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The colocalization of CGRP receptor and AMPA receptor in the spinal dorsal horn neuron of rat: A morphological and electrophysiological study

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Abstract

Both the calcitonin gene-related peptide (CGRP) receptor and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor are involved in the transmission of sensory information from primary afferent to the spinal cord. The present study found that there was a colocalization of CGRP receptor and AMPA receptor in a single spinal dorsal horn neuron in rat determined by double immunofluorescence labeling image methods. Furthermore, our results showed that the evoked discharge frequency of the wide dynamic range (WDR) neuron, one type of the dorsal horn neurons, increased significantly after micro-iontophoretic delivery of CGRP or AMPA alone tested by extracellular recording, indicating a functional colocalization of CGRP receptor and AMPA receptor in a single spinal dorsal horn neuron. The results of the present study found a morphological and functional colocalization of the CGRP receptor and AMPA receptor in a single dorsal horn neuron that involved in the transmission and modulation of sensory information from primary afferent to the spinal cord in rats.

Keywords: Calcitonin gene-related peptide (CGRP); AMPA; Micro-iontophoresis; Extracellular recording; Double immunofluorescence labeling; Wide dynamic range (WDR) neurons

Calcitionin gene-related peptide (CGRP) is a 37-amino acid neuropeptide generated from the alternative splicing of the calcitionin gene [1]. CGRP plays a wide variety of biological roles in various tissues including brain, heart, smooth and skeletal muscles [6,16]. It has been found that CGRP expresses in most of dorsal root ganglion neurons (DRG) of all sizes [15], and CGRP-containing primary afferents from DRG are abundantly distributed in the spinal laminae I, II and V where the CGRP-containing C-fibres from DRG make synapses upon sensory neurons in dorsal horn of the spinal cord [13]. CGRP receptors localize on the soma and dendrites of postsynaptic neurons in the superficial dorsal horn, as well as on the presynaptic terminals in the dorsal horn presumably serving an autoreceptor function [18]. Iontophoretic delivery of CGRP onto the spinal nociceptive neurons can increase their ongoing firing, and facilitate their response to innocuous and noxious peripheral stimulations [12]. The wide dynamic range (WDR) neuron in dorsal horn of the spinal cord receives sensory information, both nociceptive and non-nociceptive, from prepheral afferents to the spinal dorsal horn [16]. Previous studies in our laboratory demonstrated that CGRP increased the discharge frequency of the WDR neuron in dorsal horn, and the effect was blocked by the CGRP receptor antagonist CGRP8-37 [17,19].

Glutamate is the main excitatory neurotransmitter in the spinal cord and brain. Iontotropic glutamate receptors are divided into three classes, *N*-methy-D-asparate (NMDA) receptor, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor and kainite receptor [2]. Glutamate and AMPA receptor are distributed in dorsal horn of the spinal cord [4,11] involved in the main transmission of sensory information from prepheral nerve to dorsal horn of the spinal cord [9,10]).

It has been shown that the agonist of AMPA receptor, (s)-AMPA, inhibits electrically evoked CGRP release from the rat dorsal horn [3]. Ebersberger et al. demonstrated that iontophoretical administration of CGRP enhanced the AMPA-induced discharge frequency of the WDR neuron in dorsal horn of the spinal cord of rats [5]. These results strongly suggest an interac-

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tion of CGRP receptor and AMPA receptor in the dorsal horn of spinal cord. The present study was performed to explore whether there was a colocalization of CGRP receptor and AMPA receptor in the dorsal horn neurons of rat spinal cord using morphological and electrophysiological methods.

