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Upregulation of Group I metabotropic glutamate receptors in neurons and astrocytes in the dorsal horn following spinal cord injury

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Abstract

Of the glutamate receptor types, the metabotropic glutamate receptors (mGluRs) are G proteins coupled and can initiate a number of intracellular pathways leading to hyperexcitability of spinal neurons. In this study, we tested the expression of mGluRs to determine which cell types might contribute to sustained neuronal hyperexcitability in the lumbar enlargement with postoperative day (POD) 7 (early), 14 (late), and 30 (chronic phase) following spinal cord injury (SCI) by unilateral hemisection at T13 in Sprague–Dawley rats. Expression was determined by confocal analyses of immunocytochemical reaction product of neurons (NeuN positive) and astrocytes (GFAP positive) in the dorsal horn on both sides of the L4 segment. Neurons were divided into two sizes: small (<20 μm) and large (>35 μm), for physiological reasons. We report a significant increase of $mGluR_1$ expression in large and small neurons of the dorsal horn on both sides of the cord in late and chronic phases when compared to control sham groups. Expression of $mGluR_{2/3}$ significantly increased in large neurons on the ipsilateral (hemisected) side in the late phase. Expression of $mGluR_5$ significantly increased in large neurons in early, late, and chronic phases. In addition, $mGluR_1$ and $mGluR_5$ expression after hemisection was significantly increased in astrocytes in early, late, and chronic phases; whereas $mGluR_{2/3}$ did not display any significant changes. In conclusion, our data demonstrate long-term changes in expression levels of Group I mGluRs ($mGluR_1$ and $mGluR_5$) in both neurons and astrocytes in segments below a unilateral SCI. Thus, permanent alterations in dorsal horn receptor expression may play important roles in transmission of nociceptive responses in the spinal cord following SCI.

Keywords: Astrocytes; Central neuropathic pain; Metabotropic glutamate receptor; Spinal dorsal horn; Spinal cord injury

Introduction

Metabotropic glutamate receptors (mGluRs) are coupled to G-proteins and are known to modulate nociceptive transmission in the spinal cord (Fisher et al., 2002; Mills et al., 2000). These receptors include three different types: Group I, II, and III. Activation of Group I mGluR initiates intracellular pathways that induce enhanced neuronal excitability by: (1) release of calcium ions from intracellular calcium stores (Pin and Duvoisin, 1995), (2) excitation of ionotropic glutamate receptors (iGluRs) by feedback loop (Budai and Larson, 1998), and (3) inhibition of Ca^{2+}-dependent after hyperpolarization (Anwyl, 1999). However, Group II and III mGluRs modulate nociceptive transmission by inhibiting the formation of cyclic adenosine monophosphate (cAMP) (Conn and Pin, 1997).

Expression levels of mGluRs are upregulated in the spinal gray following joint inflammation (Neugebauer and Carlton, 2002), peripheral nerve injury (Fisher et al., 2002), and spinal contusion injury (Mills et al., 2001). In addition, several studies indicate that a significant increase of mGluR expression in the spinal cord is involved in chronic neuropathic pain states because the blockade of mGluR receptor action by receptor antagonists results in antinociceptive effects (Dogrul et al., 2000; Mills et al., 2000; Neugebauer et al., 1999; Yashpal et al., 2001). A shortcoming of the aforementioned studies, however, is that the changes in mGluR expression in the dorsal horn were analyzed in terms of overall density changes and as a result provide no information as to what cell types (neurons vs.

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glial) or what different classes of neurons display expression level changes. The cytoarchitecture of the spinal cord dorsal horn consists of neuronal cell populations of various sizes and shapes as well as several types of non-neuronal cells such as spinal glia (astrocytes, oligodendrocyts, microglial cells). Neuronal soma size is correlated with function: small neurons (soma diameter < 20 μm) are predominantly distributed in superficial layers (laminae I–II) and mediate pain sensation in response to heat and/or nociceptive stimuli (Leem et al., 1994, 1993) whereas large neurons (soma diameter > 35 μm) are distributed in the deeper laminae (laminae III–VI) of the dorsal horn (Willis and Coggeshall, 1991) and predominantly mediate touch or vibration information from peripheral stimulation. In addition to neuronal contributions to pain sensation, recent data demonstrate that activation of spinal glia, such as astrocytes, is critically involved in the maintenance of neuropathic pain states following neural injury (Colburn et al., 1999; DeLeo and Yezierski, 2001; Rutkowski and DeLeo, 2002; Watkins et al., 2001). Therefore, it is important to evaluate both the cellular localization (neuronal vs. glial) and size of neuronal soma that display significant changes in mGluR expression to give more insight into the mechanisms for transmission of nociceptive responses and neuropathic pain behavior syndromes such as allodynia and hyperalgesia in a model of chronic central neuropathic pain (Christensen and Hulsebosch, 1997).

