Mainstream- Fringe- and Patho- Physiology of Voltage Dependent Na\(^+\) (Na\(_v\)) Channels

Alon Meir, Ph.D.

Voltage gated Na\(^+\) (Na\(_v\)) channels mediate action potential genesis and propagation in most excitable cells. Other roles played by Na\(_v\) channels in both excitable but also in non-excitable cells highlight their role as Na\(^+\) influx mediators in response to stimuli other than strong and brief depolarization of the cell’s membrane.

The lipid membrane that surrounds the cell is impermeable to charged ions that are unequally distributed across it and this unequal distribution of charged particles generates polarization of the membrane potential (more negative inside). Ion channels are proteins that span the membrane and form channels through which these ions can pass and generate ionic electrical currents. Most channels exist in either an open or shut conformation and the transition between these two main conformations is highly controlled. Voltage sensitive channels respond to changes in membrane potential by transition to a conducting conformation.\(^1\)

Voltage dependent Na\(^+\) (Na\(_v\)) channels are sensitive molecular devices that respond to membrane depolarization with a transient Na\(^+\) influx.\(^2\) This activity switches on the action potential (AP), the principal neuronal and muscular electrical signal.\(^3\) This review focuses on Na\(_v\) channels and their role in other cellular process ranging from neuronal modulation to cancer.

Structure, Nomenclature and Distribution of Na\(_v\) Channels

Na\(_v\) channels belong to the larger protein superfamily of voltage dependent channels that also include the K\(_v\) and Ca\(_v\) channels.\(^3\) The archetypical voltage sensing K\(_v\) channel is formed by tetramerization of similar pore forming subunits and is also true for bacterial Na\(^+\) channels.\(^4\) However, in higher organisms, Na\(_v\) genes encode a protein with four similar domains, each corresponding to a single subunit in the K\(_v\) channel tetramer.\(^1\) Nine related genes encoding Na\(_v\) channels have been identified in mammals, all forming one protein family (See Table).\(^1\) In addition, a structurally similar gene corresponding to a Na\(^+\) activated channel (Na\(_X\)) which lacks the voltage sensor element, might form a second subfamily within Na\(_v\).\(^5\) However this will not be discussed in this review.

Na\(_v\) channels are expressed primarily in excitable

<table>
<thead>
<tr>
<th>Channel</th>
<th>Gene</th>
<th>Chromosome</th>
<th>TTX sensitivity</th>
<th>Main tissue distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(_v)1</td>
<td>SCN1A</td>
<td>2q23-24</td>
<td>Yes</td>
<td>CNS</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN2A</td>
<td>2q23-24</td>
<td>Yes</td>
<td>CNS (embryonic)</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN3A</td>
<td>2q23-24</td>
<td>Yes</td>
<td>CNS (embryonic)</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN4A</td>
<td>17q23-25</td>
<td>Yes</td>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN5A</td>
<td>3p21-24</td>
<td>No</td>
<td>Heart muscle</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN8A</td>
<td>12q13</td>
<td>Yes</td>
<td>CNS (neuron, glia), PNS</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN9A</td>
<td>2q23-24</td>
<td>Yes</td>
<td>PNS (neuron, Schwann), endocrine cells</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN10A</td>
<td>3p21-24</td>
<td>No</td>
<td>PNS</td>
</tr>
<tr>
<td>Na(_v)1</td>
<td>SCN11A</td>
<td>3p21-24</td>
<td>No</td>
<td>PNS</td>
</tr>
</tbody>
</table>

CNS = central nervous system, PNS = peripheral nervous system.
tissues (nervous and muscle), with certain isoform-tissue/area specificity (See Table).\textsuperscript{2,3} This point is nicely illustrated by the detection of the cardiac isoform Na\textsubscript{1,5} in human embryonic stem cell derived cardiomyocytes.\textsuperscript{2} However, recent findings demonstrate that neuronal Na\textsubscript{1,1} and Na\textsubscript{1,3} channels control the pacing of the mouse heart\textsuperscript{4,5} and that the cardiac isoform Na\textsubscript{1,5} is expressed in limbic regions of the rat brain.\textsuperscript{2,3} In addition, several reports describe the expression of Na\textsubscript{a} channels in non-excitable cells.\textsuperscript{2,3} This demonstrates that the tissue specificity of Na\textsubscript{a} channels is a broad observation, but important deviations from this pattern exist.

