

Role of Cav2.1 Channel Signaling in Glutamate-Related Brain Injury

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Abstract

Voltage-gated Ca^{2+} channels (VGCCs) play a pivotal role in intracellular processes such as neurotransmitter release, axonal outgrowth, membrane excitability, and synaptic plasticity. Three major presynaptic Cav2 channels, including the Cav2.1 channel, are involved in the mechanism underlying Ca^{2+} -dependent excitotoxicity. Glutamate is among the excitatory neurotransmitters regulated by the Cav2.1 channel. Glutamate-related excitotoxicity is a pathological process associated with seizures, traumatic brain injury, and cerebral ischemia. This review emphasizes the relationship between activation of the Cav2.1 channel and these various types of brain injury.

Keywords: Cav2.1; Glutamate; Excitotoxicity; Brain injury

Types of Brain Injury

Cerebral ischemia, seizures, traumatic brain injury. Neuroprotection has been studied worldwide in the context of brain injuries, including neurodegenerative diseases, cerebral ischemia, epilepsy and seizures, and traumatic brain injury (TBI) [1-3]. Cerebral ischemia is one of the most common causes of death in adults worldwide, resulting in necrosis and apoptosis in the brain that in turn leads to a high possibility of ischemic stroke-related disability [4-6]. Epilepsy, characterized by multiple, unpredictable seizures, is a common neuronal disease in human populations regardless of age, sex, or race. Approximately 1% of the population worldwide is known to have epilepsy, which often requires lifelong medication [7]. TBI, which is typically due to falls or motor vehicle- or sports-related accidents, is a serious health issue regardless of age [8]. Approximately 1.4 million patients visit emergency rooms in the USA each year due to TBI [9]. The need for treatment of such brain injuries appears to be dramatically increasing. Therefore, in this paper, we review the role of the Cav2.1 channel in cerebral ischemia, seizures, and TBI.

Voltage-Gated Ca^{2+} Channels

Structure, subtypes, and function

Voltage-gated Ca^{2+} channels (VGCCs) are located in presynapses in the central nervous system (CNS) [10-12]. Ca^{2+} influx via VGCCs regulates intracellular processes such as neurotransmitter release, axonal outgrowth, membrane excitability, and synaptic plasticity [10]. Glutamate signaling is among the types of signaling regulated by the Cav2.1 channel [13,14]. VGCCs are molecular complexes composed of four subunits that are encoded by multiple, distinct genes: α_1 , α_2/δ , β , and γ [10,11,15] (Figure 1). The α_1 subunit, which is the largest pore-forming subunit, consists of four homologous transmembrane domains (I-IV), each of which contains six membrane-spanning helices (S1-S6) and a reentrant p-loop. Cytoplasmic linkers connect the four domains. Both the cytoplasmic C- and N-termini interact with regulatory proteins [16] and play an essential role in VGCC functions and properties, such as voltage sensing and protein binding [11,15,16]. VGCCs are functionally divided into several subtypes: the Cav1 (L-type), Cav2.1 (P/Q-type), Cav2.2 (N-type), Cav2.3 (R-type), and Cav3 (T-type) channels. Cav1, Cav2.1, Cav2.2, and Cav2.3 are high voltage-gated channels, whereas Cav3 is a low voltage-gated channel. Each channel has different functions and pharmacological characteristics. The Cav1 channel, which is inhibited by dihydropyridines, phenylalkylamines, and benzothiazepines, is involved in excitation-contraction coupling, Ca^{2+}

