ABSTRACT: Hand-transmitted vibration from powered-tools can cause peripheral vasospasm and neuropathy. A rat-tail model was used to investigate whether the pattern of vibration influenced the type and severity of tissue damage. The tails of awake rats were vibrated continuously or intermittently for a total of 4 hours at 60 Hz, 49 m/s^2. Nerves and arteries were harvested immediately or 24 hours after treatment. Tails subjected to intermittent vibration showed transiently increased sensitivity to thermal stimuli. Intermittent vibration caused the most nerve injury immediately and 24 hours after vibration. Continuous vibration invoked a persistent reduction in vascular lumen size. Compared to epinephrine-induced transient vacuolation in vascular smooth muscle cells, both continuous and intermittent vibration caused greater persistence of vacuoles, indicating a vibration-induced pathological process. All vibration groups exhibited elevated nitrotyrosine immunoreactivity indicative of free-radical damage. Pattern of vibration exposure may exert a major influence on the type of vibration injury.
Muscle Nerve **34:** 197–204, 2006

COMPARISON OF CONTINUOUS AND INTERMITTENT VIBRATION EFFECTS ON RAT-TAIL ARTERY AND NERVE

SANDYA R. GOVINDARAJU, MBBS, PhD, BRIAN D. CURRY, PhD, JAMES L. W. BAIN, BA, and DANNY A. RILEY, PhD

Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA

Accepted 4 April 2006

Hand–arm vibration syndrome (HAVS) is a secondary form of Raynaud's phenomenon with cold-triggered vasospastic episodes of the digits and peripheral neuropathy. The strong association between occupational powered-tool usage and development of HAVS is well established.[2,18] There is a paucity of information about the early molecular and cellular events underlying the evolution of HAVS, which hinders the development of detection and prevention measures. In our rat-tail vibration model, exposure to 4 h of continuous vibration at 30, 60, and 120 Hz caused vasoconstriction and vacuole formation in smooth muscle and endothelial cells of the rat-tail artery.[6,7] These changes are similar to the effect of exogenous application of potent vasoconstrictors, epinephrine and norepinephrine, on medium to small resistance arteries.[8,14,20] Joris et al. proposed that the process of smooth-muscle vacuole formation is a normal physiological response of arteries to intense vasoconstriction and that this may become pathological if the vacuoles detach from cells, resulting in loss of cell membrane and content.[13] It is unknown whether greater numbers of vibration-induced vacuoles in the rat-tail artery persist for longer than those vacuoles generated by epinephrine treatment.

Depending on the task and tool, the pattern of powered-tool use during the workday varies, ranging from a few minutes to hours, with episodic breaks. The effects of pattern of hand-transmitted vibration on arteries and nerves have not been well characterized. However, it is believed that taking frequent breaks is beneficial and results in less harm than continuous exposure to vibration over long periods. Human studies have demonstrated that both continuous and intermittent vibration reduces digital blood flow.[5,15] For intermittent exposure, the reductions in blood flow were of similar magnitude for each repeated period of vibration, leading the investigators to suggest that pattern of exposure is unlikely to influence vibration injury.[5,15] To our knowledge, no animal models of hand–arm vibration have compared the effects of intermittent and continuous vibration on tissue injury.

Abbreviations: ANOVA, analysis of variance; HAVS, hand–arm vibration syndrome; ISO, International Organization for Standardization; rms, root mean square

Key words: hand–arm vibration syndrome; myelin damage; tyrosine nitration; vascular smooth muscle; vibration pattern

Correspondence to: D. A. Riley; e-mail: dariley@mcw.edu

Published online 11 May 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20578

The European Union Directive 2002 defined a daily vibration exposure limit of 5.0 m/s² rms and vibration action limit of 2.5 m/s² rms to protect workers against hand–arm vibration injury. The calculations utilized frequency-weighted acceleration values, as defined by the International Organization for Standardization (ISO 5348-1 and 5349-2). Griffin and others have challenged frequency-weighting as underemphasizing the damaging effects of higher frequencies.[12] Using the rat-tail vibration model, we previously reported arterial structural damage when vibrated at 30, 60, 120, and 800 Hz at a constant acceleration of 49 m/s² equivalent to A (8) values of 18.5, 9.2, 4.6, and 0.7 m/s², respectively.[7] The most damaging among the different frequencies tested was 60 Hz. The present study examined the morphological damage to arteries and nerves immediately and 24 h following continuous and intermittent vibration at 60 Hz and provided evidence that pattern dramatically influences vibration injury.

