Nerve compression upregulates gene expression of P2X4 receptors in peripheral nerve.

INTRODUCTION:

Pain after nerve damage is caused by the pathological changes of the nervous system, but the mechanisms are poorly understood. The past reports said that P2X4 receptors expression increased in the ipsilateral spinal cord after spinal nerve ligation (SNL) model, and P2X4 receptors were induced in the hyperactive microglia but not in the neurons or astrocytes. P2X4 receptors at the microglia induce pain at the central nervous system. We used sciatic nerve compression rat models and tried to reveal the expression of P2X4 receptors at the peripheral nerve.

MATERIALS and METHODS:

Animal model: Adult male Sprague Dawley rats (bodyweight approximately 250 g each) were anesthetized with 5% pentobarbital sodium at a dose of 40-50 mg/kg. The right sciatic nerve was exposed, and 1 cm silicon tube with an internal diameter of 1.3 mm and outer diameter of 2.0 mm was placed around the sciatic nerve. All animals were operated again at days 28, 56, and 84 after first surgery and samples were harvested.

Histological Evaluation: Operated nerves were stained with hematoxylin and eosin or Masson’s trichrome method. Electrophysiological Evaluation: Compound muscle action potential (CMAP) of the tibialis anterior muscle was measured at room temperature. Electrical pulses (supramaximal; duration, 100 ms; frequency, 1 Hz; square wave) were applied with an isolator connected to an electronic stimulator.

Wet Muscle Weight Measurements: The tibialis anterior muscles from each animal were dissected and weighted immediately (still wet). The tibialis anterior muscle weight data were calculated as percentage of total body weight.

Immunostaining: The sciatic nerves were stained with the horseradish peroxidase/DAB stain and double immunofluorescence labelling of P2X4 receptors with neurofilament/beta III tubulin.

Real time RT-PCR: Total RNA was isolated from each sciatic nerve and P2X4, NGF, BDNF, TNFα, and NAV1.9 specific primer and TaqMan probe were used. Samples were subjected to 40 cycles of amplification at 95°C for 15 sec and 60°C for 1 min, after holding stage at 50°C for 2 min 95°C for 10 min and mRNA expression changes between tube group and sham group were determined by calculating ΔCt (Ct for each target minus Ct for ACTB) for each sample.

RESULTS:

The latency of tube group was significantly longer than that of the sham group. The tube group showed significantly lower percentage of wet muscle weight of the tibialis anterior. Both hematoxylin and eosin staining and Masson’s trichrome staining showed development of intraneural edema and thickening of perineurium in the tube group. Immunohistochemistry showed increased expression of P2X4 receptors in the tube group (Figure 1, A, B). Double immunofluorescence staining with a P2X4 receptor antibody and a neurofilament/beta III tubulin antibody dimed P2X4 receptor expression by axons. Real time RT-PCR clearly demonstrated that expression level of P2X4 receptors increased in parallel with those of TNFα, NGF, and BDNF (Figure 2) in the tube group. In contrast, NAV1.9 expression was downregulated throughout the experimental period.

DISCUSSION:

Carpal tunnel syndrome is one of the most common entrapment neuropathy. Although tingling pain in the median nerve distribution is the most common patients’ complain especially in the early phase of the disease, little is known about the pain mechanism. Our previous studies demonstrated that a number of growth factors, cytokines and proteases are involved in the development of carpal tunnel syndrome and MMP-2 activity level is significantly associated with pain intensity. Frieboes LR et al reported involvement of Nav 1.8 expressed by Schwann cells. These reports suggest contribution of neuro-inflammatory interaction underlying the induction of pain in carpal tunnel syndrome.

In this study, we demonstrated that chronic compression of a nerve is associated with increased expression of a purinoceptor, P2X4, by non-neuronal cells within the nerve fascicles. In contrast, Nav1.9, a representative sodium channel which is known as a relevant target for pain, was downregulated throughout the experimental period. Previous reports showed upregulation of P2X4 receptors in microglia after nerve injury mediates BDNF release which, in turn, acts in the dorsal horn to induce the neuropathic pain. In addition, a recent study demonstrated involvement of BDNF in neuropathic pain in the peripheral nervous system. Taking the fact that gene expression of P2X4 receptors and BDNF increases in parallel into consideration, similar mechanisms to the central nervous system might exist at the peripheral nerve level.

REFERENCES: