IMPAIRED TRANSMISSION AT CORTICOTHALAMIC EXCITATORY INPUTS AND INTRATHALAMIC GABAERGIC SYNAPSES IN THE VENTROBASAL THALAMUS OF HETEROZYGOUS BDNF KNOCKOUT MICE

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Abstract—Beside its role in development and maturation of synapses, brain-derived neurotrophic factor (BDNF) is suggested to play a critical role in modulation and plasticity of glutamatergic as well as GABAergic synaptic transmission. Here, we used heterozygous BDNF knockout (BDNF$^{+/-}$) mice, which chronically lack approximately 50% of BDNF of wildtype (WT) animals, to investigate the role of BDNF in regulating synaptic transmission in the ventrobasal complex (VB) of the thalamus. Excitatory transmission was characterized at glutamatergic synapses onto relay (TC) neurons of the VB and intrathalamic inhibitory transmission was characterized at GABAergic synapses between neurons of the reticular thalamic nucleus (RTN) and TC neurons. Reduced expression of BDNF in BDNF$^{+/-}$ mice did not affect intrinsic membrane properties of TC neurons. Recordings in TC neurons, however, revealed a strong reduction in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in BDNF$^{+/-}$ mice, as compared to WT littermates, whereas mEPSC amplitudes were not significantly different between genotypes. A mainly presynaptic impairment of corticothalamic excitatory synapses in BDNF$^{+/-}$ mice was also indicated by a decreased paired-pulse ratio and faster synaptic fatigue upon prolonged repetitive stimulation at 40 Hz. For miniature inhibitory postsynaptic currents (mIPSCs) recorded in TC neurons, both, frequency and amplitude showed a significant reduction in knock-out animals, concurrent with a prolonged decay time constant, whereas paired-pulse depression and synaptic fatigue of inhibitory synapses were not significantly different between WT and BDNF$^{+/-}$ mice. Spontaneous IPSCs (sIPSCs) recorded in VB neurons of BDNF$^{+/-}$ animals showed a significantly reduced frequency. However, the glutamatergic drive onto RTN neurons, as revealed by the percentage reduction in frequency of sIPSCs after application of AMPA and NMDA receptor blockers, was not significantly different. Together, the present findings suggest that a chronically reduced level of BDNF to ~50% of WT levels in heterozygous knock-out animals, strongly attenuates glutamatergic and GABAergic synaptic transmission in thalamic circuits. We hypothesize that this impairment of excitatory and inhibitory transmission may have profound consequences for the generation of rhythmical activity in the thalamocortical network. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: neurotrophin, glutamate, development, synaptic current, thalamocortical network.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), synthesized and released by central neurons, is an eminent differentiation and survival factor for neurons (Lewin and Barde, 1996; Greenberg et al., 2009). In addition, BDNF has been shown to regulate synapse formation and synaptic plasticity (Huang and Reichardt, 2001; Poo, 2001). Numerous previous studies provided evidence that BDNF strongly influences glutamatergic and GABAergic synaptic transmission in various ways, including acute and chronic effects (for review see Gottmann et al., 2009). Acute application of exogenous BDNF, for instance, can enhance excitatory transmission in hippocampal and cortical neurons and facilitate long-term potentiation (see e.g., Lessmann et al., 1994; Levine et al., 1995; Akaneya et al., 1997; Carmignoto et al., 1997). Importantly, a similar enhancing effect has been proven for acutely released endogenous BDNF (Magby et al., 2006; Walz et al., 2006). Similarly, chronic application of BDNF can also improve glutamatergic transmission in various brain structures (see e.g., -Vicario-Abejon et al., 1998; Tyler and Pozzo-Miller, 2001). In accordance with these acute and chronic effects of BDNF, altered transmission and reduced synaptic plasticity was observed at glutamatergic synapses in a BDNF knock-out (KO) mouse line in the hippocampus (Korte et al., 1995; Pozzo-Miller et al., 1999) and in the neocortex (Bartoletti et al., 2002; Abidin et al., 2006). Depending on brain structure and stage of

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Abbreviations: BDNF, brain-derived neurotrophic factor; eEPSCs, evoked EPSCs; EGTA, ethylene glycol tetraacetic acid; eIPSCs, evoked IPSCs; EPSCs, excitatory postsynaptic currents; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HF, high frequency; IPSCs, inhibitory postsynaptic currents; ISIs, interstimulus intervals; LTS, low threshold spike; mEPSCs, miniature EPSCs; mIPSCs, miniature IPSCs; PPR, paired-pulse ratio; RTN, reticular thalamic nucleus; sIPSCs, spontaneous IPSCs; SSC, steady state current; VB, ventrobasal complex; WT, wildtype.

development, acutely applied BDNF can either reduce the efficacy of GABAergic synaptic transmission via postsynaptic modifications (see e.g., Frerking et al., 1998; Brunig et al., 2001), or can enhance GABAergic transmission via presynaptic modifications (see e.g., Wardle and Poo, 2003; Jovanovic et al., 2004; for a recent review see Gottmann et al., 2009). Chronic action of BDNF in hippocampus and neocortex regulates the efficacy of GABAergic synapses either by enhancing formation of presynaptic terminals or increasing presynaptic release probability (see e.g., Baldelli et al., 2005; Kohara et al., 2007). Importantly, also endogenously released BDNF has been shown to regulate GABAergic synaptic transmission in the hippocampus (Gubellini et al., 2005; Kohara et al., 2007; Kuczewski et al., 2008), thus stressing the physiological importance of BDNF signaling also for GABAergic synapses.

