institute for Occupational Safety and Health to Johns Hopkins University, where the physiological analysis was performed (Krajnak et al., 2006).

**Vasoconstrictor Responses.** Vasoconstrictor responses of arterioles from control and vibrated rat paws were recorded simultaneously (Krajnak et al., 2006). In brief, digital arteries from control and vibrated paws were dissected out and placed in cold Krebs-Ringer-bicarbonate solution: 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 25.0 mM NaHCO₃, and 11.1 mM glucose (control solution). Arteries were cannulated at both ends with glass micropipettes, secured using 12-0 nylon monofilament suture, and placed in a microvascular chamber (Living Systems Instrumentation, Burlington, VT). The chamber was superfused with control solution, maintained at 37°C, pH 7.4 (gassed with 16%
Transmissibility of the vibration stimulus (ratio of the measured velocity on the paw to the measured velocity on the platform) plotted for different areas of the paw at a function of vibration frequency and amplitude. Transmissibility in the digits and palm was at or somewhat greater than 1.0 relative to the platform across most frequencies and amplitudes. At 100 m/s² rms and the higher frequencies, transmissibility in the wrist was less than 1.0 (dampened) compared with the digits and palm. A vibration frequency of 125 Hz and an amplitude of 49 m/s² rms was chosen for exposures in this study because transmissibility was at or slightly above 1.0 in the palm and digits and because this frequency has been associated with the greatest risk of HAVS in humans. Results are expressed as means ± S.E.M. for n = 3.

O₂-5% CO₂-balance N₂), and the artery segment was pressurized to a constant transmural pressure of 60 mm Hg (Krajnak et al., 2006). The chamber was placed on the stage of an inverted microscope (X10, Nikon Eclipse TS100; Nikon, Tokyo, Japan) and connected to a video camera (CCTV camera; Sony, Tokyo, Japan). The vessel image was projected onto a video monitor, and the internal diameter was continuously determined by a video dimension analyzer (Living Systems Instrumentation) and recorded using a BIOPAC data acquisition system (Santa Barbara, CA). For experiments involving endothelium-denuded arteries, a 1-ml air bubble was passed through the system (Santa Barbara, CA). For experiments involving endothelial denudation was conducted in the absence and presence of the nitric-oxide synthase (NOS) inhibitor, N°-nitro-L-arginine methyl ester (L-NAME; 100 μM), or catalase (1000 units/ml), which degrades extracellular hydrogen peroxide (H₂O₂). When inhibitors were used, the preparations were incubated with the drugs for 30 min before and then during exposure of the arteries to the agonist. Only one inhibitor was assessed in each blood vessel. Time control experiments, in which the arteries were incubated for a similar time without inhibitors, demonstrated that sequential concentration-effect curves to phenylephrine and to 5-HT were similar (data not shown). After all vasoconstriction curves were recorded, the perfusion solution was switched to calcium-free control solution (containing 3 mM EGTA), and passive diameter at 60 mm Hg was recorded.

Nitric Oxide. Nitric oxide (NO) activity of digital arteries from control and vibrated paws was determined using the NO-sensitive fluorescent probe 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM DA) (Invitrogen, Carlsbad, CA) (Santhanam et al., 2007; Ryo et al., 2008). Digital arteries were pinned in a Silastic-coated dish in HEPES-buffered saline solution (HBS): 10.0 mM HEPES, 134.0 mM NaCl, 6.0 mM KCl, 1.0 mM MgCl₂, 10.0 mM glucose, and 2.0 mM CaCl₂. Arteries were incubated with DAF-FM DA (5 μM in HBS) for 45 min at 37°C. The probe was then removed, and the arteries were allowed to stabilize for 10 min (in HBS) before commencing imaging. Arteries were imaged using a fluorescence microscope (Nikon Eclipse 80i), ×10 objective (numerical aperture, 0.3), and 10-ms exposure times. The number of exposures was limited to prevent bleaching. To determine the influence of NOS inhibition, arteries were treated with L-NAME (100 μM) before, during, and after the incubation with DAF-FM DA. The average change in fluorescent intensity from the L-NAME-treated background was calculated for each group.

