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Functional roles of the glial glutamate transporter (GLAST) in emotional and cognitive abnormalities of mice after repeated phencyclidine administration

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Abstract
Alterations of the glutamatergic system components, including *N*-methyl-d-aspartate (NMDA) receptors are relevant to the pathophysiology of schizophrenia. Repeated phencyclidine (PCP) administration induces several schizophrenia-like psychobehavioral abnormalities and decreases extracellular glutamate levels, which are associated with increased levels of glial gluta-

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mate and aspartate transporter (GLAST) in the prefrontal cortex (PFC) of mice. In the present study, we investigated the functional roles of GLAST in the emotional and cognitive abnormalities in mice following repeated PCP administration by using GLAST heterozygous (+/−) mice, since GLAST mutant mice are a useful tool for elucidating the contribution of glutamate dysfunction to the pathophysiology of schizophrenia. PCP-administered GLAST wild-type (+/+) mice showed enhancement of immobility in a forced swimming test, impairments of visual recognition memory in a novel object recognition test, decrease in high potassium (K^+)-induced extracellular glutamate release, and overexpression of GLAST and S100 proteins in the PFC, compared to saline-administered GLAST$^{+/+}$ mice. Such behavioral and neurochemical abnormalities were not observed in PCP-administered GLAST$^{+/-}$ mice. In conclusion, these results clearly suggest that genetic GLAST dysfunction and glial activation play important roles in the development of emotional and cognitive abnormalities in PCP-administered GLAST$^{+/+}$ mice.

1. Introduction

It has been hypothesized that dysfunction of the glutamatergic system is involved in the pathophysiology of psychiatric disorders, such as schizophrenia and mood disorders (Carlsson et al., 1997; Carlsson et al., 1999; Cui et al., 2014; Frankle et al., 2003; Kiselycznyk et al., 2011). Abundant pharmacological evidence has demonstrated that *N*-methyl- d-aspartate (NMDA) receptors are implicated in the pathophysiology of schizophrenia (Carlsson et al., 1999; Noda et al., 2009). Phencyclidine (PCP), a non-competitive NMDA receptor antagonist, has been shown to induce schizophrenia-like psychoses representing positive symptoms, negative symptoms, and cognitive impairments in humans (Javitt and Zukin, 1991). Thus, PCP-administered animals have been utilized as animal models of schizophrenia (Enomoto et al., 2005; Jentsch et al., 1997a, 1997b; Mandillo et al., 2003). These animals exhibit hyperlocomotion as an index of positive symptoms (Noda et al., 2009) and social behavioral impairments in a social interaction test and enhanced immobility in a forced swimming test as indices of negative symptoms (Noda et al., 1995a, 1997). They also show sensorimotor gating deficits and cognitive impairments in several learning and memory tests (Hida et al., 2014b; Mouri et al., 2007; Nagai et al., 2009). Some of the behavioral abnormalities, such as negative-like behaviors and/or cognitive impairments after withdrawal from repeated administration of PCP, appear to be sensitive to second-generation antipsychotics, in agreement with the clinical findings, which improve the negative symptoms and the cognitive impairments in schizophrenia (Nabeshima et al., 2006; Nagai et al., 2009; Noda et al., 1997; Zhang et al., 2013).

Glial glutamate and aspartate transporter (GLAST; excitatory amino-acid transporter 1) and glial glutamate transporter-1 (GLT1; excitatory amino-acid transporter 2) are described as glial glutamate transporters that regulate extracellular glutamate concentration by rapidly clearing glutamate from the extracellular fluid (Eulenburg and Gomeza, 2010; Gomez-Galan et al., 2013; Thomassen et al., 1985). Clinical studies show, an increased incidence of a rare genetic variant in the human gene encoding GLAST (Walsh et al., 2008) and an increased level of GLAST mRNA in the prefrontal cortex (PFC) of schizophrenia (Parkin et al., 2018). Simpson et al. (1998) investigated postmortem tissue of schizophrenics and reported that the number of binding sites and protein levels of glutamate transporters were increased in the PFC. Repeated PCP administration decreases extracellular glutamate levels and high potassium (K^+)-induced glutamate release, which are associated with increased levels of GLAST but not GLT1, and expression and activation of glial cells in the PFC of mice (Murai et al., 2007).

Microinjection of DL-threo-β-benzyloxyaspartate (DL-TBOA), a potent glutamate transporter blocker, enhances extracellular glutamate levels in the PFC and attenuates the psychobehavioral abnormalities induced in PCP-administered mice (Murai et al., 2007). It is possible that DL-TBOA attenuates the psychobehavioral abnormalities associated with activation of the glutamatergic system in the PFC by inhibition of GLT1 because DL-TBOA inhibits not only GLAST but also GLT1 (Shimamoto, 2008), which is the major glutamate transporter present in the PFC (Eulenburg and Gomeza, 2010). However, the functional roles of GLAST in the psychobehavioral abnormalities induced by repeated administration of PCP are not yet clear.

GLAST homozygous (−/−) mice, but not GLAST heterozygous (+/−) mice show a significant increase in the total distance traveled during exposure to a novel open field as well as an increase in cognitive impairment measured by an instrumental visual discrimination task (Karlsson et al., 2009). GLAST mutant mice are a useful tool for elucidating the contribution of glutamate dysfunction to the pathophysiology of schizophrenia (Karlsson et al., 2009; Watase et al., 1998). In the present study, we investigated the functional roles of GLAST in the emotional and cognitive abnormalities induced by repeated PCP administration using GLAST$^{+/-}$ mice, but not GLAST$^{-/-}$ mice, because GLAST$^{+/-}$ mice did not show any baseline abnormalities (Supplemental Fig. S1).

2. Experimental procedures

2.1. Animals

Male and female adult (8-week-old) GLAST heterozygous (+/−) mice and GLAST wild-type (+/+) mice, which were provided by Professor Kohichi Tanaka (Laboratory of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University: TMDU) (Karlsson et al., 2009; Watase et al., 1998) were used. The mutant mice were obtained by crossing F13