In the corticothalamic loop of the thalamocortical system glutamatergic synapses between cortical pyramidal cells of layers V and VI and relay neurons of the ventrobasal complex (VB), and GABAergic synapses between neurons of the reticular thalamic nucleus (RTN) and VB neurons, are the structural basis for the control of intrathalamic oscillations (Huguenard and McCormick, 2007). The knowledge regarding BDNF-dependent modulation of synapses in the thalamus is sparse. Recently, a BDNF-dependent modulation of synaptic plasticity at excitatory glutamatergic inputs from the mammillary bodies into the anterior thalamus has been described (Tsanov et al., 2009). However, further BDNF-dependent mechanisms of synaptic modulation have not yet been investigated in the thalamic network, although layer V and VI pyramidal cells, which also project into the thalamus, strongly express BDNF in juvenile and adult rodents (Murer et al., 2001; Patz and Wahle, 2006), and BDNF is highly expressed in the VB of rats during postnatal (P3–P21) development (Mooney and Miller, 2011). Furthermore, TrkB receptor mRNA and TrkB receptor protein are clearly expressed in the ventrobasal thalamus of adult rats (Avwenagha et al., 2006). It remains to be demonstrated, however, whether the BDNF of cortical pyramidal cells is transported to their thalamic targets in the RTN and VB and whether it is released in an activity-dependent manner. Interestingly, BDNF is anterogradely transported from layer V/VI pyramidal cells of the motor-cortex to the striatum and released onto striatal neurons, indicating a functional role during development and maintenance (Altar et al., 1997; Conner et al., 1997).

In a first step to characterize BDNF-dependent mechanisms at corticothalamic excitatory and inhibitory synapses, we used a heterozygous BDNF KO (BDNF$^{+/-}$) mouse model (Korte et al., 1995), showing reduced BDNF expression to 50–60% of levels observed in wildtype (WT) animals (Genoud et al., 2004; Abidin et al., 2006). Homozygous BDNF$^{-/-}$ mice were not used because they show pronounced retardation of overall growth and of neuronal development, and usually die within 2 weeks after birth (Korte et al., 1995). Studies

using the heterozygous mouse model have demonstrated changes in glutamatergic synaptic transmission in the hippocampus (Patterson et al., 1996; Pozzo-Miller et al., 1999) and the visual cortex (Bartoletti et al., 2002; Abidin et al., 2006). BDNF$^{+/-}$ mice have also been successfully used to describe changes in the efficiency of GABAergic inhibition in the visual cortex (Abidin et al., 2008). In our study, we found a significant decrease of the efficiency of both glutamatergic and GABAergic transmission at excitatory and inhibitory synapses in the VB, suggesting reduced glutamate and GABA release onto VB neurons.

EXPERIMENTAL PROCEDURES

Animals

The BDNF$^{+/-}$ mice used in the present study are characterized by a chronic reduction in the expression of BDNF to approx. 50% (Korte et al., 1995; Abidin et al., 2006). Wildtype littermates were used as control animals. Mice were genotyped by polymerase chain reaction (PCR) from tail tissue as described earlier (Abidin et al., 2008). All experiments were carried out in accordance with the European Committees Council Directive (86/609/EEC) and approved by the Local Animal Care Committee (Landesverwaltungsamt Sachsen-Anhalt).

Slice preparation

BDNF$^{+/-}$ and WT-mice at the age of postnatal day 14–19 (P14–P19) were anesthetized with isoflurane and decapitated. The brain was rapidly removed and placed in chilled (4 °C) saline, containing the following (in mM): 195 sucrose, 10 dextrose, 20 PIPES, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 10 MgSO$_4$, 0.5 CaCl$_2$, pH 7.25 with NaOH. Horizontal slices of 250 μm thickness were cut with a vibratome (TPI, St. Louis, USA), hemi-sected and placed in a pre-heated (33 °C) submersion chamber for 20 min in standard ACFS containing (in mM): 120 NaCl, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 24 NaHCO$_3$, 2 MgSO$_4$, 2 CaCl$_2$, 10 dextrose, bubbled with 95% O$_2$/ 5% CO$_2$ to a final pH of 7.35.

Electrophysiology

Tissue slices were allowed to recover for at least 90 min before they were transferred to a submerged recording chamber mounted on the stage of an upright microscope (Axioskope 50, Zeiss, Germany) equipped with a 40× water immersion objective (LUMPlanFI, Olympus, Japan). Single slices were placed in the submersion chamber, fixed by a silk mesh, and perfused continuously at a rate of 2 ml/min at room temperature (23–25 °C) with ACSF. Whole-cell patch-clamp recordings were performed on TC neurons of VB under visual control by use of infrared differential interference contrast (DIC) videomicroscopy (Dodt and Zieglgansberger, 1990). Patch pipettes were pulled from borosilicate glass (GC150TF-10, Clark Electromedical Instruments, Pangbourne, UK) to resistances of 2.5–4 MΩ. Access resistance amounted to about 5–10 MΩ. Errors attributable to series resistance were <5 mV. The standard pipette solution used for voltage-clamp contained (in mM): 107 Csgluconate, 13 CsCl, 10 HEPES, 11 EGTA, 0.07 CaCl$_2$, 1 MgCl$_2$, 3 MgATP, 0.5 NaGTP, pH adjusted to 7.25 with KOH. For current-clamp recordings, pipettes were filled with (in mM): 120 Kgluconate, 10 HEPES, 5 EGTA, 2 MgCl$_2$, 0.5 CaCl$_2$, 2.5 KCl, 3 MgATP, pH 7.2 with KOH. A liquid junction potential of 10 mV was corrected off-line (Neher, 1992). Excitatory postsynaptic currents (EPSCs) were recorded