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Brain-derived neurotrophic factor modulates cell excitability in the mouse medial nucleus of the trapezoid body

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Abstract

Neurotrophins are a large class of trophic factors located throughout the central nervous system. While the role of neurotrophins in neuronal survival and axon guidance is well known, their secondary role in modulating synaptic transmission and cell firing properties is largely unexplored. In this study we examined the expression of neurotrophins in the mouse medial nucleus of the trapezoid body (MNTB) and investigated the effect of exogenous brain-derived neurotrophic factor (BDNF) application on the firing properties of MNTB principal cells. The expression levels of nerve growth factor, BDNF, neurotrophin-3, neurotrophin-4/5 and major receptor tyrosine kinase B was found to be moderate to high at postnatal day 12, indicating that the neurotrophins may have a role following synaptogenesis. A 2-h exposure to exogenous BDNF (100 ng/mL) had a significant effect on principal cell firing properties and voltage-gated potassium currents. Importantly, preincubation in BDNF increased the incidence of multifiring and rebounding cells, and significantly increased the number of action potentials fired in response to a single depolarizing step. BDNF exposure also significantly decreased underlying voltage-gated potassium currents, including both the low- and high-voltage-activated components. Our data show that the neurotrophins, specifically BDNF, may have a novel role in modulating cell excitability in the auditory brainstem.

Introduction

The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), and their tyrosine kinase receptors (TrK)A–C are found in both neurons and glia throughout the central nervous system (Riley *et al*., 2004). Neurotrophic signalling is known to be important for axon and synaptic targeting and neuronal survival. However, in several areas of the brain neurotrophins have also been shown to affect neuronal firing properties. In the hippocampus, BDNF bound to TrKB can induce membrane depolarization and action potentials by interacting with $Na_v1.9$ (Blum *et al*., 2002). Moreover, TrKB is coupled to phospholipase-C, which can increase release of calcium from IP3 stores, and TrKB activation down-regulates the KCC2 potassium–chloride transporter, making inhibition less effective (Kovalchuk *et al*., 2004). Finally, TrKB signalling can activate and/or modulate a host of ion channels, including voltage-gated and inward-rectifying potassium channels and transient receptor proteins (Rose *et al*., 2004). However, few studies have examined neurotrophin presence and signalling in auditory-related nuclei. In the auditory brainstem, the anteroventral cochlear nucleus, medial nucleus of the trapezoid body (MNTB) and medial and lateral superior olives are involved in localization of high- and low-frequency sounds using interaural timing and intensity differences. In the present study we examined neurotrophin and TrKB expression and function in the mouse MNTB, an auditory brainstem nucleus in the interaural level difference pathway. Specifically, we investigated the hypothesis that exogenous BDNF exposure can affect principal cell firing properties in the MNTB. Our results show that the neurotrophins can significantly increase cell excitability by modulating voltage-gated potassium (Kv) currents.

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Materials and methods

Brainstem slice electrophysiology

CBA mice aged postnatal day (P)12–14 were decapitated in accordance with local Australian National University ethics guidelines. Brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) for cutting, containing (in mM): KCl, 3; $NaHCO_3$, 26.2; NaH_2PO_4, 1.25; $MgCl_2$, 5; $CaCl_2$, 1; glucose, 10; and sucrose, 218. Coronal brainstem slices, 180–225 μm thick, were prepared and incubated for 40 min in normal ACSF containing (in mM): KCl, 3; $NaHCO_3$, 26.2; NaH_2PO_4, 1.25; $MgCl_2$, 1; $CaCl_2$, 2; and glucose, 10; at 36 °C. Neurons were visualized with infrared differential interference contrast optics and all electrophysiological recordings were performed at room temperature (22–25 °C). Slices were continually superfused during recordings with normal ACSF (oxygenated with 95% O_2 : 5% CO_2). Electrodes had a tip resistance of 3–6 MΩ and were filled with a potassium gluconate-based internal solution containing (in mM): KGlu, 122.5; KCl, 17.5; NaCl, 9; $MgCl_2$, 1; HEPES, 10; EGTA, 0.2; ATP-Mg, 3; and GTP-Tris, 0.3. During whole-cell voltage clamp ($V_m = -60$ mV) recordings, series resistance was <10 MΩ and compensated by $\geq 80\%$. In the BDNF condition, BDNF (100 ng/mL; Chemicon, Temecula, CA, USA) was added to the slice incubation well for 2 h prior to recording, allowing it to fully penetrate the tissue. Voltage-gated potassium currents were recorded in the presence of 1 μM tetrodotoxin (Alomone, Jerusalem, Israel), 10 μM strychnine hydrochloride (Sigma), 0.5 mM Ca^{2+} and