METHODS

Animal Groups. Male Sprague–Dawley rats (303 ± 12 g) were randomly assigned to five groups (n = 7/group): nonvibrated sham-vibration control; continuous vibration with immediate sampling; continuous vibration with sampling at 24 h after treatment; intermittent vibration with immediate sampling; and intermittent vibration with sampling at 24 h after treatment. The tails of the vibrated animals were exposed to 4 h of cumulative vibration at 60 Hz, with 49-m/s² acceleration (unweighted) delivered either continuously or intermittently (cycles of 10-min vibration followed by 5-min rest). For all rats, tail reflex withdrawal times to noxious heat stimulation were measured before, immediately after, and 24 h after vibration. Sham control rats were put into the rat-tail vibration apparatus for 4 h but not vibrated.[6] Rats were removed from the apparatus and tested for tail-flick reflex latency times within 5 min of cessation of vibration. Three tail-flicks were timed and averaged for each time-point. Rats in the continuous 24-h and intermittent 24-h postvibration groups were returned to vivarium housing for 24-h recovery before repeat tail-flick testing.

An additional 17 rats, weighing 274 ± 17 g, were anesthetized. Their proximal tail skin was incised to expose the ventral tail artery, which was exogenously treated with 1 mM epinephrine (n = 11) in Hank's physiological solution (Gibco, Grand Island, New York) or vehicle (n = 6) for 15 min. The concentration of epinephrine was based on a similar, previously published study addressing the efficacy of exogenous epinephrine in inducing vacuolation in the rat-tail artery.[8] Arteries from 5 rats treated with epinephrine and all 6 rats treated with vehicle were harvested immediately. The remaining 6 epinephrine-treated animals were processed 24 h later.

Animal treatment, surgical interventions, and husbandry procedures were approved by our institutional animal care committee and complied with the Laboratory Animal Welfare Act.

Behavioral Evaluation and Tissue Processing. Rats in the vibration apparatus were observed for signs of stress and scored as follows: 1, unilateral periocular porphyrin (reddish-black discoloration of fur caused by secretions from the eye); 2, bilateral porphyrin; 1, loose stool; 1, excessive grinding of teeth. A total score of 4 indicated the highest stress, and 0 represented no signs of stress.

For tissue harvest, rats were deeply anesthetized by intramuscular injection of a mixture of ketamine (72 mg/kg), xylazine (12 mg/kg), and acepromazine (0.09 mg/kg), and tail segments 5–8 were excised. Segments 5, 6, and 8 were immersion-fixed in 4% paraformaldehyde for 2.5 h at room temperature before ventral arteries and nerves were removed. The fixed tissues were cryoprotected at 20°C in a graded series of buffered sucrose (10% for 20 min, 20% for 45 min, and 30% at 5°C for 24 h), quick frozen in Freon 22, and stored in liquid nitrogen for light microscopy. Segment 7 was immersion-fixed in 4% glutaraldehyde and 2% paraformaldehyde for 2.5 h at room temperature for electron microscopy. Arteries and nerves were removed and postfixed with 1.3% osmium tetraoxide before standard embedding in epoxy resin.

Tissue Staining and Analysis. Semithin epoxy sections (0.5 μm) of arteries and nerves were cut with glass knives, stained with toluidine blue, and digitally imaged for morphometric analyses. Artery lumen size was expressed as the ratio of the lumen circumference to internal elastic membrane length × 100 using version 1.28 Image J software (National Institutes of Health, Bethesda, Maryland).[7,8,11] The total number of vacuoles (2–12 μm in size) in the endothelial and smooth muscle cell layers was counted in each artery cross-section.[7,8]

The total numbers of myelinated fibers in full, semithin cross-sections of tail nerves were counted. The percentage of disrupted myelinated axons per nerve showing abnormally intense toluidine blue staining and focal areas of thickening of the myelin sheath were quantitated using the Image J program. Intraneural edema was defined as an increase above the

sham-vibration in the interstitial space between myelinated axons in semithin cross-sections of nerves. Interstitial area was defined by employing the exclusion thresholding procedure of MetaMorph 5.2 (Universal Imaging Corp., West Chester, Pennsylvania).

For higher resolution of structural changes in arteries and nerves, selected regions in the semithin sections were thin-sectioned (~70 nm), picked up on grids, heavy metal–contrasted, and examined with a JEOL 100 CXII electron microscope.

Frozen tissues were used for immunohistochemical detection of nitrated tyrosines using anti-nitrotyrosine antibodies (1:250; Upstate, Lake Placid, New York) on 6-μm cryostat sections of arteries. Biotin-tagged secondary antibodies and Alexa fluor-455 streptavidin tertiary reagents (Molecular Probes, Eugene, Oregon) were used to visualize the signal. Primary antibody was omitted for the negative control, and other sections were treated with peroxynitrite before immunostaining to provide a positive control.

Statistical Analysis. A one-way analysis of variance (ANOVA) followed by Newman–Keuls pairwise test and Student's *t*-test were used to compare means of the different groups. Differences were considered significant at $P < 0.05$. Values are presented as mean ± SEM.