To evaluate the changes of mGluR expression in the lumbar spinal dorsal horn and gain insight into nociceptive response below the site of injury ("below level" pain; Siddall et al., 2002), we examined the mGluR expression in both neurons and astrocytes caudal to a spinal injury 7, 14, and 30 days following T13 unilateral spinal hemisection. We have previously reported preliminary data that mGluR expression significantly increased in the lumbar dorsal horn following T13 spinal hemisection (Gwak et al., 2003a).

Materials and methods

Spinal cord injury (SCI)

A total of 48 rats were used in this study with 24 rats (12 hemisection and 12 sham control) for neuronal analysis and 24 (12 hemisection and 12 sham control) rats for astrocytic analysis, respectively. Additionally, both hemisection and sham groups were divided into 3 different groups with postoperative day (POD) 7 (early phase), 14 (late phase), and 30 (chronic phase, $n = 4$, each group) to study $mGluR_1$, $mGluR_5$, and $mGluR_{2/3}$ expression in both neurons and astrocytes. Unilateral SCI was produced by transverse hemisection of the spinal cord at T13 of male Sprague–Dawley rats (200–250 g, Gwak et al., 2003b, Fig. 1). Experimental procedures were reviewed by the UTMB Animal Care and Use Committee and were consistent with the NIH *Guide for the Care and Use of Laboratory Animals*. Briefly, using sodium pentobarbital anesthesia (50 mg/kg, i.p.) with maintenance level of 2% isoflurane inhalation with mask, a laminectomy was performed on vertebral segments, T11–12. The spinal cord was unilaterally hemisected at the T13 spinal level with a # 11 scalpel blade without damage to the major dorsal vessel or its branches using a surgical microscope (KAPS, Germany). An insulin syringe with a 28-gauge needle was inserted dorsoventrally at the midline of the cord and the syringe was pulled laterally to ensure the completeness of the hemisection which was confirmed with surgical microscope inspection and by immediate post-recovery behavioral observations by motor deficits only in the ipsilateral hindlimbs. Rats displaying evidence of motor deficits on the contralateral (uninjured) hindlimbs were excluded from the study as this is the result of over-hemisection. As a control group, sham surgeries were performed by laminectomy on the T11–T12 vertebrae after which the dura was cut.

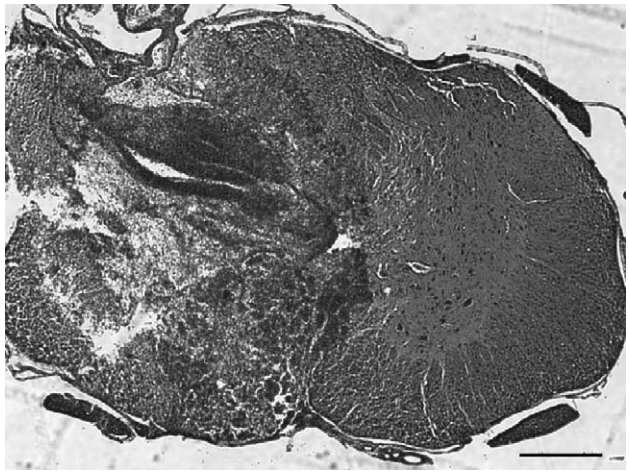

Fig. 1. The histology of spinal hemisection. Unilateral spinal hemisection displayed damage to the dorsal and ventral horn, dorsal column, intermediate zone, white and gray matters, and Lissauer's tract. Hematoxylin and eosin staining. Scale bar: 500 μm.

Immunofluorescence

To test for changes of mGluR expression in both neurons and astrocytes in the lumbar spinal dorsal horn following transverse hemisection, co-localization immunofluorescence was used and the density of reaction product was measured on both ipsilateral (injured) and contralateral (uninjured) sides in the caudal (L4) region after T13 spinal hemisection and compared to sham controls. In addition, the spinal dorsal horn neurons were divided into two different sized neurons: small (<20 μm) and large (>35 μm) neurons (Cervero and Iggo, 1980; Rexed, 1952), distributed in superficial and deep laminae, respectively (see Willis and Coggeshall, 1991). Note that some authors divide the dorsal horn into superficial (laminae I–II), intermediate (laminae III–IV), and deep layers (laminae V–VI) (Shimoyama et