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Homeostatic plasticity of GABAergic synaptic transmission in mice lacking GAT1 ☆

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Abstract

GABA transporter-1 (GAT1) plays a key role in GABA reuptake, and deletion of GAT1 leads to a largely increased GABA-induced tonic conductance in the $GAT1^{-/-}$ mice. We hypothesized that homeostatic plasticity of $GABA_A$ receptor-mediated inhibition takes place to balance the increased tonic inhibition and maintains stability of the nervous system. In this study, we employed the loss of righting reflex assay and compared the behavioral difference of three animal models, mice with acute, partial, and permanent GAT1 deficiency, to confirm our hypothesis. Our data demonstrated that both acute and partial block of GAT1 increased the sensitivity of mice to GABAergic sedative/hypnotic drugs, whereas permanent GAT1 dysfunction in the $GAT1^{-/-}$ mice decreased the sensitivity to some extent. These results confirmed our presumption about the down-regulation of phasic GABAergic transmission in the GAT1 knockout mice. Moreover, electrophysiological measurements performed on slices from motor cortex suggested that it was the reduced GABA release, but not change of postsynaptic GABA receptors, which led to the down-regulation of phasic inhibition in $GAT1^{-/-}$ mice.

Keywords: GABA transporter; Tiagabine; Knockout

GABA is the principal inhibitory neurotransmitter in the CNS, and altered GABAergic synaptic inhibition is thought to be implicated in a variety of nervous system disorders [1]. GABA released from presynaptic terminals is rapidly cleared from the vicinity of the synaptic cleft by pre-synaptic and glial GABA transporters to terminate synaptic response. This maintains moderate extracellular GABA levels and thus prevents excessive activation of GABA receptors. Therefore, GABA transporters are believed to play a key role in regulating GABAergic transmission [2]. Four distinct GABA transporters (GAT1–4) have been identified, and each of them has a unique regional distribution and transports GABA with varying affinities [3]. Among these transporters, GABA transporter subtype 1 (GAT1) is the most abundant one and contributes more than 75% of the GABA reuptake activity in the CNS [4,5]. It was reported that knockout of GAT1 in mice leads to enhanced extracellular GABA levels resulting in an increased $GABA_A$ receptor-mediated tonic conductance in several brain regions [4,6]. Such an excessive tonic inhibition, however, puts the nervous system into a state of

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imbalance. Hence, according to the concept of homeostatic plasticity [7–9], it is presumed that some homeostatic mechanisms, such as down-regulation of phasic $GABA_A$ receptor-mediated inhibition, might take place in the GAT1 knockout mice to balance the increased tonic inhibition. Our previous studies have shown that mice genetically lacking GAT1 function displayed altered behavioral response to ethanol compared with that of wild-type mice [5], suggesting a neuroadaptive process had occurred in the GABAergic system. However, it is well known that the mechanisms of ethanol action are quite complex and a wide variety of ion channels and neurotransmitter receptors are involved in ethanol-induced behaviors [10]. To address this question, several GABAergic sedative/hypnotic drugs which exert their effects by specifically enhancing the activation of $GABA_A$ receptors were used to further validate the hypothesis.

An α-selective drug, diazepam [11], a β-selective drug, etomidate [12–14], and sodium pentobarbital, a drug with activities on several channels [15,16], were tested in this study. Ketamine, an effective general anesthetic target on NMDA receptors [17], was used as a control because of its small effect on GABAergic transmission. In the present study, sensitivity of mice to those sedative/hypnotic drugs was assessed using the traditional loss of righting reflex (LORR) assay, and three animal models were used to compare with wild-type mice. Mice pretreated with tiagabine, a selective GAT1 inhibitor, were used to mimic acute GAT1 dysfunction. The $GAT1^{+/-}$ mice were used as an animal model with partial GAT1 deficiency. In addition, the GAT1 knockout mice were used as an animal model with permanent GAT1 dysfunction.

Materials and methods

Animals. GABA transporter subtype 1 (GAT1) null mutant mice were generated in our laboratory as previously reported [5]. To minimize the possible effects of diversity in genetic background, GAT1 knockout heterozygotes ($GAT1^{+/-}$) were selected for sib mating to produce wild-type ($GAT1^{+/+}$), heterozygous ($GAT1^{+/-}$), and homozygous ($GAT1^{-/-}$) littermates for all drug treatment experiments. Only male mice were used throughout this study.

