The voltage-gated potassium channel subunit Kv4.2 is a pore-forming α subunit 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 Nav1. 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.

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


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...
density in HEK293 cells (8). Upregulation of A-type potassium current density by Navβ1, 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 Navβ1 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 channels being inserted in the cell membrane, which in turn leads to decreased excitability.

To determine if Navβ1 regulates native neuronal Kv4-encoded A-type potassium current, they used a shRNA approach to knockdown Navβ1 in cultured cortical neurons. Acute knockdown of Navβ1 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 Navβ1 in rat neonatal ventricular myocytes (9). These results demonstrate that acute loss of Navβ1 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 Navβ1 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 Navβ1-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 Navβ1 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

References

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGF) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGF or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships
   Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today’s Date: 3/28/12

2. First Name Jennifer Last Name Kearney Degree Ph.D.

3. Are you the Main Assigned Author? ☑ Yes ☐ No

If no, enter your name as co-author:

4. Manuscript/Article Title: Voltage-gated ion channel accessory subunits: sodium, potassium, or both? and A Mutation Hot-Spot for Benign Infantile Epilepsy

5. Journal Issue you are submitting for: 13.1

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.
**Section #3 Relevant financial activities outside the submitted work.**  
Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td></td>
<td></td>
<td>X</td>
<td>Gilead Sciences</td>
<td>Investigator-initiated grant (role: Co-I)</td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>Gilead Sciences</td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.  
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

**Section #4 Other relationships**  
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- [ ] No other relationships/conditions/circumstances that present a potential conflict of interest.  
- [ ] Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.  
*Epilepsy Currents* Editorial Board