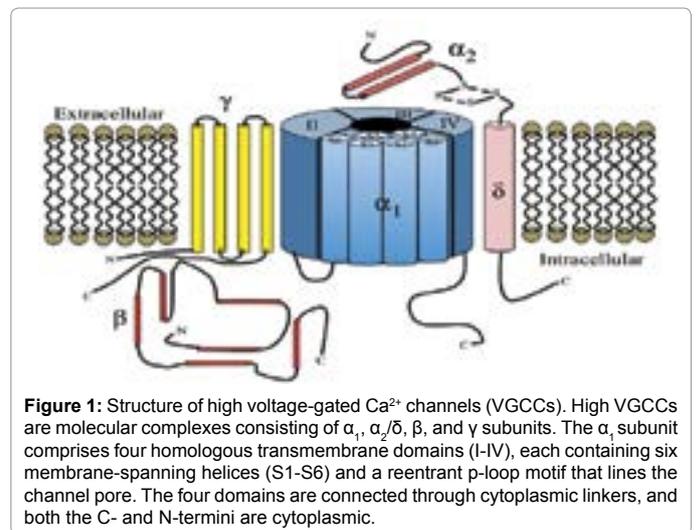


Figure 1: Structure of high voltage-gated Ca^{2+} channels (VGCCs). High VGCCs are molecular complexes consisting of α_1 , α_2/δ , β , and γ subunits. The α_1 subunit comprises four homologous transmembrane domains (I-IV), each containing six membrane-spanning helices (S1-S6) and a reentrant p-loop motif that lines the channel pore. The four domains are connected through cytoplasmic linkers, and both the C- and N-termini are cytoplasmic.

homeostasis, gene regulation, and neurotransmitter release. ω -agatoxin IVA (ω -aga) is a Cav2.1 channel antagonist and ω -conotoxin GVIA is a Cav2.2 channel antagonist. Both the Cav2.1 and Cav2.2 channels are important for neurotransmitter and hormone release, as well as for generating dendritic Ca^{2+} transients. The Cav2.3 channel, which is inhibited by SNX-482, is associated with repetitive firing and the generation of dendritic Ca^{2+} transients. The Cav3 channel, for which no selective antagonist has yet been identified, is involved in pacemaking and repetitive firing [16,17]. Among the various VGCCs, the Cav2.1 and Cav2.2 channels are the most involved in neurotransmitter release in the CNS, and are also associated with the mechanism underlying Ca^{2+} -dependent excitotoxicity. Each VGCC has antagonists, such as ω -aga for the Cav2.1 channel (Table 1).

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Mutations in the Cav2.1 α_1 subunit

The α_1 subunit of the Cav2.1 channel (Cav2.1 α_1) is encoded in the *CACNA1A* gene in humans. Mutations in the *Cav2.1 α_1* gene induce autosomal-dominant symptoms and disorders, including familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), spinocerebellar ataxia type 6 (SCA6), and epilepsy [10,15]. Mutations in the *Cav2.1 α_1* gene have been observed in mice (Figure 2). Several types of spontaneous mutant mice with mutations in the *cacna1a* gene on chromosome 8 have been reported, including rocker (rkr), tottering (tg), rolling Nagoya, wobbly, tottering-4j, tottering-5j, tottering-6j, and leaner (la) mice. Among these mutations, tottering-5j and wobbly

Cav subtype	Antagonist	Physiological function
Cav1 (L-type)	Dihydropyridine Phenylalkylamines Benzothiazepines	Excitation-contraction coupling Calcium homeostasis Gene regulation Neurotransmitter release
Cav2.1 (P/Q-type)	ω -agatoxin IVA	Neurotransmitter release, dendritic Ca ²⁺ transient currents
Cav2.2 (N-type)	ω -conotoxin GVIA	Neurotransmitter release Dendritic Ca ²⁺ transients Hormone release
Cav2.3 (R-type)	SNX-482	Repetitive firing Dendritic Ca ²⁺ transients
Cav3 (T-type)	No selective antagonist	Pacemaking Repetitive firing

Table adapted from: Takahashi and Niimi [16], Catterall et al. [17].

Table 1: Physiological function and pharmacology of Cav channel subtypes.

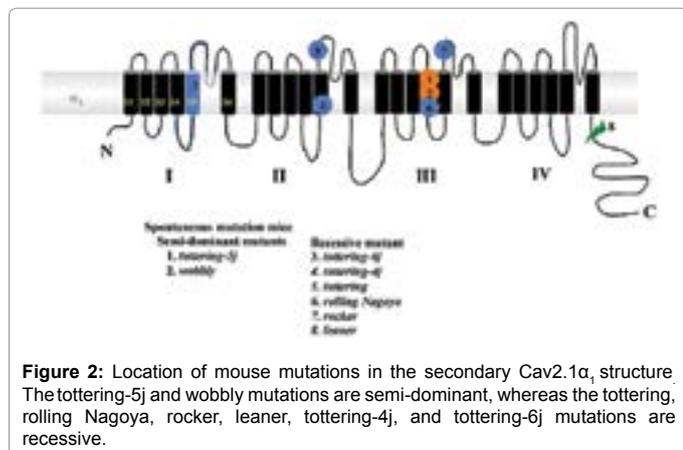


Figure 2: Location of mouse mutations in the secondary Cav2.1 α_1 structure. The tottering-5j and wobbly mutations are semi-dominant, whereas the tottering, rolling Nagoya, rocker, leaner, tottering-4j, and tottering-6j mutations are recessive.

