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Presynaptic inhibitory actions of pregabalin on excitatory transmission in superficial dorsal horn of mouse spinal cord: Further characterization of presynaptic mechanisms

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HIGHLIGHTS

- Antisense ODN reduced Ca channel α2δ-1 expression and weakened pregabalin's action.
- Nerve injury increased Ca channel α2δ-1 expression and augmented pregabalin's action.
- Pregabalin's presynaptic actions were attenuated in syntaxin 1A knockout mice.
- Syntaxin 1A is necessary for pregabalin to exert its full presynaptic inhibition.

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ABSTRACT

Pregabalin is widely used as an analgesic for the treatment of neuropathic pain. In the present experiments using mouse spinal slices, we recorded electrically evoked glutamatergic excitatory postsynaptic currents (eEPSCs) from superficial dorsal horn neurons. Pregabalin reduced the amplitude of eEPSCs, and increased the paired pulse ratio. Pregabalin also inhibited the frequency of spontaneously occurring miniature EPSCs without affecting their amplitude. Partial ligation of the sciatic nerve increased the expression of the calcium channel α2δ-1 subunit, and increased the presynaptic inhibitory action of pregabalin. Intrathecal injection of antisense oligodeoxynucleotide against the α2δ-1 subunit, decreased the expression of α2δ-1 mRNA in the spinal dorsal horn, and decreased pregabalin's action. These results provide further evidence that pregabalin exerts its presynaptic inhibitory action via binding with the α2δ subunit in a state-dependent manner. Furthermore, presynaptic actions of pregabalin were attenuated in knockout mice lacking the protein syntaxin 1A, a component of the synaptic vesicle release machinery, indicating that syntaxin 1A is required for pregabalin to exert its full presynaptic inhibitory action. These observations might suggest that direct and/or indirect interactions with the presynaptic proteins composing the release machinery underlie at least some part of pregabalin's presynaptic actions.

1. Introduction

Pregabalin (PGB) is S-enantiomer of racemic 3-isobutyl GABA [21], having structure and actions similar to gabapentin. PGB has been used effectively for the treatment of neuropathic pain associated with diabetic peripheral neuropathy, postherpetic neuralgia and other conditions [13].

The analgesic effects of PGB are shown to be mediated by its binding with the α2δ subunit of voltage-gated calcium channels [4,9,20]. This binding inhibits the influx of Ca^{2+} into the presynaptic terminals, which leads to a subsequent reduction in the release of neurotransmitters such as glutamate and substance P [3,8].

Experiments on cultured hippocampal neurons loaded with fluorescent dye in synaptic vesicles, have shown that calcium-independently occurring release of fluorescent dye is inhibited by PGB [10]. Additionally, the frequency of spontaneously occurring miniature excitatory postsynaptic currents, which is independent of presynaptic Ca^{2+} influx, is inhibited by gabapentin in the entorhinal cortex [2]. Thus, it has been proposed that PGB inhibits transmitter release by reducing presynaptic Ca^{2+} influx, and also by modulating transmitter release machinery downstream of Ca^{2+} influx.

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Several electrophysiological studies with various results have been reported in regards to the effects of PGB and gabapentin on synaptic transmission in the spinal dorsal horn [11,12,15,16]. It has been reported that gabapentin presynaptically inhibits glutamatergic synaptic transmission in normal naive rats [15]. Others have reported that the presynaptic inhibitory effects of gabapentin take place in hyperalgesic conditions but not in normal conditions [12,16]. With regard to such state-dependent effects of gabapentin and PGB, several studies have suggested the expression level of the calcium channel α2δ subunit in spinal dorsal root ganglion and in the spinal dorsal horn as one of the possible mechanisms [4,9,20].

In the present experiments using electrophysiological recordings, we investigated the effects of PGB on excitatory synaptic transmission in the superficial dorsal horn in an attempt to understand the molecular mechanisms underlying the state-dependent presynaptic inhibitory actions of PGB. Furthermore, using knockout mice lacking syntaxin 1A, which is a member of the SNARE proteins, and which forms presynaptic release machinery with SNAP-25, synaptobrevin and other presynaptic proteins, we tried to obtain clues to an understanding about whether and how PGB inhibits excitatory synaptic transmission by acting on the presynaptic release machinery.

2. Materials and methods

2.1. Animals

Animal experimental procedures were reviewed and approved by the institutional animal care and use committees at Dokkyo Medical University. The care and use of the animals were in accordance with the guidelines of the International Association for the Study of Pain [22]. Syntaxin 1A knockout mice were generated as previously described [5], and backcrossed into the C57BL/6J genetic background. Experiments were performed on 68 male mice of age 6–8 weeks.

2.2. Partial ligation of the sciatic nerve and assessment of mechanical allodynia

Mice were anesthetized with halothane for the nerve-ligation surgery and for the sham surgery. The left sciatic nerve was partially ligated according to the protocol described by Seltzer et al. [14]. The mechanical sensitivity of the hindpaw was assessed with a Von Frey filament of #3.61, which is equivalent to 0.41 g force. The number of foot withdrawals to 10 repetitive stimuli was recorded.

