

Current Literature

In Basic Science



Voltage-Gated Ion Channel Accessory Subunits: Sodium, Potassium, or Both?

The Sodium Channel Accessory Subunit Navβ1 Regulates Neuronal Excitability through Modulation of Repolarizing Voltage-Gated K⁺ Channels.

Marionneau C, Carrasquillo Y, Norris AJ, Townsend RR, Isom LL, Link AJ, Nerbonne JM. *J Neurosci* 2012;32:5716–5727.

The channel pore-forming α subunit Kv4.2 is a major constituent of A-type (I_A) potassium currents and a key regulator of neuronal membrane excitability. Multiple mechanisms regulate the properties, subcellular targeting, and cell-surface expression of Kv4.2-encoded channels. In the present study, shotgun proteomic analyses of immunoprecipitated mouse brain Kv4.2 channel complexes unexpectedly identified the voltage-gated Na⁺ channel accessory subunit Navβ1. Voltage-clamp and current-clamp recordings revealed that knockdown of Navβ1 decreases I_A densities in isolated cortical neurons and that action potential waveforms are prolonged and repetitive firing is increased in *Scn1b*-null cortical pyramidal neurons lacking Navβ1. Biochemical and voltage-clamp experiments further demonstrated that Navβ1 interacts with and increases the stability of the heterologously expressed Kv4.2 protein, resulting in greater total and cell-surface Kv4.2 protein expression and in larger Kv4.2-encoded current densities. Together, the results presented here identify Navβ1 as a component of native neuronal Kv4.2-encoded I_A channel complexes and a novel regulator of I_A channel densities and neuronal excitability.

Commentary

The voltage-gated potassium channel subunit Kv4.2 is a pore-forming α subunit of A-type potassium channels. Kv4.2, encoded by the *KCND2* gene, is broadly expressed in the central nervous system and is predominantly localized on neuronal dendrites, with increased density on distal dendrites (1). A-type potassium channels are important regulators of neuronal excitability, modulating the resting membrane potential, action potential repolarization, repetitive firing rates and backpropagation of action potentials. Dynamic regulation and trafficking of A-type channels is also thought to contribute to synaptic plasticity and remodeling (2). Alterations in Kv4.2 expression and A-type potassium current have been observed in various rodent models of epilepsy and in tissue from temporal lobe epilepsy patients (3–5). Ion channels are regulated by interactions with various accessory proteins that can influence expression, trafficking, subcellular localization, stabilization, and biophysical properties of the channel. Several accessory subunits that interact with Kv4.2 have been identified, including Kvβ1, K⁺ Channel Interacting Proteins (KChIPs) and dipeptidyl peptidase-like proteins (DPP) (2, 6).

In the present study, Marionneau and colleagues used co-immunoprecipitation and mass spectrometry to identify additional proteins associated with native neuronal Kv4.2

complexes. They used an in-solution approach coupled with 2-dimensional liquid chromatography-tandem mass spectrometry, also known as multi-dimensional protein identification technology (MudPIT). This highly sensitive, unbiased approach enables the identification of additional binding partners that may not be observed by traditional gel-based proteomic approaches. One of the proteins they identified in neuronal Kv4.2 complexes was Navβ1, a previously identified accessory subunit of the voltage-gated sodium channel (Nav) complex. Navβ1, encoded by the *Scn1b* gene, is a multifunctional subunit that is known to act both as a cell adhesion molecule (CAM) and a modulator of Nav channel cell surface expression, kinetics and voltage-dependence (7). Although co-immunoprecipitation of Navβ1 from native neuronal Kv4.2 complexes may seem unexpected, there was suggestive evidence for interaction between Kv4.2 and Navβ1 from previous studies focused on cardiac potassium channel complexes. Deschenes and colleagues had previously demonstrated co-immunoprecipitation of Navβ1 and Kv4.3 from transiently transfected HEK-293 cells (8) and from native Kv4.2 and Kv4.3 complexes in neonatal rat ventricular myocytes (9).

To define the functional contribution of Navβ1 to Kv4.2 channel complexes, Marionneau and colleagues first performed a series of experiments in a heterologous expression system. Using whole-cell voltage-clamp recordings, they demonstrated that co-expression of Kv4.2 with Navβ1 significantly increased potassium current density compared to Kv4.2 alone. This was consistent with previous results showing that co-expression of Navβ1 with Kv4.3 resulted in increased current

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density in HEK293 cells (8). Upregulation of A-type potassium current density by Nav β 1, would be predicted to reduce cellular excitability. To determine the underlying mechanism, they performed a series of biochemical experiments. These experiments showed that co-expression with Nav β 1 increased the level of total and cell surface Kv4.2 protein by stabilizing the intracellular pool of Kv4.2, without influencing cell surface turnover. This greater availability of Kv4.2 results in more channel being inserted in the cell membrane, which in turn leads to decreased excitability.

To determine if Nav β 1 regulates native neuronal Kv4-encoded A-type potassium current, they used a shRNA approach to knockdown Nav β 1 in cultured cortical neurons. Acute knockdown of Nav β 1 resulted in reduced A-type potassium current without any change in the kinetics or voltage-dependence, consistent with decreased cell surface expression of A-type potassium channels. This was in agreement with previous results that demonstrated decreased A-type potassium currents following knockdown of Nav β 1 in rat neonatal ventricular myocytes (9). These results demonstrate that acute loss of Nav β 1 results in decreased A-type potassium current, most likely due to decreased cell surface expression of Kv4.2. This would be predicted to result in impaired membrane repolarization and increased neuronal excitability, particularly under conditions of repetitive stimulation.

Mutations in *Scn1b* have been identified in human epilepsy patients with GEFs+ and Dravet syndrome (7). Chronic loss of Nav β 1 in *Scn1b*^{-/-} knockout mice results in an epilepsy phenotype that shares features of human Dravet syndrome (7). To determine the potential physiological consequences of disruption of Nav β 1-Kv4 channel complexes, Marionneau and colleagues performed current clamp recordings of cortical pyramidal neurons from *Scn1b*^{-/-} mice. The *Scn1b*^{-/-} neurons exhibited impaired membrane repolarization as evidenced by significantly greater mean action potential decay time and widths compared to wild type. These results are similar to the observed effects of blocking A-type potassium channels (10). In response to prolonged stimulation, cortical neurons from mutant mice were hyperexcitable, exhibiting a significantly greater number of action potentials than did wild-type neurons. Interestingly, in these studies, cortical pyramidal neurons from *Scn1b*^{-/-} mice did not exhibit features indicative of a major defect in sodium currents, suggesting that decreased A-type potassium current in cortical pyramidal neurons may contribute to increased excitability and seizures in this mouse model. However, it is not clear whether this is a direct effect of loss of Nav β 1 or a secondary effect of seizures in the *Scn1b*^{-/-} mice prior to slice isolation, as alterations in Kv4.2 transcript and A-type potassium current

have been observed following seizures in other rodent models (3, 4). Additional studies will be necessary to discriminate between these possibilities.

Ion channels function in macromolecular complexes with a large number of associated proteins. Thus, the downstream consequences of ion channel subunit dysfunction or deficiency are likely to be multifold and may affect multiple neuronal currents. Application of highly sensitive proteomic approaches to additional ion channels will give us a more complete picture of the molecules present in native channel complexes and improve our understanding of neuronal excitability. Further, examining the consequences of ion channel mutations on multiple neuronal currents may provide insight into the complex changes underlying increased excitability and epileptogenesis.

by Jennifer Kearney, PhD

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