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Levetiracetam-mediated emotional behavior in heterozygous *rolling* Nagoya $Ca_V2.1$ channel mutant mice

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ABSTRACT

$Ca_V2.1$, which is highly expressed in the nervous system, plays an essential role in presynaptic neurotransmitter release. Although recent data suggest that the antiepileptic drug levetiracetam (LEV) inhibits presynaptic $Ca_V2.1$ activity, the precise physiological role of $Ca_V2.1$/LEV-regulated emotional performance has not been elucidated. We examined whether $Ca_V2.1$/LEV mediates emotional behavior using a combined pharmacologic and genetic approach. Heterozygous *rolling* Nagoya (*rol*/+) mice carrying the $Ca_V2.1\alpha_1$ mutation demonstrated normal emotional behavior. Exposure to 75 mg/kg LEV, which had no effect in wild-type controls, reduced anxiety in elevated plus maze and light–dark exploration tests and reduced depression in forced swimming and tail suspension behavioral tests in *rol*/+ mice. Similar behavioral patterns in motor activity were noted in wild-type and *rol*/+ mice injected with 0–150 mg/kg LEV. The phosphorylation of tryptophan hydroxylase at serine-58 and serotonin concentration were increased in the brainstems of *rol*/+ mice injected with 75 mg/kg LEV but not in those of wild-type controls. These results indicate that $Ca_V2.1$/LEV mediates serotonin signaling leading to alterations in emotion. Our results also indicate that a combination of subthreshold pharmacologic and genetic approaches can be used to study functional signaling pathways in neuronal circuits.

1. Introduction

Voltage-gated Ca^{2+} (Ca_V) channels play an important regulatory role in diverse neuronal functions attributed to elevated intracellular Ca^{2+} concentrations (Berridge et al., 2000; Liu et al., 2003). Ca^{2+} influx triggers neurotransmitter production and release in a cooperative process with other components of neurotransmitter-biosynthesizing enzyme activation and vesicle fusion machineries (Catterall, 1998; Mendoza et al., 2003). Given the pivotal role of Ca^{2+} channels in the control of neurotransmitter production and release, defects in the expression, localization, structure, or modulation of presynaptic Ca^{2+} channels may result in aberrant synaptic signaling leading to various patterns of neural network dysfunction. Two major Ca_V2 channel types, $Ca_V2.1$ (P/Q-type) and $Ca_V2.2$ (N-type), have critical roles in presynaptic terminals (Catterall, 1998; Yokoyama et al., 2005).

The α_1 subunit is a pore-forming component, which functions as a voltage sensor and is capable of generating channel activity (Catterall, 1999; Mikami et al., 1989). Mutations in the $Ca_V2.1$ channel α_1 subunit ($Ca_V2.1\alpha_1$) gene have been identified in ataxic mutant mice such as *rolling* mouse Nagoya, *tottering*, and *leaner* (Oda, 1973; Fletcher et al., 1996). *Rolling* mouse Nagoya carries a mutation in the voltage-sensing S4 segment of the third repeat in the $Ca_V2.1\alpha_1$ gene (Mori et al., 2000). Previously, we assessed emotion-related behavior and $Ca_V2.1\alpha_1$ mRNA expression in two- and 22-month-old mice (Takahashi et al., 2009a). Reduced anxiety and depression phenotypes were observed in 22-month-old heterozygous (*rol*/+) mice compared to age-matched wild-type (+/+) mice, suggesting that aged *rol*/+ mice can be used to delineate the interaction between $Ca_V2.1$ function and emotional performance. The mRNA expression of mutant-type $Ca_V2.1\alpha_1$ was increased in the brainstems of 22-month-old *rol*/+ mice. In contrast, no difference in behavior or expression was noted between two-month-old *rol*/+ and +/+ mice. Further, no significant difference was observed between two-month-old *rol*/+ and +/+ mice and between 22-month-old *rol*/+ and +/+ mice in motor-related behavioral tasks, including footprint and traction tests (Takahashi et al., 2009b), suggesting that *rol*/+ mice possess no epileptic or ataxic phenotypes. These findings suggest that *rol*/+ mice show age-related emotional changes but not epileptic or ataxic changes, and that mutant-type $Ca_V2.1\alpha_1$ expression in two-month-old *rol*/+ mice has a sensitive subthreshold dose to emotional performance.

