Neuroscience 260 (2014) 265–275

LACK OF BDNF EXPRESSION THROUGH PROMOTER IV DISTURBS EXPRESSION OF MONOAMINE GENES IN THE FRONTAL CORTEX AND HIPPOCAMPUS

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Abstract—Brain-derived neurotrophic factor (BDNF) is implicated in the pathophysiology of psychiatric conditions including major depression and schizophrenia. Mice lacking activity-driven BDNF expression through promoter IV (knock-in promoter IV: KIV) exhibit depression-like behavior, inflexible learning, and impaired response inhibition. Monoamine systems (serotonin, dopamine, and noradrenaline) are suggested to be involved in depression and schizophrenia since many of the current antidepressants and antipsychotics increase the brain levels of monoamines and/or act on monoamine receptors. To elucidate the impact of activity-driven BDNF on the monoamine systems, we examined mRNA levels for 30 monoamine-related genes, including receptors, transporters, and synthesizing enzymes, in KIV and control wild-type mice by using quantitative reverse-transcription polymerase chain reaction (qRT-PCR). mRNA levels were measured in the frontal cortex and hippocampus, which are regions related to depression and schizophrenia and where promoter IV is active. The frontal cortex of KIV mice showed reduced levels of mRNA expression for serotonin receptors 1b, 2a, and 5b (5HTR1b, 5HTR2a, 5HTR5b), dopamine D2 receptors (DRD2), and adrenergic receptors alpha 1a and 1d (AdRα1a and AdRα1b), but increased levels for serotonin synthesizing enzyme, tryptophan hydroxylase (TPH), and dopamine D4 receptor (DRD4) when compared to control wild-type mice. The hippocampus of KIV mice showed decreased levels of 5HTR5b. Our results provide causal evidence that lack of promoter IV-driven BDNF disturbs expression of monoaminergic genes in the frontal cortex and hippocampus. These disturbed expression changes in the monoamine systems may mediate the depression- and schizophrenia-like behavior of KIV mice. Our results also suggest that antidepressant and antipsychotic treatments may actually interfere with and normalize the disturbed monoamine systems caused by reduced activity-dependent BDNF, while the treatment responses to these drugs may differ in the subject with reduced BDNF levels caused by stress and lack of neuronal activity.

Key words: BDNF, promoter IV, monoamine systems, gene expression, frontal cortex, hippocampus.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a major neuronal growth factor in the brain, promotes neurogenesis, neuronal maturation, and synaptic plasticity (Thoenen, 1995). Reduced BDNF levels are observed in the frontal cortex and hippocampus of patients with depression (Dwivedi et al., 2003) and schizophrenia (Weickert et al., 2003; Hashimoto et al., 2005) as well as in stressed animals (Smith et al., 1995). While *Bdnf* expression is regulated by at least nine promoters (Aid et al., 2007), promoter IV (previously classified as promoter III) is the most responsive to neuronal activity and induces activity-driven *Bdnf* expression (Shieh et al., 1998; Tao et al., 1998). Reduced promoter IV function has been observed under stress (Tsankova et al., 2006; Fuchikami et al., 2009) and is implicated in major depression (Keller et al., 2010; Hing et al., 2012) and schizophrenia (Wong et al., 2010). We recently showed causal evidence that lacking promoter IV-driven BDNF expression (knock-in promoter IV: KIV) (Sakata et al., 2009) leads to defective behavioral phenotypes that reflect the symptoms of depression, anxiety, and schizophrenia (Sakata et al., 2010, 2013a). KIV mice display depression-like behavior (decreased exploratory activity in the open field test, stress-induced despair in the tail suspension test and learned helplessness test, and anhedonia in the sucrose preference test), anxiety-like behavior (novelty suppressed feeding), inflexibility in reversal learning (in the Morris water maze and fear extinction), and impaired response inhibition (in the passive avoidance test). KIV mice display otherwise normal learning and memory (e.g., in Morris water maze, fear conditioning test, and active avoidance test) and visual and motor functions (Sakata et al., 2013a).

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Abbreviations: AdR, adrenergic receptor; BDNF, Brain-derived neurotrophic factor; Ct, cycle threshold; DAT, dopamine transporter; DRD2, dopamine D2 receptor; DRD4, dopamine D4 receptor; EDTA, ethylenediaminetetraacetic acid; EPSC, excitatory postsynaptic current; FC, frontal cortex; FDR, false discovery rate; HIP, hippocampus; IPSC, inhibitory postsynaptic current; KIV, knock-in promoter IV; NET, norepinephrine transporter; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SE, standard error of mean; SSRI, selective serotonin reuptake inhibitor; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; WT, wild-type.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.12.013

It should be noted that many other lines of BDNF mutant mice do not present depression-like behavior (Duman and Monteggia, 2006), possibly because region-specific BDNF reduction (dorsal brain regions as opposed to ventral regions) by an endogenous promoter, such as promoter IV, would cause depression. The critical roles of activity-dependent promoter IV in the behavior related to psychiatric symptoms suggests an intriguing ''activity-dependent BDNF hypothesis of psychiatric conditions'' whereby neuronal activity induces promoter IV-driven BDNF expression, which in turn increases neuronal activity and neurogenesis/maturation to maintain active brain function. Any disruption to activity-dependent promoter IV-driven BDNF expression, such as by reduced neuronal activity, mutations in the promoter region, and/or epigenetic processes through stress, would decrease neuronal activity and function, thereby leading to psychiatric conditions such as depression (Sakata, 2011).