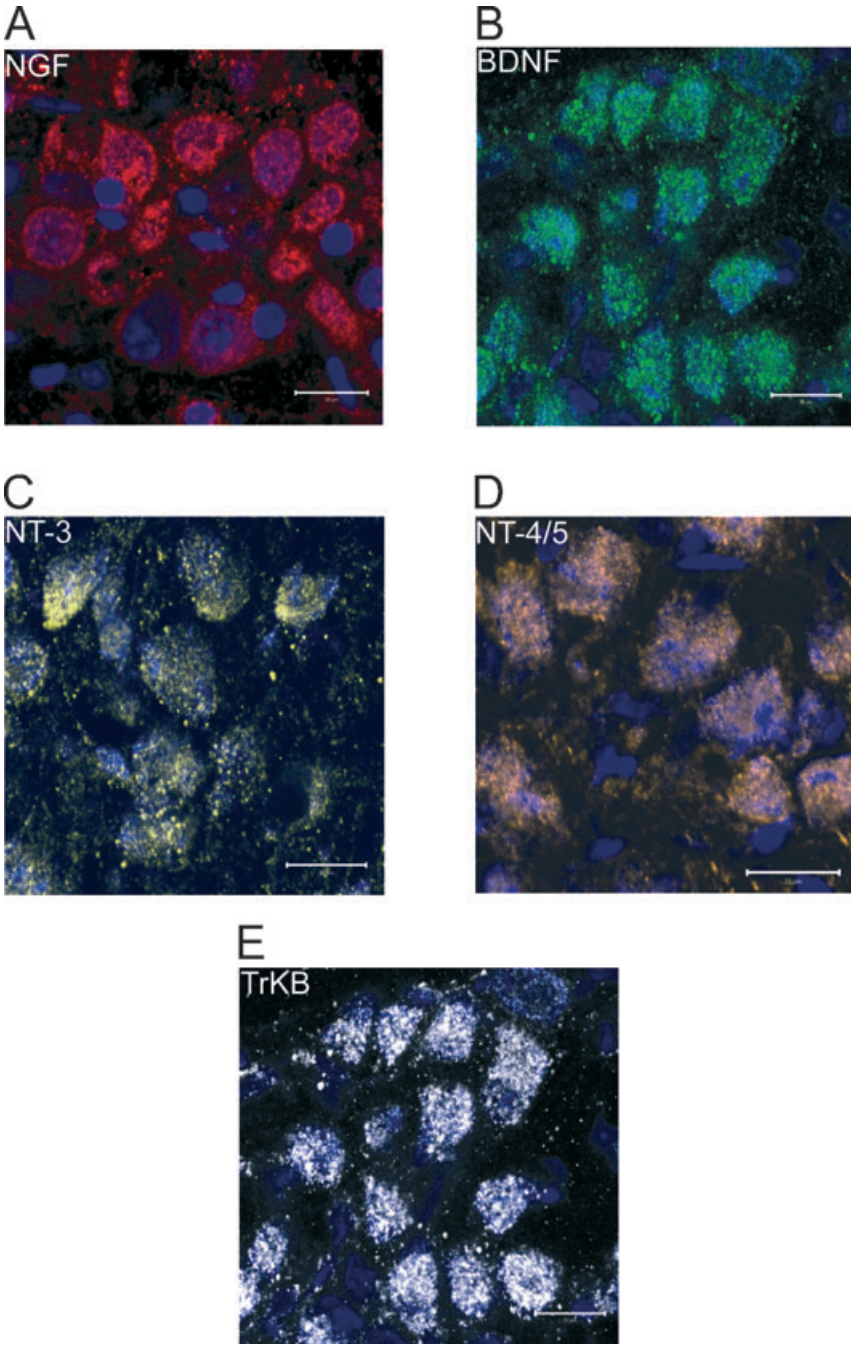

FIG. 1. Neurotrophin and TrKB expression in the mouse MNTB was medium to high at P12. (A–D) Expression of neurotrophins NGF (red), BDNF (green), NT-3 (yellow) and NT-4/5 (orange) in MNTB principal cells (blue NeuroTrace® labelling) at P12. (E) Expression of neurotrophin receptor TrKB (white) in MNTB principal cells (blue NeuroTrace® labelling) at P12. Note the diffuse punctate labelling and colocalization with the cell soma. Scale bars, 20 μm.