The anti-epileptic drug levetiracetam (LEV) inhibits presynaptic $Ca_V2.1$ function (Lee et al., 2009). However, the precise physiologic role of $Ca_V2.1$/LEV-regulated synaptic function in emotional performance at the system level remains unclear. There may be a subthreshold dose of LEV that triggers an alteration in emotion-related behavior in two-month-old *rol*/+ mice but not in wild-type controls.

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In the present study, we conducted emotion-related behavioral tests and analyzed the levels of the serotonin-biosynthesizing enzyme tryptophan hydroxylase (TPH) and serotonin in the brainstems of two-month-old $Ca_V2.1$ mutant *rol*/+ mice using various concentrations of LEV.

2. Materials and methods

2.1. Animals

All procedures involving animals were approved by the Animal Experiments Committee of RIKEN. All animals were cared for humanely in accordance with institutional guidelines for animal experimentation. The *rolling* Nagoya mouse strain, which was found among descendants of a cross between strains SIII and C57BL/6 (Oda, 1973), was provided by the RIKEN BioResource Center with support from the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Male +/+ and *rol*/+ F1 progeny were derived from a cross between +/+ and *rol*/+ mice and genotyped by PCR using tail DNA (Takahashi et al., 2009a). 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-h/12-h light/dark cycle (lights on from 08:00 to 20:00) at 23 ± 1 °C and 55 ± 5% humidity. All behavioral analyses were conducted between 09:00 and 16:00 by a well-trained experimenter who was blinded to the mouse strains. Anxious behavior was studied using the elevated plus maze (Pellow et al., 1985) and light–dark exploration (Crawley, 1981) behavioral tests. Depressive behavior was studied using the forced swimming (Porsolt et al., 1978) and tail suspension (Steru et al., 1985) tests. The mice were moved into the behavioral testing room at least 1 h before testing. We examined the levels of TPH and TPH phosphorylated at serine-58 (p-TPH) by Western blot analysis and used high-performance liquid chromatography (HPLC) to examine the monoamine concentrations. Separate groups of two-month-old male mice were used for the behavior, expression, and monoamine concentration tests.

2.2. Drug

LEV (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% NaCl and injected intraperitoneally 30 min before behavioral testing in a final volume of 5 ml/kg. The doses (25–150 mg/kg) used were within the range reported to produce emotional alterations in various rodent models (Gower et al., 2003; Klitgaard and Pitkänen, 2003; Lamberty et al., 2003).

2.3. Open field test

Motor activity was measured by placing individual mice in a clear Plexiglas box (L×W×H: 30×20×15 cm). The box was then positioned in a frame on which infrared beams (Scanet SV-10, Tokyo, Japan) were mounted. The light intensity in the experimental room was 60 lux. Beam interruptions were summed over 5 min. The following mice were used in the open field test: 0 mg/kg LEA-injected +/+ ($n=10$), 0 mg/kg LEA-injected *rol*/+ ($n=10$), 25 mg/kg LEA-injected +/+ ($n=10$), 25 mg/kg LEA-injected *rol*/+ ($n=10$), 75 mg/kg LEA-injected +/+ ($n=10$), 50 mg/kg LEA-injected +/+ ($n=10$), 50 mg/kg LEA-injected *rol*/+ ($n=10$), 75 mg/kg LEA-injected *rol*/+ ($n=10$), 150 mg/kg LEA-injected +/+ ($n=10$), and 150 mg/kg LEA-injected *rol*/+ mice ($n=10$).