Reactive Oxygen Species. Digital arteries were pinned in a Silastic-coated dish in HBS and incubated with the reactive oxygen species (ROS)-sensitive fluorescent probe, 5-(and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein (DCDHF) (Invitrogen), 5 μM, for 30 min (37°C) (Santhanam et al., 2007; Ryo et al., 2008). The probe was then removed, and the arteries were allowed to stabilize for 10 min (in HBS) before commencing imaging. Arteries were imaged using a fluorescent microscope (Nikon Eclipse 80i), ×10 air objective (numerical aperture, 0.3), and 10-ms exposure times. Only three images were captured from each DCDHF-loaded artery to prevent generation of ROS or bleaching of the DCDHF signal, and the average of these three images was used for all calculations. To determine the effects of inhibition of NOS or of extracellular H₂O₂, arteries were treated with L-NAME (100 μM) or catalase (1000 U/ml) before, during, and after the incubation with DCDHF. In control and vibrated arteries, DCDHF fluorescence was virtually abolished by treatment with the antioxidant N-acetylcysteine (20 mM) (data not shown). The average fluorescent intensity was calculated for each group.

Reagents. Unless stated otherwise, all reagents were from Sigma-Aldrich (St. Louis, MO). Stock solutions of drugs were prepared fresh each day and stored at 4°C during the experiment. Drugs were dissolved in distilled water, with the exception of DAF-FM DA and DCDHF, which were dissolved in dimethyl sulfoxide. All drug concentrations are expressed as final molar concentration.

Calculations and Statistics. All data are expressed as means ± S.E.M., where n refers to the number of animals in each group. Transmissibility was calculated for each location by dividing the velocity at that location by the velocity at the reference point. Transmissibility values greater than 1.0 indicate vibration amplification, and values less than 1.0 indicate attenuation. These values were averaged across rats for each location and plotted as a function of frequency and magnitude.

Myogenic tone was calculated as the percentage difference in diameter at a transmural pressure of 60 mm Hg when arteries were side (0.001–1 μM) were obtained after constriction of the arteries to ~50% of the stable baseline diameter with phenylephrine.
superfused with Ca\(^{2+}\)-free versus Ca\(^{2+}\)-replete control solution. Vasomotor responses were expressed as a percentage change in the baseline diameter before administering vasoconstrictor agonists. Concentration-response curves were compared by analyzing areas under the curves. Statistical evaluation was performed using Student’s *t* test for either paired or unpaired observations. When more than two means were compared, analysis of variance was used followed by Dunnett or Bonferroni multiple comparison tests (GraphPad Software Inc., San Diego, CA). A probability of *P* < 0.05 was accepted as statistically significant for all comparisons.

**Results**

**Biodynamic Analyses.** Vibration transmissibility from 32 to 250 Hz was at or slightly above 1.0 in the digits and palm at 9.8 and 49 m/s\(^2\) rms (Fig. 2). At 100 m/s\(^2\) rms and at

\[ \text{Fig. 3. Effects of vibration on dilation to the endothelium-dependent agonists acetylcholine (A and B) and substance P (C and D) or the endothelium-independent agonist sodium nitroprusside (SNP) (E) in untreated (A, C, and E) and L-NAME (100 \(\mu\)M)-treated (B and D) digital arteries. Arterial constriction was titrated to approximately 50% baseline diameter (B) by the } \alpha_1\text{-AR agonist phenylephrine (PE) before administering vasodilator agonists. Because of decreased reactivity of arteries from vibrated paws, higher concentrations of phenylephrine were required to constrict vibrated compared with control arteries. Vasomotor responses were expressed as percent constriction from baseline diameters (before administering phenylephrine) and are presented as means ± S.E.M. (n = 4–11).} \]
the higher frequencies, transmissibility in the palm and wrist was less than 1.0.

**Functional Analyses.** For the functional studies, rats in the vibrated group were exposed to a 4-h period of vibration at 125 Hz and constant acceleration of 49 m/s² root mean squared. At a transmural pressure of 60 mm Hg, the baseline diameters of control and vibrated digital arteries were similar (211 ± 6 and 196 ± 6 μm, respectively, n = 18; P = N.S.). In the absence of constrictor agonists, these arteries did not display significant basal tone.

**Vibration Does Not Affect Vasodilator Agonist Responses.** Vasodilator responses to the endothelial agonists, acetylcholine or substance P (0.001–1 μM), were analyzed during constriction of arteries to phenylephrine (by ~50% baseline diameter). There was no difference in vasodilation concentration-effect curves between control and vibrated arteries in response to either agonist (P = N.S.), whether assessed in the absence or presence of the NOS inhibitor l-NAME (100 μM) (Fig. 3). l-NAME (100 μM) inhibited (Fig. 3) and endothelial denudation abolished vasodilator responses to each agonist (data not shown). The endothelium-independent dilator sodium nitroprusside (0.001–1 μM) caused concentration-dependent relaxation of digital arteries that was similar between control and vibrated paws (Fig. 3) (P = N.S.).