2.5 mM Mg^{2+}. Cells were held at −100 mV for 750 ms to relieve all Kv channels from inactivation before being stepped to test potentials from −100 to +30 mV. With this protocol the steady-state current at +10 mV represents the total mixed Kv current, while the steady-state current at −40 mV is 90% mediated by low-threshold Kv channels. To isolate high-threshold Kv currents, cells were held at −30 mV before being stepped from −100 mV to +30 mV. The high-threshold steady-state current was measured at +30 mV (Brew & Forsythe, 2005). During whole-cell current-clamp, recordings were made in Iclamp fast mode with an output gain (α) multiplier of 5. Current-clamp steps included 15 steps from −300 to +400 pA in 50-pA increments to examine rebound spiking and multi- vs. single-firing properties. All currents and potentials were recorded and filtered at 10 kHz with an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) then digitized at 20 kHz. Analysis was carried out offline using Axograph 4.8 (Axon Instruments).

Immunohistochemistry

Brainstem slices 180–225 μm thick were prepared as for electrophysiology from CBA mice at P12–14, then fixed for 2 h at room temperature in 4% paraformaldehyde and stored in PBS. Sections were blocked with

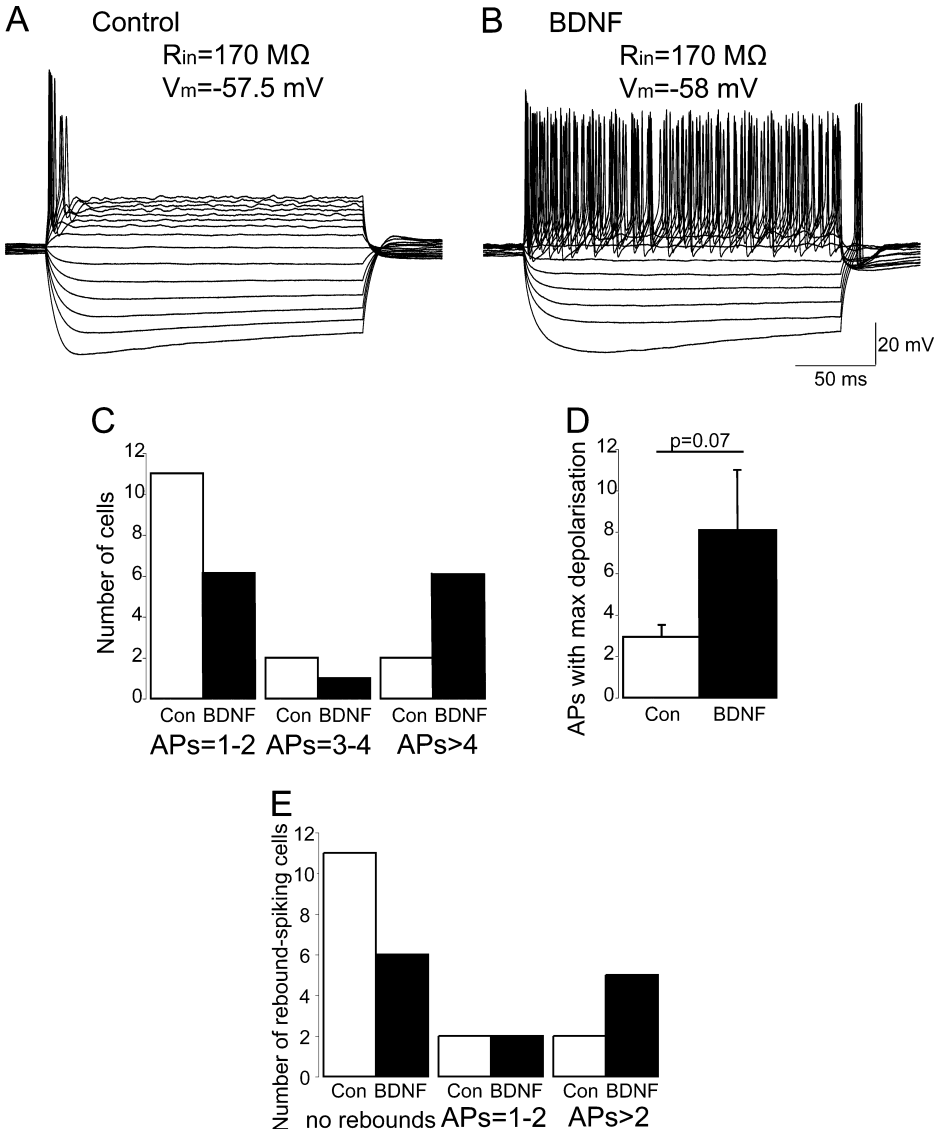

FIG. 2. A 2-h exposure to BDNF increased the incidence of multifiring and rebound spiking principal cells. (A) Control traces of current injections from −300 to +400 pA, showing single-firing at depolarized potentials and lack of rebound spiking following hyperpolarizing potentials. (B) Traces after a 2-h BDNF exposure, showing multifiring and rebound spiking. (C) Representative bar graph showing that the incidence of multifiring increases after BDNF exposure. (D) Representative bar graph showing increase in number of action potentials (APs) fired in response to maximal current injection following BDNF exposure. Statistical significance was $P = 0.07$ with nonparametric analysis. (E) Representative bar graph showing that the incidence of rebound spiking increased following BDNF incubation, especially in cells that also showed multifiring properties. APs, action potentials; R_{in}, input resistance; V_m, resting membrane potential.

