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Editorial

Hyperexcitability in adult mice with severe deficiency in $Na_V1.2$ channels

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Abstract: Epilepsy is one of the most common neurological diseases. Epileptic individuals are faced with seizures, which are largely caused by enhanced neuronal excitability and/or decreased neuronal inhibitory activity. *SCN2A* encodes a neuronal voltage-gated sodium channel, $Na_V1.2$ that is primarily found in excitatory neurons throughout the brain. $Na_V1.2$ is most concentrated within the principal neurons of the corticostriatal circuit, which includes pyramidal neurons in the medial prefrontal cortex and medium spiny neurons in the striatum. In the early stage of adult development, the $Na_V1.2$ channel plays critical roles in generation and propagation of action potentials in these neurons. Gain of Function variants of *SCN2A* results in unprovoked seizures and epilepsy, while loss-of-function variants of *SCN2A* is a leading cause for autism spectrum disorder as well as intellectual disability. Previous studies have shown that full deletion of *Scn2a* gene in mice is lethal and partial disruption of *Scn2a* gene (less than 50%) leads to inhibition of neuronal excitability. A recent study from Dr. Yang's laboratory revealed an unexpected result from mice with severe $Na_V1.2$ deficiency and they demonstrated that severe deletion of *Scn2a* gene (around 68% gene disruption) in $Na_V1.2$ triggers neuronal hyperexcitability in adult mice. Their findings may explain the puzzling clinical observation that certain individuals with $Na_V1.2$ deficiency still develop unprovoked seizure. With the knowledge that using sodium-channel blockers simply exacerbates the seizure, the need for understanding the intrinsic nature of the $Na_V1.2$ channel provides an important research topic in the future.

Keywords: $Na_V1.2$, epilepsy, neuronal excitability, Scn2a, voltage-gated potassium channel

Introduction

Voltage-gated sodium channels (VGSCs) are essential in generating action potentials (APs) in excitable cells such as neurons [1, 2]. These VGSCs are composed of α and β subunits [1, 2]. The core protein of the voltage-gated sodium channel (VGSC) is the α subunit that comprises the functional centers of VGSC and consists of four highly similar transmembrane domains [2]. VGSC α subunits reveal all the machinery responsible for channel cell surface expression, ion conduction, voltage sensing, gating, and inactivation [1, 2]. However, VGSC β subunits present unique gating mechanisms, regulate cellular excitability, affect brain development, reveal distinct channel pharmacology, and have functions that are independent of the α subunits [1, 2]. In humans, the four variations of the α subunits in $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.8$ are encoded by the *Scn1a, 2a, 3a, and 8a genes*, respectively [3]. Each of the VGSC has an adult and neonatal isoform created through alternative splicing of exon 5 [1, 2]. The influx of sodium ions through the integral membrane proteins of the channel causes the initiation of an action potential (AP) [2]. $Na_V1.2$, specifically, is highly expressed in medium spiny neurons (MSNs) located in the caudate nucleus and the putamen (CPu) in the striatum [4]. Conventionally, mutations that cause a loss of function (LoF) are associated with a loss of excitability in diseases such as autism spectrum disorder, whereas a gain of function (GoF) is associated with an increase of excitability in epilepsy [5]. Mutations causing LoF are associated with mutations in the N-terminal amino acid residues leading to suppression of the VGSC [6]. However, contrary to normal belief, severe deficiency in $Na_V1.2$ can lead to incre-

ased neuronal excitability in certain studies [7]. Studies have shown that the homozygous *Scn2a*$^{-/-}$ *mice died early [8, 9]; while heterozy*gous *Scn2a*$^{+/-}$ mice with 50% expression of *Scn2a* lived till adulthood [8] and revealed seizures in some adult mice [6]. Studies also showed unchanged APs and reduced excitability in pyramidal neurons recorded from brain slices of prefrontal cortex (PFC) in heterozygous adult mice [9]. 50% deletion of *Scn2a* gene in adult mice is associated with decreased neuronal excitability and does not always lead to an altered phenotype causing seizures [9]. Therefore, a severe disruption of *Scn2a* in $Na_V1.2$ might explain the change of neuronal excitabilities in epilepsy and certain phenotypes associated with GoF mutants in principal neurons of the corticostriatal circuit.

