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Role of Cav2.2-mediated signaling in depressive behaviors

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Abstract

Neuronal voltage-gated calcium channels (VGCCs) including Cav2.2, mediate the mechanisms involved in the presynaptic release of neurotransmitters. The role of Cav2.2 in neural circuits underlying depression remains poorly understood. In this study, intracerebroventricular injection of the Cav2.2 inhibitor ω-conotoxin GVIA (5 pg/side) in mice increased depression-like behavior in the forced swimming and tail suspension tests. These results suggest that Cav2.2-mediated signaling plays a role in depressive behaviors.

Introduction

Voltage-gated Ca^{2+} (Cav) channels play an important role in regulating diverse neuronal functions attributed to elevated intracellular Ca^{2+} concentrations [1,2]. Cav channels are molecular complexes comprising α1, β, α2-δ, and γ subunits [1]. The α1 subunit is essential for channel function and determines fundamental channel properties [1]. Genes encoding 10 pore-forming α1 subunits and several splice variants have been identified and characterized [3].

At the presynaptic terminal, Cav2.2 (N type) channels mediate Ca^{2+}-dependent exocytotic release of neurotransmitters [4]. Ca^{2+} influx *via* these channels triggers neurotransmitter release in a cooperative process with other components of the vesicle fusion machinery [5]. Given the pivotal role of Ca^{2+} channels in controlling neurotransmitter 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. Cav2.2 channels have been reported to influence the release of dopamine [DA; 6–8], serotonin [5-HT; 9], glutamate [10], gamma-aminobutyric acid [11], acetylcholine [12], and norepinephrine [NE; 13] from central neurons. In terms of clinical relevance, imbalances of neurotransmitters are strongly associated with depression [14]. According to the monoamine hypothesis, depression can be ascribed to deficits in the major monoamine neurotransmitters (DA, 5-HT, and NE). As Cav2.2 channels are involved in the regulation of neurotransmitter release, administration of a Cav2.2 blocker is expected to result in depressive behavior.

In mice that received intracerebroventricular (i.c.v.) injections of ω-conotoxin GVIA, a Cav2.2 inhibitor, baseline levels of DA and 5-HT were reduced in the striatum and frontal cortex [15]. The Cav2.2 inhibitor also induced depressive behavior, as measured by the forced swimming test [16] and tail suspension test [17].

In the present study, the relationship between Cav2.2-mediated signaling and depression was investigated further. Mice were treated with i.c.v. injections of ω-conotoxin GVIA, and depression was assessed using forced swimming and tail suspension tests.

Materials and methods

Mice

All animal procedures were approved by the Animal Experiments Committee of Shanghai Jiao Tong University and RIKEN. C57BL/6J mice were provided by Charles River Japan (Kanagawa, Japan). The mice were given free access to water and food pellets (CRF-1; Oriental Yeast Co. Ltd., Tokyo, Japan) and 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. We used separate groups of 2-month-old male mice for each of the behavioral tests. All experiments were conducted by investigators blinded to the treatment conditions at light phase.

Infusion

For the infusion studies, the Cav2.2 blocker, ω-conotoxin GVIA (100 pg/μL, Peptide Institute, Osaka, Japan) was dissolved in saline (vehicle). The drug dose was determined based on a previous report [15,18,19]. Non-treated mice received an equivalent volume of vehicle. Under anesthesia and using standard stereotaxic procedures, stainless-steel guide cannulae (22-gauge) were implanted into the lateral ventricle (posterior to bregma, -0.34 mm; lateral to midline, ±0.9 mm; ventral from the dura, −2.3 mm), and mice were allowed to recover for at least 1 week following surgery. The mice were briefly anesthetized with isoflurane to facilitate insertion of the injection cannula (26-gauge). Infusion into the lateral ventricle (0.1 μL/side) was accomplished at a rate of 0.05 μL/min 30 min before behavioral testing. The injection cannula was left in place for 2 min following infusion.

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Open field test

Motor activity was measured by placing individual animals 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 in 20 min.

Tail-suspension test

The apparatus consisted of a non-transparent compartment (L × W × H: 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 the 2nd and 7th min. The light intensity in the experimental room was 150 lux. The parameter recorded was the total amount of time (s) spent immobile.

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 and its hindlimbs appeared immobile, with only small movements of the forepaws used to keep the head above water. The light intensity in the experimental room was 150 lux. The behavior was recorded with a video camera for 7 min. Immobility was recorded between the 2nd and 7th min. The parameter recorded was the total amount of time (s) spent immobile.