Double immunofluorescence labeling was used to investigate the morphological colocalization of AMPA receptors and CGRP receptors in the spinal dorsal horn neurons of rats. Experiments were performed on male Wistar rats weighing 250–400 g (Experimental Animal Center, Peking University, Beijing, China). Rats were housed in cages with free access to food and water and were maintained at a room temperature of 24 °C ± 2 °C with a 12-h light/dark cycle. All experiments were conducted according to the guidelines of the International Association for the Study of Pain [20], and every effort was made to minimize both animal suffering and the number of animals used. Rats were anesthetized with intraperitoneal injection of choral hydrate (400 mg/kg), and euthanized by transcardiac perfusion (saline wash, followed by 4% paraformaldehyde, 5% sucrose in 0.1 M phosphate buffer, pH 7.4). The spinal cord was removed and post-fixed in the fixing solution for 4 h, then stored in 0.1 M phosphate buffer sodium (PBS) containing 30% sucrose for at least 24 h. Transverse spinal cord sections (free floating, 30 μm) were cut and processed for double immunofluorescence labeling. Sections were pre-incubated for 60 min in PBS containing 0.3% Triton X-100, supplemented with 10% donkey serum at room temperature, followed by incubation with rabbit anti-CGRP type 1 receptor against a sequence near the C-terminal of human CGRP type 1 receptor (1:500; Wuhan Boster Biological Technology Co., Ltd., Wuhan, China) and monoclonal mouse antibody against an extracellular epitope (amino acid 175–430) of GluR2 subunit (1:1000; Chemicon International, Temecula, California, USA) diluted in PBS-Triton at 4 °C for 24 h. After careful washing with PBS, Cy3-conjugated donkey anti-mouse (1:200; Jackson, West Grove, PA, USA) and Cy2-conjugated donkey anti-rabbit (1:200; Jackson, West Grove, PA, USA) secondary antibodies were incubated for 1 h at room temperature in dark. The double-labeling results were analyzed by using fluorescence microscope (Olympus BH2-RFCA, Olympus, Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo) combined with microscope camera (Olympus DP70 Digital Microscope Camera, Olympus).

As shown in Fig. 1, GluR2 subunits immunostaining (red fluorescence) was observed in most of the dorsal horn neu-

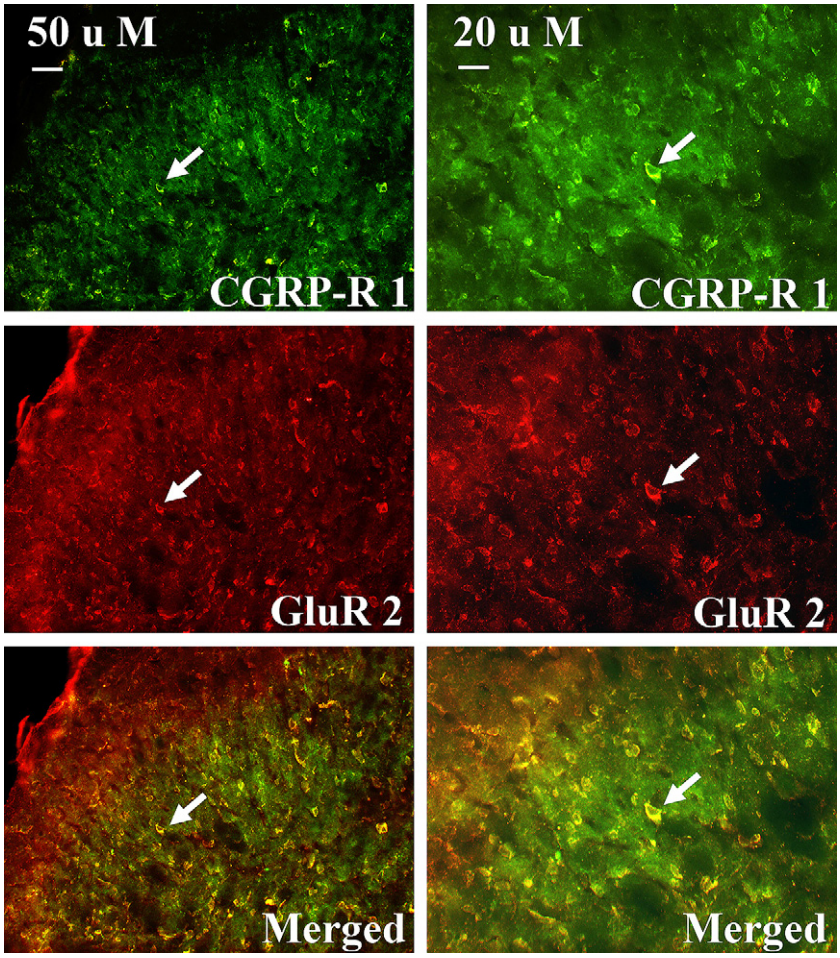

Fig. 1. Colocalization of AMPA receptor and CGRP receptor in the dorsal horn of rat spinal cord. Immunofluorescent images showed the distribution of CGRP type 1 receptor (green) and GluR2 subunits (red) in dorsal horn tissue slices by double immunofluorescence labeling with antibodies against CGRP type-1 receptor and GluR2 subunit of AMPA receptor.