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Histone Acetylation in Microglia Contributes to Exercise-Induced Hypoalgesia in Neuropathic Pain Model Mice

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Abstract: Physical exercise can attenuate neuropathic pain (NPP), but the exact mechanism underlying exercise-induced hypoalgesia (EIH) remains unclear. Recent studies have shown that histone hyperacetylation via pharmacological inhibition of histone deacetylases in the spinal cord attenuates NPP, and that histone acetylation may lead to the production of analgesic factors including interleukin 10. We intended to clarify whether histone acetylation in microglia in the spinal dorsal horn contributes to EIH in NPP model mice. C57BL/6J mice underwent partial sciatic nerve ligation (PSL) and PSL- and sham-runner mice ran on a treadmill at a speed of 7 m/min for 60 min/d, 5 days per week, from 2 days after the surgery. PSL-sedentary mice developed mechanical allodynia and heat hyperalgesia, but such behaviors were significantly attenuated in PSL-runner mice. In immunofluorescence analysis, PSL surgery markedly increased the number of histone deacetylase 1-positive/CD11b-positive microglia in the ipsilateral superficial dorsal horn, and they were significantly decreased by treadmill-running. Moreover, the number of microglia with nuclear expression of acetylated H3K9 in the ipsilateral superficial dorsal horn was maintained at low levels in PSL-sedentary mice, but running exercise significantly increased them. Therefore, we conclude that the epigenetic modification that causes hyperacetylation of H3K9 in activated microglia may play a role in producing EIH.

***Perspective:** This article presents the importance of epigenetic modification in microglia in producing EIH. The current research is not only helpful for developing novel nonpharmacological therapy for NPP, but will also enhance our understanding of the mechanisms and availability of exercise in our daily life.*

***Key words:** Neuropathic pain, exercise-induced hypoalgesia, treadmill-running, histone deacetylase 1, acetylated histone H3K9.*

The term epigenetics refers to processes that lead to stable/heritable changes in gene function without any concomitant DNA sequence changes.[14] Epigenetic mechanisms include modifications of genomic DNA or histones and the action of small regulatory noncoding RNAs. Histones are DNA-packing globular proteins that can undergo post-translational modification at specific sites at their N-terminus, including acetylation, methylation, phosphorylation, and ubiquitination.[16] In particular, histone acetylation is controlled through the dynamic interplay of related enzymes, histone acetyltransferases and histone deacetylases (HDACs). Acetylation at histone lysine residues via histone acetyltransferases relaxes chromatin structures and promotes the transcription of target genes, whereas deacetylation of histone lysine residues via HDACs causes chromatin condensation and, consequently, transcriptional repression of the target genes.[14,20,47] Neuropathic pain (NPP) is an intractable form of chronic pain, which is produced by damage to and pressure on peripheral and central nervous systems. Recent studies have shown that pharmacological inhibition of HDACs in the spinal cord of NPP animal models improves pain-related behaviors by reducing

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HDAC1 and enhancing histone acetylation,[6,13,33] and have also suggested that epigenetic modification plays important roles in producing and attenuating NPP.[15]

Physical exercise has been established as an effective means to reduce chronic pain.[3,5,10,32,44,45] For instance, treadmill-running was shown to reduce pain hypersensitivities in chronic constriction injury (CCI) model mice, and it was suggested that inhibition of microgliosis in the spinal dorsal horn may contribute to this exercise-induced hypoalgesia (EIH).[11] In addition, treadmill-running in spinal nerve ligation model rats increases endogenous opioids in periaqueductal gray matter and rostroventral medulla, and it was suggested that the descending pain inhibitory system may play an important part in producing EIH.[48] Moreover, swimming in partial sciatic nerve ligation (PSL) model mice attenuates pain behaviors via the reduction of proinflammatory cytokines in the injured sciatic nerve.[34] Thus, physical exercise improves NPP through alterations of cells that constitute the spinal dorsal horn, brainstem, and injured peripheral nerve, but the exact mechanisms underlying EIH are not fully understood.

