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Peripheral nerve injury decreases the expression of metabolic glutamate receptor 7 in dorsal root ganglion neurons

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HIGHLIGHTS

- mGluR7 is expressed in peptidergic and large DRG neurons but not in IB4$^+$ neurons.
- mGluR7 is anterogradely transported from DRG cell body to the peripheral site.
- After peripheral nerve injury, the expression of mGluR7 is down-regulated.

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ABSTRACT

Group II and III metabolic glutamate receptors (mGluRs) are responsible for the glutamate-mediated postsynaptic excitation of neurons. Previous pharmacological evidences show that activation of mGluR7 could inhibit nociceptive reception. However, the distribution and expression patterns of mGluR7 after peripheral injury remain unclear. Herein we found that mGluR7 was expressed in the rat peptidergic dorsal root ganglion (DRG) neurons and large neurons, but rarely in isolectin B4 positive neurons. Sciatic nerve ligation experiment showed that mGluR7 was anterogradely transported from cell body to the peripheral site. Furthermore, after peripheral nerve injury, mGluR7 expression was down-regulated in both peptidergic and large DRG neurons. Our work suggests that mGluR7 might be involved in the regulation of pathological pain after peripheral nerve injury.

1. Introduction

Glutamate is one of the most prominent excitatory transmitters in the nervous system. According to the mechanisms by which their activation gives rise to the postsynaptic current, glutamate receptors could be divided into two different groups, ionotropic glutamate receptors (iGluRs) and metabolic glutamate receptors (mGluRs) [17]. Different from the iGluRs, mGluRs belong to the seven transmembrane G-protein coupled receptors and have no channel activity. They could activate the downstream ion channels through a G-protein mediated signaling pathway [5]. Based on their signal transduction pathways and pharmacological profiles, mGluRs could be divided into three groups, groups I–III [2]. Both iGluRs and mGluRs have been shown to modulate the synaptic plasticity [4].

More and more evidences suggest that glutamate pathway is involved in the nociceptive perception and chronic pain. Vesicular glutamate transporter 2 (VGLUT2) has been proved to be involved in the TRPV1 thermal nociception and normal itch response [9,12]. VGLUT3, expressed in unmyelinated TRPV1-negative neurons, is critical for mechanical hypersensitivity caused by injury [20]. After sciatic nerve transection, the expression level of VGLUT1 and VGLUT2 is both down-regulated [1]. Pharmacological evidence shows that inhibition of the NMDA and AMPA, two major iGluRs, could significantly relief neuropathic pain and enhance the antinociceptive effects of the electroacupuncture [11,26]. Blocking the peripheral mGluR activity could increase the capsaicin-induced nociceptive behaviors and nociceptor activity [3]. Administration of AMN082, agonist of mGluR7, could significantly attenuate neuropathic pain and inflammatory pain, and enhance the analgesic effects of morphine [6,16].

Previous studies have shown that mGluR7 is widely expressed in central nervous system and peripheral nervous system [8,10], yet the expression profile of mGluR7 in the DRG neurons after peripheral nerve injury is still unclear. In this paper, our study shows that mGluR7 is expressed in peptidergic and large DRG neurons, but

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rarely in isolectin B4 (IB4) positive neurons. Sciatic nerve ligation experiment indicates that mGluR7 could be delivered to the peripheral terminal. The expression of mGluR7 was down-regulated after axotomy. The above evidences suggest the involvement of mGluR7 in regulation of pathological pain after peripheral nerve injury.

2. Materials and methods

2.1. Animals and surgery

Animal care was performed in accordance with the policy of Society for Neuroscience (USA) for neuroscience research. The experimental protocol was approved by the Animal Care and Use Committee of Huzhou Central Hospital. Male Sprague-Dawley (SD) rats (~200 g, Animal Center of Zhejiang Academy of Medical Sciences) were housed in the 12:12 h light–dark cycle room. To generate the neuropathic rat model, sciatic nerve was axotomized according to the literature [24]. In brief, after SD rats were anesthetized, 3–5 mm sciatic nerve from the left limb was transected. Rats were used for further experiments on the 2nd and 7th day after surgery. For the sciatic nerve ligation, sciatic nerve was exposed and ligated after SD rats were anesthetized. Rats were used for further experiments on the 1st day after surgery.

2.2. Immunohistochemistry

After deep anesthesia, the L4–L5 DRGs were dissected and fixed in 4% paraformaldehyde for 4 h, following by cryoprotection in 20% sucrose in PBS. Samples were then sectioned on a cryostat at 10 μm and mounted onto microscope slides (Fisher Scientific) for staining. Tissue was incubated in PBS with 0.3% Triton X-100 plus 5% bovine serum albumin (BSA, Sigma) for 1 h at room temperature. Primary antibodies diluted in PBS with 0.3% Triton X-100 and 1% BSA were added and incubated overnight at 4 °C. After washed three times using PBS for 30 min, secondary antibodies were added and incubated with samples for 2 h at room temperature. After the slides were mounted, images were acquired with confocal microscope. The following primary antibodies were used: mGluR7 (1:500, Millipore-Chemicon), calcitonin gene-related peptide (CGRP; 1:500, Sigma), Substance P (SP; 1:500, Neuromics), IB4 (1:500, Sigma), neurofilament 200 (NF200; 1:1000, Sigma) and Vanilloid receptor (TrpV1; 1:100, Santa Cruz).

