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Developmental and degenerative modulation of GABAergic transmission in the mouse hippocampus

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ABSTRACT

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter involved in synaptic plasticity. GABAergic transmission is also implicated in developmental and degenerative processes in the brain. The goal of the present study was to understand the developmental and degenerative regulation of GABAergic transmission in the mouse hippocampus by examining changes in GABA receptor subunit mRNA levels and GABA-related protein expression during postnatal development of the hippocampus and trimethyltin (TMT)-induced neurodegeneration in the juvenile (postnatal day [PD] 24) and adult hippocampus (PD 56). During postnatal development, the mRNA levels of GABA A receptor ($GABA_AR$) subunits, including α1, α4, β1, β2, and δ; GABA B receptor ($GABA_BR$) subunit 2; and the expression of GABA-related proteins, including glutamic acid decarboxylase, vesicular GABA transporter (VGAT), and potassium chloride cotransporter 2 increased gradually in the mouse hippocampus. The results of seizure scoring and histopathological findings in the hippocampus revealed a more pronounced response to the same administered TMT dose in juvenile mice, compared with that in adult mice. The mRNA levels of most GABA receptor subunits in the juvenile hippocampus, excluding $GABA_AR$ subunit β3, were dynamically altered after TMT treatment. The mRNA levels of $GABA_AR$ subunits γ2 and δ decreased significantly in the adult hippocampus following TMT treatment, whereas the level of $GABA_BR$ subunit 1 mRNA increased significantly. Among the GABA-related proteins, only VGAT decreased significantly in the juvenile and adult mouse hippocampus after TMT treatment. In conclusion, regulation of GABAergic signaling in the mouse hippocampus may be related to maturation of the central nervous system and the degree of neurodegeneration during postnatal development and TMT-induced neurodegeneration in the experimental animals.

1. Introduction

γ-Aminobutyric acid (GABA) is involved in neurogenesis, neuronal migration, plasticity, and neuronal networking in central nervous system (CNS) (Behar et al., 1996; Pallotto and Deprez, 2014; Taketo and Yoshioka, 2000). GABAergic functions are mediated by GABAergic transmission (Berg et al., 2013; Bovetti et al., 2011) via various factors, including alterations in GABA receptor (GABAR) subunits and GABA-related proteins (Bettler et al., 2004; Brooks-Kayal et al., 1998; Smith and Olsen, 1995). GABA A receptors ($GABA_ARs$), which are hetero-pentameric chloride channels mediating fast synaptic inhibition, have been implicated in anxiety, muscle tension, epileptogenic activity, and memory functions (Arslan et al., 2014; Olsen and Avoli, 1997; Rudolph and Mohler, 2004; Sieghart and Sperk, 2002). $GABA_ARs$ form the integral GABA-gated chloride channels composed of five subunits selected from at

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least 19 $GABA_AR$ subunits, including α1–6, β1–3, γ1–3, δ, ϵ, θ, π, and ρ1–3 (Fritschy and Mohler, 1995; Sieghart and Sperk, 2002; Tretter et al., 1997). Numerous subunit combinations are possible; however, the major fundamental receptor complexes contain at least one member of the α, β, and γ subunit classes (Farrant and Nusser, 2005; Rudolph and Mohler, 2006; Sieghart et al., 1999; Whiting, 2003). GABA B receptors ($GABA_BRs$) are widely expressed in the CNS and have been implicated in neurological and psychiatric disorders (Barnard et al., 1998). $GABA_BRs$ form a heterodimer composed of two subunits; $GABA_BR$ subunit 1 is the major functioning subunit but must associate with the $GABA_BR$ subunit 2 for normal functioning (Barnard et al., 1998; Fritschy et al., 2004; Jones et al., 1998).

Several proteins in the GABAergic cascade influence GABAergic transmission (Taketo and Yoshioka, 2000; Walls et al., 2010). Glutamate decarboxylase (GAD) is a rate-limiting enzyme that catalyzes decarboxylation of glutamate to GABA that is distributed abundantly in GABAergic interneurons (Houser, 2007). GAD is involved in the regulation of glutamate and GABA balance, giving it an important role in CNS function (Martin and Rimvall, 1993; Walls et al., 2010). GAD has two isoforms, called GAD65 and GAD67, which have different properties and distributions (Erlander et al., 1991; Hendrickson et al., 1994). GAD67 is distributed throughout the cytosol and catalyzes GABA synthesis under resting conditions, whereas GAD65 is localized to axonal terminals and catalyzes GABA synthesis under more intense neuronal activity (Erlander et al., 1991; Patel et al., 2006; Tian et al., 1999). The vesicular GABA transporter (VGAT) mediates the active accumulation of GABA and glycine into synaptic vesicles and contributes to inhibitory neurotransmission by mediating the vesicular GABA concentration (Chaudhry et al., 1998; McIntire et al., 1997). Moreover, potassium-chloride cotransporter 2 (KCC2) is specifically distributed in neurons to regulate neuronal intracellular chloride gradient by extruding chloride, and it may also act as a modulator of neuroplasticity (Rivera et al., 1999; Vinay and Jean-Xavier, 2008; Watanabe et al., 2009). Previous studies have suggested that changes in KCC2 expression might be tightly correlated with changes in GABAergic signaling (Briggs and Galanopoulou, 2011; Ganguly et al., 2001).