2.4. Elevated plus maze test

The apparatus consisted of two open arms (30×5 cm) and two closed arms (30×5×15 cm) that extended from a common central platform (5×5 cm). A small raised lip (0.3 cm) around the perimeter of the open arms prevented the mice from falling. The apparatus was made of Plexiglas with a gray floor and walls, and was elevated 45 cm above the floor. At the beginning of each experiment, a mouse was placed on the center platform. The mice were allowed to explore the apparatus freely for 5 min under 20 lux of illumination. Behavior was recorded with an overhead video camera. Arm entry was defined as four legs entering one of the maze arms. The number of transitions between the arms, the number of entries into open arms, and the time spent in open arms were measured. The following mice were used in the elevated plus maze test: 0 mg/kg LEA-injected +/+ ($n=12$), 0 mg/kg LEA-injected *rol*/+ ($n=12$), 25 mg/kg LEA-injected +/+ ($n=12$), 25 mg/kg LEA-injected *rol*/+ ($n=12$), 50 mg/kg LEA-injected +/+ ($n=12$), 50 mg/kg LEA-injected *rol*/+ ($n=12$), 75 mg/kg LEA injected +/+ ($n=12$), 75 mg/kg LEA-injected *rol*/+ ($n=12$), 150 mg/kg LEA-injected +/+ ($n=12$), and 150 mg/kg LEA-injected *rol*/+ mice ($n=12$).

2.5. Light–dark exploration test

The apparatus consisted of two compartments: a dark compartment (15×10×20 cm) and a light compartment (20×15×20 cm). The dark compartment had a lid on top and was made of black Plexiglas, whereas the light compartment was open at the top and was made of white Plexiglas. A black Plexiglas tunnel (10×7×4.5 cm) separated the dark box from the light box. The light intensity in the experimental room was 100 lux. A mouse was placed in the light compartment and its behavior was recorded on a videotape over a 5-min period. The number of transitions between the compartments and the time spent in the light compartment were measured. A mouse with all four paws in the light compartment was considered to be fully in the light compartment. The following mice were used in the light–dark exploration test: 0 mg/kg LEA-injected +/+ ($n=12$), 0 mg/kg LEA-injected *rol*/+ ($n=12$), 25 mg/kg LEA-injected +/+ ($n=12$), 25 mg/kg LEA-injected *rol*/+ ($n=12$), 50 mg/kg LEA-injected +/+ ($n=12$), 50 mg/kg LEA-injected *rol*/+ ($n=12$), 75 mg/kg LEA injected +/+ ($n=12$), 75 mg/kg LEA-injected *rol*/+ ($n=12$), 150 mg/kg LEA-injected +/+ ($n=12$), and 150 mg/kg LEA-injected *rol*/+ mice ($n=12$).

2.6. Forced swimming test

Each mouse was placed in a 19-cm glass cylinder (11.0 cm in diameter) containing 13 cm of water at 23 ± 1 °C. A mouse was deemed immobile when it floated, its hindlimbs appeared immobile, and only small movements of the forepaws were used to keep the head above water. The light intensity in the experimental room was 150 lux. The behavior of the mice was recorded with a video camera for 7 min. Immobility was recorded at 2 and 7 min. The parameter recorded was the total amount of time (s) spent immobile. The following mice were used in the forced swimming test: 0 mg/kg LEA-injected +/+ ($n=10$), 0 mg/kg LEA-injected *rol*/+ ($n=10$), 25 mg/kg LEA-injected +/+ ($n=10$), 25 mg/kg LEA-injected *rol*/+ ($n=10$), 75 mg/kg LEA-injected +/+ ($n=10$), 75 mg/kg LEA-injected *rol*/+ ($n=10$), 150 mg/kg LEA-injected +/+ ($n=10$), and 150 mg/kg LEA-injected *rol*/+ mice ($n=10$).

2.7. Tail-suspension test

The apparatus consisted of a non-transparent compartment (15.0×16.0×25.0 cm) with a hook (4.0 cm in length). The distance between the hook and floor was 21 cm. Each mouse was hung from the hook using adhesive tape placed 2 cm from the end of its tail, and its behavior was recorded with a video camera for 7 min. The immobility time was evaluated between 2 and 7 min. The light intensity in the experimental room was 150 lux. The following mice were used in the tail suspension test: 0 mg/kg LEA-injected +/+