11TH INTERNATIONAL CONFERENCE ON HAND-ARM VIBRATION

ACUTE VIBRATION EXPOSURE REDUCES NITRIC OXIDE (NO) CONCENTRATIONS AND NO-MEDIATED VASODILATION

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Abstract

The goal of this study was to determine if vibration affects nitric oxide (NO)-mediated vasodilation in the ventral tail arteries of rats after exposure to a single bout of vibration. Male Sprague Dawley rats were restrained and their tails were exposed to a single 4 h bout of vibration at 125 Hz, 49 m/s^2 r.m.s, or control conditions. Vascular levels of the synthetic enzymes, nitric oxide synthase (NOS)-1 and -3 were measured along with vascular NO concentrations. The sensitivity of the ventral artery to NO-induced vasodilation was also assessed in vitro. Exposure to a single bout of vibration reduced NOS-1 and NO concentrations in the ventral tail artery of vibrated animals, and resulted in a reduction in NO-mediated vasodilation that did not appear to be dependent upon endothelial cell activity. The reduced sensitivity of exposed arteries to NO-mediated vasodilation may make these blood vessels more susceptible to damage during subsequent exposures to vibration.

1. Introduction

Exposure to hand-transmitted vibration through the use of power or pneumatic hand tools can result in vascular, sensory and muscular dysfunction that is collectively referred to as hand-arm vibration syndrome (HAVS; reviewed in [1]). The hallmark symptom of HAVS is cold-induced vasospasms that result in finger blanching (or vibration white finger; VWF), similar to the cold-induced finger blanching seen in people with primary Raynaud's phenomenon [2]. Although many studies have described the vascular and neural pathology associated with HAVS, the etiology of this disorder is still not well understood.

We recently demonstrated that exposure to a single bout of vibration results in an increased sensitivity of the rat ventral tail artery to α2C-adrenoreceptor-mediated vasoconstriction [3]. This specific receptor also mediates cold-induced vasoconstric-

tion in cutaneous arteries [4]. Thus, cold and vibration synergistically could act through this receptor to induce the blanching and cyanosis that are characteristic of VWF [5]. However, chronic exposure to vibration also appears to affect factors that mediate vasodilation [6-8]. Nitric oxide (NO) is a potent vasodilator that is synthesized by vascular smooth muscle, skeletal muscle, endothelial cells and peripheral nerves [9-11]. Changes in NO-mediated vasodilation and NO concentrations have been associated with vascular dysfunction in patients with HAVS [7], and in animals exposed to chronic vibration [8]. However, it is unclear if these changes in NO and NO activity lead to vascular dysfunction, or are a result of the injury caused by chronic exposure to vibration and the accompanying bouts of vasoconstriction. The goal of this study was to use a rat tail model of HAVS to determine if changes in NO and NO-mediated vasodilation occur with acute exposures to vibration, and thus potentially contributes to the development of vibration-induced vascular dysfunction.

2. Materials and Methods

2.1 Animals and Exposure Apparatus

Male Sprague-Dawley CVF rats (6 weeks of age; Hilltop Lab Animals, Inc, Scottdale, PA) were used for all exposures. All rats were maintained in a colony room with a 12:12 light/dark cycle and with food and water available *ad libitum* at the National Institute for Occupational Safety and Health (NIOSH) facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Rats were allowed to acclimate to the colony room for 1 week before being used in any experiments. All procedures were approved by the NIOSH Animal Care and Use Committee and were in compliance with Public Health Service Policy.

The shakers, amplifiers and software used for the vibration exposure previously were described [3]. Vibration platforms were mounted onto each shaker. Platforms were aluminum, 50 mm x 25 mm (major and minor radii) elliptical platforms, which were 12.7 mm thick, tapered down to 6.35 mm at the ends, and had 14 mm wide extensions that lengthen the entire platform from the middle ellipse to 170 mm. The platforms oscillated in a vertical direction. Identical platforms also were used for control animals.