10% donkey serum and 0.3% Triton X-100 in 0.1 M PBS. Sections were then incubated overnight (∼ 16 h) at 4 °C in blocking solution containing primary antibodies directed against one of four different neurotrophins or one neurotrophin receptor (NGF, BDNF, NT-3, NT-4/5 or TrKB) at 5–10 μg/mL (antineurotrophins) and 1 : 1000 (anti-TrKB; Chemicon). To maximize tissue use, in some cases slices were coincubated with one antineurotrophin antibody and anti-TrKB; subsequent individual labelling (using only one antineurotrophin or anti-TrKB antibody) indicated that there were no confounding effects of double immunostaining. After washing in PBS, an Alexa 488- (antineurotrophins) or Alexa 555- (anti-TrKB) conjugated secondary antibody (1 : 1000) was added to the sections for 1 h at room temperature. Neurons were subsequently labelled with a fluorescent 640/660 Nissl stain (NeuroTrace®, Molecular Probes, Eugene, OR, USA). Slices were coverslipped and viewed with a confocal microscope (Zeiss Axioskop 2 FS mot; Oberkochen, Germany). Single-plane and *z*-stack images (1-μm slices) were obtained using 10× and 40× Plan-Neofluar objectives (Zeiss) and a computer equipped with LSM 510 software (Thornwood, New York, USA). Neurotrophin antibody specificity was confirmed by Western blot.

Results are expressed as mean ± SEM. Statistical significance was determined using one-tailed Student's *t*-tests, although the Mann–Whitney test was used as indicated; unless specified, $P < 0.05$ was taken to be the lowest level of significance.

Results

Neurotrophic factors and TrKB receptors were highly expressed postnatally

The presence of the BDNF, NGF, NT-3 and NT-4/5 proteins and the BDNF and NT-4/5 receptor TrKB was examined in the mouse MNTB at P12. Neurotrophin immunoreactivity was strong at P12

