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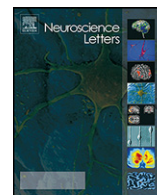

Blockade effects of BIBN4096BS on CGRP-induced inhibition on whole-cell K^+ currents in spinal dorsal horn neuron of rats

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ABSTRACT

Calcitonin gene-related peptide (CGRP) plays an important role in the transmission and modulation of nociceptive information in the spinal cord. BIBN4096BS, a nonpeptide CGRP receptor antagonist, has been shown to be efficiency in clinical migraine treatment. The present study was performed to investigate the effects of BIBN4096BS on the CGRP-induced inhibition to whole-cell K^+ currents in spinal wide dynamic range (WDR) neuron of rats. Application of BIBN4096BS inhibited the neuronal activity of WDR neurons in lumbar dorsal horn of the spinal cord in rats tested by extracellular recording method. Furthermore, CGRP induced inhibition on whole-cell K^+ currents in cultured dorsal horn neurons of rats tested by whole-cell patch-clamp recording, and the effect was significantly blocked by BIBN4096BS. The results indicate that BIBN4096BS may produce antinociceptive effects at the spinal level in rats.

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Calcitonin gene-related peptide (CGRP) plays an important role in nociceptive information processing or pain modulation [1,2,24]. In the superficial dorsal horn of spinal cord, CGRP exists widely in the primary afferents and CGRP receptor were also found in the bodies and dendrites of postsynaptic neurons [4,20,24]. It has been demonstrated that the peripheral noxious stimulation induces CGRP-containing afferent fibers to release CGRP in dorsal horn of the spinal cord [5,19,24]. Wide dynamic range (WDR) neurons, a population of cells in the spinal dorsal horn, respond to various noxious or innocuous stimuli such as touch, press and pinch, which play an important role in the spinal transmission of pain-related information [17,23]. The previous reports and results from our laboratory have demonstrated that the activity of WDR neuron increased significantly after application of CGRP in rats in vivo [9,25]. It is known that voltage-dependent K^+ channel plays an important role in regulation of neuron activity [11,12]. Zona et al. reported that CGRP reduced the whole-cell K^+ current of nerve cells in dorsal horn of the spinal cord [28].

BIBN4096BS, a nonpeptide CGRP receptor antagonist, has been underwent clinical trials for migraine treatment [6,14,15]. A variety of preclinical and clinical experiments have demonstrated that BIBN4096BS is highly selective and efficacious in the treatment of migraine attacks [14,15]. Dorsal horn of the spinal cord also is pivotal in the transmission of nociceptive information from peripheral tissues to the brain [3]. As CGRP can activate the spinal neurons and facilitate the spinal nociceptive transmission [3,9,24,25], the present study was performed to investigate the effects of BIBN4096BS on the CGRP-induced inhibition of whole-cell K^+ currents in spinal WDR neuron of rats.

The neuronal discharge of the dorsal horn neuron was recorded in male Wistar rats (220–250 g; Laboratory Animal Center of Academy of Military Medical Science, Beijing, China). The experiments were conducted according to the guidelines of the International Association for the Study of Pain [27], and every effort was made to minimize both the animal suffering and the number of animals used.

We chose the methods of electro-recording in vivo and drug delivery according to the previous reports [10,22,26]. The rat was anesthetized with intraperitoneal injection of choral hydrate (400 mg/kg, maintained with intermittent doses of 40 mg/(kg h)). After a tracheal cannula was inserted, the animal was placed in a stereotaxic frame, and a laminectomy was performed to expose the L2–L4 dorsal surface of the spinal cord. The vertebral column was stabilized by vertebral and hip clamps. The exposed spinal cord between L2 and L4 was placed on a curved metal saddle, gently lifted 0.5 mm from the vertebral canal, and covered with warm, fluid paraffin oil (37 °C) to prevent it from drying in a pool made of skin flaps. After careful removal of the covering paraffin oil and cerebrospinal fluid with filter paper, BIBN4096BS (100 μl in volume) was then applied directly onto the dorsal surface of

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the recording segments. The concentration of BIBN4096BS was selected according to the results of previous behavioral experiments. By intrathecal administration of 0.01, 0.1 and 1 μg/μl of BIBN4096BS, we have found that BIBN4096BS could induce a dose-dependent antinociception effects in intact rats at spinal level (data was not shown). So, the similar concentration of BIBN4096BS was used in the electrophysiological experiments.