RESULTS

Behavioral Observations. Intermittent vibration caused an average stress level of 1.7 ± 0.3. The animals exhibited a startle reflex with brief vocalization at the onset of each bout of vibration. The duration of vocal response increased with time, suggesting growing discomfort over the 4 h of cumulative exposure. Immediately after vibration, there was hypersensitivity and vocalization to light touch of the tail. The hypersensitivity was absent 24 h later. The level of stress in rats after continuous vibration was 0.2 ± 0.1, and there was no hypersensitivity. None of the sham-vibrated animals showed signs of stress. When compared to the sham, there were no changes in tail-flick response times after continuous vibration exposure (sham, 3.1 ± 0.1 s; continuous immediate, 3.0 ± 0.1 s; and continuous 24-h survival, 3.1 ± 0.1 s). Intermittent vibration significantly reduced tail-flick response times by 20% immediately after vibration (2.4 ± 0.1 s), indicating thermal sensitization. Response time returned to preexposure level 24 h after intermittent exposure (3.1 ± 0.2 s).

Changes in Arteries. Immediately following 4 h of continuous vibration, arterial lumen size (38.1 ± 2.5) was significantly smaller than in the sham-vibrated animals (49.0 ± 4.2) (Figs. 1a and b and 2a). Remarkably, lumen size at 24 h after continuous

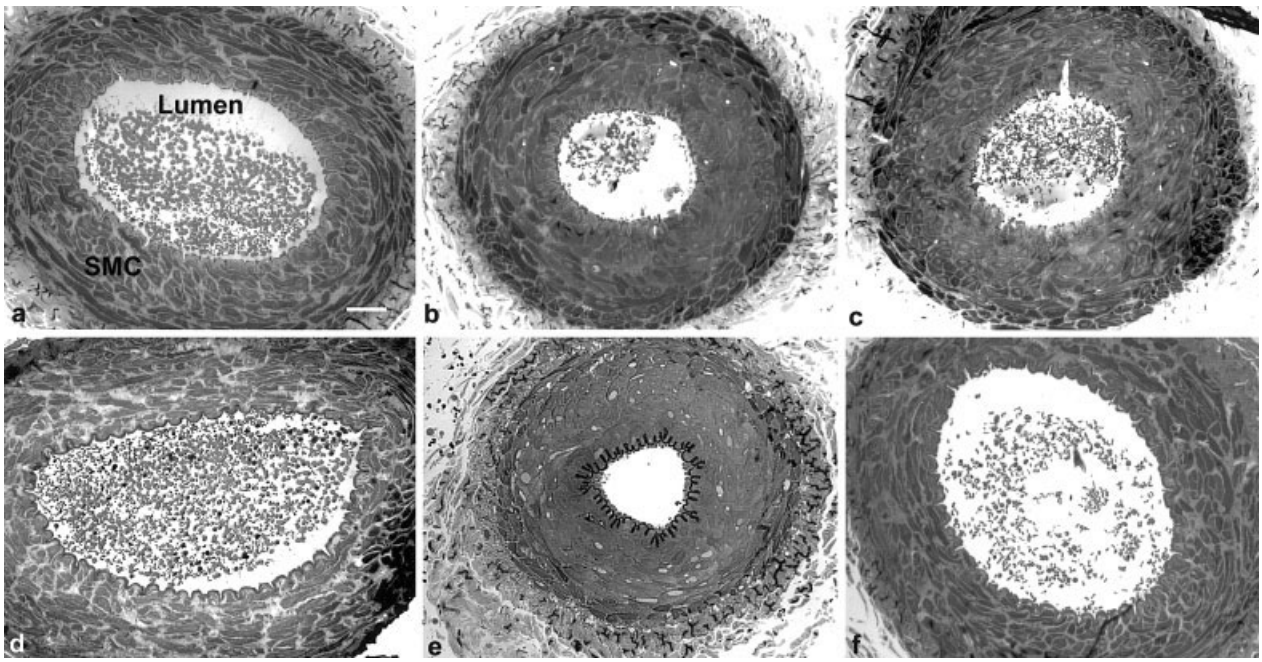

FIGURE 1. Semithin cross-sections of ventral-tail arteries stained with toluidine blue. Compared to the nonvibrated sham **(a)**, the lumen size is smaller for the continuously vibrated rats processed immediately **(b)** and 24 h after vibration **(c)**. Arteries vibrated intermittently and sampled either immediately (illustrated) or 24 h later do not have smaller lumens **(d)**. Epinephrine causes a decrease in lumen size immediately after application **(e)**. The arteries are dilated 24 h after epinephrine application **(f)**. SMC, smooth-muscle cells. Bar in **a** is 40 μm for all panels.