Prior to experimental exposures, all rats were placed in Broome-style restrainers 4 hrs/day for 5 days to acclimate them to restraint. Rats were allowed to walk into the restrainers, head first, and their tail was gently threaded through a hole in the removable hatch. The hole in the hatch had been enlarged so that the rats' tails were not held in an awkward position in relationship to their bodies. Restraint control rats were treated in a manner identical to the vibrated rats except that the platforms holding the control tails were placed on isolation blocks, instead of shakers. All shakers and control platforms were placed in ventilated, sound attenuated chambers. On the day of an experiment, each rat's tail was secured to a vibrating or stable platform with 4 elastic straps (6.35 mm wide) that were pulled over the tail and fastened over screws secured into the side of the platform. Care was taken to make sure that the tail was secured to the platform without compressing the tissue. All animals were exposed to a single 4 h bout of tail vibration (125 Hz, acceleration of 49 m/sec^2

r.m.s.) or restraint control. Chamber temperatures were monitored throughout the exposure to assure the temperature remained stable for all exposures (average ± sem chamber temperature; 22° C ± 0.13). All exposures were performed between 0900 and 1300 h. Animals were euthanized with by pentobarbital injection (100 mg/kg, i.p.).

2.2 Biological Measures and Analyses

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to measure transcript levels for the synthetic enzymes, nitric oxide synthase (NOS)-1 and NOS-3 in dissected ventral tail arteries. Transcript specific primers and probes used were from the rat Universal probe set (Roche, Indianapolis, IN USA). All PCR reactions were performed in the ABI Prism 5700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Waltham, MA USA) using 96 well optical reaction plate with optical caps (both from Perkin-Elmer Applied Biosystems). The critical threshold value for each sample was obtained, and used to calculate fold changes in transcript levels between groups.

NOS-1 and NOS-3 protein concentrations were measured in ventral tail arteries using Western blot analyses. NOS-1 bands were identified using the polyclonal anti-NOS-1 antibody (Cayman Chemicals, Ann Arbor, MI USA) and NOS-3 bands were identified using a polyclonal anti-NOS-3 antibody (Cayman Chemicals) diluted 1:1000. Bands were visualized by chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL USA) and exposed to film. Scanned images of the blots were analyzed with Scion Image (Scion Corporation, Frederick, MD USA) gel densitometry analysis software.

Changes in NOS in the tail arteries were most prevalent 24 h after the exposure. Therefore, we measured nitrate/nitrite concentrations (a measure of NO levels) in the tail artery 24 h after the exposure. Total nitrate/nitrite concentrations were measured in artery tissue homogenates using the nitrate/nitrite colormetric Assay Kit (Cayman Chemicals), and total protein concentrations were measured using the BCA protein assay (Pierce). Tissue concentrations were expressed as μM nitrate/nitrite per μg protein.

To determine if vibration exposure affects NO-mediated vasodilation, arteries were dissected from tails and mounted in a microvessel chamber (Living Systems, Burlington, VT, USA). The vessel was maintained at 37°C in HEPES bicarbonate solution and held at a constant pressure of 60 mm/Hg. Arteries were constricted to approximately 50% of their resting diameter using the α-1 adrenoreceptor agonist, phenylephrine (dose 10^{-6} M; Sigma, St Louis, MO USA). Dose-response curves to the NO donor, S-nitroso-N-acetylpenicillamine, (SNAP; Sigma) or the vasodilating factor acetylcholine (ACh; Sigma) were generated by increasing the concentrations of these agonists in half log increments (from 10^{-9} to 10^{-5} M). Changes in the diameter of the vessel were measured by placing the microvessel chamber on an inverted microscope, capturing vessel images using a video camera and using a video dimension analyzer (Living systems) and Data-Q Instruments software (Akron, OH USA) to continually monitor the internal diameter of the artery.

Fold changes in NOS transcript levels and NOS concentrations in arteries were analyzed using 2-way (treatment x time) ANOVAs. Changes in nitrate/nitrite concentrations were analyzed using a 1-way ANOVA. Dose-response curves showing the internal diameter an artery at each treatment dose were constructed for each artery using a non-linear regression model (Prism GraphPad, San Diego, CA USA), and then groups curves were produced. Group diameter data was analyzed using 1-way ANOVA with animal as a random variable. Differences with $p < 0.05$ were considered significant.

3. Results

Transcript levels and protein concentrations of NOS-1 (neuronal form) and NOS-3 (endothelial form) in the ventral artery were measured in the ventral tail arteries of rats exposed to 4 h of vibration or restraint, and euthanized 1 or 24 h after the exposure. NOS-1 and NOS-3 transcript levels in the ventral arteries were not affected by vibration (n = 8 animals/group, data not shown). However, NOS-1 protein concentrations in the artery were reduced 24 h after the vibration exposure (Figure 1A; $F(1, 17) = 6.03$, $p < 0.03$). NOS-3 protein levels were more variable in all groups of rats, and although there appears to be a decrease in restraint control and vibrated animals 24 h after the exposure, this difference was not significant (Figure 1B).