To maintain the body temperature at physiological level, a heating plate was placed under the rat so that rectal temperature remained from 35.5 to 37.5 °C. The discharge frequency of the dorsal horn neuron in the lumbar enlargement of the spinal cord was recorded extracellular. Glass microelectrode filled with 2 M NaCl (8–12 MΩ) was inserted into the dorsal horn of the spinal cord with a hydraulic microdriver. The electrode was advanced into the recording sites ranged from 200 to 1000 μm below the dorsal surface of the spinal cord at L2–L4 levels. The signals were displayed on an oscilloscope. When a stable recording from a single dorsal horn neuron was observed, the neuron type was defined. Briefly, the neuron was identified by means of natural stimulation (brush, touch, press, and pinch). The receptive field was tested by brushing with a soft hair brush, pressing with a blunt probe, or pinching the skin with toothed forceps. The WDR neuron responded to all those models of stimulation, and to pinching to a greater degree than the other methods [23,25]. Once a WDR neuron was identified, the drug was applied onto the surface of spinal cord at the recording segments, and the evoked discharge was recorded. The recorded discharge was used to plot frequency histograms with the MacLab system. Each histogram showed the discharge frequency in a 1-min period, and the average frequency per minute was calculated. The average discharge frequency obtained before drug application was regarded as the basal discharge frequency. The discharge frequencies recorded during subsequent experiments were expressed as percentage changes of the basal discharge frequency (% change of discharge frequency). The recordings were performed for more than 30 min, and the data obtained before and 0, 5, 10, 20, and 30 min after the chemicals application were taken for statistical analysis.

Primary cultures of rat dorsal horn neurons were prepared from 1-day-old newborn rats. Fresh dorsal horn tissues were dissociated with 0.25% trypsin at 37 °C for 20 min. Trypsin was then inactivated by 5% decomplemented fetal bovine serum (FBS, HyClone). The triturated homogenate was filtered through 130 and 70 μm filters. The flow-through was centrifuged for 5 min to pellet cells. The pellet was then washed twice by Dulbecco's modified Eagle's medium with 0.25% sodium bicarbonate, 0.2% HEPES, 100 units/ml antibiotic Pen-Strep (Gibco) with 5% FBS. Cells were seeded on poly-L-lysine coated plates at the density of 1×10^5 cells/ml. The plated cells attach to the coverslips within 24 h and neurons develop synaptic connections in about 3–4 days. Ten micromolar of cytarabine was added to culture media 24 h after neuron plating at to inhibit glial cell growth. The cultures were maintained at 37 °C and with 5% CO_2. Culture media were changed every 96 h.

The recording pipettes were pulled on a micropipettes puller (P-97, Sutter Instruments, Novato, CA) from borosilicate glass capillaries, then fire-polished to a resistance of 3–5 MΩ. The bath solution contained (in mM) 130 NaCl, 5.4 KCl, 1 $MgC1_2$, 2 $CaC1_2$, 10 HEPES and 25 D-glucose; pH was adjusted to 7.35 with NaOH. All recordings were carried out in the presence of 1 μM tetrodotoxin. And the electrodes were filled with (in mM) 120 KCl, 2 $MgC1_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES, 20 D-glucose and 2 Na_2ATP; pH was adjusted to 7.25 with KOH. All chemicals were from Sigma (CA, USA) unless indicated. Patch-clamp recording used an EPC-10 double amplifier with Pulse software (HEKA Instruments, Germany). Raw signals were filtered at 2.9 kHz. Neurons were clamped at −70 mV, and then depolarized to +100 mV with 10 mV increments. Each pulse lasted 150 ms and interpulse interval was 300 ms. Leak

