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Research report

Airway-related vagal preganglionic neurons express brain-derived neurotrophic factor and TrkB receptors: Implications for neuronal plasticity

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Abstract

Recent evidence indicates that brain-derived neurotrophic factor (BDNF) is present in neurons and may affect neurotransmitter release, cell excitability, and synaptic plasticity via activation of tyrosine kinase B (TrkB) receptors. However, whether airway-related vagal preganglionic neurons (AVPNs) produce BDNF and contain TrkB receptors is not known. Hence, in ferrets, we examined BDNF and TrkB receptor expression in identified AVPNs using in situ hybridization and immunohistochemistry. BDNF protein levels were measured within the rostral nucleus ambiguus (rNA) region by ELISA. We observed that the subpopulation of AVPNs, identified by neuroanatomical tract tracing, within the rNA region express BDNF mRNA, BDNF protein, as well as TrkB receptor. In addition, brain tissue from the rNA region contained measurable amounts of BDNF that were comparable to the hippocampal region of the brain. These data indicate, for the first time, that the BDNF–TrkB system is expressed by AVPNs and may play a significant role in regulating cholinergic outflow to the airways.

Theme: Autonomic regulation
Topic: Respiratory regulation

Keywords: Neurotrophins; Trk receptors; Central control of airway smooth muscle tone; Rostral nucleus ambiguus; Vagal parasympathetic preganglionic neurons; Airways; Ferret

1. Introduction

In the last decade, enormous progress has been made in understanding the role that neurotrophins, including brain derived neurotrophic factor (BDNF), play in development, neuronal survival, differentiation, synapse formation and stabilization, and structural and functional neuronal plasticity in many networks [10,11,35,41,44,47,55–59], including the brainstem and spinal cord respiratory related pathways [3,4,54]. For example, intermittent hypoxia provokes a serotonin-dependent increase in the BDNF protein synthesis that is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxic stress [3].

Recent findings, obtained through an array of techniques in normal and transgenic animals, provide insight into the modulatory mechanisms of BDNF at central synapses [7,8,13]. BDNF signaling evokes both short- and long-term periods of enhanced synaptic transmission, acting pre- and postsynaptically [57]. Results indicate that BDNF-producing neurons respond to stimulation with increased synthesis and elevated release of BDNF [3,5,56,61] that in turn enhances quantal neurotransmitter release [55,56] such as glutamate [12,22,45,49]. The elevated levels of BDNF increase

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expression and activity of glutamatergic receptors [37,43], facilitate glutamatergic synaptic transmission [13,42], and unmask the silent synapses [36]. Furthermore, it has been shown that a positive feedback between acetylcholine and the BDNF exists in the rat hippocampus [40]. BDNF released may cause rapid excitation of neurons via activation of high-affinity tyrosine kinase B (TrkB) receptors [38], producing postsynaptic long-term potentiation [3,41,48,57]. Therefore, BDNF–TrkB receptors could participate in centrally induced increase in cholinergic outflow to the airways.

In order to define the role of BDNF in the plasticity of the neuronal network linked to regulation of cholinergic outflow to the airways, first it should be shown that BDNF and TrkB receptors are present in the network regulating cholinergic outflow to the airways. Therefore, the aim of the present study was to test our hypothesis that the BDNF and/or TrkB receptors are expressed by airway-related vagal preganglionic neurons (AVPNs) at the mRNA and protein levels.

In these studies, we characterized BDNF and TrkB receptor expression by parasympathetic premotor cells that innervate extrathoracic trachea using neuroanatomical and molecular techniques. Results showed for the first time that AVPNs innervating the airways produce BDNF and express TrkB receptors, suggesting that BDNF–TrkB receptor signaling pathway may play a role in neuronal plasticity, modulating AVPN discharge and cholinergic outflow to the airways.

2. Methods

2.1. Animals

Experimental protocols were approved by the Institutional Animal Care and Use Committees at Case Western Reserve and Howard University. To minimize gender-related differences, all experiments were performed in male European ferrets, *Mustella putorius furo* (600–900 g). In the present study, six healthy male ferrets were used.

