1. INTRODUCTION

Both epidemiological (1) and experimental studies (2) suggest that the ISO 5349 frequency weighting curve may not place enough weight on exposure to mid-range vibration frequencies (i.e., 100-500 Hz). Data from rat vibration studies demonstrated the risk of developing vibration-induced vascular dysfunction is greater in rats exposed to vibration at 125 or 250 Hz than in rats exposed to vibration at 62.5 Hz (3). Vibration also affects peripheral nerve function. However, the frequency-dependent effects of vibration on injury to the peripheral nervous system have not been examined. The goal of this study was to characterize the frequency-dependent effects of repeated vibration exposures (i.e., 10 days) on peripheral nerve function and biology.

2. METHODS

2.1. Exposure

Male Sprague-Dawley rats [Hla:(SD) CVF rats; 6 weeks of age at arrival; Hilltop Lab Animals, Inc, Scottdale, PA] were used in this study. Animals were maintained in an AALAC-accredited vivarium under a 12:12 LD cycle (lights on 0700 h) with food and water available ad libitum. Rats were acclimated to the laboratory for 1 week prior to the beginning of the experiment.

On the first day of the experiment, rats were restrained in Broome style restrainers. Each rat had their tail secured to a platform as previously described (4). Rats (N=6/group) were exposed to vibration at 62.5, 125 or 250 Hz (49 m/sec² r.m.s.), restraint-control, or cage control conditions for 10 consecutive days. Restraint-control rats had their tails secured to a platform mounted on isolation blocks. Cage control rats were maintained in their home cages. Following the last exposure, rats were anesthetized using pentobarbital (100 mg/kg, i.p.) and euthanized by exsanguination 10 days following the exposure.

Tail nerves and dorsal root ganglia (DRG, from the L5-6 regions of the spine) were dissected and frozen for analyses of transcript expression or for immunohistochemistry. An additional segment of the nerve was embedded in JB4, sectioned and stained for histological analyses.

All procedures were approved by the NIOSH Animal Care and Use committee and were in compliance with CDC and NIH guidelines for the care and use of laboratory animals.

2.2. Current Perception Threshold (CPT)

Aβ, Aδ, and C fiber functions were assessed using transcutaneous electrical stimulation at 3 different frequencies (2000, 250 or 5 Hz to test different fiber types). Thresholds were measured prior to vibration or restraint exposures on days 1 and 9 of the study.

To perform the CPT, a stimulating electrode was attached near the C18 region of the tail and a dispersing electrode was attached approximately 1cm above that. Electrical stimulation was gradually increased (0.5 amp increments at 2000 Hz and 0.1 amp increments at 250 and 5 Hz) until the rat flicked its tail.

2.3. Gene Expression

Changes in gene expression were measured in the ventral tail nerve and DRG using total rat genome arrays to identify candidate genes (Illumina Rat Expression Arrays) and changes were verified using quantitative PCR as previously described (3).

2.4. Morphology

Segments of the ventral tail nerve were embedded in JB4 and 2 µm transverse sections were cut on a microtome. Sections were stained with Sudan Black to assess changes in myelinated nerve number and myelin thickness. Other sections were stained with methylene blue to count infiltration of mast cells. Frozen segments of nerve were sectioned and immunostained for albumin to determine if vibration resulted in edema in nerves.

2.5. Data Analyses

CPT data were analyzed using 2 (day) x 5 (treatment) ANOVAs, with subjects added as a random variable. Histological and gene data were analyzed using 1-way ANOVAs. Differences with p < 0.05 were considered significant.
3. RESULTS

There were no significant changes in the CPTs at 250 or 5 Hz. However, between days 1 and 9, there was a significant decrease in the 2000 Hz CPT in rats exposed to vibration at all frequencies (Figure 1).

There were few significant changes in gene expression in either the tail nerve or DRG after 10 days of vibration exposure. However, nerves from rats exposed to vibration at 250 Hz displayed a significant reduction in myelin thickness (Figure 2) and an increase in the area stained for albumin (Figure 3). These changes in swelling and myelin thickness were not accompanied by a change in the number of myelinated nerves or a change in the number of mast cell.

4. DISCUSSION AND CONCLUSIONS

- After 9 days of vibration exposure, rats displayed an increased sensitivity to 2000 Hz stimuli during the CPT. This suggests that in vibrated rats, Aβ fibers are more sensitive to electrical stimulation. Changes in perception thresholds are early indicators of injury.

- Myelin thickness and albumin staining (i.e. edema) were altered in nerves from rats exposed to vibration at 250 Hz, but not at the other frequencies.

- These data suggest that there are frequency dependent changes in peripheral nerves after 10 days of vibration exposure. However, vibration at all frequencies appears to have an effect on Aβ nerve function.

- These data are consistent with previous findings suggesting that greater weight should be given to mid-range frequencies (100-500 Hz) in the ISO 5349 frequency weight curve.

REFERENCES


