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BRAIN RESEARCH

Research Report

The impact of short-lasting repeated vibrations on retrograde axonal transport, the expression of CGRP and parvalbumin in lower lumbar dorsal root ganglia

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ABSTRACT

A prolonged exposure to vibration stimuli triggers pathological changes with many later manifested symptoms. Early vibration-induced changes are still not very well explored. Therefore, short 30 min vibration period per day with frequency 60 Hz repeated for 10 days was used, and the retrograde axonal transport from the sciatic nerve, the expression of calcitonin gene-related peptide (CGRP) and parvalbumin (PV) were studied in the dorsal root ganglia (DRGs) corresponding to lower lumbar spinal levels. Repeated vibration markedly decreased (25 and 34%) the accumulation of retrogradely transported Fluorogold to spinal motor neurons, whereas a significant increase (35 and 25%) was seen in the DRG primary sensory neurons corresponding to the L4 and L5 spinal level. Immunohistochemical studies showed a significant reduction of CGRP-positive small-sized neuronal cells in both DRGs. Fluoro-Jade labeling revealed that marked loss of CGRP-imunoreactive DRG sensory neurons is not due to neuronal degeneration. CGRP protein expression determined by Western blot analysis and optical density measurement, and NGF level measured by ELISA have been decreased, markedly only at the L4 DRG. PV protein expression was not affected by short repeated vibrations. Our results indicate that (a) short-lasting repeated vibrations affect the retrograde axonal transport in the DRG sensory neurons differently than in spinal motor neurons; (b) a decreased NGF-dependent CGRP production in the DRG primary sensory neurons plays an important role in early vibration-induced pathological mechanisms.

1. Introduction

A prolonged hand-arm vibration is a common stress stimulation coming from the use of hand-held power tools and is the cause of serious health problems like hand-arm vibration syndrome (HAVS). Damage from HAVS can be prevented, but once it occurs than it is permanent. There is no therapy at present for neurological symptoms other than removal from

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Abbreviations: HAVS, hand-arm vibration syndrome; CGRP, calcitonin gene related peptide; PV, parvalbumin; DRGs, dorsal root ganglia; NGF, nerve growth factor

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vibration exposure. HAVS is a disease that involves circulatory, musculoskeletal, motor and sensory disturbances, including a reduction or loss of sense of touch and pain perception. However, early pathological mechanisms in the vibration injury process are still not fully clarified.

Active transport along the axon is the foundation for survival and metabolism of the neuron. Alterations in axonal transport induced by various pathological conditions can lead to neuronal stress and even to cell death (Perlson et al., 2010). Although, retrograde axonal transport disruption after repeated vibrations was detected in motor fibers of sciatic nerve (Yan et al., 2005), it is not know whether a retrograde transport is affected in primary sensory neurons or not.

Calcitonin gene-related peptide (CGRP) as well as parvalbumin (PV) is highly expressed in selective subpopulation of dorsal root ganglion (DRG) neuronal cells, and they are rarely colocalized (Carr et al., 1989). CGRP is a marker of neuronal plasticity in primary sensory neurons and is expressed by small- to medium-sized primary sensory neurons, predominantly by small ones (Verge et al., 1989). Most of the small DRG neurons are involved in nociception (Aoki et al., 2004). PV is one of the calcium binding proteins extensively distributed in the DRG predominantly in large-sized neuronal cells (Carr et al., 1989). PV-immunoreactive neurons project their axons intimately to muscle spindles and coil themselves around intrafusal muscle fibers (Celio, 1990). It strongly suggests that PV-immunoreactive DRG neuronal cells are included in muscular proprioception. The precise function of PV is unknown, but its participation in calcium buffering (Baimbridge et al., 1982), modification of neuronal excitability to synaptic input (Baimbridge et al., 1985) or mediation of calcium dependent events was well documented (Berchtold et al., 1984).

The present study was focused on retrograde axonal transport, CGRP and PV expression after short-lasting repeated vibrations in the DRGs corresponding to lower lumbar spinal cord segments.

2. Results

2.1. *Fluorogold labeling—the detection of retrograde axonal transport*

The impact of vibrations on axonal retrograde transport was significant. Fluorogold labeling within lower lumbar spinal cord segments was clearly seen only in ventral motor neurons (Fig. 1). A quantitative measurement revealed that a retrograde axonal transport of Fluorogold in lower lumbar ventral horn motor neurons after 10 day vibrations was markedly diminished about 25% in the L4 segment and 34% in the L5 segment (Fig. 1). A reversed effect of short-term repeated vibrations was detected in corresponding DRGs. Retrogradely transported Fluorogold was accumulated under physiological conditions exclusively in DRG neuronal cells (predominantly in small-sized ones), but it also appeared in neuropil after vibrations (Fig. 2A–D). Visual examination and density quantification showed a significant increase of Fluorogold labeling in both L4 and L5 DRGs (35% and 25% increase, respectively) (Fig. 2E,F).

2.2. *CGRP and PV immunostaining—density measurement and cell counting*

CGRP-immunohistochemical labeling in the DRGs corresponding to the L4 and L5 spinal segments was distributed predominantly in small-sized primary sensory neurons, but some medium-sized ones and sensory fibers were immunopositive as well (Fig. 3A–D). Densitometric measurement of CGRP-immunoreactivity revealed a statistically significant decrease of CGRP expression in the L4 DRG (Fig. 3E). Cell counting showed a marked decline of small-sized CGRP-immunoreactive neuronal cells by about 23% after repeated vibrations in both DRGs (Fig. 3G, H).

PV-immunolabeling in lower lumbar DRGs was found mainly in some fibers belonging to medial part of dorsal roots and large-sized neuronal cells, but also in few medium-sized ones (Fig. 4A–D). Neither density measurement of PV-IR nor large-sized immunopositive cell counting indicated a statistically significant modification after repeated vibrations (Fig. 4E–H).

2.3. *Western blot analysis*

Western blot analysis of CGRP protein expression confirmed results determined by immunohistochemical quantification. Short-lasting repeated vibration reduced the CGRP expression in both DRGs, however, statistically significant decline was seen only in the L4 DRG (Fig. 5).

2.4. *Fluoro-Jade labeling—the detection of neuronal degeneration*

Fluoro-Jade stains the cell bodies, dendrites, axons and axon terminals of degenerating neurons, but does not stain healthy neurons, myelin, vascular elements or neuropil (Schmued et al., 1997). Cells of the meninges exhibit an affinity for the dye. No Fluoro-Jade labeling was found in the DRGs indicating that no neuronal cell degeneration occurs in primary sensory neurons after short-lasting repeated vibrations (Fig. 6).

2.5. *NGF level determination*

NGF level in the DRGs was measured by rat sandwich ELISA NGF kit (Chemicon, MA, USA). Short-lasting repeated vibration induced a statistically significant reduction of NGF level under basal level only in the DRG corresponding to the L4 spinal level (Fig. 7).

3. Discussion

Active retrograde axonal transport has a very important role in the maintenance and survival of specific neuronal population. Yan et al. (2005) recently reported that retrograde axonal transport in the sciatic nerve motor fibers of experimental animals vibrated 5 h/day for 10 days is disrupted, and that the effect of repeated vibrations is cumulative. These animals have shown progressive weakness of the hind limbs and difficulty in walking (Yan et al., 2005). The present study shows that vibrations performed each day for 30 min markedly reduced retrograde axonal transport to ventral motor