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Effects of brain-derived neurotrophic factor (BDNF) on activity mediated by NMDA receptors in rat spinal cord cultures

Jean-Christophe Legrand, Pascal Darbon*, Jürg Streit

Department of Physiology, University of Bern, Bühlplatz 5, CH-3012 Bern, Switzerland

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Abstract

Brain-derived neurotrophic factor (BDNF) is involved in the differentiation and the survival of neurons. It has also been shown to be associated with the regrowth of neurons of damaged spinal cord and the modulation of ionic currents by acting on sodium channels and NMDA receptors through tyrosine kinase B (TrkB) receptors. We investigated the effects of BDNF on rhythm generation induced by disinhibition in dissociated cultures from embryonic rat spinal cord (E14), with extracellular multisite recordings (MultiElectrode Arrays, MEAs) or intracellular patch-clamp recordings. Exogenous BDNF had only minor effects on the bursting by increasing the activity during the burst. This increase of activity is suggested to be mediated by a potentiation of the postsynaptic NMDA receptors because it has been found that BDNF potentiates the NMDA-evoked depolarization in cultures incubated with BDNF for 10 min. Possible direct effects of BDNF on sodium channels were also investigated by local application of BDNF to the soma of patched neurons but no depolarization was observed. Long-term application of BDNF strongly decreased the activity during the burst and also the number of active electrodes, possibly due to a decrease in network density.

Keywords: Neuronal network; NMDA receptor potentiation; Multielectrode array

Brain-derived neurotrophic factor (BDNF) is a neurotrophin, which is known to be implicated in differentiation and survival of cells. Neurotrophins are required for the development of neurons in the peripheral and central nervous system. In the spinal cord, neurotrophins could influence many aspects of neuronal activity that result in new synaptic connections by modifying neurotransmitter release and synaptic structures [15]. However, neurotrophins are not only implicated in development. In the spinal cord, BDNF enhanced NMDA-mediated dorsal root-evoked responses [10]. BDNF is also implicated in activity-dependent plasticity of motoneuronal NMDA receptors [2]. Moreover, BDNF is involved in the regeneration of motor axons [4] and of spinal tracts after lesion [16].

Responses to BDNF are mediated by two types of receptors, the specific high affinity tyrosine kinase receptor B (TrkB) and the non-specific low affinity pan-neurotrophin receptor ($p75^{NTR}$). In contrast to the $p75^{NTR}$, which is known to be involved in cell death, it is well established that TrkB receptors are involved in regeneration [4], phosphorylation of NMDA receptors [2] and survival.

Recently, BDNF has been considered as a neurotransmitter. In the hippocampus, BDNF can act within milliseconds on one type of sodium channels (Nav 1.9) by a direct coupling to TrkB receptors [3,9]. It remains unclear, however, whether this finding can be generalized to other preparations and especially to spinal cord.

Our model of rhythmogenesis induced by disinhibition of spinal cord cultures is mainly based on glutamatergic receptors, which support the recurrent excitation of the network [12], and ionic channels, which contribute to intrinsic spiking [5]. With regard to the known effects of BDNF on channels and receptors, it is of interest to investigate the effects of BDNF on rhythm generation in spinal cord networks.

Cultures were prepared from the spinal cord of rats at embryonic age 14. Animal care was in accordance with guidelines approved by Swiss local authorities. The cultures were prepared as described previously [20]. The cells were plated on Multi-Electrode Arrays (MEAs) or on glass coverslips at a density of 150,000 or 75,000/150 μl, respectively. MEAs were produced as described previously [21]. The cultures were incubated in a 5% CO_2/95% air atmosphere at 36.5 °C. Serum-free Neurobasal™ medium (Gibco) supplemented with B 27 and Glutamax (both Gibco) was used for the MEA cultures. The cultures on cover-

* Corresponding author. Tel.: +41 31 631 87 48; fax: +41 31 631 46 11.
E-mail address: darbon@pyl.unibe.ch (P. Darbon).

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slip were kept in a MEM Eagle's Medium supplemented with 10% fetal bovine serum, 0.2% glucose, B27 and Glutamax. Half of the medium was changed weekly. Recordings were made in a chamber mounted on a microscope (Nikon) from cultures of 4–12 weeks in vitro age. The medium was replaced by an extracellular solution containing (in mM): NaCl, 145; KCl, 4; $MgCl_2$, 2; $CaCl_2$, 2; HEPES, 5; Na-pyruvate, 2; glucose, 5 at pH 7.4. All recordings were made at room temperature (22–26 °C).

MEA signals were sampled at 6 kHz using custom made virtual instruments within Labview® (National Instruments) as described previously [20]. Detection of the extracellularly recorded action potentials and further analysis were done offline in the software package IGOR (WaveMetrics) as described previously [21]. Signal detection was based on standard deviation with the threshold set at three times the noise level of each electrode. Network activity plots show the total activity of all selected electrodes within a sliding time window of 10 ms, shifted by 1 ms. Data are expressed as median and range. Statistics are based on non-parametric tests and were done with GB-Stat (Dynamic Microsystems). A Wilcoxon test was used to compare the effects of a treatment with the control taken previously in the same culture. A Mann–Whitney test was used to compare the effects of a treatment in a pool of cultures and the effects of a control treatment in another pool of cultures.