Histology

Histological verification of the cannula locations was performed at the end of behavioral testing. Mice were perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde (PFA). After extraction from the skull, the brains were post-fixed in 4% PFA and then transferred to a 30% sucrose solution until sectioning. Coronal sections (40 μm thick, taken every 120 μm) were cut on a cryostat (-16°C) and mounted on glass microscope slides. After drying, the sections were stained with cresyl violet.

Statistical analysis for behavioral results

The data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were conducted using Excel Statistics 2006 (SSRI, Tokyo, Japan). The data were analyzed using analysis of variance (ANOVA). Tukey's test was performed when appropriate. The results were considered significant at 5% or lower probability of error.

Results

This study examined the effect of ω-conotoxin GVIA on depressive behavior. Two groups of male mice were given i.c.v. injections of either ω-conotoxin GVIA (5 pg/side) or vehicle.

In the open field test, no significant difference was observed in motor activity between vehicle-injected and ω-conotoxin GVIA-injected mice (Figure 1). In both the tail suspension and forced swimming tests, ω-conotoxin GVIA-injected mice had significantly longer immobile times than vehicle-injected mice (Figure 2 and 3).

Mice with injection needle placements outside of the boundaries of the target areas were excluded from behavioral analyses (data not shown).

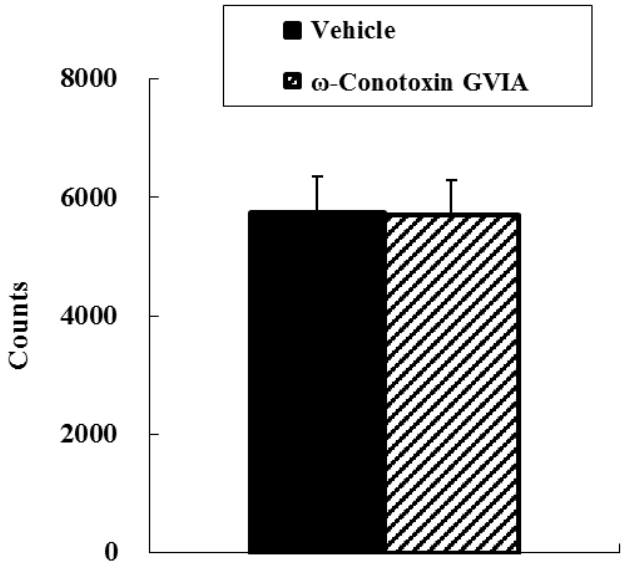

Figure 1. Open field test. The vehicle-injected or ω-Conotoxin GVIA-injected mice (n=10 each) were allowed to explore the field freely for 20 min.

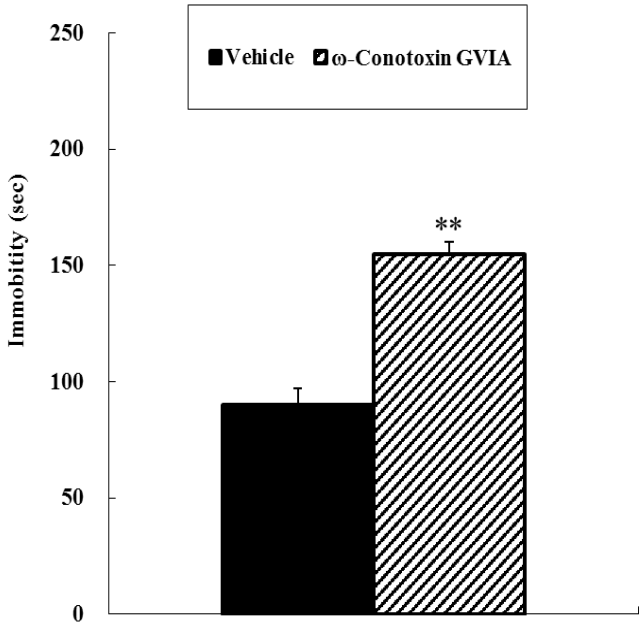

Figure 2. Tail suspension test. The vehicle-injected or ω-Conotoxin GVIA-injected mice (n=10 each) were suspended by the tail. Time spent immobile (s) was evaluated during the 2nd to 7th minutes. The data are presented as means ± standard error of the mean (SEM). $^{**}P$ <0.01 compared with the appropriate control (Tukey's test).