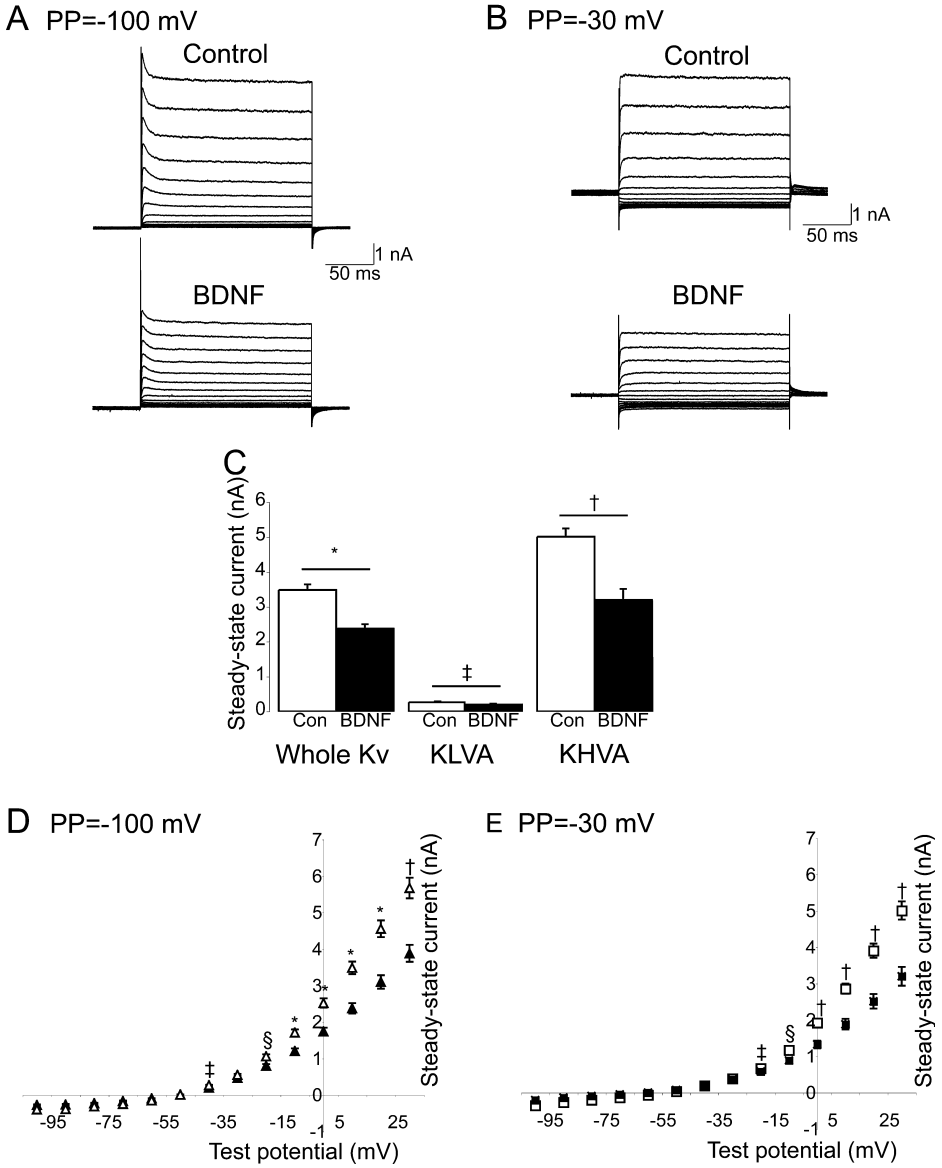

FIG. 3. A 2-h exposure to BDNF significantly decreased outward potassium currents. (A) Steady-state Kv currents in control (top) and BDNF conditions (bottom) recorded from −100 to +30 mV after being held at a prepotential (PP) of −100 mV. (B) Steady-state Kv currents in control (top) and BDNF conditions (bottom) recorded from −100 mV to +30 mV after being held at a PP of −30 mV. Note in both A and B the strong decrease in outward currents following BDNF exposure. (C) Summary bar graphs of the whole, low- and high voltage-activated components of the Kv currents, showing a significant decrease in all three in the BDNF condition. (D) Steady-state currents plotted against test potential after being held at −100 mV, showing a significant decrease in outward potassium currents after BDNF exposure. (E) Steady-state currents plotted against test potential after being held at −30 mV, showing a significant decrease in outward potassium currents after BDNF exposure. Open symbols indicate control condition, filled symbols indicate BDNF-exposed condition. $^{*}P < 0.0001$, $^{\dagger}P < 0.0005$, $^{\S}P < 0.005$ and $^{\ddagger}P < 0.05$. KHVA, high voltage-gated potassium current; KLVA, low voltage-gated potassium current.