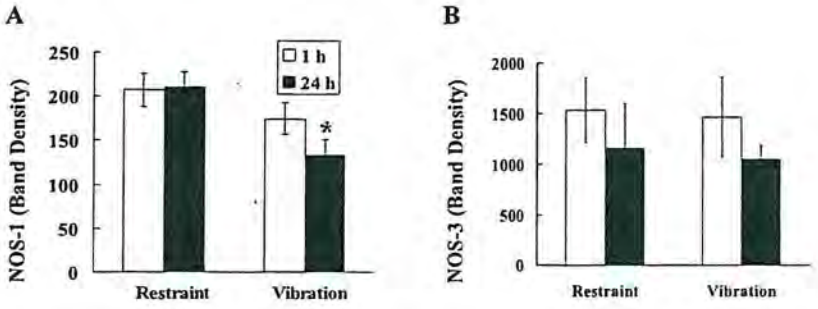

Figure 1 - NOS-1 (A) and NOS-3 (B) protein concentrations in the ventral tail arteries of rats exposed to vibration or restraint control. Vibration resulted in a significant decrease in NOS-1 protein concentration in the ventral tail arteries of rats 24 h after the exposure (*$p < 0.05$). There were no significant differences in NOS-3 concentrations in arteries from restraint control and vibrated rats.

Nitrate/nitrite concentrations, measured in the ventral arteries 24 h after exposure (n = 8 animals/group) were also reduced in vibrated arteries compared to arter-

ies from cage control rats ($p < 0.05$). Nitrate/nitrite concentrations were not significantly different between cage and restraint controls. (Figure 2).

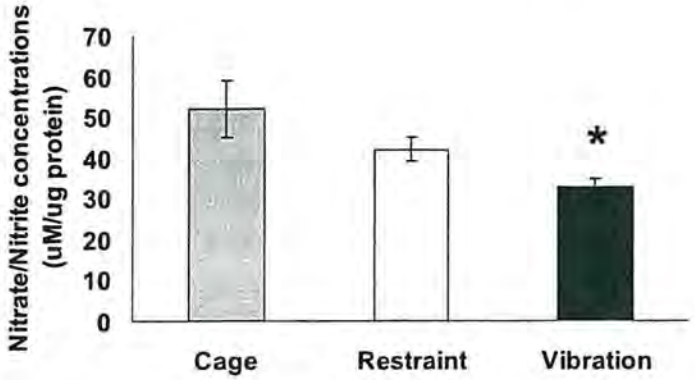

Figure 2 - Nitrate/nitrite concentrations in the ventral tail arteries of rats 24 h after exposure to vibration or control conditions. Nitrate/nitrite concentrations in arteries of rats exposed to vibration were significantly lower than concentrations in cage control rats (*$p < 0.05$).

To determine if changes in NO concentrations were associated with changes in the sensitivity of arteries to NO-mediated vasodilation, dilation in response to increasing doses of SNAP and ACh were assessed in arteries from rats exposed to restraint control or vibrated conditions, and euthanized 24 h after the exposure. The response to ACh was tested because this neurotransmitter, which is released by autonomic inputs to peripheral vessels, stimulates the release of NO from endothelial cells. NO in turn can act on vascular smooth muscle or endothelial cells to induce vasodilation. The dose-response curves of the ventral arteries to SNAP are presented in figure 3 A below. Vibration resulted in a shift in the dose-response curve to SNAP, with vibrated arteries generally being less sensitive to the dilating effects of SNAP than arteries from restraint control rats (main effect of exposure; $F(1, 10) = 6.80$, $p < 0.05$). However, the dose-dependent vasodilation in response to ACh was not different in arteries from restraint control and vibrated rats (Figure 3B), suggesting that endothelial-mediated vasodilation was preserved in these animals.

