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BDNF-mediated modulation of glycine transmission on rat spinal motoneurons

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HIGHLIGHTS

- BDNF did not produce a direct excitatory or inhibitory effect on the motoneurons.
- BDNF dose-dependently increased the glycinergic transmission in the motoneurons.
- BDNF-induced enhancement of the glycinergic transmission was mediated by the activation of TrkB receptors.
- BDNF and its receptors TrkB had an extensive expression in the motoneurons.

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ABSTRACT

BDNF has a widespread distribution in the central and peripheral nervous systems, suggesting that BDNF may play a role in the regulation of motor control. However, the direct actions of BDNF on the motoneurons and their underlying mechanisms are still largely unknown to date. Therefore, by using whole-cell patch clamp recordings, quantitative RT-PCR and immunocytochemistry, the present study was designed to investigate the effects of BDNF on electrical activity and glycinergic transmission on the motoneurons and the underlying receptor mechanism. The results reveal: (i) BDNF did not produce a direct excitatory or inhibitory effect on the motoneurons; (ii) BDNF dose-dependently increased the glycinergic transmission on the motoneurons; (iii) glycinergic transmission on motoneurons was a direct postsynaptic effect; (iv) BDNF-induced enhancement of the glycinergic transmission was mediated by the activation of TrkB receptors; and (v) BDNF and its receptors TrkB had an extensive expression in the motoneurons. These results suggest that BDNF is directly involved in the regulation of glycinergic transmission on the motoneurons through postsynaptic TrkB receptors. Considering that the glycinergic synaptic transmission of motoneurons mainly comes from Renshaw cells, the important inhibitory interneurons of spinal cord, we speculate that BDNF may play an important role in the information integration in the spinal cord and participate in the sensitivity of motoneurons.

1. Introduction

Brain-derived neurotrophic factor (BDNF), belonging to the neurotrophic family of growth factors, has a widespread distribution in the central and peripheral nervous systems, including subcortical motor structures, such as the cerebellum, basal ganglia, brainstem vestibular nuclei and even the spinal cord [1,2]. More and more works indicate that BDNF holds a key position in central motor structures and the deficiency of BDNF could lead to severe motor deficits, such as depression, Alzheimer's disease, epilepsy, and drug addiction [3–6]. In the spinal cord, reports show that BDNF heightens the sensitivity of motoneurons to excitotoxic insults through activation of TrkB [7]. However, the direct actions of BDNF on the inhibitory neurotransmission and the underlying mechanisms are still largely unknown.

Spinal motoneurons locate in the ventral horn and directly control the contraction of skeletal muscle. Movement from simple reflex, rhythmic locomotion to complex voluntary movement is the result of a highly organized and precise pattern of activity of many populations of motoneurons in the spinal cord and brainstem. So the motoneuron is the final common pathway of motor system and motor command. Previous studies revealed that BDNF modulate the glutamatergic transmission on motoneurons, and Renshaw cells utilize the neurotransmitter glycine as an inhibitory substance [8]. Therefore, the purpose of the paper is to investigate whether glycinergic transmission is modulated by BDNF. The results

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demonstrate that BDNF enlarge the outward currents induced by glycine on motoneurons.

2. Methods

2.1. Spinal slice preparations

Under sodium pentobarbital (40 mg/kg) anesthesia, 32 Sprague-Dawley rats aged 14–24 d of either sex were decapitated, the spinal cord was carefully exposed and removed into ice-cold artificial cerebrospinal fluid (ACSF; composition in mM: NaCl, 138.0; KCl, 1.35; $NaHCO_3$, 21.0; NaH_2PO_4, 0.58; $MgCl_2$, 1.16; $CaCl_2$, 1.26; glucose, 10.0, pH 7.4) [9,10] equilibrated with 95% O_2 and 5% CO_2. The spinal cord was transected at L2 to L5 and gently removed from the vertebral column. Then, according to the rat brain atlas of Paxinos and Watson [11], the coronal spinal slices (300–400 μm in thickness) were prepared with a vibroslicer (VT 1000S; Leica). The slices were incubated in the ACSF solution equilibrated with 95% O_2 and 5% CO_2 at 30 °C for at least 1 h and then used for whole-cell patch-clamp recordings.

2.2. Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were performed on motoneurons with borosilicate glass pipettes (2–5 MΩ) filled with an internal solution (composition in mM: 130 K-gluconate, 10 KCl, 10 HEPES, 1.0 EGTA, 0.1 $CaCl_2$ and 2.0 MgATP (pH adjusted to 7.25 with 1 M KOH). During recording sessions, motoneurons were visualized with an Olympus BX51WI microscope (Japan). Patch-clamp recordings were acquired with an Axopatch-700B amplifier (Axon Instruments, USA) and the signals were fed into a computer through a Digidata-1440A interface (Axon Instruments, USA) for data capture and analysis (pClamp 10.0, Axon Instruments, USA). Recordings of whole-cell currents were lowpass filtered at 2 kHz and digitized at 10 kHz and recordings of membrane potentials were lowpass filtered at 5 kHz and digitized at 20 kHz. Neurons were held at a membrane potential of −60 mV and then characterized by injection of rectangular voltage pulse (5 mV, 50 ms) to monitor the whole-cell membrane capacitance, series resistance and membrane resistance. Neurons were excluded from the study if the series resistance was not stable or exceeded 20 MΩ.

