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Research article

Activated leukocyte cell adhesion molecule is involved in excitatory synaptic transmission and plasticity in the rat spinal dorsal horn

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HIGHLIGHTS

- ALCAM is involved in the modulation of excitatory synaptic transmission.
- ALCAM is required for LTP at primary afferent-dorsal horn neuron synapses.
- ALCAM is expressed in the dorsal horn of the spinal cord.

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ABSTRACT

Activated leukocyte cell adhesion molecule (ALCAM), a member of type I transmembrane immunoglobulin superfamily of cell adhesion molecule, is expressed in the surface membrane of various cell types including neurons. In the spinal cord dorsal horn (DH), the first gate for the sensory and pain transmission to the brain, the expression and function of ALCAM have not been known yet. Therefore, we here investigate the synaptic function of ALCAM in the substantia gelatinosa (lamina II) of the spinal DH, as well as its expression in the DH. Bath-application of ALCAM/Fc or CD6/Fc, the recombinant human IgG_1-Fc chimeric proteins, specifically potentiated C-fiber-mediated excitatory synaptic transmission and predominantly increased spontaneous release of glutamate. In addition, the development of long-term potentiation, a form of synaptic plasticity, at excitatory synapses was significantly inhibited in the presence of the recombinant proteins. The functional roles of ALCAM in the spinal DH were further supported by immunohistochemical analysis; it showed that ALCAM intensely expressed through laminae I/II with the exception of lateral portion of the dorsal part of inner lamina II and distinctly co-localized with molecular markers of C-fibers, such as peptidergic calcitonin gene-related protein and transient receptor potential vanilloid type 1 and non-peptidergic isolectin B4. This study, for the first time, suggests the modulatory roles of ALCAM in the excitatory synaptic transmission and plasticity in the rat spinal DH.

1. Introduction

Cell adhesion molecules (CAMs) provide the correct interaction between distinct cell types that is required for proper development and structural and functional integrity of tissues and organs in multicellular organisms [1]. Many CAMs in the central nervous system (CNS) fall into four groups, namely, cadherins, immunoglobulin superfamily (IgSF), integrins and neurexins and neuroligins [2]. Activated leukocyte cell adhesion molecule (ALCAM; also called CD166) belongs to the type I transmembrane IgSF, comprising two variable- and three constant-types of Ig extracellular domains, followed by one transmembrane region and a variable short cytoplasmic tail [3,4]. ALCAM was originally found as a ligand of CD6 [3], the transmembrane surface receptor of the scavenger receptor cysteine-rich protein superfamily, and thus, its extracellular domain interacts heterophilically with CD6 (ALCAM-CD6) as well as homophilically with itself (ALCAM-ALCAM) to facilitate both homo- and heterotypic cell-cell clustering [5]. In the CNS, CAMs are known to be important for the formation of synapses and implicated in neuronal clustering and layering, axon fiber assembly and neuron-glia interaction. On the other hand, recent studies indicate that synaptically localized CAMs also modify synaptic receptor function and modulate synaptic plasticity [6].

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In the spinal cord, ALCAM is expressed in a subset of motor neurons in the ventral horn and in a subset of sensory dorsal root ganglion (DRG) neurons, where it plays a role in specific targeting of axons with other IgSF CAMs during development of sensory-motor circuits [7]. In contrast, the expression and function of ALCAM are not clearly demonstrated in the spinal cord dorsal horn (DH), the first CNS gate for the transmission of sensory and pain signals from the periphery to the higher brain, although its expression seems to be prominent in sensory axons of developing spinal cord [7–9]. Therefore, in this study, we investigated the expression and function of ALCAM in the spinal DH, of which little is known to date. First, to investigate functional roles of ALCAM in the spinal DH, recombinant mouse ALCAM (or CD6)/human IgG_1 Fc chimeric proteins (ALCAM/Fc or CD6/Fc) were tested for their effect on synaptic transmission and plasticity at excitatory synapses, because no agonists or antagonists of ALCAM are available currently. Next, we attempted to clarify which sensory fibers express the ALCAM using double immunostaining with well-known markers for sensory neurons, such as calcitonin gene-related protein (CGRP), isolectin B4 (IB4) and transient receptor potential vanilloid type 1 (VR1).

2. Materials and methods

2.1. Animals

Young adult (3–4 week-old) or adult (8 week-old, 250–300 g) Sprague Dawley rats (Samtaco Inc., Osan, R. O. Korea) were maintained under a 12-h light/12-h dark cycle and provided with food and water *ad libitum*. The animal experiments were approved by the Animal Care and Use Committee of Kyungpook National University in accordance with NIH Publication No. 8023 on animal care. All efforts were made to minimize animal suffering and reduce the number of animals used.

2.2. Blind whole-cell patch clamp recordings

Spinal cord slices lumbar (L)4 or 5 were prepared from young adult rats (3–4 week-old; males or females). After laminectomy under deep urethane anesthesia (1.5 g/kg, i.p.), the spinal cord was dissected out and quickly transferred to a petri dish filled with pre-oxygenated ice-cold Krebs' solution (composition in mM: 117 NaCl, 3.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 25 NaHCO3, and 11 glucose; 95% O_2/5% CO_2). After removal of membranes (dura, arachnoid and pia) and unnecessary roots, L4 or L5 slices (400–450 μm) were cut transversely in the preoxygenated ice-cold Krebs' solution on a vibratome (Vibratome 1000$^+$; Vibratome, St. Louis, MO, USA). Slices with an attached dorsal root were kept in a beaker, containing the Krebs' solution with continuous oxygenation at room temperature (24–25 °C), for at least 1 h to recover from damages during cutting. A slice was then placed in a submerged recording chamber, and perfused with the preoxygenated Krebs' solution (2–3 ml/min; at room temperature).

