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Research Report

Altered long-term synaptic plasticity and kainate-induced Ca^{2+} transients in the substantia gelatinosa neurons in GLU_{K6}-deficient mice

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Abstract

Functional kainate receptors are expressed in the spinal cord substantia gelatinosa region, and their activation contributes to bi-directional regulation of excitatory synaptic transmission at primary afferent synapses with spinal cord substantia gelatinosa neurons. However, no study has reported a role(s) for kainate receptor subtypes in long-term synaptic plasticity phenomena in this region. Using gene-targeted mice lacking glutamate receptor 5 (GLU_{K5}) or GLU_{K6} subunit, we here show that GLU_{K6} subunit, but not GLU_{K5} subunit, is involved in the induction of long-term potentiation of excitatory postsynaptic potentials, evoked by two different protocols: (1) high-frequency primary afferent stimulation (100 Hz, 3 s) and (2) low-frequency spike-timing stimulation (1 Hz, 200 pulses). In addition, GLU_{K6} subunit plays an important role in the expression of kainate-induced Ca^{2+} transients in the substantia gelatinosa. On the other hand, genetic deletion of GLU_{K5} or GLU_{K6} subunit does not prevent the induction of long-term depression. These results indicate that unique expression of kainate receptors subunits is important in regulating spinal synaptic plasticity and thereby processing of sensory information, including pain.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Excitatory amino acid receptors: physiology, pharmacology and modulation

Keywords: Kainate receptor; Spinal cord; LTP; GLU_{K6}

1. Introduction

Kainate receptors (KARs), a subtype of the ionotropic glutamate receptors, are composed of homomeric and heteromeric configurations of five cloned subunits: glutamate receptor 5 (GLU_{K5}), GLU_{K6}, GLU_{K7}, GLU_{K1} and GLU_{K2} [17]. These subunits may exist as certain homomeric assemblies, although native receptors are likely to be heteromeric assemblies. The GLU_{K5} and GLU_{K6} subunits among them undergo RNA editing (glutamine/arginine (Q/R)) that determines single-channel conductance [18,49] and Ca^{2+} permeability [6,7,12]. In the substantia gelatinosa (SG), lamina II of the spinal cord dorsal horn (DH), the region which integrates noxious afferent information from periphery [55], GLU_{K5}-KARs are mainly localized at primary afferent terminals where they regulate glutamate release [19,27,28,37,57]. However, the possibility of both pre- and postsynaptic localization of GLU_{K6} was reported recently [28,57]. The postsynaptic GLU_{K6} may contribute to excitatory postsynaptic currents [35], modulation of excitatory transmission [28,57] and Ca^{2+} permeability of KARs [26,53].

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Involvement of KARs in synaptic plasticity has been reported in hippocampal mossy fiber pathways [4,5,9,32,44] and recently in amygdala [30]. In the mossy fiber pathways, a GLU_{K5}-selective antagonist, (3*S*,4a*R*,6*S*,8a*R*)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid (LY 382884), prevents induction of long-term potentiation (LTP) ([4]; but see also [5]), whereas the magnitude, but not the induction, of LTP is reduced in the GLU_{K6}, but not GLU_{K5}, knockout mice [9]. In the amygdala, the deletion of GLU_{K5} subunit did not affect LTP induction or maintenance, but the LTP was blocked in GLU_{K6} knockout mice. These findings suggest that KAR subunits may be involved in the induction or expression of LTP. However, the possible involvement of the KAR subtypes in the induction or expression of long-term synaptic plasticity in the spinal SG has not been addressed, as yet.

Change of intracellular free calcium concentration ($[Ca^{2+}]_i$) is a possible mechanism that operates in the induction and expression of LTP [38]. Although Ca^{2+} permeability of KARs in the SG neurons is still unknown [33], it has been demonstrated in hippocampal [26,46], cortical neurons [13] and cerebellar granule and glial cells [6,42]. In rat dorsal root ganglion (DRG), Ca^{2+}-permeable KARs are expressed and functional in embryonic and neonatal period but switch to being Ca^{2+}-impermeable after the first postnatal week [33]. On the other hand, in the mouse spinal SG, the existence, the functional role and the specific subunit composition of Ca^{2+}-permeable KARs have not been studied as yet [53]. Hence, by using wild-type, GLU_{K5} and GLU_{K6} knockout mice, we investigated here the involvement of KAR subunits in the cytosolic $[Ca^{2+}]_i$ changes and in the induction or expression of LTP and long-term depression (LTD) in the spinal SG neurons.