2.3. Immunofluorescence

Postnatal rats (18 d old) were deeply anesthetized with pentobarbitone (80 mg/kg, i.p.) and transcardially perfused with 50 ml of saline followed by 300 ml of paraformaldehyde (4% in 0.1 M phosphate buffer). L2 to L5 spinal cord tissue was dissected, post-fixed in 4% paraformaldehyde (2 h at 4 °C), and transferred to 20% sucrose (overnight at 4 °C) and then 30% sucrose (overnight at 4 °C). Tissue was then blocked in OCT embedding compound on dry ice. Transverse sections through the spinal cord (25 μm thickness) were cut using a freezing microtome (CM 1850S, Leica) and mounted on gelatin-coated slides. The slices were rinsed in phosphate-buffered saline containing 0.1% Triton X-100 (PBST) and then incubated in 10% normal bovine serum in PBST for 30 min. Sections were incubated overnight at 4 °C with primary antibodies to BDNF and TrkB (rabbit anti-rat BDNF polyclonal antibody, 1:400; Abcam, UK; goat anti-rat TrkB polyclonal antibody, 1:300; Abcam, UK.). After a complete wash in PBS, the sections were incubated in the Alexa 594-conjugated donkey anti-rabbit (1:2000; Life Technologies, USA) and Alexa 488-conjugated donkey anti-goat (1:2000; Life Technologies, USA), for 2 h at room temperature in the dark. The slides were washed and mounted in mounting medium. All micrographs were taken with an inverted laser scanning confocal microscope (LSM 510; Zeiss).

2.4. Quantitative real-time RT-PCR

Tissues from the ventral horn of the spinal cord were collected from coronal brain slices of Sprague-Dawley rats (18 d old) according to the rat brain atlas of Paxinos and Watson [11]. RNA extraction was done using TriZol reagent (Invitrogen, USA) according to the manufacturer's instructions. A 1 μg aliquot of total RNA was used for the first-strand cDNA synthesis according to the protocol of M-MLV Reverse transcriptase (Promega, USA). Real-time PCR was then performed using iQ SYBR Green SuperMix (Bio-Rad, USA) in a 20 μl reaction mixture containing 10 μl of 2× master mix, 2 μl of cDNA, 2 μl of each primer (5 μM) and 4 μl of distilled water. The reaction was carried out in a Bio-Rad CFX-96 Real-time PCR System (USA) using the following parameters: 95 °C for 3 min to activate the hot-start iTaq DNA polymerase, followed by 40 cycles at 95 °C for 15 s, 60 °C for 25 s and 72 °C for 1 s. The PCR program was completed by a melting temperature analysis. For quantification, the quantity of the target gene was expressed relative to the amount of the reference gene (*gapdh*) to obtain a normalized target expression value. For negative controls, cDNA was replaced with water. The following forward and reverse primers were used: mBDNF sense: 5′-CGGATCCGCTGCAAACATGTCCATG-3′; mBDNF antisense: 5′-GCCACTATCTI′CCCCTTTTAATGG-3′; mTrkB sense: 5′-TGCTGTGGTGGTGATTWCTCTGTG-3′; mTrkB antisense: 5′-GTTCTCTCCTACCAAGCAGTTCCGG-3′; GAPDH sense: 5′-TCACCACCATGGAGAAGGC-3′; GAPDH antisense: 5′-GCTAAGCAGTTGGTGGTGCA-3′. All the sequences are all from previous reports [12,13].

2.5. Drugs

In all experiments, the drugs were applied by bath application. BDNF purchased from Alomone Labs (Jerusalem, Israel); highly selective antagonist for TrkB from Tocris UK; tetrodotoxin (TTX) and glycine from Sigma USA. All drugs were stored frozen and were dissolved in distilled water, and dilutions were freshly prepared in ACSF and equilibrated with 95% O_2 and 5% CO_2 before perfusing the slices. We bathed the slices with glycine to stimulate the recorded neurons. TTX (0.3 μM, Alomone Labs, Israel) was used to confirm and isolate the postsynaptic effect of glycine.

2.6. Data analysis and statistics

All data were analyzed with Origin 8.0 (MicroCal Software, USA) and expressed as means ± SEM. Student's *t*-test was employed for statistical analysis of the data and *P*-values of <0.05 were considered to be significant.

3. Results

3.1. Motoneuron identifications

A total of 40 identified motoneurons were selected from 27 Sprague-Dawley rats (12–24 d old). All the motoneurons from the ventral horn have a diameter over 30 μm (Fig. 1A and B). A bipolar concentric electrode (FHC, USA) was placed close to the ventral rootlets for antidromic activation of the recording neurons in the ventral horn of rats. Only by appearance of an antidromic spike potential following stimulation of the ventral rootlets were neurons identified as motoneurons (Fig. 1C). In the present study, motoneurons selected for study had resting membrane potentials of −55 to −80 mV, were silent at rest, had action potentials of 60–90 mV