A variety of stage-specific changes are caused by intra/extracellular alterations in the developing CNS, including a reversed intracellular chloride gradient and GABA-mediated tonic activation (Egawa and Fukuda, 2013; Farrant and Nusser, 2005). The differences between rat and mouse brains, including the hippocampus, are demonstrated in the composition of respective GABAR subunits. For example, expression of the $GABA_AR$ subunits α1, β1, and γ2 is stronger in the rat brain, whereas expression of subunits α4 and δ is higher in the mouse brain (Hortnagl et al., 2013). However, there is a relative lack of knowledge regarding GABAR and GABA-related proteins in the mouse hippocampus during postnatal development, as compared to the situation in the rat hippocampus. Therefore, in the present study, expression changes in GABAR subunit mRNA and GABA-related proteins were investigated to understand modulations in GABAergic transmission in the developing mouse hippocampus.

Trimethyltin (TMT) is an organo-tin compound that induces neurotoxicity in the mammalian limbic system, particularly the hippocampus, in experimental animals (Lattanzi et al., 2013; Lee et al., 2014). Exposure to TMT induces neurodegeneration and causes extensive neuronal loss, cognitive impairment, and behavioral dysfunction, including aggressiveness, epileptic seizure, and ataxia (Bertram and Cornett, 1994; Besser et al., 1987; Ishida et al., 1997; Kim et al., 2014a). In rats, pyramidal neurons of the hippocampal *cornu ammonis* subfield are most affected by TMT, whereas dentate gyrus (DG) neurons in mice are primarily affected (Balaban et al., 1988; Bruccoleri et al., 1999). Previous studies suggested that TMT intoxication induces both histopathological damage to the hippocampus and clinical symptoms, including seizure (Kim et al., 2014b). TMT may induce its neurotoxic effects by disturbing neuronal glutamatergic and GABAergic transmission (Kruger et al., 2005; Zuo et al., 2009). Nishimura et al. (2001) reported alterations in GABAR subunit mRNA and GAD65/67 expression using in situ hybridization in adult rat hippocampi treated with TMT. However, semi-quantitative alterations in the expression levels of GABAR subunit mRNAs and GABA-related proteins in TMT-induced hippocampal degeneration in mice remain unclear.

Differential GABAergic transmission is necessary for neuronal development, and it can be affected by neurodegenerative drugs, such as TMT. Furthermore, the duration of neurotoxin exposure may influence the magnitude of the after-effects, including behavioral defects and neuropathology (Freeman et al., 1994; Nitecka et al., 1984; Rice et al., 1981). Therefore, a comparison of changes in the juvenile and adult GABAergic systems is important to investigate the CNS response to neurodegenerative insults. In the present study, we investigated alterations in GABAR subunit mRNA levels and the expression of GABA-related proteins in the mouse hippocampus during postnatal development and TMT-induced neurotoxicity to understand modulation of GABAergic transmission in the developing and degenerating mouse hippocampus.

2. Materials and methods

2.1. Experimental design and animals

Three experiments were conducted to investigate changes in GABAergic transmission (Fig. 1): experiment 1 examined postnatal developmental changes in GABAergic transmission, and experiments 2 and 3 examined neurodegenerative changes in GABAergic transmission during TMT-induced hippocampal neurodegeneration in juvenile (postnatal day (PD) 24) and adult (PD 56) mice, respectively. Eight pregnant (gestational day 17) C57BL/6 mice were obtained from Daehan Biolink (Chungbuk, South Korea), and mice on PD 3, 7, 14, 21, and 56 ($n = 8$/group) were used for the developmental study. Juvenile C57BL/6 male mice (PD 24) and adult C57BL/6 male mice (PD 56) were divided into five groups for the TMT treatment study ($n = 5$–8/juvenile mouse group; $n = 8$–9/adult mouse group) as the vehicle-treated control (4 days after vehicle treatment) and TMT-treated groups (1, 2, 4, and 8 days after TMT treatment). 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 protocols used in this study (approval no. CNU IACUC-YB-2012-18). All experiments were conducted to minimize the number of animals used and the suffering caused.

2.2. Drug treatment and behavioral examination

TMT (Wako, Osaka, Japan) was dissolved in sterile 0.9% (w/v) saline. The time-dependent effects of TMT on the juvenile and adult mouse hippocampus were examined after intraperitoneal administration of TMT (2.6 mg/kg body weight). The vehicle-treated controls were injected with 0.9% saline (10 mL/kg body weight). Behavioral tests were conducted in a brightly lit area (40 × 40 cm; 250 lux) 1, 2, and 4 days after the TMT treatment (Fig. 1). Behavioral changes were scored by the degree of seizure, 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).