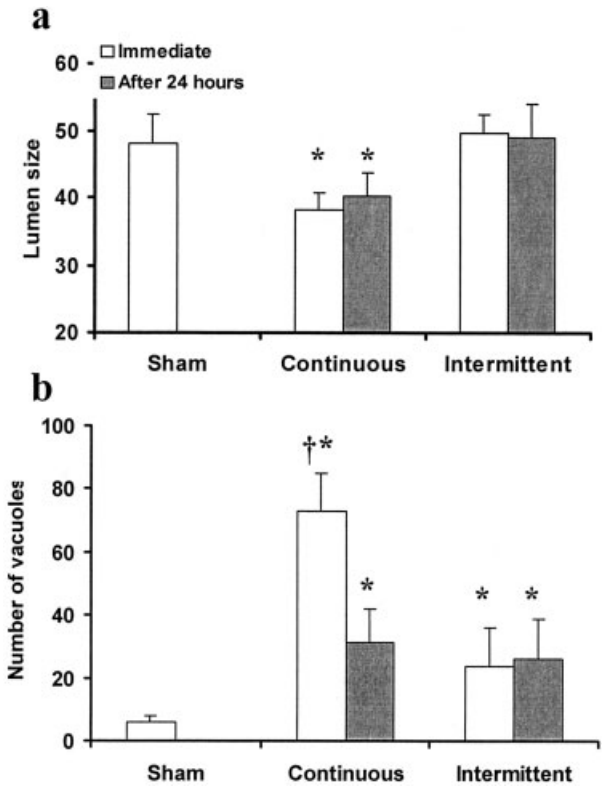

FIGURE 2. Lumen size and vacuole numbers in arteries. **(a)** Arterial lumen size is the ratio of lumen circumference to internal elastic membrane length × 100. Immediately and 24 h after continuous vibration, vascular lumen size is significantly smaller (*$P < 0.05$) compared to sham and intermittent vibration groups. **(b)** All vibration groups have significantly higher total numbers of vacuoles (endothelial and smooth muscle) compared to the sham-vibration group (*$P < 0.05$). The group sampled immediately after continuous vibration has a significantly (†$P < 0.05$) higher total number of vacuoles compared to the other vibration groups.

vibration (40.3 ± 3.4) remained smaller than in the sham-vibrated group (Figs. 1a, c and 2a). Intermittent vibration did not alter lumen size from sham, either immediately (49.6 ± 2.7) or 24 h after vibration (48.9 ± 5.1) (Figs. 1a, d and 2a). Exogenous application of Hank's physiological solution did not alter lumen sizes when compared to sham-vibration and did not cause vacuoles in the endothelial or smooth muscle cells. Arteries had the smallest lumen sizes (28.3 ± 3.5) immediately after epinephrine application (Fig. 1e). In contrast to continuous vibration, epinephrine-treated arteries were dilated at 24 h, with lumen sizes averaging 60.9 ± 6.9 (Fig. 1f).

Although all vibration groups demonstrated vacuoles in the endothelial and smooth-muscle cells, arteries harvested immediately after continuous vibration had a significantly ($P < 0.05$) higher total number (endothelial and smooth muscle) of vacuoles (76.1 ± 16.1) than the sham-vibrated group (6.1 ± 1.9), intermittent vibration group sampled immediately (24.8 ± 12.3), or the groups studied 24 h after continuous (31.3 ± 10.9) or intermittent vibration (26.0 ± 13.0) (Fig. 2b).

The greatest numbers of endothelial vacuoles were observed after continuous vibration (immediate, 26.5 ± 10.1; 24 h after, 7.3 ± 4.1) and epinephrine treatment (immediate, 13.5 ± 3.9; 24 h after, 5.6 ± 2.7). Intermittent groups demonstrated very few (immediate, 3.3 ± 2.6; 24 h after, 4.3 ± 1.5) endothelial vacuoles.

Epinephrine caused the largest number of smooth-muscle cell vacuoles immediately after application, whereas intermittent vibration generated fewer vacuoles than continuous vibration immediately after exposure (Table 1). When allowed to recover for 24 h, 48% of vacuoles in the smooth muscle persisted after continuous vibration, as opposed to only 18% of epinephrine-induced vacuoles (Table 1). For intermittent vibration, there was no reduction in vacuole numbers (endothelial and smooth muscle) when allowed to recover for 24 h (Table 1).

Arteries in all four vibrated groups exhibited higher immunoreactivity than the sham-vibrated group for nitrotyrosine moieties (Fig. 3). The immunolabeling was present in both the endothelial and smooth-muscle cell layers (Fig. 3b–e). Omission of the primary antibody reduced immunostaining to the sham control level. Peroxynitrite treatment of sham-vibrated sections generated positive immunostaining in the endothelial and smooth-muscle cells (data not shown).

Myelinated Axon Changes. On average, there were 1223 ± 145 myelinated axons in the tail nerves at the level of segment C7. In semithin sections of the sham-vibrated rats, the myelin was uniformly stained (Fig. 4a). Myelinated axons stained darker with toluidine blue after both patterns of vibration. An example from the intermittent group sampled immediately is shown in Figure 4b. Ultrastructural anal-

Table 1. Smooth-muscle cell vacuoles in arteries.