(Fig. 1A–D). The labelling appeared as small diffuse puncta colocalized with the cell soma. There was no obvious membranous labelling or synaptic localization, suggesting a subcellular cytosolic location. Moreover, this pattern of labelling was similar with all neurotrophin antibodies, showing that BDNF, NGF, NT-3 and NT-4/5 were all located within cells of the MNTB at an early postnatal age. Qualitatively, TrKB immunoreactivity appeared to be more intense than that of any of the neurotrophins (Fig. 1E). Again, TrKB labelling appeared to colocalize with the cell soma, with no clear synaptic or membranous colocalization. Therefore, it is clear that there was a considerable amount of neurotrophin and TrKB receptor labelling in the second postnatal week in the MNTB of mice. As this high expression occurs after the initial period of synaptic targeting and neuronal survival, the neurotrophins may play an unknown role in neurotransmission or cell excitability.

Two-hour exposure to BDNF affected postsynaptic firing properties

We examined the effect of BDNF exposure on single-firing and rebound spiking in principal cells. In the control condition (2-h postslicing incubation in normal ACSF), 11 out of 15 cells fired only one or two action potentials in response to a single 200-pA current injection (Fig. 2A). A further two out of 15 fired three or four, and a final two out of 15 fired more than four action potentials. However, in the BDNF condition (2-h postslicing incubation in ACSF with

100 ng/mL BDNF), six out of 13 cells fired one or two action potentials, one fired three or four, and six cells fired more than four action potentials in response to depolarization (Fig. 2B). This indicates a three-fold increase in the number of cells firing abnormally after BDNF pretreatment, as well as a reduction in the number of cells that fired only one or two action potentials, characteristic of principal cells (Fig. 2C). The average number of action potentials in response to the maximal current injection (400 pA) was 3 ± 1 in normal and 8 ± 3 in BDNF-treated animals ($n = 15$ for control, $n = 13$ for BDNF; $P = 0.07$ with nonparametric Mann–Whitney test; Fig. 2D). BDNF exposure did not significantly affect resting membrane potential or input resistance (V_m was −56 ± 1 mV in control, $n = 15$, and −57 ± 1 mV in the BDNF condition, $n = 13$, ns; R_{in} was 225 ± 17 MΩ in control and 225 ± 20 MΩ in the BDNF condition; $n = 13$, ns). We also investigated the presence of rebound spikes after the principal cell was hyperpolarized with current injections between −300 and −50 pA. The majority of cells in the control condition (11 out of 15) did not exhibit rebound spikes, while only six out of 13 cells in the BDNF condition did not show rebound spikes. In cells that fired only one or two action potentials during depolarizing steps, two each from the control and BDNF conditions showed rebound spiking. Although not all multifiring cells were also rebound spiking, in cells that fired more than two action potentials when depolarized, two out of four cells (50%) from the control condition exhibited rebound spikes while five out of seven cells (71%) from the BDNF condition showed the same (Fig. 2E). This indicates that following BDNF exposure there was an increase in the presence of both multifiring and rebound spiking among principal cells in the MNTB.

BDNF significantly decreased outward Kv currents

Potassium currents were recorded in the presence of tetrodotoxin, strychnine, 0.5 mM Ca^{2+} and 2.5 mM Mg^{2+} (Fig. 3A and B). Interestingly, the whole Kv current steady-state amplitude was significantly different between the control and BDNF conditions, with an average of 3.48 ± 0.14 nA in control and 2.37 ± 0.13 nA after BDNF treatment ($P < 0.0001$; $n = 10$ for control, $n = 10$ for BDNF). Part of this large difference was due to changes in low-threshold Kv currents. In the control condition, low-threshold Kv currents averaged 267 ± 28 pA while in the BDNF condition they were 202 ± 21 pA ($P < 0.05$; $n = 10$ for control, $n = 10$ for BDNF). The high-threshold component was also affected. Following BDNF incubation, the average high-threshold steady-state current decreased from 5.00 ± 0.21 nA in control to 3.20 ± 0.31 nA after BDNF treatment ($P < 0.0005$; $n = 10$ for control, $n = 8$ for BDNF; Fig. 3C). Plots showing the current–voltage relationship of Kv activation reveal that outward potassium currents were significantly depressed in the BDNF condition at more depolarized potentials. It is also clear that, despite similarities, when cells were held at −100 mV and then stepped to different test potentials, there was a significant reduction in currents after BDNF incubation as early as −40 mV (Fig. 3D). When cells were held at −30 mV, the difference in steady-state amplitude was significant at −20 mV and more depolarized potentials (Fig. 3E). These data indicate that a 2-h BDNF incubation had significant effects on both the low- and high-threshold Kv currents.