The nine human Na\textsubscript{a} channel genes are clustered on different chromosomes. Apparently structural, functional and pharmacological characteristics separate the channels encoded on chromosome 3 from all the others. This is manifested mainly by Tetrodotoxin (TTX) sensitivity demonstrated by all but the Na\textsubscript{1,5}, 1.8 and 1.9 isoforms (See Table).\textsuperscript{2,3}

### Na\textsubscript{a} Channels as AP Initiators

Depolarization of the resting membrane potential is caused by the accumulation of positive charges on the inner interface of the plasma membrane. Thus, Na\textsubscript{a} inflow results in membrane depolarization. This positively charged ion influx causes further depolarization of the membrane, which activates neighboring channels. This mechanism forms a positive feedback loop that lies at the heart of membrane excitability, as it forms the rising phase of most APs.\textsuperscript{1} AP is a regenerative voltage spike, traveling along the cell membrane, by means of ionic currents and activation of voltage dependent ion channels (K\textsubscript{v}, Na\textsubscript{a}, and Ca\textsubscript{2+}, channels, co-localized with Na\textsubscript{a}, repolarize the cells’ membrane, terminating the signal). In neurons, AP travel rapidly for long distances along axons, transmitting cellular coded information.

Thus, most of the nine human genes encoding Na\textsubscript{a} channels participate in AP generation in neurons and muscle cells.\textsuperscript{2,3} The combination of Na\textsubscript{a} channel isoforms expressed by an excitable cell determines parameters such as AP firing frequency, which are in the basis of neuronal coding. Indeed, different cell types express different combinations of Na\textsubscript{a} channels (See Table) and alterations in such expression patterns occur in different pathophysiological conditions. For example, changes in the levels of functional Na\textsubscript{a} channel expression forms the basis of changes in conduction properties of DRG neurons associated with chronic pain phenomena.\textsuperscript{4} Recently, it was shown that the ubiquitin system participates in the regulation of Na\textsubscript{1,5} channel functional membrane expression in the heart,\textsuperscript{14} adding another dimension to the regulation of cardiac excitability.

---

**Expression of Na\textsubscript{1,1} in Mouse Cerebellum**

Immunohistochemical staining of Na\textsubscript{1,1} channel with Anti-Na\textsubscript{1,1} antibody (ASC-001) in mouse cerebellum. (A) The distribution of Na\textsubscript{1,1} (red) forms a band (arrow) in the molecular layer (Mol), close to the Purkinje cell bodies. (B) Purkinje nerve cells are stained with mouse anti Parvalbumin (green). (C) Confocal merge of Na\textsubscript{1,1} and Parvalbumin.

**Expression of Na\textsubscript{1,2} in Mouse Hippocampus**

Immunohistochemical staining of Na\textsubscript{1,2} channel with Anti-Na\textsubscript{1,2} antibody (ASC-002) in mouse hippocampus. (A) Na\textsubscript{1,2} channel (red) in dendrites of pyramidal neurons in the CA3 region. (B) staining of Purkinjne nerve cells with mouse anti Parvalbumin (green) demonstrates the restriction of Na\textsubscript{1,2} to dendrites (arrows) outside the pyramidal layer (P). (C) Confocal merge of Na\textsubscript{1,2} and Parvalbumin.

**Expression of Na\textsubscript{1,3} in Rat Brain**

Immunohistochemical staining of Na\textsubscript{1,3} channel in rat brain using Anti-Na\textsubscript{1,3} antibody (ASC-004). Na\textsubscript{1,3} was visualized with immuno-peroxidase methods and final brown-black diaminobenzidine color product. There was strong staining of some axonal groups such as the mossy fibers in hippocampus (A) and cortico-striatal fibers (B).

---

**Western blotting of rat newborn brain membranes:**

1. Anti-Na\textsubscript{1,1} antibody (ASC-001) (1:200).
2. Anti-Na\textsubscript{1,1} antibody, preincubated with the control peptide antigen.

**Western blotting of rat brain membranes:**

1. Anti-Na\textsubscript{1,2} antibody (ASC-002) (1:200).
2. Anti-Na\textsubscript{1,2} antibody, preincubated with the control peptide antigen.

**Western blotting of rat newborn brain membranes:**

1. Anti-Na\textsubscript{1,3} antibody (ASC-004) (1:200).
2. Anti-Na\textsubscript{1,3} antibody, preincubated with the control peptide antigen.
Na+

Channel Influxes Near the Resting Membrane Potential and in Non-Excitable Cells

Usually Na+ channel influxes are brief and transient, generally activating within 1ms after depolarization and terminating within 10 ms (while the stimulus is still on, a process called inactivation). Thus, AP arise from the brief activity of Na+ channels, which respond to the extreme depolarization generated by AP. Nevertheless, several mechanisms and Na+ channels may give rise to different activities, when the membrane voltage is at rest or during mild depolarization. This corresponds both to neuronal excitability and to the emerging role of Na+ channels in non-excitable tissue physiology and pathophysiology. Both of these roles are briefly discussed below.