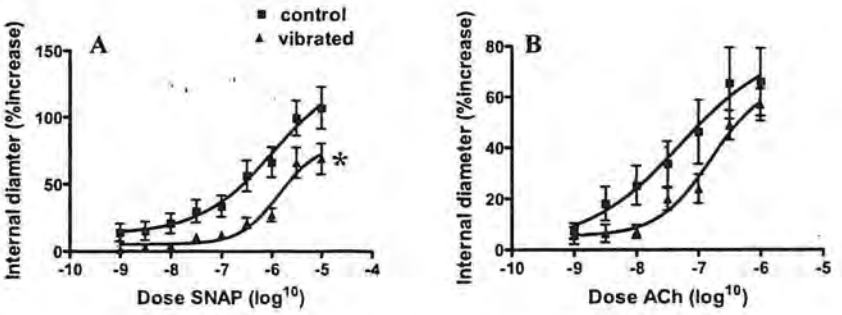

Figure 3 - Dose-dependent vasodilation of ventral tail arteries in response to SNAP (A) and ACh (B). Arteries from vibrated animals were less sensitive to the dilating effects of the NO donor, SNAP, than arteries from control animals (* $p < 0.05$). In contrast, the dose-dependent re-dilation to ACh was not different in arteries from vibrated and restraint control rats.

4. Discussion

Concentrations of the vasodilator, NO are reduced in the ventral tail artery of rats 24 h after exposure to a single 4 h bout of vibration. This reduction in NO is associated with a decrease in NOS-1 but not NOS-3 concentrations in the ventral artery. Thus, peripheral blood vessels may stay constricted after vibration exposure because NO concentrations and NO synthesis are reduced for at least 24 h after the exposure. Reductions in NO activity can be caused by decreases in available oxygen levels or to increased oxidative activity [12]. A number of labs have demonstrated that exposure to single bout of vibration can result in an increase in oxidative activity [13] and increased concentrations of reactive oxygen species [14] in the ventral artery. Thus, we hypothesize that vibration-induced reductions in NOS-1 and NO are due to increased oxidative activity in vascular tissue after vibration exposure.

The ability of the NO agonist, SNAP, to stimulate re-dilation of constricted vessels was also reduced in vibrated arteries. SNAP acts as an NO donor, and therefore responses to SNAP are not dependent upon de novo tissue generation of NO. Instead, application of this compound allows us to characterize the response of arteries to equivalent doses of NO. Therefore, changes in the response of arteries to SNAP are solely indicative of reductions in the ability of the artery to respond to NO, and not due to changes in NO release by the tissue.

In contrast, the ability of the ACh to induce dilation was not altered. ACh induces vasodilation by stimulating the release of NO from vascular endothelial cells. The failure to see a change in ACh-mediated vasodilation suggests that after a single exposure to vibration, endothelial-induced vasodilation may be preserved., but, vasodilation directly mediated by sensory or autonomic inputs may be inhibited. Fu-

ture studies will determine if repeated exposures to vibration also alter endothelial-induced vasodilation.

5. Conclusions

Based on these data and data from our previous studies [9], we conclude that vibration enhances noradrenergic-induced vasoconstriction through α2C-adrenoreceptor-mediated mechanisms, but that the reduced ability of peripheral vessels to recover from vibration-induced constriction [14] may be the result of reductions in NO concentrations and NO activity in vascular tissue. Reductions in NO-mediated vasodilation, which are apparent for at least 24 h after the exposure, may make peripheral arteries more susceptible to the physiological effects of subsequent bouts of vibration, and eventually lead to the vascular pathology underlying VWF.

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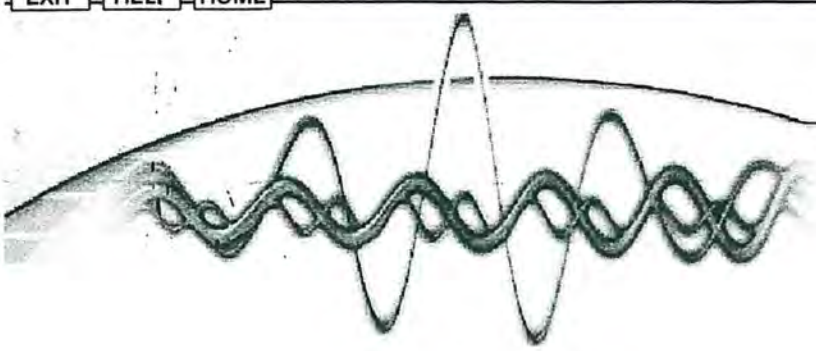

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