**Vibration Depresses Vasoconstrictor Agonist Responses.** Vasoconstrictor responses to the α₁-AR agonist phenylephrine (0.001–0.3 μM) or 5-HT (0.001 to 0.03 μM) were significantly inhibited in vibrated compared with control digital arteries (Fig. 4) (P < 0.001). α₂-AR activation by UK 14,304 did not cause significant constriction of these arteries (data not shown).

**Role of the Endothelium.** Endothelial-denudation decreased constriction to phenylephrine or 5-HT in control (P < 0.05) but not vibrated arteries and eliminated the vibration-induced decrease in agonist responses (Fig. 5).

**Role of NO.** The NOS inhibitor, l-NAME (100 μM), increased vasoconstriction to phenylephrine or 5-HT in control (P < 0.05) but not control arteries and abolished the vibration-induced depression in vasoconstrictor responses (Fig. 6). NO activity, determined using the NO-sensitive fluorescent probe DAF-FM DA, was significantly reduced in arteries from vibrated paws compared with control paws (P < 0.05) (Fig. 7A). Catalase also abolished the vibration-induced depression in vasoconstrictor stimuli, which was prevented by endothelial denudation or inhibition of NOS, suggesting that it might be mediated by increased activity of endothelium-derived NO. However, NO activity, assessed using the fluorescent probe DAF-FM, was actually reduced in vibrated arteries whereas the endogenous levels of ROS, assessed using the ROS-sensitive probe DCFH, were increased in vibrated arteries. The vibration-induced increase in levels of ROS was abolished by inhibition of NOS or by inactivation of H₂O₂ with catalase. Catalase also abolished the vibration-induced depression in vasoconstrictor responses. The results suggest that, after vibration, endothelial NOS generates ROS, leading to increased levels of H₂O₂ and depressed vasoconstrictor responses. Therefore, oxidative stress, which may result from uncoupling of endothelial NOS, appears to be an early response to vibration injury and could be an important early event in the pathogenesis of HAVS.

**Vibration and Oxidant Stress**

**Fig. 4.** Effects of vibration on vasoconstrictor responses to phenylephrine (A) or 5-HT (B) in endothelium-intact digital arteries. Vasoconstrictor responses are expressed as a percentage of the baseline internal diameter and are presented as means ± S.E.M. (n = 5). Vasoconstrictor responses were significantly different between control and vibrated paws for each agonist. ***P < 0.001.

caused a pronounced depression in arterial reactivity to vasoconstrictor stimuli, which was prevented by endothelial denudation or inhibition of NOS, suggesting that it might be mediated by increased activity of endothelium-derived NO. However, NO activity, assessed using the fluorescent probe DAF-FM, was actually reduced in vibrated arteries whereas the endogenous levels of ROS, assessed using the ROS-sensitive probe DCFH, were increased in vibrated arteries. The vibration-induced increase in levels of ROS was abolished by inhibition of NOS or by inactivation of H₂O₂ with catalase. Catalase also abolished the vibration-induced depression in vasoconstrictor responses. The results suggest that, after vibration, endothelial NOS generates ROS, leading to increased levels of H₂O₂ and depressed vasoconstrictor responses. Therefore, oxidative stress, which may result from uncoupling of endothelial NOS, appears to be an early response to vibration injury and could be an important early event in the pathogenesis of HAVS.