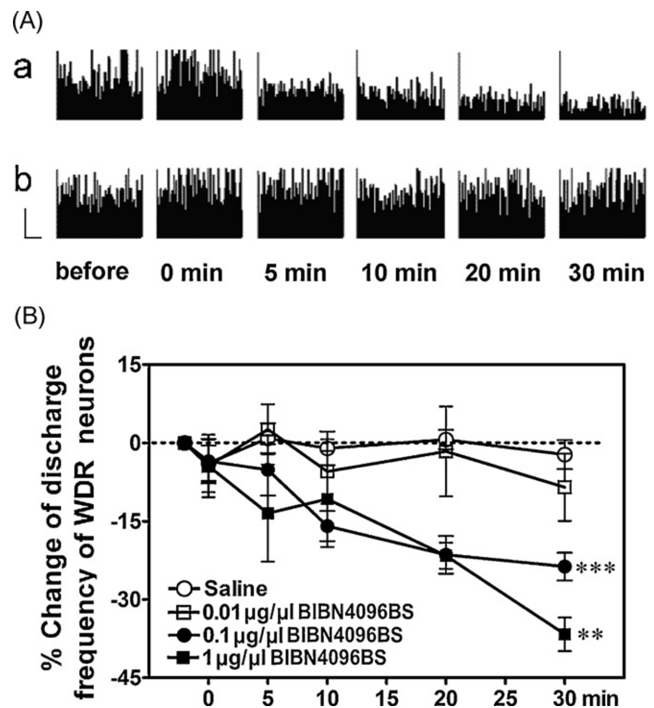

Fig. 1. Blockade effect of BIBN4096BS on the discharge frequency of wide dynamic range (WDR) neurons in dorsal horn of the spinal cord. Before application of BIBN4096BS, we recorded the spontaneous discharge of WDR neurons at least 1 min for base line, then applied BIBN4096BS at 0 min and kept recording for 30 min. After washing the dorsal surface by 0.9% saline, the discharged of WDR neuron recovered. Then using 0.9% saline to displace BIBN4096BS, and discharge of the same WDR neuron was recorded for 30 min as control group. (A-a) Administration of 1 μg BIBN4096BS induced significant decrease in the discharge frequency of a WDR neuron; (A-b) Administration of 0.9% saline induced no significant effect on the discharge frequency of the same WDR neuron. (B) Changes in discharge frequency of WDR neurons after applying 0.9% saline or BIBN4096BS in 30 min. The statistical difference between groups was evaluated by two-way ANOVA. Values represent means ± SEM, $^{**}p < 0.01$ and $^{***}p < 0.001$.

currents and liquid junction potentials were compensated by the recording software. All experiments were performed at room temperature. The data for whole-cell recording were analyzed with the Pulsefit software.

BIBN4096BS was provided kindly from Dr. Henri Doods (Boehringer Ingelheim, Germany) [7]. CGRP was purchased from Bachem (Heidelberg, Germany). BIBN4096BS was dissolved in dimethylsulphoxide (DMSO), and was diluted with perfusion buffer to the final concentrations for the experiments. The concentrations of DMSO in the working solution were so low (<0.1%) that they had no effect on any other parameters. Data is presented as mean ± SEM. The difference between groups was determined by two-way analysis of variance (ANOVA). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were considered as significant differences.

We first investigated the influence of BIBN4096BS on the neuronal activity of WDR neurons in dorsal horn of the spinal cord in rats. Administration of 0.01 μg/μl ($n = 7$; $F = 0.77$, $p = 0.39$), 0.1 μg/μl ($n = 6$; $F = 30.7$, $p < 0.001$) and 1 μg/μl ($n = 4$, $F = 11.18$, $p < 0.01$) of BIBN4096BS induced dose-dependent decreases in the discharge frequency of the WDR neuron compared with the saline group ($n = 9$), as shown in Fig. 1A. As shown in Fig. 1B, BIBN4096BS inhibited the spontaneous discharge of WDR neurons in a dose-dependant manner and the effects reached a maximum level at 30 min after administration.

The whole-cell K^+ currents in cultured rat dorsal horn neurons were evoked by a 150-ms depolarizing voltage steps from −60 mV to +100 mV. The whole-cell K^+ currents in one group of cultured rat dorsal horn neurons without treatment were recorded as a control

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