INTERPRETING HAVS BASED ON RAT TAIL VIBRATION INJURY DATA

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Introduction

Hand arm vibration syndrome (HAVS) is a vasospastic, neurodegenerative and musculoskeletal disease. The pathology of late stage disease persists after quitting vibration tool use. HAVS onset and severity is highest for percussive tools.[1] One 12 min exposure to riveting hammer vibration damaged nerve endings in rat tails.[2] Nerve fibers were assayed structurally by protein gene product 9.5 (PGP9.5) nerve immunostaining. PGP9.5 binds an ubiquitin pathway protein present in all types of nerve fibers, but PGP9.5 levels may change with injury and alter identification consistency. Markers specific for nerve subtypes are required to relate changes to functional alterations. The present study of hammer vibration was conducted to validate that complete cross sections of the tail could be cut and stained for nerve subtypes and mast cells.

Methods

Adult male rats were distributed into riveting hammer and sham vibrated groups (n=6/group). Rats were restrained 12 min with their tails taped to a vibration platform that remained stationary for the sham controls. After treatment, rats were euthanized, and tail tissues were chemically fixed for histological analysis of nerves (PGP9.5, neuropeptide Y, calcitonin gene related protein) and mast cells (avidin, Alcian Blue/Safranin O).[2] Single sections were double-stained for nerve fibers and mast cells to permit assessment of the degree of physical overlap. Microscope images were taken and analyzed by computer-assisted morphometry.

Results

Figure 1 shows perivascular NPY-positive nerve fibers and avidin-positive mast cells.

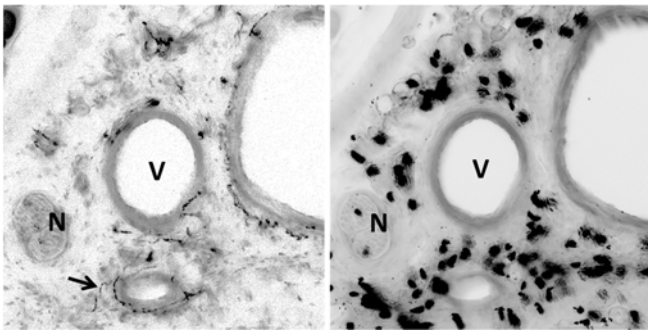

Figure 1. The images are of a double-stained cross section showing nerve bundles (N), veins (V) and terminal nerve fibers. The arrow points to neuropeptide Y immunoreactive nerve fibers ramifying on the vascular smooth muscle layer (left). Imaging the section for avidin binding (right) reveals the high concentration of dark-stained mast cells in the perivascular connective tissue. The nerve endings and mast cells are closely associated.

Figure 2 illustrates CGRP-positive nerve fibers and avidin-positive mast cells. NPY fibers and mast cells are numerous in the perivascular regions and exhibit overlapping distributions. CGRP fibers are much less numerous, but, compared to NPY, there is a closer association with mast cells (Fig 2). Some mast cells appear CGRP stained. Overlap of nerve fibers and mast cells in tissues processed after vibration was slightly higher in vibrated compared to sham tissues.

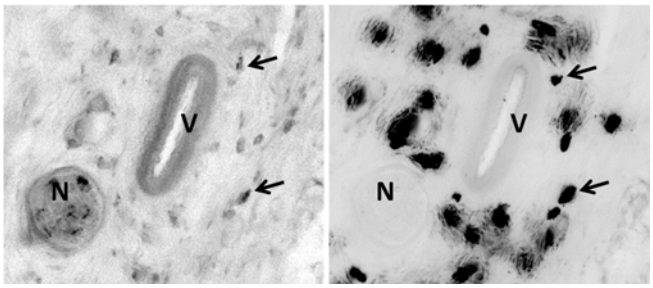

Figure 2. The left panel was immunostained for calcitonin gene related protein (CGRP), and the right panel was avidin stained for mast cells. The nerve bundle (N) contains CGRP positive nerve fibers. CGRP fibers are not numerous and infrequently contact blood vessels (V). Some mast cells (arrows) appear immunoreactive for CGRP.

Discussion

PGP9.5, NPY and CGRP staining of nerve fibers and avidin-positive mast cells is feasible in rat tail sections. PGP9.5 stains all nerve types which permits assessing the global effects. NPY and CGRP reveal subtype specific changes. NPY fibers richly innervate arteries, arterioles and veins. NPY potentiates sympathetic nerve vasoconstrictive effects.[3] Vibration white finger is likely driven by somatosympathetic reflex activation, increased α2c adrenergic receptor expression and endothelin-1 release. CGRP stimulates relaxation of vascular smooth muscle. Mast cells moderate vasoconstriction by releasing proteases that degrade vasoactive neuropeptides and endothelin-1. The present study shows CGRP in some mast cells, but it is unknown whether this represents associated nerve fibers and/or uptake with re-release during vasoregulation. Mast cells secrete histamine that inhibits vasoconstriction via H2 receptors on smooth muscle cells. Mast cells are key players in vasoregulation. Chronic exposure to vibration is predicted to decrease mast cell number and result in untempered vasospastic activity.

Previously, we demonstrated destruction of terminal nerve fibers by impact vibration.[2] Nerve fibers can regenerate after damage. Regenerating axons sprout more terminal branches than normal, but eventually branches are lost. Repeated nerve injury can invoke death of the nerve cell body and failure to regenerate. We predict that multiple day exposure to vibration will decrease the total, PGP9.5 positive innervation. Loss of sensory fibers leads to loss of feeling. Nerve fibers of different types regenerate to different degrees. Vasoconstriction fiber (NPY) regeneration is expected to be higher than (CGRP) vasorelaxant fibers. This shifts in favor of vasoconstriction, a possible cause of white finger. It is hypothesized that the number of mast cells declines during weeks of vibration. Fewer mast cells means less histamine-induced vasodilation and protease inactivation of NPY and endothelin-1, favoring vasospasm. Repeated injury and smooth muscle remodeling in resistance arteries is expected to thicken the vessel wall and narrow the lumen and reduce blood flow.[4, 5] The neural and vascular changes from long term vibration are envisioned to generate the persistent vasospastic and neurodegenerative HAVS.

References

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