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Research Report

Changes in VGLUT2 expression and function in pain-related supraspinal regions correlate with the pathogenesis of neuropathic pain in a mouse spared nerve injury model

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ABSTRACT

Vesicular glutamate transporters (VGLUTs) control the storage and release of glutamate, which plays a critical role in pain processing. The VGLUT2 isoform has been found to be densely distributed in the nociceptive pathways in supraspinal regions, and VGLUT2-deficient mice exhibit an attenuation of neuropathic pain; these results suggest a possible involvement of VGLUT2 in neuropathic pain. To further examine this, we investigated the temporal changes in VGLUT2 expression in different brain regions as well as changes in glutamate release from thalamic synaptosomes in spared nerve injury (SNI) mice. We also investigated the effects of a VGLUT inhibitor, Chicago Sky Blue 6B (CSB6B), on pain behavior, c-Fos expression, and depolarization-evoked glutamate release in SNI mice. Our results showed a significant elevation of VGLUT2 expression up to postoperative day 1 in the thalamus, periaqueductal gray, and amygdala, followed by a return to control levels. Consistent with the changes in VGLUT2 expression, SNI enhanced depolarization-induced glutamate release from thalamic synaptosomes, while CSB6B treatment produced a concentration-dependent inhibition of glutamate release. Moreover, intracerebroventricular administration of CSB6B, at a dose that did not affect motor function, attenuated mechanical allodynia and c-Fos up-regulation in pain-related brain areas during the early stages of neuropathic pain development. These results demonstrate that changes in the expression of supraspinal VGLUT2 may be a new mechanism relevant to the induction of neuropathic pain after nerve injury that acts through an aggravation of glutamate imbalance.

Abbreviations: VGLUT, vesicular glutamate transporter; SNI, spared nerve injury; CSB6B, Chicago Sky Blue 6B; EAAT, excitatory amino acid transporter; PAG, periaqueductal gray; mPFC, medial prefrontal cortex; ACSF, artificial cerebrospinal fluid; PB, phosphate-buffer; PBS, phosphate-buffered saline; 4-AP, 4-aminopyridine; L-*trans*-2,4-PDC, L-*trans*-2,4-Pyrrolidine dicarboxylic acid

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1. Introduction

Glutamatergic transmission and homeostasis are regulated by a glutamate transporter system that includes excitatory amino acid transporters (EAATs) and vesicular glutamate transporters (VGLUTs). EAATs remove glutamate from the neuronal synapse into glia and neurons to terminate excitatory signaling, whereas VGLUTs transport glutamate into vesicles prior to exocytotic release. Three distinct isoforms of VGLUTs have been identified (Ni et al., 1994; Takamori et al., 2001; Schafer et al., 2002), with VGLUT1 and VGLUT2 accounting for most of the presumed excitatory glutamatergic terminals in the central nervous system, whereas VGLUT3 is diffusely distributed in the brain, defining a discrete subpopulation of non-glutamatergic neurons (Kaneko and Fujiyama, 2002; Schafer et al., 2002).

In adult rodents, VGLUT2 is most abundant in the diencephalon, brainstem, and spinal cord (Sakata-Haga et al., 2001; Kaneko and Fujiyama, 2002; Landry et al., 2004). The characteristic distribution pattern of VGLUT2 seems to be generally coincident with the nociceptive pathways, suggesting that VGLUT2 is involved in the signaling of pain. In addition, several studies in heterozygous mice have demonstrated that VGLUT2 deficiency results in attenuation or deletion of some neuropathic pain symptoms, unlike the effects of a VGLUT1 signaling impairment (Moechars et al., 2006; Leo et al., 2009). However, these results from genetically modified mice may be confounded by multiple factors, such as adaptation or compensation during development. Moreover, no study has investigated in wild-type animals possible time-dependent changes in VGLUTs in the central nervous system during the pathophysiological process of neuropathic pain development. The supraspinal regions are especially understudied in this connection.