Vibration-induced soft tissue damage is believed to be dependent on the amount of tissue stress and strain, which can be estimated by calculating the transmissibility of the vibration stimulus to the tissue (Dong et al., 2005b; Wu et al., 2006). The transmission of vibration to the paw was frequen-
This generalized decrease in contractile activity would be consistent with a proposed vibration-induced disruption of smooth muscle cell integrity (Curry et al., 2002). However, the vibration-induced depression in constriction was prevented by endothelial denudation, indicating that it resulted from altered activity of endothelial rather than smooth muscle cells. Although endothelial cells can express serotonergic receptors, functional α₁-ARs are not present on arterial endothelium. Therefore, the vibration-induced, endothelium-dependent depression in constrictor responses would be consistent with basal rather than stimulated activity (by phenylephrine or 5-HT) of an endothelial mediator. Indeed, vibration did not alter the magnitude of endothelium-dependent relaxations evoked by the endothelial activators, acetylcholine or substance P. The vibration-induced depression in vasoconstriction was also prevented by inhibition of NOS with L-NAME, which would be consistent with a vibration-induced increase in basal NO activity. It is surprising that the basal activity of NO, assessed using the fluorescent probe DCDHF, were dramatically increased in vibrated
arteries. The vibration-induced increase in levels of ROS was abolished by L-NAME, suggesting that in vibrated arteries, NOS was actually associated with the generation of ROS. Active NOS is a homodimer that generates NO and L-citruline from L-arginine. When deprived of its cofactor tetrahydrobiopterin or substrate L-arginine or exposed to oxidant stress, including peroxynitrite, NOS can uncouple to the monomeric form, which acts as an NADPH oxidase and generates superoxide rather than NO (Heinzel et al., 1992; Xia et al., 1996; Rosen et al., 2002; Kuzkaya et al., 2003). The vibration-induced increase in DCDHF fluorescence was abolished by catalase, which inactivates H2O2 (1000 U/ml) or by catalase, which inactivates H2O2. DCDHF is more sensitive at detecting H2O2 compared with controls (*, P < 0.05). The vibration-induced increase in DCDHF fluorescence was abolished by the NOS inhibitor L-NAME (100 μM) or by catalase (1000 U/ml), which catalyzes the degradation of H2O2 (##, P < 0.01; untreated vibrated arteries versus treated vibrated arteries). Data are expressed as means ± S.E.M. for n = 7 (untreated control and untreated vibrated arteries) or n = 4 (other groups).

Fig. 7. Fluorometric analysis of NO (A) and ROS (B) activity in control and vibrated digital arteries. Fluorescence is expressed as arbitrary fluorescent units. A, DAF-FM DA fluorescence was significantly reduced in vibrated paws compared with controls (*, P < 0.05), n = 3 for all groups. B, conversely, DCDHF fluorescence was significantly increased in vibrated paws compared with control (**, P < 0.01). The vibration-induced increase in DCDHF fluorescence was abolished by the NOS inhibitor L-NAME (100 μM) or by catalase (1000 U/ml), which catalyzes the degradation of H2O2 (##, P < 0.01, untreated vibrated arteries versus treated vibrated arteries). Data are expressed as means ± S.E.M. for n = 7 (untreated control and untreated vibrated arteries) or n = 4 (other groups).

Responses in vibrated arteries. This indicates that the basal activity of NOS-derived mediators causes relaxation in vibrated but not control arteries. Endothelial denudation inhibited vasoconstrictor responses in control arteries but had no effect on constriction in vibrated arteries. This indicates that the net basal effect of the endothelium in control arteries is amplification of vasoconstriction, consistent with the generation of an endothelium-derived contractile mediator, whereas there is no net basal effect in vibrated arteries. In vibrated arteries, inhibition of NOS with L-NAME increased vasoconstrictor responses to phenylephrine comparing with control arteries (P < 0.05). Catalase increased vasoconstriction to phenylephrine or 5-HT in vibrated (P < 0.01) but not control arteries. After catalase, vasoconstrictor responses were similar in digital arteries from control and vibrated rat paws. Vasoconstrictor responses are expressed as a percentage of the baseline internal diameter and are presented as means ± S.E.M. (n = 6). Endothelium-intact, vibrated arteries demonstrated blunted responses to the vasoconstrictor agonists compared with control arteries (P < 0.05). Catalase increased vasoconstriction to phenylephrine or 5-HT in vibrated (P < 0.01) but not control arteries. After catalase, vasoconstrictor responses were similar in digital arteries from control and vibrated arteries. *** P < 0.0001; ** P < 0.001; * P < 0.05; N.S., not statistically significant, with brackets and symbols indicating comparisons.

Fig. 8. Effects of catalase (1000 U/ml), which degrades extracellular H2O2, on vasoconstrictor responses to phenylephrine (A) or 5-HT (B) in digital arteries from control and vibrated rat paws. Vasoconstrictor responses are expressed as a percentage of the baseline internal diameter and are presented as means ± S.E.M. (n = 6). Endothelium-intact, vibrated arteries demonstrated blunted responses to the vasoconstrictor agonists compared with control arteries (P < 0.05). Catalase increased vasoconstriction to phenylephrine or 5-HT in vibrated (P < 0.01) but not control arteries. After catalase, vasoconstrictor responses were similar in digital arteries from control and vibrated arteries. *** P < 0.0001; ** P < 0.001; * P < 0.05; N.S., not statistically significant, with brackets and symbols indicating comparisons.

Although endothelial denudation or inhibition of NOS each prevented the vibration-induced depression in vasoconstrictor responses, they had distinct effects on the concentration-effect curves to vasoconstrictor agonists in control and vibrated arteries. Inhibition of NOS had no effect on vasoconstrictor responses in control arteries but amplified responses in vibrated arteries. This indicates that the basal activity of NOS-derived mediators causes relaxation in vibrated but not control arteries. Endothelial denudation inhibited vasoconstrictor responses in control arteries but had no effect on constriction in vibrated arteries. This indicates that the net basal effect of the endothelium in control arteries is amplification of vasoconstriction, consistent with the generation of an endothelium-derived contractile mediator, whereas there is no net basal effect in vibrated arteries. In vibrated arteries, inhibition of NOS with L-NAME increased vasoconstrictor responses in endothelium-containing arteries but had no effect in endothelium-denuded arteries (data not shown), thereby revealing an endothelium-dependent constrictor influence (compare with Figs. 5 and 6). Therefore, although there appears to be no net basal effect of the endothelium in vibrated arteries, this actually reflects a balanced activity of endothelium-derived contracting and dilating mediators. The major difference between control and vibrated arteries and the reason for the interesting divergent shifts in the concentration-effect curves appears to be the dramatic
increase in endothelial (and NOS-dependent) dilator activity in vibrated arteries and maintained activity of endothelium-derived contractile factors in control and vibrated arteries. The endothelium-derived dilator in vibrated arteries appears to be H$_2$O$_2$, whereas the nature of the contracting factor was not pursued.

Vibration increased endothelial dilator activity under basal conditions but did not change the magnitude of endothelium-dependent relaxation to endothelial agonists, acetylcholine, and substance P. Although NOS uncoupling can reduce endothelium-dependent responses to these agonists, the NOS-dependent generation of H$_2$O$_2$ can also effectively substitute for NOS-derived NO enabling maintained relaxation (Cosentino and Katrusić, 1995). The selective vibration-induced increase in endothelial dilator activity under basal conditions could result from persistent basal activation of the endothelium in vibrated arteries or from different subcellular compartments of NOS.

A vibration-induced diminution in constrictor activity may be a somewhat surprising finding because HAVS is characterized by exaggerated vasoconstriction (Weir and Lander, 2005). However, clinical and in vitro studies have also demonstrated diminished responses to vasconstrictor stimuli in individuals or in isolated blood vessels exposed to vibration (Ekenvall and Lindblad, 1986; Lindblad et al., 1986; Chen et al., 1994). Although low levels of H$_2$O$_2$ may act as a temporary compensatory mechanism to maintain vasodilatation and decrease vasoconstriction, sustained exposure to high levels of oxidants could contribute to disease progression. NO is vasoprotective and acts to inhibit platelet aggregation and adhesion and vascular remodeling and inflammation, whereas H$_2$O$_2$ and other oxidants can initiate pathological changes in the vasculature, stimulating inflammatory genes, vascular remodeling, and enhanced vascular stiffening (Boulden et al., 2006; Förstermann, 2006; Förstermann and Münnzel, 2006). Indeed, NOS uncoupling and subsequent oxidative stress are thought to contribute to many cardiovascular disorders including hypertension, atherosclerosis and diabetes (Cosentino et al., 1998; Oelze et al., 2000; Hink et al., 2001; Landmesser et al., 2003; Förstermann, 2006; Förstermann and Münnzel, 2006) and may be an important early step in the vasculopathy of HAVS. Chronic exposure to vibration injury, by causing persistent oxidant stress and loss of NO activity, could then contribute to structural and functional pathological changes in these blood vessels and disease progression. Therapy targeted toward preserving NO activity may be beneficial in this disease process.

Acknowledgments

We thank Dr. Ren Dong for assistance with the biomechanical measurements and helpful comments on the manuscript.

References


Address correspondence to: Dr. Nicholas A. Flavahan, Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University, Ross Research Building, Room 370, 720 Rutland Ave., Baltimore, MD 21205. E-mail: nflavah1@jhmi.edu