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Developmental and degenerative modulation of brain-derived neurotrophic factor transcript variants in the mouse hippocampus

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is regarded as an important factor for neurogenesis, synaptic plasticity, and neuronal network organization in brain circuits. However, little is known about the regulation of BDNF transcript variants in the hippocampus during postnatal development and following chemically induced neurotoxicity. In the present study, we examined the expression of individual BDNF transcript variants in the mouse hippocampus on postnatal day (PD) 3, 7, 14, 21, and 56, as well as in the adult hippocampus 1, 2, 4, and 8 days after trimethyltin (TMT) treatment. During postnatal development, the expression levels of common BDNF-coding transcripts and BDNF transcript variants increased gradually in the hippocampus, but the temporal patterns of each exon transcript showed significant differences. In the TMT-treated hippocampus, the levels of common BDNF-coding transcripts and exon I, IIC, III, VII, VIII, and IXA transcripts were significantly increased 1 day post-treatment. These observations suggest that the differential regulation of BDNF exon transcripts may be associated with neuronal and synaptic maturation during postnatal development, and neuronal survival and synaptic plasticity in chemically induced neurodegeneration.

1. Introduction

Brain-derived neurotrophic factor (BDNF) plays important roles in the morphological and physiological functions of neuronal cells (Bibel and Barde, 2000; Chao et al., 2006). During brain development, BDNF supports the growth, differentiation, and survival of immature neurons (Jones et al., 1994; McAllister et al., 1997; Schecterson and Bothwell, 1992). In addition, in the adult brain, BDNF is involved in synaptic plasticity-related processes, which are associated with learning and memory and long-term potentiation (LTP) (Bramham and Messaoudi, 2005; Tyler et al., 2002; Yamada et al., 2002). However, the dysregulation of BDNF expression may be related to neurological disorders, such as depression, schizophrenia, bipolar disease, Alzheimer's disease, Parkinson's disease, and Rett syndrome (Allen et al., 2011; Angelucci et al., 2005; Autry and Monteggia, 2012; Duman and Aghajanian, 2012; Liu et al., 2005; Martinowich et al., 2007; Neves-Pereira et al., 2002; Sklar et al., 2002). Furthermore, BDNF depletion induces abnormal brain development and mental retardation in mice (Schwartz et al., 1997).

BDNF translation is a complex and precise mechanism that is regulated from approximately 22 BDNF transcript variants (Lubin et al., 2008; Timmusk et al., 1993). Each BDNF transcript consists of one 5′ non-coding exon among 11 BDNF exons (exons I, IIA, IIB, IIC, III, IV, V, VI, VII, VIII, and IXA) and the common 3′ BDNF-coding exon IX, which has two polyadenylation sites. Several studies have shown that the regulation of BDNF transcript variants is closely related to brain development (Aid et al., 2007; Pattabiraman et al., 2005; Schwartz et al., 1997; Timmusk et al., 1994). For example, Pattabiraman et al. (2005) reported that the expression of BDNF exon IV transcript was important during postnatal development. However, there have been few investigations of the expression of multiple BDNF transcript variants.

Trimethyltin (TMT) is a potent neurotoxic organotin compound that affects the limbic system, particularly the hippocampus (Chang et al., 1982; Lattanzi et al., 2013). TMT-induced neurodegeneration

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Table 1
Primer sequences for real-time RT-PCR analysis.

Gene	Accession no.	Primer sequence		Tm (°C)	Product size (bp)	Primer efficiency
BDNF	NM_001048142.1	FWD	5′-TGGCTGACACTTTTGAGCAC-3′	55	188	93%
		RVS	5′-GTTTGCGGCATCCAGGTAAT-3′			
Exon I	EF125669.1	FWD	5′-TGAGAGTTGAAGCTTTGCGG-3′	55	189	93%
		RVS	5′-ATTGTGGCTTTGCTGTCCTG-3′			
Exon IIC	EF125672.1	FWD	5′-TTTGGTCCCCTCATTGAGCT-3′	55	159	95%
		RVS	5′-TTCTTTGCGGCTTACACCAC-3′			
Exon III	EF125681.1	FWD	5′-TCTATCATCCCTCCCCGAGA-3′	55	123	93%
		RVS	5′-AACTGGGCTCAAGGAAGCAT-3′			
Exon IV	EF125673.1	FWD	5′-AGCATGAAATCTCCCAGCCT-3′	55	213	98%
		RVS	5′-CGGTCCCCAAGGTTCTAGAC-3′			
Exon VI	EF125674.1	FWD	5′- GGGCTTGGAGAAGGAAACCG-3′	55	197	93%
		RVS	5′- GGTCCACACAAAGCTCTCGG-3′			
Exon VII	EF125683.1	FWD	5′-CTGTCACCTGCTCTCTAGGG-3′	55	118	95%
		RVS	5′-AGTTCCGCAGACCCTTTCAG-3′			
Exon VIII	EF125684.1	FWD	5′-CAACTGGATGTGTGGAACCA-3′	55	128	97%
		RVS	5′-AGTGTGTGGGTAGATGCCAA-3′			
Exon IXA	EF125685.1	FWD	5′-ATTTGTGTCCCCTGCAGCT-3′	55	151	94%
		RVS	5′-GTGGGAAGGAAGCAGAGACA-3′			
18s rRNA	NM_007393.3	FWD	5′-GGGGAGTATGGTTGCAAAGC-3′	55	190	95%
		RVS	5′-CGCTCCACCAACTAAGAACG-3′			

Abbreviations: BDNF, brain-derived neurotrophic factor; FWD, forward; RVS, reverse; Tm, melting temperature.

Table 2
Results of two-way ANOVA to determine the effects of TMT treatment on changes in the levels of BDNF transcript variants for each dependent variable.

Study	Figure	Time	Exon	Interaction
Postnatal development	Fig. 1B	$F(4, 315) = 71.10 P < 0.001$	$F(8, 315) = 18.58 P < 0.001$	$F(32, 315) = 3.307 P < 0.001$
TMT treatment	Fig. 2B	$F(4, 351) = 35.34 P < 0.001$	$F(8, 351) = 6.558 P < 0.001$	$F(32, 351) = 2.995 P < 0.001$

Abbreviations: ANOVA, analysis of variance; TMT, trimethyltin; BDNF, brain-derived neurotrophic factor.

is closely related to excitotoxicity through disturbance of brain glutamate metabolism and the GABAergic system (Chang, 1986, 1990). Representative clinical features of TMT intoxication include aggressiveness, temporal lobe seizure, and ataxia (Bertram and Cornett, 1994; Besser et al., 1987; Ishida et al., 1997). Previous studies have shown that TMT-induced neurotoxicity significantly increases BDNF expression *in vivo* and *in vitro* (Andersson et al., 1997; Viviani et al., 2005). In addition, it has been reported that kainic acid (KA), which induced neuronal excitotoxicity like TMT (Tandon et al., 1999), significantly upregulates the levels of common BDNF-coding transcripts and BDNF exon I and IV transcripts in the rat hippocampus (Aid et al., 2007; Sathanoori et al., 2004). However, nothing is known concerning the differential regulation of BDNF transcript variants during TMT-induced neurotoxicity.

In the present study, we investigated the expression of BDNF transcript variants in the mouse hippocampus to elucidate the differential regulation of hippocampal BDNF transcript variants during postnatal development and TMT-induced neurotoxicity using real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR).

2. Materials and methods

2.1. Animals, drug treatment and tissue sampling

Eight pregnant (gestational day 17) C57BL/6 mice were obtained from Daehan Biolink (Chungbuk, South Korea). Mice on postnatal day (PD) 3, 7, 14, 21, and 56 ($n = 8$ per group) were used for the developmental expression study. For preparation of RNA extracts, eight mice per group were sacrificed on each PD, and the hippocampi were rapidly dissected out and stored at −80 °C.

For the TMT treatment study, adult C57BL/6 male mice (8 weeks old) were divided into five groups ($n = 9$ per group): vehicle-treated controls and TMT-treated groups (1, 2, 4, and 8 days after treatment). TMT (trimethyltin hydroxide; Wako, Osaka, Japan) was dissolved in sterile 0.9% (w/v) saline. The time-dependent effects of TMT (2.6 mg/kg) on the adult mouse hippocampus were examined after intraperitoneal administration (i.p.) of TMT (10 mL/kg body weight). The vehicle-treated controls were injected with 0.9% saline (10 mL/kg body weight). Tremor/seizure tests were performed in brightly lit areas (40 × 40 cm; 250 lx). Behavioral changes were scored as follows: (1) aggression; (2) weak tremor; (3) systemic tremor; (4) tremor and spasmodic gait; and (5) death (Yang et al., 2012; Yoneyama et al., 2008). For preparation of RNA extracts, nine mice per group were sacrificed 1, 2, 4, and 8 days after TMT administration, and the hippocampi were rapidly dissected out and stored at −80 °C. To confirm that the clinical symptoms induced by TMT treatment were accompanied by neurodegeneration in the hippocampus, brains were processed for paraffin embedding after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) using routine protocols 1, 2, 4, and 8 days after TMT administration ($n = 3$ per group).

The care and handling of animals conformed to all current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). The Institutional Animal Care and Use Committee of Chonnam National University approved all of the protocols used in the present study (approval no. CNU IACUC-YB-2012-18). All of the experiments were conducted in a manner minimizing the numbers of animals used and the suffering caused.

2.2. Tissue preparation, RNA extraction and cDNA synthesis

Total RNA was isolated from the hippocampus using an RNAeasy® Lipid Tissue Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The concentrations of RNA samples were ascertained by measuring the optical density using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific Inc., Waltham, MA). Next, first-strand complementary DNA (cDNA) was prepared using Random Primers (Takara Bio, Tokyo, Japan) with Superscript™ II Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA was diluted to 8 ng/μL with RNase-free water and stored at −80 °C.

2.3. Primer preparation and primer efficiency determined

The gene sequences of BDNF transcript variants were obtained from the National Center for Biotechnology Information (NCBI) database, and the primer sequences are indicated in Table 1. The specificities of primer sequences were confirmed by NCBI – Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov). These primers were manufactured by Bioneer Co. (Daejeon, Korea). The efficiency of each primer was measured by plotting a linear equation, for the threshold cycle value (Ct), against the function of log[10] of the serial diluted control template mass (1, 5, 10, 50, and 100 ng); the slope(s) of the linear equation was subsequently substituted into the following formula: $E = 10^{(1/-s)} - 1$, where E equates to primer efficiency (Ginzinger, 2002). The results of the efficiency measurement were shown in Table 1: the efficiencies of all primers were above 90%.