2.3. Western blot

L4–L5 DRGs from normal and axotomized rats were dissected after rats were deep anesthetized. Total proteins were extracted using the RIPA buffer (Cell Signaling). Samples were then separated by SDS-PAGE, transferred and probed with primary antibodies overnight at 4 °C. After reacted with secondary antibodies for 1 h at room temperature, samples were visualized with chemiluminescence (GE Healthcare Life Sciences). Primary antibodies against mGluR7 (1:500, Millipore-Chemicon) and actin (1:5000, Abcam) were used.

2.4. RT-PCR

After deep anesthesia, L4–L5 DRGs were dissected and stored in −80 °C. RNA from normal and axotomized rat DRGs was extracted with Trizol (Invitrogen) according to the protocol. Reverse transcript was then performed using M-MLV reverse transcriptase (Promega). The following primers were used for PCR to detect the mRNA expression: for mGluR7, 5′-TCAGTGGCGCTGGGAATGCT-3′ and 5′-GCCAGGCTGGGTGACAGAAT-3′; for GAPDH, 5′-ACAGCAAC AGGGTGGTGGAC-3′ and 5′-TTTGAGGGTGCAGCGAACTT-3′.

3. Results

Previous studies have shown that mGluR7 is widely expressed in central nervous system and peripheral nervous system [8,10]. However, the detailed expression profile of mGluR7 in dorsal root ganglion neurons is still not quite clear. Immunohistochemistry experiment shows that 85.11% ± 2.19% DRG neurons could express mGluR7. To identify the subpopulation of mGluR7 positive DRG neurons, we analyzed co-expression of mGluR7 with SP, CGRP, IB4, TRPV1 and NF200. Double immunolabeling shows that 86.08% ± 3.35% SP positive neurons and 91.78% ± 2.96% CGRP positive neurons could express mGluR7. Almost all the NF200 positive large DRG neurons are mGluR7 positive. In contrast, only 21.77% ± 3.51% IB4 positive neurons could express mGluR7 (Fig. 1). These data suggest that mGluR7 is specifically expressed in the peptidergic neurons and large DRG neurons. Recent studies suggested that group II/III mGluRs could exert endogenous activity-dependent modulation of TRPV1 receptors on peripheral nociceptors [3]. It has been shown that TRPV1 exclusively expressed in peptidergic and IB4 positive nociceptors [13]. To test the relationship between mGluR7 and TRPV1, we colabeled mGluR7 and TRPV1 in rat DRG neurons. We found that 71.09% ± 3.26% TRPV1 positive neurons could express mGluR7 (Fig. 1). These results indicate that mGluR7 might be involved in regulation of TRPV1 in DRG neurons.

Former experiments showed that intraplantar-injected antagonists of group II/III mGluRs could increase the capsaicin-induced nociceptive behaviors [3]. To test whether mGluR7 could be transported to the peripheral site and exert its function, the sciatic nerve was ligated for 1 day to accumulate the proteins. Immunohistochemistry shows that mGluR7 was accumulated at the proximal site of DRG neurons. SP, which is anterogradely transported peptide and released at the nerve terminal, was also accumulated at the proximal site (Fig. 2). The above data suggest that mGluR7 could be delivered to the afferent terminals at the peripheral site and exert its function.

To gain further insights into the mechanisms of mGluR7 regulation, we investigated the expression level of mGluR7 after sciatic nerve axotomy. RT-PCR experiment shows that mRNA level of mGluR7 is decreased 7 days after injury (Fig. 3A). Western blot was then performed and shows that the protein level is also decreased after axotomy (Fig. 3B). To study the expression profile of mGluR7 after nerve injury, immunostaining was carried out. As shown in Fig. 3C, the intensity of immunostaining is dramatically decreased 7 days after surgery. Moreover, only 55.87% ± 3.83% DRG neurons could express mGluR7, which is significantly lower than the normal DRG neurons (85.11% ± 2.19% are mGluR7 positive). Thus, mGluR7 is down-regulated after peripheral nerve injury. Downregulation of mGluR7 suggests the involvement of mGluR7 in the regulation of pathological pain after peripheral nerve injury.

4. Discussion

In this study, we uncovered the distribution profile of mGluR7 in the peripheral nervous system. Our results indicated that mGluR7 is specifically expressed in peptidergic and large DRG neurons, but rarely in IB4 positive DRG neurons. It has been proved that peptidergic and IB4 positive fibers innervate different sites in the epidermis and project to the different lamina in the dorsal spinal cord. Peptidergic nerve endings exclusively terminate in the underlying stratum spinosum and mainly project to the lamina I and outer lamina II in the spinal cord, while IB4 positive fibers terminated about 10 μm from the stratum corneum and mainly project to the inner lamina II of dorsal spinal cord [21,27]. Thus, mGluR7