The involvement of BDNF in psychiatric conditions has been proposed for more than a decade (Duman et al., 1997; Nestler et al., 2002; Lewis et al., 2005); however, the detailed neurotransmitter mechanisms linking reduced BDNF to defective behavior remain unclear. An involvement of the monoamine system is suggested in depression and schizophrenia because drug treatments with antidepressants and antipsychotics act on monoamine systems: antidepressants increase extracellular monoamine levels by blocking monoamine transporters and monoamine oxidase, while antipsychotics act on monoamine receptors including D2-like dopamine receptors, serotonin receptors, and adrenoceptors. We hypothesized that changes in the monoaminergic system may underlie the reduced activity-driven BDNF condition that leads to the defective behavioral phenotypes reminiscent of psychiatric conditions—depression-like behavior, inflexible learning, and reduced response inhibition. In this study, we examined the effect of activity-driven BDNF through promoter IV on gene expression levels in the monoamine systems including serotonin, dopamine, and noradrenaline. We measured mRNA levels of genes for monoamine receptors, transporters, and synthesizing enzymes using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) in KIV mice compared with control wild-type (WT) mice. We focused on the frontal cortex and hippocampus, which are the regions related to depression where promoter IV is active (Sakata, 2011).

EXPERIMENTAL PROCEDURES

Animals

The generation of BDNF-KIV (BDNF promoter IV-green fluorescent protein knock-in) mice has been described previously (Sakata et al., 2009). Briefly, 129/sv embryonic stem cells with C57BL/6J blastocytes were used for generating chimeric mice, which were crossed to C57BL/6J females for >12 generations. Eight pairs of adult, age- and gender-matched (2–3 months old; four males and four females) KIV and control WT littermates were used. All animals were housed in a group (2–5 mice per cage) in a normal 12:12-h dark–light cycle and had *ad libitum* access to food and water. All animal experiments were approved by the University of Tennessee Laboratory Animal Care and Use Committee and were done in accordance with the NIH guidelines.

Tissue collection

Animals were euthanized by intraperitoneal (i.p.) injections of 90 mg/kg ketamine and 10 mg/kg of xylazine. Frontal cortex and hippocampus were removed and immediately frozen within 6 min after the euthanasia and stored at −80 °C until RNA extraction.

RNA extraction and qRT-PCR

Each tissue was homogenized by pipetting and QIAshredder (Qiagen, Valencia, CA, USA) and used for total RNA extraction. Total RNA was extracted and purified using the RNeasy Kit (Qiagen) with on-column DNase (Qiagen), following the manufacturer's instructions. RNA was quantified using the NanoDrop spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). One μg of total RNA was reverse transcribed into single-strand cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Indianapolis, IN, USA) following the manufacturer's protocol. qRT-PCR was performed using BioMark as described previously (Reiner et al., 2012). Briefly, 0.5 μl of the cDNA was preamplified in a mixture with 1.25 μl of pooled primers (final concentration, 200 nM), 2.5 μl of 2× PreAmp Master Mix (Applied Biosystems, Carlsbad, CA, USA), and 0.75 μl of water. The cycling program consisted of 95 °C for 10 min, followed by 14 cycles of 95 °C for 15 s and 60 °C for 4 min. At the end, the reactions were diluted 1:5 with Tris-EDTA buffer (10 mM Tris–HCl pH 8 and 1 mM EDTA). The qPCR was performed in 96.96 dynamic array chips (Fluidigm, South San Francisco, CA, USA) following the manufacturer's instructions. Each assay inlet of the M96 Dynamic Array contained 5 μl of an assay mix containing 1 μM forward primer, 1 μM reverse primer, 1 μM Universal Probe Library (UPL) probe, and 1× assay reagent (Fluidigm, PN85000736). The sample inlets contained 5 μl of a sample mix consisting of 2.25 μl of the preamplified sample, 2.5 μl of 2× Universal TaqMan Master Mix (Applied Biosystems), and 0.25 μl of 20× Sample Loading Solution (Fluidigm, PN85000735). The cycling program was 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 1 min at 60 °C. The cycle threshold (Ct) values were obtained using the BioMark Gene Expression Data Analysis after automatic inspection for quality. In a pre-assay, we verified the reliability of Ct values obtained with the BioMark system by comparing with Ct values obtained the 96 well qRT-PCR Light-Cycler system (Roche Applied Science): the difference in Ct values of the same sample was an average of 0.3 in 48 readings (average 1.7% error of Ct values 17–27). Relative gene expression values were determined by using the $2^{-\Delta\Delta Ct}$ method of Livak and Schmittgen (2001). Six