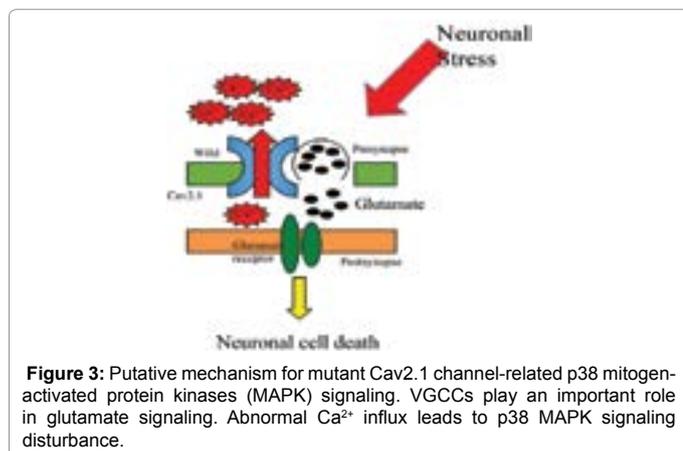


Figure 3: Putative mechanism for mutant Cav2.1 channel-related p38 mitogen-activated protein kinases (MAPK) signaling. VGCCs play an important role in glutamate signaling. Abnormal Ca²⁺ influx leads to p38 MAPK signaling disturbance.

are semi-dominant and the others are recessive [10]. The mutant mice show symptoms similar to those of humans.

Glutamate

Glutamate, the major endogenous excitatory neurotransmitter, is coupled to the glutamatergic system in the CNS [14]. Glutamate binds to post-synaptic glutamate receptors, including ionotropic glutamate, kainate, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and N-methyl-D-aspartate (NMDA) receptors [18]. Glutamate-induced excitotoxicity is considered an important mechanism underlying neuronal death in ischemia, CNS trauma, and epilepsy [19]. Neuronal stress leads to excessive release of glutamate, which over activates post-synaptic glutamate receptors and causes neurodegeneration [20]. Many studies have reported that NMDA is involved in excitotoxicity [21-23], while AMPA is involved in neuronal cell death related to oxygen/glucose deprivation [24], ischemia [25], seizures [26], and TBI [27]. Kainate, a well-known cause of epilepsy, induces persistent high-amplitude, high-frequency spikes [28].

Studies on the protective role of the Cav2.1 channel against brain injury

Seizure

The Cav2.1 channel is among the VGCCs most involved in synaptic transmission. Abnormalities in the Cav2.1 channel affect cellular and neuronal networks, leading to seizure [29-30]. The Cav2.1 channel is involved in glutamate signaling [13,14].

Kainate injection has been used in experimental models of seizures [31-33]. Kainate binds to both kainate and AMPA receptors [31,32]. Following kainate-induced seizures, neuronal loss in the hippocampus is observed, along with the appearance of reactive astrocytes and delayed neuronal cell death [34]. A study using Cav2.1 channel mutant mice reported that seizures and neuronal damage were reduced in animals injected with kainate compared to wild-type controls, suggesting that the kainate/AMPA signaling gateway may be suppressed by Cav2.1 channel dysfunction, leading to resistance against excitotoxicity [26]. In the same study, the expression of p38 was not increased in kainate-injected Cav2.1 mutant mice relative to kainate-injected +/+ mice. These results suggest that changes in the threshold for glutamate release, via depolarization-induced Ca²⁺ influx through the mutant Cav2.1 channel, induce abnormal glutamate receptor function and postsynaptic p38 mitogen-activated protein kinases (MAPK) signaling cascades (Figure 3). The seizure scores of kainate-injected heterozygous p38 knockout mouse are significantly lower than those of +/+ mice [34]. Kim et al. [35] reported that inhibition of p38 MAPK significantly attenuates both neuronal loss in the hippocampus and the accompanying gliosis. This accords with studies that have shown that the p38 MAPK pathway is involved in seizures and apoptotic processes [34,36].