Severe deficiency in $Na_V1.2$ channels triggers hyperexcitability in adult mice

A recent study reported in *Cell Reports* from Dr. Yang's laboratory suggested that a severe deficiency of *Scn2a* gene in mouse results in a counterintuitive increase in neuronal excitability, leading to seizures and epilepsy [10]. Using a gene trap (gt) approach, they generated a mouse line with severe deficiency of *Scn2a* gene (around 68% disruption). The caudate nucleus and CPu are the most important brain regions that are related to *Scn2a* absence causing seizures [11]. Unexpectedly, they found a significantly increased neuronal excitability in striatal principal MSNs from the gt mouse. AP firing was increased in the MSNs from gt mice compared to neurons from wild-type (WT) mice along with a more depolarized resting membrane potential (RMP). They also detected a higher voltage threshold, reduced AP amplitude, increased fast after-hyperpolarization (AHP), and elevated half-width values in MSNs from *Scn2a* gt mice. All characteristics point towards an enhanced neuronal excitability. They then examined if changed RMP is, in fact, a determining factor in hyperexcitability and found that even with the fixed membrane potential (MP), increase of excitability was detected along with altered AP waveform in gt mice. Their results suggest that severe $Na_V1.2$ deficiency results in increased neuronal excitability in adult mice, which is accompanied by a higher voltage threshold in striatal MSNs.

Further, they found that severe *Scn2a* disruption mice also contain a rescue element that allows for restoration of *Scn2a* expression. Via a "tm1a" cassette flanked by Frt sites, it is possible to restore *Scn2a* expression via an adeno-associated virus (AAV) delivery of codon-optimized Flp (FlpO). AAV-FlpO transduction was found to have a reduced LacZ signal as well as a significant elevation of $Na_V1.2$ protein expression. Moreover, transduction also allowed for significant elevation in $Na_V1.2$ channel expression. The severe *Scn2a* gene deficiency mice transduced with AAV-FlpO were found to have decreased excitability of striatal MSNs. Moreover, within the pyramidal neurons of the mPFC the excitability was able to be reversed by FlpO restoration of the *Scn2a* gene. To differentiate whether the hyperexcitability was due in part to intrinsic properties or result from a disrupted circuit, an AAV injection to a few MSNs was performed, and transduced neurons had a greatly reduced excitability in comparison to non-transduced neurons in the same brain slices. The results demonstrate that the $Na_V1.2$ hyperexcitability is due to the intrinsic nature of the neurons rather than its surrounding environment.

VGSCs are highly localized in brain neurons and are important influencers of neuronal excitability [1, 2]. It is, therefore, that they have been shown to play a critical role in the genesis and alleviation of epilepsy [2, 6]. Genetic studies on patients with epilepsy have identified more than 700 mutations among the genes that encode for VGSCs attesting to their role in pathogenesis [12, 13]. By using RNA-sequencing, about 900 genes were severely impacted in the *Scn2a* gt mice in comparison to their WT littermates [10]. They also found that the expression of *Scn2a* gene was indeed reduced to around 32% in severe $Na_V1.2$ deficiency mice [10]. $Na_V1.6$ and $Na_V1.2$ are two major sodium channels that are thought to have a compensatory relationship thus if one's expression decreases the other will increase [11]. For example, an upregulation of $Na_V1.2$ was found in $Na_V1.6$-deficent mouse model, indicating a compensatory relationship [12]. Surprisingly, they found a slightly decreased expression of $Na_V1.6$ in *Scn2a* gt mice, which did not reveal statistical significance [10]. However, results from the RNA-sequence analysis, qPCR, and whole-cell voltage-clamp record-

ings of the channel currents were all pointing towards a profound downregulation of multiple potassium channels including $K_V1.1$ and $K_V1.2$ in the MSNs with severe $Na_V1.2$ deficiency. By using pimaric acid (PiMA), a non-selective potassium channel opener such as $K_V1.1$ to $K_V2.1$, on gt mice, they found that MSNs had their excitability returned to normal levels as seen in WT MSNs. Further, they used a selective $K_V1.1$ opener, called 4-trifluoromethyl-L-phenylglycine (4TFMPG). Interestingly, different from PiMA, 4TFMPG did not restore the input resistance, AP voltage threshold, amplitude, AHP, or half-width values in MSNs from gt mice. Lastly, there was an increase in $K_V1.1$ and $K_V1.2$ expression due to treatment by FlpO causing some restoration of the *Scn2a* gene, which proves that the potassium channels are influenced by $Na_V1.2$ expression. Collectively, these data suggest that brain neurons have a dynamic adaptation mechanism to regulate gene expression in response to the changes of *Scn2a* expression level, and downregulation of potassium channels contributes to enhanced neural excitability in severe $Na_V1.2$ deficient mice.