Mice were maintained under specific-pathogen-free conditions up to at least 12–18 weeks at the time of analysis. In each experiment, the age and weight of mice were matched. Mice were group-housed (3–5 per cage) under a 12-h light/dark cycle (7:00 AM to 7:00 PM lights on) and provided ad lib access to food and water. All experiments were conducted in an isolated behavioral-testing room in the animal facility to avoid external distractions. All mice were allowed to recover for at least 10 days between each drug treatment. Each group of mice was not used more than twice or for different drugs. Drug testing was randomized. The animal experiments were approved by the Institutional Animal Care and Use Committee.

Drugs. Diazepam and ketamine injections were obtained from Shanghai Xudong Haipu Pharmaceutical Co. Ltd. Etomidate injection was obtained from B. Braun Melsungen. Sodium pentobarbital was obtained from Sigma–Aldrich, and tiagabine from R&S Pharmchem Co.,Ltd. Both were dissolved in sterile saline.

Intraperitoneal dosing of diazepam (20 mg/kg), etomidate (20 mg/kg), sodium pentobarbital (50 mg/kg), and ketamine (100 mg/kg) was based on related literature [18,19], and was determined in pilot experiments. In co-injection experiments, mice were pretreated with tiagabine (10 mg/kg) or saline for 15 min, and then the second drug was administered. All drugs were administered intraperitoneally (i.p.) at 0.01 ml/g b.wt.

Loss of righting reflex (LORR). Sensitivity to the selected drugs was typically determined using the loss of righting reflex (LORR) assay. Mice were injected with the drug and were tested for LORR by placing them in the supine position in V-shaped troughs. Then, the mice remained undisturbed in the trough until they regained their righting reflex. The regain of righting reflex was judged by the observation that the mice could accomplish the action of turning over two times within 30 s. The latency to LORR was defined as the time needed from injection to onset of the LORR. Duration of LORR was defined as time counted from loss of the reflex to the function regained.

The experiments were performed between 9:00 AM and 1:00 PM. During all sleep time assays, room temperature was set as 24 °C. Unless otherwise noted, mice that failed to lose the righting reflex (misplaced injections) or had a sleep time greater than two standard deviations from the group mean were excluded from the analysis.

Electrophysiology. Experiments were performed on 300 μm motor cortex slices from wild-type littermates and GAT1 knockout mice (3–5 weeks). After decapitation, the brain was removed and placed in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) at 4 °C. Coronal brain slices were cut with a vibratome (Leika VT 1000S) and maintained at room temperature (23–25 °C) in a holding chamber filled with oxygenated ACSF. After an equilibration period of at least 1 h, a single slice was transferred to the recording chamber, where it was held between two nylon nets and continuously perfused with oxygenated ACSF (23–25 °C) at a flow rate of 2.5–3 ml/min. The ACSF for incubation and recording was composed of (in mM) 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 11D-glucose, aerated with 95% O_2 and 5% CO_2 at a final pH of 7.4 (osmolarity: 289–295 mOsm).

Whole-cell recordings were made in the layer II/III of the motor cortex. Pyramidal neurons were visually identified using an infrared differential interference contrast video microscope (BX51WI, Olympus) with a 40× water immersion objective. Under voltage-clamp conditions, all cells were held at −70 mV. The access resistance was <30 MΩ, and results were discarded if it changed by more than 20%. The ionic composition of the internal solution for voltage-clamp recording was (in mM) 140 CsCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 2 Mg–ATP, 0.3 Na–GTP, and 2 QX314. To record spontaneous inhibitory postsynaptic currents (sIPSCs), 10 μM CNQX and 20 μM AP-V were added to the bath to block glutamatergic transmissions. Pentobarbital was dissolved in water and directly added into the bath in a final concentration of 100 μM. Signals were amplified (Axoclamp 200B), digitized at 10 kHz (Digidata 1320A/D converter), and analyzed off-line using the Clampfit program in pCLAMP 9.0 (all from Axon Instruments).

Data analysis. The results were represented as the mean ± SEM. Data were reported in seconds for latency time and in minutes for duration time of LORR, respectively. The statistical software program OriginPro 7.0 (OriginLab Corporation, MA) was used throughout. To evaluate differences between groups, analysis of variance (one-way ANOVA with Bonferroni post hoc analysis) and Student's t test were carried out. For all statistical comparisons, $p < 0.05$ was considered significant. Wild-type, heterozygous, and homozygous GAT1-deficient mice were designated as $GAT1^{+/+}$, $GAT1^{+/-}$, and $GAT1^{-/-}$, respectively.

Results

Significantly increased effects of sedative/hypnotic drugs in tiagabine-pretreated $GAT1^{+/+}$ mice

To determine the effect of acute block of GAT1 on the action of the sedative/hypnotic drugs, tiagabine was used to pretreat wild-type mice in the LORR assay to mimic acute GAT1 dysfunction.