Whole-cell patch-clamp recordings were performed in the substantia gelatinosa (SG, lamina II) area of the spinal DH, which was identified as a translucent band under a dissecting microscope. The resistance of borosilicate glass patch pipettes (1.5 mm o.d., World Precision Instruments Inc., Sarasota, FL, USA) was 8–12 MΩ when filled with internal solution (composition in mM: 110 Cs2SO4, 0.5 CaCl2, 2 MgCl2, 5 EGTA, 5 HEPES, 5 TEA, 5 ATP-Mg salt). Excitatory postsynaptic currents (EPSCs) were evoked at the holding potential (V_h) of −70 mV by electrical stimulation at 30 s interval of the attached dorsal root connected to a suction electrode with an S48 square pulse stimulator (Natus Neurology Inc., RI, USA). (+)-Bicuculline (5 μM) and strychnine (2 μM) were added to the Krebs' solution to block inhibitory postsynaptic currents (IPSCs) mediated by ionotropic $GABA_A$ and glycine receptors, respectively. Currents were amplified with Multiclamp 700A (Molecular Devices, Sunnyvale, CA, USA), filtered at 1–2 kHz, digitized at 5 kHz and collected and analyzed using pClamp 10 software (Molecular Devices, Sunnyvale, CA, USA). EPSCs were determined as 'monosynaptic' if they had constant latencies and no failure during repetitive 10–20 Hz stimulation, as 'Aδ-fiber' if their conduction velocities were more than 1.5 m/s and their stimulus intensities were less than 100 μA/0.1 ms, and as 'C-fiber' in the case of the conduction velocities of less than 1.5 m/s with higher stimulus intensities than Aδ-fibers. After stable baseline recordings of EPSCs (~5 min), ALCAM/Fc or CD6/Fc (R & D systems, MN, USA) were bath-applied, or a protocol of long-term potentiation (LTP) induction was applied in the presence of one of the recombinant proteins. The LTP protocol consisted of 2 Hz repetitive stimulation of 120 pulses paired with postsynaptic depolarization to +30 mV, and was applied within 10 min after accomplishing whole-cell configuration to prevent washout of induction machinery. Each neuron was recorded in a different slice.

2.3. Immunohistochemistry

Adult male rats were deeply anesthetized, and perfused through the ascending aorta with normal saline followed by ice-cooled Zamboni's fixative. The L5 segment of the spinal cord was dissected out, postfixed in Zamboni's fixative for 2 h, and then immersed in 30% sucrose for cryoprotection. A freezing microtome set at 30 μm was used to prepare transverse sections of spinal cord, which were collected in phosphate-buffered saline and processed for immunofluorescence [10]. For dual-immunofluorescent staining of ALCAM and CGRP, or ALCAM and VR1, the sections were hybridized with a mixture of ALCAM (goat, 1:5000; R&D Systems, Minneapolis, MN, USA) and CGRP (rabbit, 1:10,000; Peninsula Laboratory Inc., San Carlos, CA, USA) or ALCAM and VR1 (guinea pig, 1:2000; EMD Millipore, Billerica, MA, USA) antibodies overnight at 4 °C. The sections were washed and incubated for 1 h at room temperature with biotinylated horse anti-goat IgG (1:200; Vector Labs, Burlingame, CA, USA), followed by a signal amplification step with Alexa 594-conjugated streptavidin (1:200; Invitrogen, Carlsbad, CA, USA) for detection of ALCAM for 1 h. After several washes, the sections were exposed to goat anti-rabbit Alexa 488 antibody (1:200; Invitrogen) or anti-guinea pig Alexa 488 antibody for detection of CGRP or VR1, respectively, for 2 h. For colocalization of ALCAM and IB4, the sections were incubated simultaneously with fluorescein-conjugated IB4 (10 μg/ml; Sigma, St. Louis, MO, USA) and ALCAM antibody. After several washing, the sections were incubated with biotinylated anti-goat IgG, followed by Alexa 594-conjugated streptavidin. The stained sections were coverslipped with Vectashield mounting medium (Vector Labs). Double staining images were acquired using a confocal laser scanning microscope (model LSM 5; Carl Zeiss Microimaging Inc., Thornwood, NY, USA).

2.4. Data analysis

All the data were expressed as mean ± standard error of the mean (SEM). One-way ANOVA or Student's *t*-test was used to determine a statistical significance. Particularly, the magnitudes of EPSC amplitudes (in percent of control) at 20–25 min after the LTP protocol (2 Hz), among three groups, were assessed by Student's *t*-test with Bonferroni corrections. A *P*-value of <0.05 or <0.01 was considered statistically significant.

3. Results

To investigate whether ALCAM has any functional role in excitatory synaptic transmission, the effects of recombinant mouse