2. Materials and methods

2.1. Slice preparation

All experiments were performed under the approval of University Animal Care and Use Committee that follows ethical guidelines of the National Institutes of Health and the International Association for the Study of Pain, and every effort was made to minimize the number of animals used. Transverse spinal cord slices were obtained from 48- to 120-day-old wild-type (129SvEv) and GLU_{K5} knockout ($GLU_{K5}^{-/-}$; 129SvEv) or $GLU_{K6}^{-/-}$ (a hybrid 129SvEv/C57BL/6) mice, which were provided by Dr. S.F. Heinemann (The Salk Institute, USA), as previously described [57]. Lumbosacral spinal cord with long (8–15 mm) dorsal roots was removed from a mouse under deep isoflurane anesthesia. Slices (400–500 μm) were cut from L4–L5 spinal segments with attached dorsal root in an oxygenated (95% O_2, 5% CO_2) Krebs–bicarbonate solution (4 °C) containing (mM): 124 NaCl; 5 KCl; 1.2 KH_2PO_4; 2.4 $CaCl_2$; 1.3 $MgSO_4$; 26 $NaHCO_3$; 10 glucose; pH 7.4, on a vibratome and were placed for at least 1 h in a holding chamber (36 ± 1 °C) to recover. A single slice was then submerged in a recording chamber and superfused (3 ml/min) with oxygenated recording medium (34 °C; same composition to the incubation solution, except for 128 mM NaCl and 1.9 mM KCl).

2.2. Intracellular recording

A single fiberglass (outer diameter and inner diameter, 1.00 and 0.58 mm, respectively; AM Systems, Carlsborg, WA, USA) microelectrode (4 M potassium acetate, pH 7.2; 140–220 MΩ) was placed in the SG region of the slices. Neurons were impaled by oscillating capacity compensation circuit of an amplifier (Axoclamp2A, Axon Instruments, Foster City, CA, USA). Digidata1200 and pClamp (version 6; Axon Instruments) software were used for data acquisition and analysis. Most recordings were obtained from neurons with a stable resting membrane potential (≤−60 mV) and with overshooting action potentials. Input resistance was monitored by passing a hyperpolarizing current pulse of 0.05 nA (duration, 200–300 ms) across the cell membrane and measuring the voltage deflection produced. Bridge balance was monitored throughout experiments and corrected when necessary. Synaptic potentials were evoked by electrical stimulation of primary afferent fibers with a bipolar platinum electrode positioned on the dorsal root. Single shocks at a fixed suprathreshold strength (0.01–0.5 ms, 5–35 V) yielding a 5–15 mV excitatory postsynaptic potentials (EPSPs) were repeated at 2-min intervals for at least 10 min before and 30 min period after tetanic stimulation of primary afferents. Each change of EPSP in peak amplitude was expressed as a percentage of baseline control. Primary-afferents-evoked EPSPs were classified on the basis of conduction velocity and stimulus intensity as previously reported [57]. Briefly, primary afferents conducting at velocity above 15 m/s were classified as Aβ, whereas those conducting between 1.5 and 15 m/s were classified as Aδ, and those conducting below 1.5 m/s as C fiber. The minimum stimulus intensities and durations used to activate Aδ and C fibers were 3 V/0.1 ms and 5 V/0.5 ms, respectively. Identification of the Aδ-fiber-evoked EPSPs as monosynaptic EPSPs was based on their short and constant latencies and absence of failures with a repetitive stimulation at frequency of 10 Hz. The shapes and amplitudes of monosynaptic EPSPs were similar in different trials when dorsal root was stimulated at a constant intensity. Because C-fiber-activated responses with long latencies could not easily be distinguished as ‘monosynaptic’ or ‘polysynaptic’ due to frequent failures during repetitive 10 Hz stimulation, only EPSPs showing no failures at low frequency (~1 Hz) stimulation, which are presumably monosynaptic C-fiber-evoked EPSPs, were analyzed. After a stable baseline recording (at least 10 min), high-frequency stimulation (HFS; 100 Hz for 1 s, 3 times at 10-s interval)