Antiepileptic drugs, such as Lamotrigine and Levetiracetam, Cav2.1 channel blockers, have been used for treating seizure successfully [37]. However, these drugs don't solely block Cav2.1 channels. Lamotrigine, Cav3 channel, Cav2.1-2.3, sodium channel, A-type potassium channel inhibitor, is a broad-spectrum antiepileptic drug [37]. It is used for monotherapy and also for adjunctive therapy. Levetiracetam inhibits high VGCCs such as, Cav2.1 and Cav2.2. It also inhibits potassium current and reduces GABA turnover rate [37]. Clinical trials regarding Cav2.1 channel selective inhibition have not been widely conducted. Further studies focusing on Cav2.1 inhibition is warranted.

Traumatic brain injury

TBI induces both primary and secondary brain damage. Primary (mechanical) damage, for example from a cryogenic stimulus, can damage the brain cortex, whereas secondary damage involves astrocytes and neurons. Abnormal release of neurotransmitters, including glutamate, induces excitotoxicity; TBI is associated with glutamate signaling [38-40].

In a study that induced brain injury using a cryogenic method, Cav2.1 channel mutant mice had smaller lesions compared to +/- mice; the degree of neuronal damage and number of reactive astrocytes were also lower the Cav2.1 channel mutants [41]. These results indicate that mutations in the Cav2.1 channel can protect against the excitotoxicity induced by cryogenic brain injury and decreased Ca²⁺ influx by attenuating astrocyte reactivation [41]. In addition, in that same study, administration of ω -aga into the lateral ventricle 24 h after cryogenic injury had a therapeutic effect, shown by smaller lesions as well as fewer degenerated cells and reactive astrocytes [41]. Further study on the behavioral effects of ω -aga treatment is warranted. That same study also showed that neuronal cells in vehicle-treated mice showed greater levels of pp38 expression compared to mice treated with ω -aga. Similar to kainate-induced seizures, these results indicate that the Cav2.1 channel is involved in the p38 MAPK signaling cascade (Figure 3).

In clinical trial, administrating Cav1 channel antagonist improved outcome when injected acutely [42]. Clinical trial regarding Ziconotide, Cav2.2 channel blocker, was conducted, however due to the side effect the study was halted [42]. Future studies are needed for Cav2.1 channel antagonist.

Cerebral ischemia

Cerebral ischemia is defined as a level of blood flow to the brain that is insufficient to maintain normal cellular function [43]; this blood flow restriction leads to a massive release of glutamate. Overactivation of NMDA and AMPA receptors results in neuronal death and dysfunction [43,44]. Induced middle cerebral artery occlusion has been used in numerous cerebral ischemia models [45-48]. In such models, the caudate putamen and cerebral cortex become the ischemic core and penumbra area, respectively [43,44].

Excessive intracellular Ca²⁺ influx plays an important role in neuronal cell death following cerebral ischemia [49]. For example, Cav2.1 channel mutant mice show a smaller infarct area than wild-type mice [45]. As mentioned previously, glutamate signaling is regulated by the Cav2.1 channel [13,14]. This suggests that the protective effects of Cav2.1 channel mutations against cerebral ischemia might be due to a disturbance in glutamate signaling. Many studies have suggested that the p38 MAPK pathway plays a central role in cerebral ischemia by facilitating inflammatory responses. In one such study, changes in the glutamate excretion threshold, via mutation of the Cav2.1 channel, induced abnormal glutamate receptor function and postsynaptic p38 MAPK signaling cascades (Figure 3) [50].

Using Cav2.1 channel antagonist for treatment has not been studied in clinical level. However, a study conducted on rats demonstrated that injecting Cav2.1 blocker has protective effect against ischemia [48]. Further studies are warranted.

Conclusion

VGCCs play important roles in neurotransmitter release, neuronal survival, and calcium-dependent gene transcription. Three major

presynaptic Cav2 channels, namely the Cav2.1, Cav2.2, and Cav2.3 channels, are involved in the mechanism underlying Ca²⁺-dependent excitotoxicity [10]. While the therapeutic value of the Cav2.2 and Cav2.3 channels has been reported, little is known about the potential of the Cav2.1 channel as a target for therapeutics.

Mutations in the Cav2.1 channel had a protective effect against kainate-induced seizures, TBI, and cerebral ischemia, suggesting that the Cav2.1 channel is coupled to kainate/AMPA receptor signaling. Cav2.1 channel mutations also conferred protection against TBI and cerebral ischemia, while ω -aga treatment was effective for TBI recovery [41].

Taken together, these data suggest that Cav2.1 channel inhibitors are likely to hold the key to successful brain injury therapies in the future.

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