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Subthreshold pharmacological and genetic approaches to analyzing $Ca_V2.1$-mediated NMDA receptor signaling in short-term memory

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ABSTRACT

$Ca_V2.1$ is highly expressed in the nervous system and plays an essential role in the presynaptic modulation of neurotransmitter release machinery. Recently, the antiepileptic drug levetiracetam was reported to inhibit presynaptic $Ca_V2.1$ functions, reducing glutamate release in the hippocampus, although the precise physiological role of $Ca_V2.1$-regulated synaptic functions in cognitive performance at the system level remains unknown. This study examined whether $Ca_V2.1$ mediates hippocampus-dependent spatial short-term memory using the object location and Y-maze tests, and perirhinal cortex-dependent nonspatial short-term memory using the object recognition test, via a combined pharmacological and genetic approach. Heterozygous *rolling* Nagoya (*rol*/+) mice carrying the $Ca_V2.1\alpha_1$ mutation had normal spatial and nonspatial short-term memory. A 100 mg/kg dose of levetiracetam, which is ineffective in wild-type controls, blocked spatial short-term memory in *rol*/+ mice. At 5 mg/kg, the N-methyl-D-aspartate (NMDA) receptor blocker (+/−)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), which is ineffective in wild-type controls, also blocked the spatial short-term memory in *rol*/+ mice. Furthermore, a combination of subthreshold doses of levetiracetam (25 mg/kg) and CPP (2.5 mg/kg) triggered a spatial short-term memory deficit in *rol*/+ mice, but not in wild-type controls. Similar patterns of nonspatial short-term memory were observed in wild-type and *rol*/+ mice when injected with levetiracetam (0–300 mg/kg). These results indicate that $Ca_V2.1$-mediated NMDA receptor signaling is critical in hippocampus-dependent spatial short-term memory and differs in various regions. The combination subthreshold pharmacological and genetic approach presented here is easily performed and can be used to study functional signaling pathways in neuronal circuits.

1. Introduction

Neuronal voltage-dependent Ca^{2+} channels (VDCCs), including $Ca_V2.1$ (P/Q-type), $Ca_V2.2$ (N-type), and $Ca_V2.3$ (R-type) channels, mediate the presynaptic machinery involved in the vesicular release of neurotransmitters (Evans and Zamponi, 2006; Jarvis and Zamponi, 2007; Catterall and Few, 2008). A VDCC is a molecular complex comprising α1, α2-δ, β, and γ subunits (Catterall and Few, 2008). The α1 subunit is essential for channel function and determines fundamental channel properties (Catterall and Few, 2008). *Rolling* Nagoya mice carrying a mutation in the α_1 subunit of the $Ca_V2.1$ channel ($Ca_V2.1\alpha_1$) develop severe ataxia after about 2 weeks of age (Oda, 1973; Mori et al., 2000). Previously, we studied age-related short-term cognitive alterations in heterozygous (*rol*/+) mice without an apparent deficits using the object recognition test, object location test, and Y-maze test (Takahashi and Niimi, 2009; Takahashi et al., 2009). Although we found differences between 22-month-old *rol*/+ and +/+ mice, 2-month-old *rol*/+ mice showed no apparent decrease in spatial cognition compared to 2-month-old +/+ mice in the object location and Y-maze tests. Moreover, neither 2- nor 22-month-old *rol*/+ mice showed deficits in nonspatial cognition in the object recognition test.

Cognitive impairment is observed in epilepsy and appears most frequently in patients with complex partial seizures (Hermann et al., 1992; Thompson and Corcoran, 1992). These perturbations may result from abnormalities in the neuronal circuits important for normal processing of cognitive processes, as well as from epileptiform activity in the brain (Lynch et al., 1996). Furthermore, antiepileptic medication may lead to deterioration of normal cognitive processes (Devinsky, 1995). Levetiracetam [(S)-alpha-ethyl-2-oxo-1-pyrrolidine acetamide] is an antiepileptic drug used to treat patients with partial and generalized seizures (Cereghino et al., 2000; Grant and Shorvon, 2000). Levetiracetam has a broad spectrum of anticonvulsant activity and is effective in a spectrum of neuropsychiatric diseases (Specchio et al., 2006; Khurana et al., 2007). Levetiracetam inhibits presynaptic $Ca_V2.1$ functions, reducing glutamate release in the hippocampus (Lee et al., 2009). These results suggest that the

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administration of levetiracetam at a lower dose to 2-month-old *rol*/+ mice would result in cognitive dysfunction because the precise regulation of Ca^{2+} signaling through $Ca_V2.1$ plays an important role in neuronal circuits. However, the specific physiological role of $Ca_V2.1$-regulated synaptic functions in cognitive performance has not been elucidated.

In this study, to examine the importance of $Ca_V2.1$-mediated N-methyl-D-aspartate (NMDA) receptor signaling in spatial or nonspatial short-term memory, we administered the object location and Y-maze tests for spatial memory and the object recognition test for non-spatial memory to 2-month-old *rol*/+ mice treated with levetiracetam and (+/−)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), a NMDA receptor antagonist.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Animal Experiments Committee of RIKEN and were handled in accordance with the Institutional Guidelines for Experiments using Animals. The *Rolling* Nagoya mouse strain was provided by the RIKEN BioResource Center with the support of the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology in Japan. Male +/+ and *rol*/+ F1 progeny were derived from a cross between *rol*/+ mice and genotyped by PCR using tail DNA (Takahashi and Niimi, 2009). The mice were given free access to water and food pellets (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and were housed under a 12/12-h light/dark cycle (lights on from 08:00 to 20:00) at 23 ± 1 °C and 55 ± 5% humidity.