Perspective

Studies from Dr. Yang's laboratory pointed out that the paradoxical nature of severe $Na_V1.2$ deficiency results in hyperexcitability of principal MSNs in the striatum and pyramidal neurons in the mPFC, commonly associated with GoF mutations within the *Scn2a* gene [10]. A moderated deficiency of *Scn2a* gene (around 50% gene deletion) results in the traditional association with decreased neuronal excitability [8], while a severe deficiency of *Scn2a* gene (around 68% disruption) diverges from the traditional thought-process by inducing hyperexcitability, increasing the chances of seizures and epilepsy [10]. Na_V channels include isoforms of $Na_V1.1$-1.6, therefore, tissue specific hyperexcitability by different Na_V isoform should be investigated in the future. For example, $Na_V1.4$ is widely present in skeletal muscle whereas $Na_V1.6$ is highly expressed in the central nervous system [2]. Future research might determine the relative susceptibility of the hyperexcitability of various tissues containing different isoforms of VGSCs such as $Na_V1.6$. Through RNA editing, tissue-specific isoforms of the sodium channel were created via site-directed mutagenesis that was performed using the altered sites II in vitro mutagenesis system [14]. Using this approach, 9 different Na_V channels were able to be replicated for functional analysis, all differ in terms of their respective α subunit [14]. To differentiate the different variants based on their different gating properties, various electrophysiological properties of the channels could be detected such as the voltage dependence of activation and steady-state inactivation. For future research, the approach outlined by the creation of these Na_V channels can be used to determine whether the specificity of the intrinsic nature of the channels may play any roles. Employing these tissue-specific Na_V channels and pairing them with *Scn2a* gene disruption procedures can determine which isoforms might be involved in hyperexcitability, and thus might contribute to the late-onset seizures as seen in severe deficiency of the *Scn2a* gene. Complete LoF mutations in $Na_V1.1$ cause severe myoclonic epilepsy of infancy [15]; thus, the epileptic events that were seen in severe deficiencies of *Scn2a* gene could be explained because of the gradual gradient nature of the associated symptoms of the increased mutation of the $Na_V1.1$ channel. Moreover, the first group of Na_V channel isoform genes including $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.7$, on chromosome 2 in humans and mice are sensitive to tetrodotoxin (TTX). The usage of TTX has effectively contributed to suppression of seizures in low-dose prescriptions, via inhibition of polysynaptic activity and/or antidromic firing [16]. However, the second group of genes including $Na_V1.5$, $Na_V1.8$, and $Na_V1.9$, present on chromosome 3p21-24, are TTX-resistant channels [1, 2]. How to determine the second group of Na_V channels in the hyperexcitability should be studied in the future. Using fibroblast growth factor homologous factors (FHFs) which can bind to intracellular sites of VGSCs, it was demonstrated that the voltage dependence of the channel was able to be increased, allowing for a slower rate of inactivation [17]. Therefore, there is a possibility to reverse or engineer the inactivation of the Na_V channels via the FHFs. Addressing severe deficiency of VGSCs, the FHFs-Na_V channel complex binding could be structurally analyzed to see which domain of the Na_V channel is responsible for the binding. Structural analysis can provide insights into design of various drugs and pharmaceutical agents for more

effective binding, which might treat the diseases with the severe deficiency of $Na_V1.2$ that is seen within the late-onset seizures and epilepsy that has not been seen traditionally. One of the key findings in the present studies [10] was the fact that the compensatory reduction in potassium channel expression or current could be the underlying mechanism as to why the paradoxical hyperexcitability of neurons in $Na_V1.2$ severe deficient mice can be seen [10]. Thus, pharmaceutical modulation of certain potassium channels could be an effective strategy for the severe $Na_V1.2$ deficiency. The *Kcna1* and *Kcna2* channels have been established as a major regulator of neuronal excitability within the central and peripheral nervous systems [1-3]. One of the common conditions associated with down-regulation of the *Kcna1* channel is episodic ataxia type 1 which can be characterized by spastic contractions of the head, legs, and arms. Previous study has suggested that acetazolamide as a carbonic anhydrase inhibitor for treatment of glaucoma has been found in usage for channelopathies such as episodic ataxia type 2, in which there is a positive modulation of potassium channel [18]. Therefore, future research for regulation of the associated hyperexcitability via acetazolamide and other drugs for certain potassium channels that aim to bring neuronal excitability back to normal might be an effective strategy.

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Disclosure of conflict of interest

None.

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