Groups	Immediate	24 hours after exposure
Continuous vibration	49.6 ± 11.4	24.0 ± 10.9*
Intermittent vibration	21.5 ± 11.5	21.8 ± 11.7
Epinephrine treatment	140.3 ± 34.7	25.7 ± 10.5†

*For each treatment, vacuole numbers are compared immediately and after 24 h. Vacuole numbers decreased significantly 24 h after continuous vibration (*P < 0.05) and epinephrine treatment (†P < 0.01) but remained unchanged after intermittent vibration.*

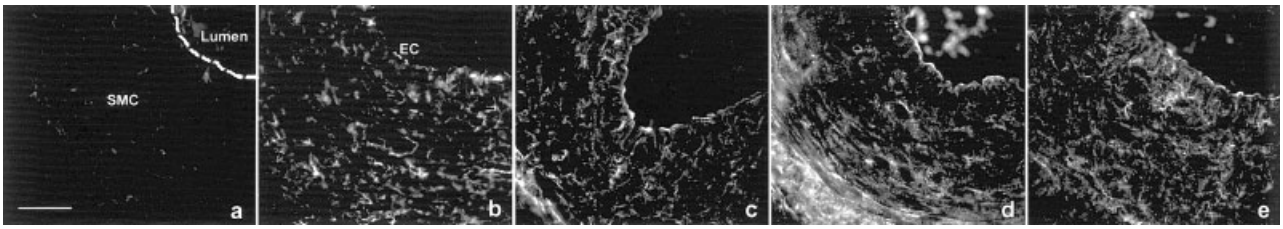

FIGURE 3. Anti-nitrotyrosine immunoreactivity in arteries. Compared to sham vibration **(a)**, arteries sampled after continuous **(b)** immediate, **(c)** 24 h after] and intermittent [**(d)** immediate, **(e)** 24 h after vibration exhibit higher immunoreactivity for nitrotyrosine. Dotted line in **(a)** marks the location of the nonstained endothelium. Immunostaining is pronounced in the endothelial and smooth-muscle cell layers. EC, endothelial cells, SMC, smooth-muscle cells. Bar in **a** is 50 μm for all panels.

ysis of myelinated fibers in the sham-vibration group showed predominantly compact packing of myelin membrane layers (Fig. 4c). Disrupted myelinated fibers in the vibrated groups showed focal areas of widening and delaminating in the myelin sheath (Fig. 4d). The decompacted myelin had increased intermembrane area for toluidine blue staining in semithin cross-sections and, most likely, accounted for the darker staining of disrupted fibers. Intermittent vibration generated significantly more axons with disrupted myelin (47.0 ± 1.9%) than continuous (28.6 ± 1.7%) when sampled immediately after vibration (Fig. 5a). Twenty-four hours later, the percentage of disrupted fibers was unchanged in the intermittent vibration group (45.3 ± 5.7%) but that in the continuous vibration group had increased to 36.2 ± 1.8% (Fig. 5a).

Immediately after continuous vibration, the interstitial area between the myelinated fibers was not significantly different from the sham-vibrated group (continuous vibration, 8.4 ± 1.3 μm^2; sham-vibration, 8.9 ± 4.4 μm^2). The intermittent-immediate group demonstrated significantly greater interstitial area between myelinated axons (26.0 ± 3.7 μm^2), indicative of edema. Both patterns demonstrated significant edema 24 h after exposure (continuous vibration, 25.3 ± 5.5 μm^2; intermittent vibration, 25.4 ± 3.4 μm^2) (Figs. 4b, d and 5b).

DISCUSSION

Effects of Vibration on the Artery. Blood-flow changes and vascular morphology in both human and animal models indicate that short durations (≤30 min) of vibration cause vasoconstriction, with resumption of blood flow immediately after cessation of vibration.[3,4,6,21] In the rat-tail model, arteries harvested immediately and 24 h after a 4-h bout of continuous vibration had lumen sizes smaller than in the sham-vibrated group. A 15-min application of epinephrine produced intense vasoconstriction, in excess of that induced by vibration; but, when allowed to recover for 24 h, epinephrine-treated arteries dilated. Pharmacologically induced vasoconstriction of a short duration, such as 5 min, is readily reversible on removal of the stimulus, but continuous vasoconstriction for 4 h is not.[16] Remodeling of the vascular smooth muscle in response to prolonged vasoconstriction prevents vasodilation.[16] Persistence of small lumens 24 h after vibration is consistent with this finding, indicating that the vibration effects last at least 1 day and that the artery adapts to maintain a smaller lumen. When vascular smooth-muscle cells contract and shorten for long periods, they reorganize their focal adhesions and increase overlap with adjacent smooth-muscle cells to maintain the smaller lumen while returning to preconstriction cell length.[16] A 5-min constriction is not long enough for remodeling, whereas a 4-h sustained vasoconstriction is. The persistence of smaller lumens after 4 h of continuous vibration indicates that the artery remained constricted throughout vibration exposure and remodeled. In contrast, vascular lumen sizes in animals treated with intermittent vibration were not different from those in the sham-vibrated group. As the magnitude of blood flow recovery is not compromised with increasing numbers of intermittent periods of vibration,[5] the "rest intervals" may have allowed reversal of vasoconstriction at the end of each period of vibration and negated smooth-muscle remodeling. This attribute of intermittent vibration may be beneficial.