Discussion

Our results show that the neurotrophins and the TrKB receptor are strongly expressed in the mouse MNTB at P12. Furthermore, experiments using exogenous BDNF application indicate that BDNF signalling affects principal cell excitability and Kv currents. Significantly, a 2-h exposure to BDNF decreased Kv currents, leading to an increased incidence of multifiring and rebound spiking in MNTB principal cells. A reduction in the Kv outward currents, particularly low-voltage-activated currents, would increase the incidence of multifiring cells by allowing the cell to remain more depolarized following an action potential. The increase in rebound spiking could be the result of an interaction with the hyperpolarizing-activated I_h current (Leao *et al*., 2006). Therefore the dual effect of BDNF on multifiring and rebound spiking would greatly increase cell excitability in response to both depolarization and hyperpolarization. This provides the first evidence of a role for the neurotrophins in modulation of cell excitability in the MNTB.

Previous studies have shown that the neurotrophins are present postnatally in all auditory brainstem nuclei. In particular, both the rat and gerbil have been shown to have medium to high levels of BDNF, NT-3 and NT-4 in the MNTB, and the TrK receptors, especially TrKB, have high immunoreactivity in the MNTB from P7 to P30 (Hafidi *et al*., 1996; Hafidi, 1999; Tierney *et al*., 2001). These results are in agreement with the data shown in this study in mice, indicating that the high level of neurotrophins and TrKB in the MNTB are conserved between species. The fact that the neurotrophins remain highly expressed for extended periods of time well after synaptogenesis and early neuronal survival indicates that they may have a physiological function in auditory brainstem synaptic transmission. Here we show evidence that neurotrophic signalling can modulate cell excitability and Kv currents. In the MNTB, these currents are predominantly mediated by Kv3.1 and Kv1.1/1.2 channels (Wang *et al*., 1998; Dodson *et al*., 2002; Macica *et al*., 2003). It is possible that BDNF exposure activates TrKB receptors, causing phosphorylation of the two channel subtypes and decreasing their steady-state outward currents. TrKB activation has been shown to modulate potassium channels by this mechanism in other areas of the brain. The inward-rectifying Kir3 current is suppressed by TrKB activation, due to tyrosine phosphorylation of the channel (Rogalski *et al*., 2000). In the rat olfactory bulb, short-term BDNF exposure significantly decreases the Kv1.3-mediated current in a phosphorylation-dependent manner (Tucker & Fadool, 2002). While the results shown here are the first to illustrate an effect of BDNF on Kv1.1/1.2 and Kv3.1, tyrosine phosphorylation of the Kv1.1 channel can decrease Kv1.1 currents irreversibly in oocyte systems (Imbrici *et al*., 2000). Furthermore, in the MNTB Kv3.1 is phosphorylated basally, and activity-dependent dephosphorylation increases the Kv3.1 current while phosphorylation decreases it (Song *et al*., 2005). It is also possible that BDNF exposure activates the pan-neurotrophic receptor p75 rather than TrKB. Previous studies have shown, in dorsal root ganglion neurons, that NGF exposure increases action potential number in response to depolarization by activating p75 and decreasing potassium channel conductance downstream of the ceramide signalling pathway (Zhang *et al*., 2002; Zhang & Nicol, 2004). In conclusion, this study has shown that exogenous BDNF significantly affects principal cell excitability and potassium currents. Future experiments will aim to uncover the physiological mechanisms and locations of BDNF release and action in the mouse MNTB.

Abbreviations

ACSF, artificial cerebrospinal fluid; BDNF, brain-derived neurotrophic factor; Kv, voltage-gated potassium (current or channel); MNTB, medial nucleus of the trapezoid body; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; P, postnatal day; TrK, tyrosine kinase receptor.

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