Whole cell patch clamp recordings were obtained from neurons in cultures on glass coverslips with an Axoclamp 2B amplifier (Axon Instruments). The patch pipettes were filled with a solution containing (in mM): K-gluconate, 100; KCl, 20; HEPES, 10; Mg-ATP, 4; Na_2-GTP, 0.3; Na_2-phosphocreatine, 10; pH 7.3 (with KOH). The pipettes had a resistance of 3–5 MΩ. No series resistance compensation was applied. Native resting membrane potentials were in the range of −40 to −60 mV. Signals were digitized using pClamp software (Axon Instruments). They were analyzed offline using custom made programs in IGOR. BDNF was locally applied with a microinjector system (microinjector 5242, Eppendorf) using injection pipettes with one or two channels, or BDNF was bath applied. The chemicals were purchased from Sigma unless otherwise stated.

Measurement of the concentration of BDNF was realized with an ELISA kit (BDNF E_{max} Immunoassay system, Promega) by taking the medium from cultures 7 days after the last change of medium.

Neutralization of the endogenous BDNF was made by adding 4 μg/ml of anti-BDNF antibody purchased from R&D system [14] 12–24 h before recording.

Long-term application of BNDF was realized by adding BDNF (25, 50 or 100 ng/ml) to the culture medium for the whole culturing period (4–8 weeks). Medium was changed once a week. The control experiments were realized by applying BSA in the same number of experiments for the same duration. As no effects of the duration of the culturing period or the concentration of BDNF on any of the parameters were found, the experiments were pooled.

In cultures on MEAs, electrical activity of the network was measured under disinhibition (blockade of glycinergic and GABAergic receptors with 1 μM strychnine and 20 μM bicuculline) to induce a rhythmic synchronous bursting [20]. Bursting consisted of periods of simultaneous activity at all the electrodes selected (bursts) followed by periods with low rates of asynchronous activity (intervals). These patterns were characterized by three parameters describing the network bursting (burst rate, burst duration, interval duration) and two parameters describing the spiking activity (burst spike rate, interval spike rate). We investigated the effect of acute application of a saturating dose of exogenous BDNF (200 ng/ml) on burst generation. As shown in Fig. 1 and Table 1, BDNF, under disinhibition, had no obvious effect on the rhythmic activity. The rhythm is neither disrupted nor become more irregular. There is no significant difference in the CVs for the burst period between control and BDNF (in %, control 27.2 (13.6–39.2), BDNF: 24.0 (17.3–34.5), $n = 6$, $p = 0.6$). Statistical analysis of the parameters describing the network and spiking activities revealed only a significant increase by 11.7% of the burst spike rate ($p = 0.028$, $n = 6$, Table 1).

Effects on spike rate during the burst could be explained by an effect on neuronal excitability as reported by Kafitz et al. [9]. Therefore, we investigated the action of BDNF on single neurons in spinal cord cultures using patch-clamp whole cell recordings, in the presence of strychnine (1 μM), bicuculline (20 μM), CNQX (10 μM) and APV (50 μM) to isolate the neurons from their synaptic inputs. Local application of BDNF (200 ng/ml) onto the soma of patched neurons induced no response in all 12 experiments (Fig. 2A), whereas the application of high K^+ (10 mM) on the same cells produced an immediate strong depolarization, proving the effectiveness of our puffing system (Fig. 2A). Bath application of BDNF (200 ng/ml) also failed to induce any change in membrane potential in 7/7 cells. These experiments show that BDNF has no direct effect on neuronal excitability.

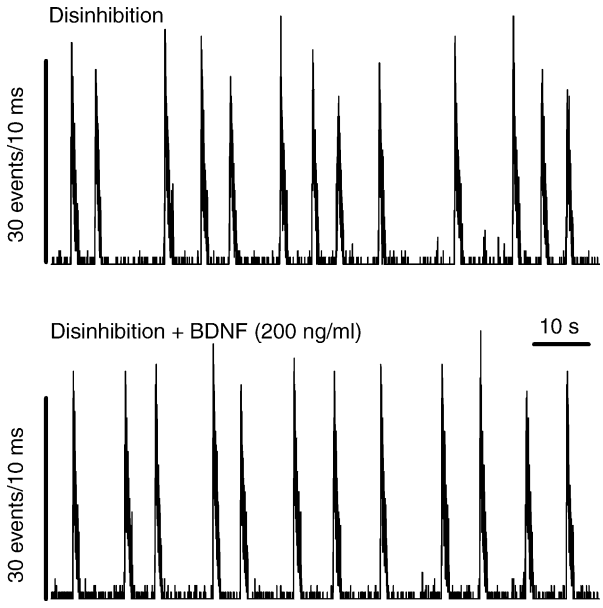

Fig. 1. Effects of exogenous BDNF on rhythm induced by disinhibition; example of network activity plots obtained under disinhibition before and after BDNF application (200 ng/ml). Note the small increase in burst amplitude under BDNF.