In the transmission of pain, nociceptive information from the spinal cord terminates in the thalamus, which is a key relay station for transmission to the cerebral cortex and periaqueductal gray (PAG), which are implicated in descending pain modulation (Millan, 2002). As to affect and cognition in pain processes, the amygdala modulates cortical functions associated with pain-induced mood disorders (Yalcin et al., 2014), while the medial prefrontal cortex (mPFC) is crucial for pain-related perception (Metz et al., 2009). Importantly, the thalamus, in which 90% of the excitatory synaptic response depends on VGLUT2, has been demonstrated to exhibit immediate reorganization after partial nerve ligation (Brüggemann et al., 2001) and abnormal discharge patterns in neuropathic pain models (Guilbaud et al., 1990). In addition, it has been reported that reduction or loss of VGLUT2 expression leads to reduction in the quantal size of glutamate release in thalamic neurons and is associated with attenuation of neuropathic pain in vivo (Moechars et al., 2006).

In this study, we investigated temporal changes in VGLUT2 expression in pain-related brain areas in SNI mice by immunohistochemistry. In addition, Chicago Sky Blue 6B (CSB6B) was used as a pharmacological tool to examine whether inhibition of VGLUT activity would result in attenuation of thalamic glutamate release and pain behaviors. These results provide further evidence that VGLUT2 is associated with neuropathic pain.

2. Results

2.1. SNI-induced mechanical allodynia

The 25 mice that received SNI surgery were divided into 5 subgroups: SNI-0.5 d, SNI-1.0 d, SNI-1.5 d, SNI-3.0 d, and SNI-7.0 d. We then validated that all the mice developed mechanical hypersensitivity to von Frey stimulation and no significant difference existed among these subgroups at the corresponding time points. Thus, only behavioral data in the SNI-7.0 d group are shown (Fig. 1). Repeated-measures two-way ANOVA showed significant effects of treatment ($F[1,32]=1025$; $p<0.001$) and a Bonferroni post hoc test revealed a significant decrease in the mechanical withdrawal threshold of the hind paws ipsilateral to the SNI surgery sides on postoperative days 0.5 ($p<0.001$), 1.0 ($p<0.001$), 1.5 ($p<0.001$), 3.0 ($p<0.001$), and 7.0 ($p<0.001$).

2.2. Changes in VGLUT2 expression in pain-related brain areas after SNI

The specificity of the VGLUT2 antibody has been validated by the pre-adsorption study (Supplementary Fig. 1). In the analyzed supraspinal areas, VGLUT2 immunostaining is punctuate, compatible with nerve terminals in the vicinity of neuronal cell bodies. Fig. 2 shows the changes in VGLUT2 expression at different times after SNI. Two-way ANOVA shows a significant effect of treatment in thalamus ($F[1,40]=20.81$, $p<0.001$) and PAG ($F[1,40]=4.67$, $p<0.05$). The Bonferroni post hoc test shows significant differences between sham and SNI mice in thalamus ($p<0.001$) and PAG ($p<0.01$) on postoperative day 1. Although two-way ANOVA does not show a significant effect of treatment in ipsilateral or contralateral amygdala, planned post hoc comparisons reveal a significant difference between sham and SNI mice on postoperative day 1 (contra, $p<0.01$; ipsi,

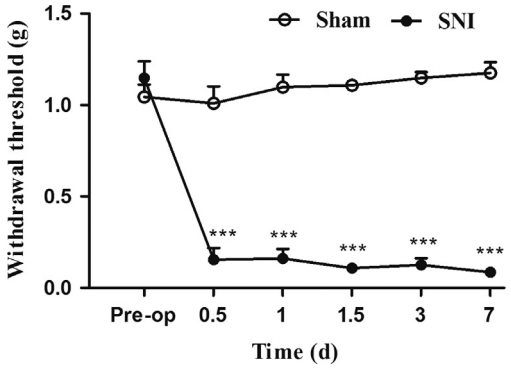

Fig. 1 – Spared nerve injury (SNI) reduces paw withdrawal threshold. This was demonstrated by stimulating the sural nerve territory with a series of von Frey filaments. The mechanical threshold of SNI mice was significantly lower than that of the sham mice on postoperative days 0.5, 1.0, 1.5, 3.0, and 7.0, expressed as means ± SEM. Note that the spacing of the time points is not to scale in this and subsequent figures $n=5$; $^{***}p<0.001$ compared with the sham control.