2.3. Preparation of spinal cord slices

Animals were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally, and segments at the lumbosacral level of the spinal cord were removed. A microslicer (Dosaka EM) was used to cut transverse slices (350–450 μm) in Krebs' solution at 4 °C. The Krebs' solution was equilibrated with 95% O_2 and 5% CO_2. The solution contained the following ingredients (in mM): NaCl, 113; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4, 1; $CaCl_2$, 2; $MgCl_2$, 1; D-glucose, 11.

2.4. Tight-seal whole-cell recordings

The spinal slices were mounted in a recording chamber on a microscope stage (Axioskop FS-II, Zeiss), and continuously perfused with Krebs' solution. Conventional tight-seal whole-cell recordings were obtained from neurons located in the superficial dorsal horn (lamina II) under visual control using infrared-differential interference contrast optics and a CCD video camera (IR-CCD 2741; Hamamatsu Photonics), as described previously [6]. The pipettes were filled with a solution of the following composition (in mM): K gluconate, 123; KCl, 14; Na gluconate, 2; EGTA, 1; HEPES, 10; and the pH of the solution was neutralized to 7.4 with KOH.

The currents were recorded in the voltage-clamp mode at a holding potential of −70 mV, using an Axopatch 200B patch-clamp amplifier (Axon Instruments). The data were sampled using a Digidata 1440 interface (Axon Instruments). A PCLAMP 10 (Axon Instruments) and Mini Analysis 6.0.3 (SynaptoSoft) were used to analyze the data.

2.5. Electrical stimulation-evoked EPSCs (eEPSCs) and spontaneously occurring miniature EPSCs (mEPSCs)

The external solution routinely contained strychnine (Sigma, 2–5 μM) and bicuculline (Sigma, 10 μM). Electrical stimulation was applied using a glass pipette filled with 1 M NaCl with its tip (diameter, ca 3 μm) placed at the dorsolateral margin of the spinal cord, 100–200 μm away from the recorded neuron. With a square pulse of 0.1 ms duration, stimulus intensity was adjusted so that an EPSC of similar amplitude was evoked in each experiment. Spontaneous mEPSCs were isolated by adding tetrodotoxin (TTX, 0.3 μM) to the external solution. Both eEPSCs and mEPSCs were abolished by 6-cyano-7-nitroquinoxaline-2,3-Dione (CNQX, 5 μM, data not shown), and thus identified as glutamatergic. To obtain a paired-pulse ratio (PPR), paired stimulations were applied (interstimuli interval of 50 ms).

2.6. Intrathecal injection of antisense oligodeoxynucleotide against the α2δ-1 subunit

For knockdown of calcium channel α2δ-1 expression in the spinal cord, antisense oligodeoxynucleotides (ODN) against α2δ-1 were intrathecally (IT) administered. The following ODNs were used (Gene Tools, Philomath, OR, USA): a morpholino antisense ODN against α2δ-1 (5′-CAGCAGGCAGCCAGCAGCCATCTCC-3′) and a control nonsense ODN (5′-CCTCTTACCTCAGTTACAATTTATA-3′). Transfections were carried out using JetPEI (Polyplus-transfection SA). The animals were anesthetized with halothane, and the ODN was injected between the fifth and sixth lumbar vertebrae, by using a microsyringe with a 30-gauge needle, according to the procedure described by Hylden and Wilcox [7].

2.7. Quantitative reverse transcription-polymerase chain reaction

To assess the effects of sciatic nerve ligation and IT injection of antisense ODN on the expression of the α2δ-1 subunit mRNA, the superficial dorsal horn ipsilateral to the ligated sciatic nerve was excised from the L4 to L5 spinal slices under a binocular stereoscopic microscope. Total RNA was isolated using an ISOGEN reagent (Nippongene, Japan). Reverse transcription (RT) was performed with random hexamers by using a SuperScript cDNA synthesis kit (Invitrogen). Following RT, the cDNA was subjected to a real-time polymerase chain reaction (PCR) by using the Mastercycler ep realplex 2 system (Eppendorf, Hamburg, Germany). The cDNAs for α2δ-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were amplified by using commercially available TaqMan gene expression assays (Mm00486607 and Mm99999915, respectively; Applied Biosystems). The relative expression of α2δ-1, normalized to that of GAPDH, was determined by using the comparative $2^{-\Delta\Delta Ct}$ method (Applied Biosystems).

The von Fray measurements were made every day from 2 days before surgery (sham operation or nerve ligation) through up to 10 days after surgery, until the animals were sacrificed for whole-cell recordings or mRNA analysis. The IT injections of antisense ODN were made three times (3, 5, 7 days after surgery). The whole-cell recordings or mRNA analysis were performed 10 days after surgery.