2.2. Injection of CT-b into the extrathoracic trachea

Under pentobarbital anesthesia (50 mg/kg, ip), the tracheas of ferrets were injected with cholera toxin β subunit (CT-b) as previously described [27,29]. CT-b was injected along the tracheal wall beginning with the third intercartilaginous space. After 5 days, the animals were re-anesthetized with pentobarbital (50 mg/kg, ip), mechanically ventilated with oxygen, and perfused through the left ventricle with 0.1 M sodium phosphate-buffered saline (PBS, pH 7.4) containing 10,000 U of heparin. This was subsequently followed by a 4% paraformaldehyde in PBS, pH 7.4. The brains were removed and stored in the same fixative for 8–12 h. Since paraformaldehyde fixation may decrease antigenicity of some molecules, one ferret was perfused with a fixative shown to preserve the antigenic properties of BDNF and TrkB receptors (2% paraformaldehyde in 0.07% sodium phosphate buffer, pH 7.2, containing 0.2% parabenzoquinone) [10]. A series of transverse 40-μm sections of cryoprotected (30% sucrose in PBS) brain tissue were cut from the level of the spino-medullary junction to the rostral border of the pons using a Bright OTF cryostat (Hacker Instruments Inc., Faifield, NJ).

2.3. BDNF and TrkB receptor in situ hybridization

In situ hybridization on sequential frozen tissue sections was performed using digoxigenin-labeled cRNA probe to detect BDNF or TrkB mRNA expression in AVPNs. The rat BDNF plasmid was obtained from Regeneron Pharmaceutical, NY) and amplified as previously described [10]. The plasmid template was linearized by digestion with *Bam*H1 or *Hin*dIII to generate the corresponding sense or antisense transcripts, under T3 or T7 promoters, respectively. Post-restriction digest (cDNA template) was treated with proteinase K and was phenol-chloroform extracted.

For TrkB receptor mRNA in situ hybridization, mouse TrkB cDNA fragment encoding the intracellular domains specific to tyrosine kinase containing isoform-specific region (from nucleotide 1386 to nucleotide 2054 inserted in pBluescript plasmid) was used to generate digoxigenin-labeled cRNA probes (gift from Dr. Baoji Xu, Georgetown University, Washington, DC). The validity and specificity of the cDNA fragment were tested and published previously [26,64]. The plasmid template was linearized by digestion with *Bam*H1 or *Xho*1 to generate the corresponding sense or antisense transcripts under T3 or T7 promoters respectively. Post-restriction digest (cDNA template) was treated with proteinase K and was phenol-chloroform extracted.

The digoxigenin-labeled sense and antisense probes for BDNF and TrkB were synthesized using a Dig RNA labeling kit (Roche Molecular Biology). In brief, the reaction mixture contained 40 mM Tris–HCl, pH 8.0, 6 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM spermidine, 10 mM NaCl, 1 unit/μl RNase inhibitor, and 1 mM ATP, GTP, CTP with 0.65 mM UTP and 3.5 mM DIG-111-UTP. The product was treated with DNAse and the labeled cRNA probe was recovered by ethanol precipitation. Tissue sections were briefly treated with protease (125 μg/ml, Sigma, St. Louis, MO) and acetylated with 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine–HCl. Pretreated tissue sections were incubated with hybridization solution containing 20 mM Tris–HCl buffer (pH 7.4), approximately 5 μg of cRNA probe, 0.5 mg/ml tRNA, 0.1 M DTT, 50% formamide, 0.3 M NaCl, 10 mM NaH_2PO_4 (pH 8.0), 5 mM EDTA, 10% Dextran sulfate, and 1× Denhardt's solution (Sigma). Sense or antisense probes were hybridized with tissue sections overnight on microslides at 55 °C in a humidified chamber. Following hybridization, the slides were washed for 30 min each at 55 °C in 5× SSC (0.3 M NaCl/0.03 M sodium citrate, pH 7.0) and 2× SSC containing 10 mM DTT and treated with RNAse. The slides were then washed in 2× SSC containing 50%