Arteries after continuous vibration and epinephrine treatment, which had smaller lumen sizes, had more endothelial vacuoles than the sham-vibration and intermittent vibration groups. Mechanical trauma due to pinching of endothelial cells between the tight folds of internal elastic membrane during vasoconstriction may play a major role in vacuole formation in the endothelium. Some of the endothelial vacuoles may be enhanced subendothelial spaces formed by osmotic damage resulting from

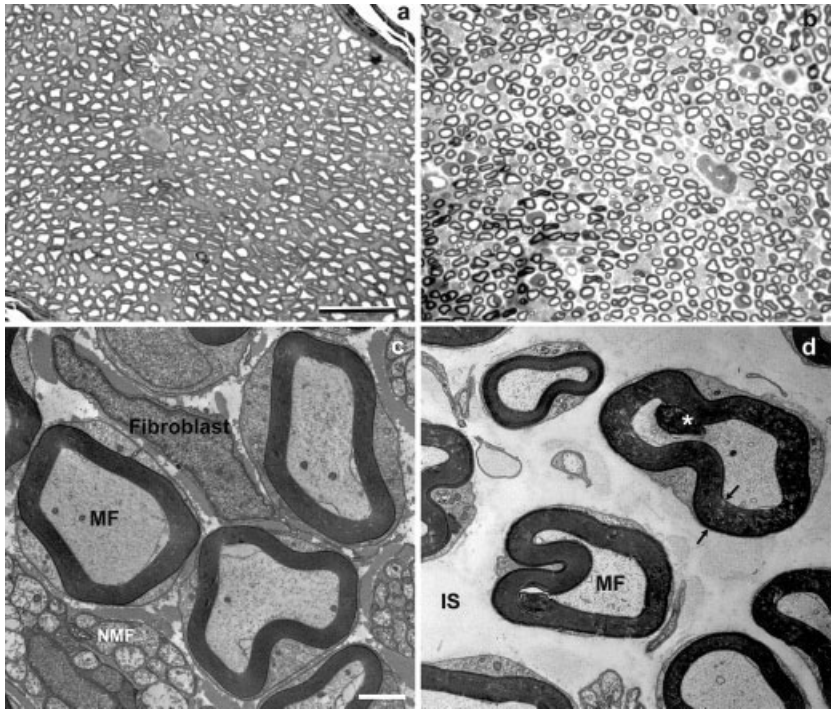

FIGURE 4. Light- and electron-microscopic images of tail nerves. **(a)** The semithin cross-section of the tail nerve from a sham-vibrated rat demonstrates that the myelin is evenly stained with toluidine blue, and there is very little interstitial space between nerve fibers. **(b)** Following both patterns of vibration, immediately and 24 h after vibration, the myelin stains darker with toluidine blue and exhibits focal thickening. The interstitial area is enlarged, indicating edema. An example from the intermittent vibration group sampled immediately is illustrated. **(c)** At the electron-microscopic level, the myelin membranes are compact except for tiny foci of separation in the sham-vibrated control nerves. Myelinated (MF) and nonmyelinated fibers (NMF) are closely packed. **(d)** Vibrated nerves immediately and 24 h after vibration exhibit larger and more extensive areas of separation of the myelin membranes (arrows), and frequently the myelin sheaths show decompaction (*). There is also increased interstitial space (IS) between fibers when vibrated. An example from the intermittent vibration group sampled immediately is illustrated. Bar in **a** is 40 μm for **a** and **b**; bar in **c** is 0.5 μm for **c** and **d**.

lifting of cells due to repeated mechanical displacement of tissue during vibration.[6,7]

We have previously observed the presence of double membrane-limited vacuoles within adjacent smooth-muscle cells, formed during vibration-induced vasoconstriction.[6–8] These vacuoles are envisioned to protrude from the cell surface during contraction and connect to the parent cell by a narrow neck. The vacuoles are structurally similar to those induced by norepinephrine.[14] All the vacuoles formed after intermittent vibration, and 48% of those formed after continuous vibration, persisted 24 h after exposure, whereas only 18% of vacuoles persisted 24 h after epinephrine induction without vibration. The higher percentages of vacuoles persisting after vibration exposure suggest that the repeated mechanical displacement of the tissue causes breakage of the slender necks and detachment of vacuoles from cells. The protrusions that were not sheared off may have been resorbed into the cell during relaxation, as proposed by Joris et al. for norepinephrine-induced vacuoles.[14] If vacuole formation is a normal physiological response of contracting smooth-muscle cells, vibration renders this response pathological by causing detachment of these processes from cells. Detachment results in loss of the cell membrane surrounding the vacuole and may compromise cell function. Interestingly, all three treatments (epinephrine, continuous vibration, and intermittent vibration) yielded similar absolute numbers of vacuoles in the smooth-muscle cells 24 h after treatment (Table 1). This may be coincidental or indicate the existence of a subpopulation of smooth-muscle cells highly susceptible to vasoconstrictive damage.

