Altered Expression of Sodium Channels Distribution in the Dorsal Root Ganglion after Gradual Limb Lengthening of the Rat Sciatic Nerve: A Precursor of Chronic Nerve-Stretch Disorder

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Introduction: Limb lengthening has become a popular method but this technique has the potential to cause peripheral nerve injury. It is believed that gradual nerve elongation is the cause of this form of injury. Clinically it is known that the early symptoms of neuropathy induced by limb lengthening is pain/paresthesia along the distribution of a sensory nerve[1]. Previous studies have identified that the underlying pathophysiology of disorders after a nerve injury is closely associated with the altered expression of the voltage-gated sodium channels (VGSCs) in the dorsal root ganglion (DRG). These studies involving neuropathic states have suggested pain/paresthesia is modulated by a subset of VGSCs, including downregulation of tetrodotoxin-resistant (TTX-R) sodium channels (Nav1.8, Nav1.9) and/or upregulation of tetrodotoxin-sensitive (TTX-S) sodium channels (especially Nav1.3) in nerve injury animal models[2-4]. To our knowledge, no previous studies have focused on changes in the expression of VGSCs in DRG following chronic nerve-stretch injury. The purpose of this study was to perform histological and electrophysiological investigations in a gradually elongated nerve and to analyze the expression of sodium channel mRNA in the DRG; a rat limb-lengthening experimental model was used for the purpose of the investigation.

Materials and Methods: Male Sprague Dawley rats at the age of 10 weeks were used. All procedures were approved by the Osaka Medical College Animal Care and Use Committee. The right femur of rat were gradually lengthened by 15 mm at the rate of 0.5 mm/day (group L, n=8) with an external fixator-distractor (M103; Orthofix). The control groups comprised sham (group S, n=5) and naïve (group N, n=8) rats. On the next day after limb lengthening is completed, compound muscle action potential (C-MAP) were recorded. After the L4, 5 DRG tissue samples were harvested, total copy number of mRNA for Nav1.8, Nav1.9, and Nav1.3 were measured with real-time RT-PCR. The gene was normalized to GAPDH expression. After transcardially perfusion, sciatic nerve was harvested and embedded with Epon. Longitudinal thin sections were stained with toluidine blue and examined under a light microscope. After L4 DRG was harvested, immunostaining for Nav1.8 protein were performed with anti-Nav1.8 polyclonal antibody. Each section was graded blindly by three independent investigators. Relative expression was graded as unobservable (-), low (+), moderate (++), high (+++). The results of electrophysiological and real-time RT-PCR analyses are presented as mean values and the standard error mean. Differences between the groups were compared using analysis of variance. After immunohistochemical assay, the Chi-square test was used to compare the relative expression. Values of P < 0.05 were considered to be significant.

Results: On X-ray, The mean lengthening rate of right femurs was lengthened 34.1% compared to that observed in the left femurs. The amplitude of C-MAP in group L was significantly reduced when compared with those observed in groups S and N. The amount of reduction was 24% in comparison with that observed in group S (P<0.0001) and 22% in comparison with that observed in group N (P = 0.001). The latency of C-MAP was significantly delayed as well. The amount of delay was 34% (P<0.0001) and 25% (P<0.0001), respectively. No significant difference was recognized between the control groups (Fig. 1). Real-time RT-PCR analysis showed Nav1.8 mRNA expression in group L was significantly decreased in comparison to both the control groups (P<0.05 respectively). Nav1.9 mRNA expression of group L was significantly decreased in comparison to that of the group S. When Compared to group N, significant difference was not detected but a decreasing tendency of mRNA Nav1.9 was observed between group L and group N (P=0.059). With regard to Nav1.3 mRNA expression, there was no significant difference within each group (Fig.2). Histological analysis and Immunohistochemical assay showed longitudinal section of nerve fibers had lost their normal wavry structure and become straight in group L in comparison to that observed in group N. But the widening of the node of Ranvier was not observed in group L (Fig.3). With regard to Nav1.8 protein expression, group L demonstrated significantly lower expressions in the DRG than group N (p<0.021) (Fig4).

Discussion: We have demonstrated the downregulation of Nav1.8 expression in DRG by gradual nerve elongation. Meanwhile, the elongated nerves did not show apparent microstructural alterations and revealed mild conduction slowing. That is, the degree of nerve damage seemed to be so mild from histological and electrophysiological perspective. Hence, this downregulation could be a precursor of chronic nerve-stretch disorder. Although the limitation of our study is that we did not perform behavioral assessment, we speculate that the hyperexcitability caused by downregulation of Nav1.8 may be involved in the earliest pathophysiological mechanisms of neuropathy induced by limb lengthening.

Fig.1. electrophysiological analysis of C-MAP.
Values are represented as the mean ± SEM. (*P<0.05, n=5 each)

Fig. 2. Real-time RT-PCR analysis of Nav1.8, Nav1.9, and Nav1.3 expression in DRG. Values are represented as the mean ± SEM. (*P<0.05, **P<0.059, n=5 each)

Fig.3. Longitudinal section of nerve fibers stained by toluidine blue. Arrows indicate the node of Ranvier. Scale bar, 10μm.

Fig.4. L4 DRG section from group L (A, B) and group N (C, D). Neuron cell bodies were stained deeply with toluidine blue (A, C). Nav1.8-immunoreactivity of neuron cell bodies (DRG) in group L (B) was significantly lower than that in group N (D). Scale bar, 20μm.

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