In response to peripheral nerve injury, activated microglia in the spinal dorsal horn release proinflammatory cytokines, chemokines, and cytotoxic compounds, which are considered to be essential factors in the development and maintenance of NPP. In addition, active microglia also produce anti-inflammatory cytokines like interleukin (IL) 10 (IL-10). Interestingly, intrathecal administration of rat IL-10 protein or intrathecal lentiviral-mediated transfer of IL-10 can reverse the enhanced pain behaviors in CCI rat models.[22,40] Moreover, a line of evidence has shown that epigenetic modification, such as phosphorylation, acetylation, and methylation, of histone H3 at specific regions in the IL-10 promoter is an important regulatory step for IL-10 production in myeloid cells including macrophages.[23,35-37,46,52,53] These results suggest that epigenetic modifications in activated microglia in the spinal dorsal horn may participate in producing EIH, perhaps via the upregulation of analgesic factors including IL-10. However, data on the relationship of EIH and histone acetylation in microglia are still lacking. Therefore, we investigated whether histone acetylation in microglia in the spinal dorsal horn contributes to the production of EIH in NPP model mice performing treadmill-running for 60 minutes.

Methods

Preparation of NPP Model Mice

Twelve-week-old, male, C57BL/6J mice (Japan SLC, Shizuoka, Japan) were used in this study. The mice were housed 4 to 5 per cage, under a 12-hour light-dark cycle, and allowed free access to food and water. NPP model mice were prepared using PSL according to the method of Seltzer et al.[43] The mice were deeply anesthetized with 2% isoflurane to maintain painless conditions. Under sterile conditions, an incision was made in the skin on the lateral surface of the right thigh and the muscle layers were parted to expose the sciatic nerve at the midthigh level. Approximately one-third to one-half of the sciatic nerve was tightly ligated with 8-0 silk sutures (Alcon Surgical, Fort Worth, TX), and then the wound was closed with 5-0 nylon sutures (Akiyama-Seisakusho, Tokyo, Japan). Sham surgery was performed according to the same procedures, except the execution of PSL. The experiments were approved by the Animal Care Committee of Wakayama Medical University. All experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 99-158, revised 2002).

Experimental Groups and Treadmill-Running Protocol

The mice were divided into 5 groups (Fig 1): 1) naive mice: mice without any artificial surgeries, 2) sham-sedentary mice: mice without treadmill-running after sham surgery, 3) sham-runner mice: mice with treadmill-running after sham surgery, 4) PSL-sedentary mice: mice without treadmill-running after PSL surgery, and 5) PSL-runner mice: mice with treadmill-running after PSL surgery.

All mice except naive mice were accustomed to treadmill-running for 2 weeks before sham and PSL surgeries. Treadmill-running was begun at 5 PM. The treadmill-running protocol is presented in Fig 1. All mice were acclimated to the treadmill belt for 10 minutes

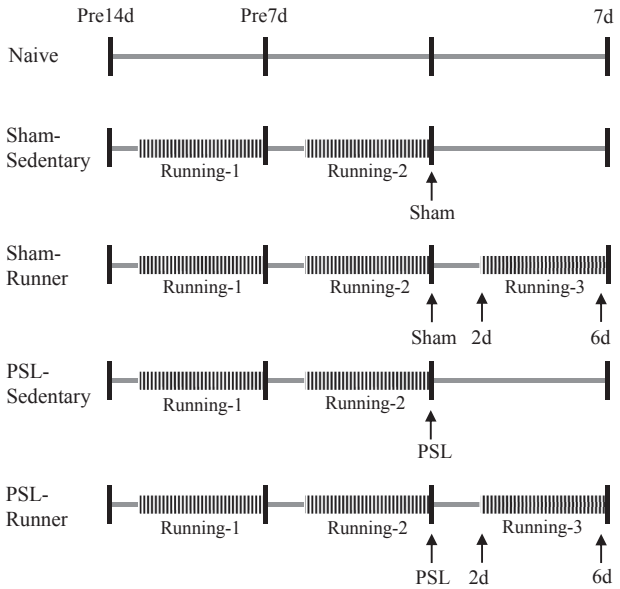

Figure 1. Experimental groups and treadmill running protocol. Adult male C57BL/6J mice were divided into 5 experimental groups. All groups except naive mice were subjected to running-1 and running-2 before PSL surgery. In the running-1 program, the mice ran at a speed of 7 m/min for 10 min/d for 5 days per week, and in the running-2 program, the mice ran at a speed of 7 m/min for 20 to 60 min/d (an increment of 10 minutes per day), 5 days per week. The PSL- and sham-runner mice ran at a speed of 7 m/min for 60 min/d, 5 days per week, from 2 days (2d) after the surgeries (running-3). Abbreviations: Pre14d, 14 days before surgery; Pre7d, 7 days before surgery; 6d, 6 days.