2.2. Drugs

Levetiracetam (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% NaCl and injected intraperitoneally 30 min before behavioral testing. CPP (Sigma-Aldrich) was dissolved in 0.9% NaCl and injected intraperitoneally 20 min before behavioral testing.

2.3. Behavioral tests

All behavioral tests were conducted between 10:00 and 16:00 by a trained experimenter who was blind to the mouse strains. We used separate groups of male 2-month-old +/+ and *rol*/+ mice for each of the behavioral tests. The mice were moved into the behavioral testing room at least 2 h before testing.

The object recognition and object location tests were performed using the reported procedures with slight modifications (Murai et al., 2007; Niimi et al., 2008). The experiments were performed at 25 lux. Each mouse performed one trial. During the acquisition phase, mice were subjected to a single 5-min session, during which two identical plastic columns (4 cm high × 5 cm diameter) fixed to the floor were placed symmetrically in the center of the arena. During the retention phase of the object recognition test, the mice were allowed to explore the open field for 10 min in the presence of two different objects, the familiar object and a novel object (triangular pyramid, 4 cm high × 5 cm base area). The recognition index [i.e., the ratio of the amount of time spent exploring any object (acquisition phase) or the novel object in particular (retention phase) divided by the total time spent exploring both objects] was used to measure nonspatial memory. During the retention phase of the object location test, the mice were allowed to explore the open field in the presence of two identical objects, a non-displaced object and a displaced object, for 10 min. The recognition index [i.e., the ratio of the amount of time spent exploring either any object (acquisition phase) or the displaced object in particular (retention phase) divided by the total time spent exploring both objects] was used to measure spatial memory.

The Y-maze test was performed using the reported procedure with slight modifications (Niimi et al., 2008). The experiments were performed at 35 lux. Before behavioral testing, the mice were placed in one of the compartments and allowed to move freely on the one of the arms for 10 min. Each mouse performed one trial. An arm entry was defined as three legs entering one of the arms, and the sequence of entries was recorded manually using videotapes. An alteration was defined as entry into all three arms with consecutive choices. The percentage of spontaneous alteration was calculated as (actual alteration/maximum alteration) × 100.

2.4. Data analysis and statistics

Data are presented as means ± standard deviation (S.D.). Statistical analyses for the behavioral tests were conducted using Excel Statistics 2006 (SSRI, Tokyo, Japan). In the object recognition and location tests, within- and between-group differences in exploration preferences were considered significant at $P<0.05$. Three-way analysis of variance (ANOVA) was used to analyze the difference between exploration preferences. *Post hoc* between-group comparisons for genotypes, doses, and phases in the tests were completed with Tukey's test. In the Y-maze test, differences between total arm entries within group, differences between percent alternations within group, and differences between groups were considered significant at $P<0.05$. Two-way ANOVA was used to analyze the difference between percent alternations and differences between total arm entries. *Post hoc* between-group comparisons for genotypes and doses in the tests were completed with Tukey's test.

3. Results

3.1. Effects of levetiracetam on the time spent exploring the displaced object in the object recognition test

We examined eight groups of mice ($n=10$ each), including four groups each of +/+ and *rol*/+ mice that were given injections of 0, 25, 100, or 300 mg/kg levetiracetam. The groups did not differ significantly in exploration time [genotype × age × phase interaction: $F(3,144)=0.424$, $P=0.736$; genotype × dose interaction: $F(3,144)=0.277$, $P=0.842$; dose × phase interaction: $F(3,144)=0.795$, $P=0.499$; genotype × phase interaction: $F(1,144)=0.073$, $P=0.788$; dose effect: $F(3,144)=0.643$, $P=0.589$; genotype effect: $F(1,144)=0.026$, $P=0.871$; phase effect: $F(1,144)=1113.200$, $P<0.001$] (Fig. 1). During the exploration sessions, all of the groups spent more time exploring the displaced object.

3.2. Effects of levetiracetam or CPP on the time spent exploring the novel object in the object location test

We examined eight groups of mice ($n=10$ each), including four groups each of +/+ and *rol*/+ mice that were given injections of 0, 25, 100, or 300 mg/kg levetiracetam. The groups differed significantly in exploration time [genotype × dose × phase interaction: $F(3,144)=24.467$, $P<0.001$; genotype × dose interaction: $F(3,144)=23.031$, $P<0.001$; dose × phase interaction: $F(3,144)=94.500$, $P<0.001$; genotype × phase interaction: $F(1,144)=29.097$, $P<0.001$; dose effect: $F(3,144)=91.864$, $P<0.001$; genotype effect: $F(1,144)=29.097$, $P<0.001$; phase effect: $F(1,144)=615.868$, $P<0.001$] (Fig. 2A). During the training session, no significant differences were found among any of the groups in terms of the time spent exploring the two objects, indicating that the mice had equal levels of curiosity and motivation for the task. During the exploration sessions, the +/+ mice given 0, 25, or 100 mg/kg levetiracetam and the *rol*/+ mice given 0 or 25 mg/kg levetiracetam showed an exploratory preference for the displaced object. The *rol*/+ mice given 100 mg/kg levetiracetam spent