In the current study, 16 bouts of intermittent vibration (10 min each, with 5 min for rest between episodes) generated fewer vacuoles than continuous vibration. We propose that smooth-muscle vacuole formation depends on the degree and duration of smooth-muscle contraction. If this is true, there are several explanations for the smaller number of vacuoles generated during intermittent vibration. First, intermittent vibration may not induce the same de-

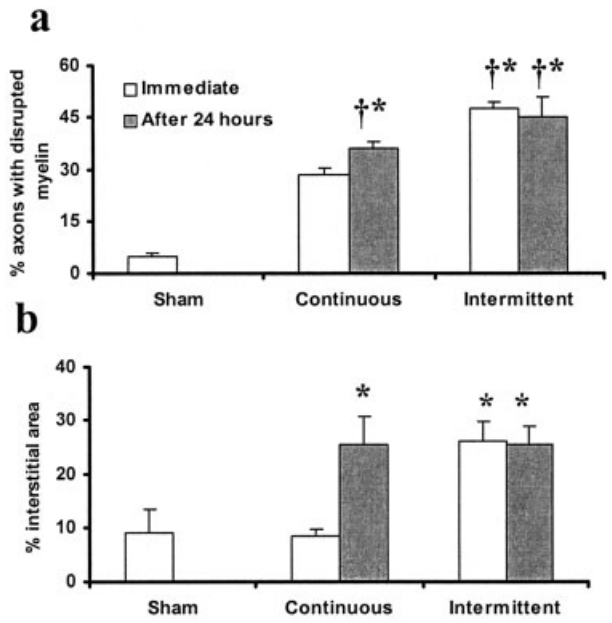

FIGURE 5. Myelin disruption and interstitial edema in nerves. **(a)** The vibrated groups have significantly ($P < 0.05$) higher percentages of disrupted myelinated fibers than the sham-vibration group. Compared to the continuous-immediate group, the percentages of damaged myelinated axons are significantly ($^{\dagger}P < 0.05$) higher in nerves sampled 24 h after continuous vibration and for both intermittent vibration groups. **(b)** The interstitial area between myelinated axons is significantly ($^{*}P < 0.05$) greater in the continuous 24-h–after, intermittent-immediate, and intermittent 24-h–after groups compared to the sham-vibration group.

gree and duration of vasoconstriction as continuous vibration. Second, the intensity of vasoconstriction induced by intermittent vibration may decrease progressively in later periods of vibration, due to contractile fatigue. Finally, vacuoles may be resorbed during the 5-min rest periods. Bovenzi et al. tested cumulative exposure times of 30 min for a range of vibration and rest periods and did not report a significant difference in blood-flow reduction or return of flow for continuous and intermittent exposures, or a significant difference between the first and subsequent periods of intermittent vibration.[5] However, this flow pattern may not hold true for a cumulative exposure of 4 h. Blood-flow measurements over longer periods of vibration are necessary to resolve the vascular responses to the pattern of vibration.

Vibration and Free Radicals. Both patterns of vibration produced free radicals as indicated by increased tyrosine nitration in arteries. The nitration persisted at 24 h after vibration, consistent with it being an irreversible chemical modification of proteins.[10] Depending on which proteins are nitrated, this post-translational modification may alter cell function.[10] It will be of interest to determine whether any of the vasoactive candidates, such as endothelin or endothelial nitric oxide synthase, which are implicated in the dysregulation of vascular tone in HAVS, undergo tyrosine nitration, specifically in their receptor binding or catalytic domains. Studies are underway to ascertain whether nitration of proteins plays a role in vibration-induced vascular and nerve dysfunction. If so, reduction of free radicals by antioxidant therapy may protect against the development of HAVS.

Effects of Vibration on Behavior. In our study, rats tolerated continuous vibration well and demonstrated minimal signs of stress. This was expected as continuous vibration is used clinically to anesthetize the skin during minor surgical procedures.[19] However, intermittent vibration generated a temporary hypersensitization to thermal stimuli and touch. The underlying mechanism for this transient sensory hypersensitivity is not known. Vibration-induced changes in rat-tail skin are being investigated to discern mechanoreceptor involvement. It would be of interest to determine whether the transient hypersensitivity becomes permanent with continued exposure.