Discussion

The neurotransmission of monoamines is thought to control emotional behavior. Biological research in depression currently involves many aspects of neurotransmitter, hormone, and vitamin

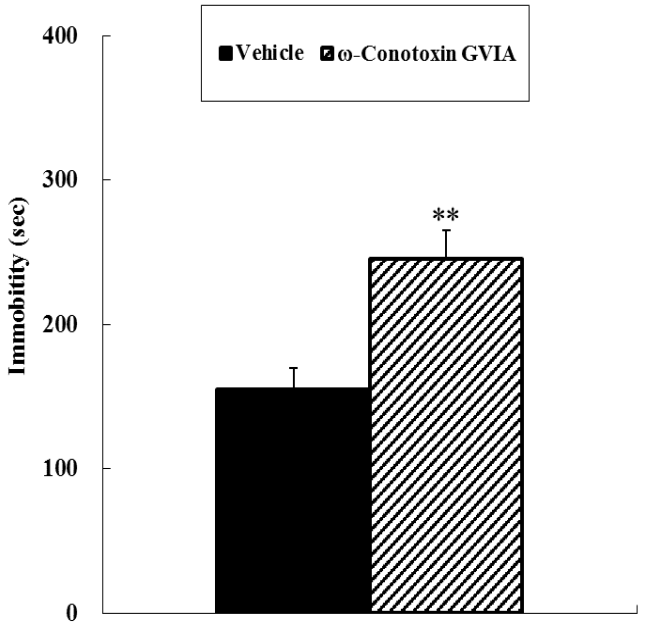

Figure 3. Forced swimming test. The vehicle-injected or ω-Conotoxin GVIA-injected mice (n = 10 each) were released in the apparatus and time spent immobile was evaluated during the 2nd to 7th minutes. The data are presented as means ± standard error of the mean (SEM). **P <0.01 compared with the appropriate control (Tukey's test).

metabolism, with metabolism of monoamine neurotransmitters being a major area of interest for over 40 years [20–23]. Neuronal Cav channels, including Cav2.1 and Cav2.2, are predominantly expressed at presynaptic neuronal terminals throughout the central nervous systems [9]. Cav channels mediate the release of neurotransmitters that are involved in depression; however, the role of different Cav channels, specifically Cav2.2, in the neural circuits underlying depression has not been explored. In the present study, we investigated the relationship between Cav2.2-mediated signaling and depression in mice that received i.c.v. injections of the Cav2.2 blocker ω-conotoxin GVIA.

We first examined the effect of ω-conotoxin GVIA on motor activity by assessing immobility in the forced swimming and tail suspension tests. The open field test revealed no significant difference in motor activity between vehicle-injected and ω-conotoxin GVIA-injected mice. In a previous study, we examined the impact of a subtle disruption of Cav2.2 channel functioning on motor activity using the activity wheel test [24]. Cav2.2 channel knockout mice showed normal activity during the light phase and increased activity during the dark phase. In the present study, conducted in the light phase, Cav2.2 channel-dependent signaling had no effect on spontaneous activity in mice. In the forced swimming and tail suspension tests, ω-conotoxin GVIA-injected mice had significantly longer immobile times (*i.e.,* increased depression-like behavior) compared with vehicle-injected mice. Overall, the results indicate that Cav2.2 channel-dependent signaling has an influence on depressive behaviors.

In our previous study, baseline levels of DA and 5-HT were reduced in the striatum and frontal cortex in mice given ω-conotoxin GVIA [15]. Although emotional behavior may be affected by multiple neurotransmitter systems, our results suggest that Cav2.2 channel dysfunction and subsequent decreases in DA and 5-HT may be at least partially responsible for the observed depressive behavior in ω-conotoxin GVIA-injected mice.

In conclusion, inhibition of Cav2.2-mediated signaling by the specific Cav2.2 blocker ω-conotoxin GVIA was found to induce behavioral deficits in the forced swimming and tail suspension tests. As Cav2.2 influences the release of DA and 5-HT [15], abnormalities in Cav2.2-mediated signal transduction may play a role in the pathophysiological mechanisms underlying depression. Additional electrophysiological studies of neurotransmitter release will help to elucidate the relationship between Cav2.2 signaling and depression.

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Conflicts of interest

The authors declare no competing interests.

Authors' contributions

WL and ET designed and supervised the research, and wrote the manuscript. YZ and KN performed the surgeries and behavioral experiments. All authors read and approved the final version of the manuscript.

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