Effects of Vibration on Nerve. A 4-h exposure to either pattern of vibration caused myelin decompaction in the tail nerves. A similar vibration exposure significantly reduced rat tail-nerve conduction velocities.[17] Pacinian corpuscles are vibroreceptors in the dermis of the skin. Their afferent innervation is with Aβ myelinated axons. Thermoreceptor and nociceptor afferents are small myelinated (Aδ, Aγ) and non-myelinated C fibers. A portion of the damaged fibers in the vibration-exposed tail nerves could be the vibroreceptor, thermal, and pain afferents. This early damage to nerves may progress with time and, over years of tool use, lead to persistent numbness and axon loss in HAVS. The tight wrapping of myelin membranes in the peripheral nervous system is maintained in part by homophilic binding of a cell adhesion glycoprotein, P_0.[9] *S*-nitrosylation of the proteolipid protein in the presence of toxic doses of nitric oxide has been proposed to cause decompaction of myelin.[1] A similar modification of myelin structural proteins in the peripheral nerve may cause unraveling of the myelin sheath in vibration injury. Alternatively, the repeated mechanical displacement of nerves imparted by vibration may be the cause of myelin disruption. Both possibilities need to be explored as possible explanations of vibration-induced nerve damage.

This work was supported by the Medical College of Wisconsin and in part by NIOSH Grant R01 OH003493. This study was presented

in part at the Experimental Biology Meeting in April 2005, San Diego, California.

REFERENCES

1. Bizzozero OA, DeJesus G, Howard TA. Exposure of rat optic nerves to nitric oxide causes protein S-nitrosation and myelin decompaction. Neurochem Res 2004;29:1675–1685.
2. Bovenzi M. Exposure–response relationship in the hand–arm vibration syndrome: an overview of current epidemiology research. Int Arch Occup Environ Health 1998;71:509–519.
3. Bovenzi M, Griffin MJ, Ruffell CM. Vascular responses to acute vibration in the fingers of normal subjects. Cent Eur J Publ Health 1995;3(suppl 3):15–18.
4. Bovenzi M, Lindsell CJ, Griffin MJ. Duration of acute exposures to vibration and finger circulation. Scand J Work Environ Health 1998;24:130–137.
5. Bovenzi M, Welsh AJ, Griffin MJ. Acute effects of continuous and intermittent vibration on finger circulation. Int Arch Occup Environ Health 2004;77:255–263.
6. Curry BD, Bain JL, Yan JG, Zhang LL, Yamaguchi M, Matloub HS, et al. Vibration injury damages arterial endothelial cells. Muscle Nerve 2002;25:527–534.
7. Curry BD, Govindaraju SR, Bain JL, Zhang LL, Yan JG, Matloub HS, et al. Evidence for frequency-dependent arterial damage in vibrated rat tails. Anat Rec A Discov Mol Cell Evol Biol 2005;284:511–521.
8. Curry BD, Govindaraju SR, Bain JL, Zhang LL, Yan JG, Matloub HS, et al. Nifedipine pretreatment reduces vibration-induced vascular damage. Muscle Nerve 2005;32:639–646.
9. Eichberg J. Myelin P0: new knowledge and new roles. Neurochem Res 2002;27:1331–1340.
10. Fries DM, Paxinou E, Themistocleous M, Swanberg E, Griendling KK, Salvemini D, et al. Expression of inducible nitric-oxide synthase and intracellular protein tyrosine nitration in vascular smooth muscle cells: role of reactive oxygen species. J Biol Chem 2003;278:22901–22907.
11. Govindaraju SR, Curry BD, Bain JL, Riley DA. Effects of temperature on vibration-induced damage in nerves and arteries. Muscle Nerve 2005;33:415–423.
12. Griffin MJ. Measurement, evaluation, and assessment of occupational exposures to hand-transmitted vibration. Occup Environ Med 1997;54:73–89.
13. Joris I, Majno G. Cell-to-cell herniae in the arterial wall. I. The pathogenesis of vacuoles in the normal media. Am J Pathol 1977;87:375–398.
14. Joris I, Majno G. Medial changes in arterial spasm induced by L-norepinephrine. Am J Pathol 1981;105:212–222.
15. Luo J, Sakakibara H, Zhu SK, Kondo T, Toyoshima H. Effect of vibration magnitude and repetitive exposure on finger blood flow in healthy subjects. Int Arch Occup Environ Health 2000;73:281–284.
16. Martinez-Lemus LA, Hill MA, Bolz SS, Pohl U, Meininger GA. Acute mechanoadaptation of vascular smooth muscle cells in response to continuous arteriolar vasoconstriction: implications for functional remodeling. FASEB J 2004;18:708–710.
17. Okada A. Physiological response of the rat to different vibration frequencies. Scand J Work Environ Health 1986;12:362–364.
18. Pyykko I. The prevalence and symptoms of traumatic vasospastic disease among lumberjacks in Finland. A field study. Work Environ Health 1974;11:118–131.
19. Smith KC, Comite SL, Balasubramanian S, Carver A, Liu JF. Vibration anesthesia: a noninvasive method of reducing discomfort prior to dermatologic procedures. Dermatol Online J 2004;10:1.
20. Van Citters RL, Wagner BM, Rushmer RF. Architecture of small arteries during vasoconstriction. Circ Res 1962;10:668–675.
21. Welsh CL. The effect of vibration on digital blood flow. Br J Surg 1980;67:708–710.