Expression of Na+1.5 in Rat Heart Cardiomyocytes

Immunohistochemical staining of Na+1.5 channel with Anti-Na+1.5 antibody (#ASC-005) in rat heart. Transversal section of the ventricular wall in the epicardium area also shows a big artery and myocardial area. DAB product is brown and counterstaining is Cresyl Violet. (A) Shows strong staining of myocytes (green arrow), no staining is evident in smooth muscle (red arrow), endothelium (black arrow), connective tissue (fibroblasts) (yellow arrow) or epicardial epithelium (blue arrows). (B) Enlargement of (A).

Expression of Na+1.8 in Subpopulations of DRG Neurons

Immunohistochemical staining of Na+1.8 channel in adult rat dorsal root ganglion (DRG) with Anti-Na+1.8 antibody (#ASC-016). Na+1.8 staining (red) was cytoplasmic and the intensity varied among DRG cells. There was a partial overlap in the distribution of Na+1.8 and neurofilament 200 (green).

Neuronal modulation by Na+ channels at rest and mild depolarization may arise from voltage dependent kinetic properties of channels such as inactivation, persistence and resurgence. These phenomena control the availability of Na+ channels before and after an AP has passed, thus, influencing the probability of an AP to emerge. Na+ channel isoforms differ in the fine-tuning of their kinetic properties. Differences may arise from intrinsic properties, or from the ability to interact with other factors. The expression patterns of the different isoforms gives rise to a large diversity of neurons with different AP firing behavior. Indeed, long lasting changes in expression patterns of Na+ channels under pathological conditions for example, is strongly correlated with changes in such coding capabilities of neurons.

Neuronal modulation may also arise from activation of channels by means other than voltage sensitivity. In mouse DRG neurons, Na+1.9 currents were found to be up-regulated by the inflammatory agent prostaglandin E2, in a G-protein dependent manner. Another important example is the activation of the TTX-resistant Na+1.9 channel by neurotrophic factors (NT). This was shown in hippocampal neurons, where BDNF via TrkB receptors and Na+1.9 channels activated a long lasting Na+ current (about 200 ms) and a similar current was fourfold longer in neuroblastoma cells. In frog sympathetic neurons, NGF upregulated both TTX-sensitive and TTX-resistant Na+ currents. On the other hand, NGF and GDNF reduced the Na+1.3 expression that accompanied DRG axotomy. These interactions may contribute to pain sensation and pathophysiology, with differential specific interactions between sets of NTs and Na+ channels. Such interactions lie at the base of differential analgesic effects of different NTs, mediated by regulation of Na+ channels in the peripheral nervous system.

Na+1.9 Expression in DRG

Immunohistochemical staining of Na+1.9 channel in rat dorsal root ganglion (DRG) with Anti-Na+1.9 antibody (#ASC-017). Cells within the DRG were stained (see solid line frame enlarged in (B)) as well as fibers and the area of entry of dorsal root into spinal cord (see dashed line frame enlarged in (C)). The counterstain in (B) and (C) is DAPI, a fluorescent dye visualized in the UV range.

Western blotting of rat DRG lysates:

1. Anti-Na+1.9 antibody (#ASC-017) (1:200).
2. Anti-Na+1.9 antibody, preincubated with the control peptide antigen.
All these subthreshold activities may be the link and explanation for the expression of these highly voltage sensitive channels in non-excitable cells that do not fire AP.

Ion channel activity is an important factor in volume regulation and the associated motility of cells. A related mechanism is suggested for the ability to migrate, acquired by transformed glial cells, on their way to spread brain cancer.  

Several Na⁺ channels were reported to be upregulated in prostate cancer cell lines. Interestingly, the magnitude of such Na⁺ current was shown to be dependent on environmental factors that are present in the cell culture serum.  

Several Naᵥ channels were reported to be upregulated in prostate cancer cell lines. 12, 27, 28 Interestingly, the magnitude of such Naᵥ current was shown to be dependent on environmental factors that are present in the cell culture serum. 29

The role played by Naᵥ channels, is suggested by the positive correlation between the rate of invasiveness and the level of Naᵥ current or expression. 12, 27, 28 Most importantly, TTX, a highly specific (for Naᵥ, but not very selective between the six sensitive isoforms), Naᵥ channel blocker, reduced these cells' invasiveness. 29 A possible role for Naᵥ channels in volume regulation was demonstrated in Jurkat T cells, where Saxitoxin (STX) sensitive Naᵥ channels play a role in apoptosis related cell shrinkage and death. 30, 31 In addition, the Naᵥ auxiliary subunit β3 (SCNB3) was found to be up-regulated in cancer cell lines in a p53 dependent manner, suggesting a role for Naᵥ channels in induced apoptosis of cancer cells. 32

These observations highlight the notion that voltage activated channels might be expressed in non-excitable cells, possibly under extreme cellular conditions.

### Naᵥ Channelopathies

Involvement of Naᵥ channels in pathophysiological conditions may arise either from genetic alterations in channel activity or from changes in functional expression levels.

Mutations in coding for Naᵥ channel proteins cause of several hereditary neuronal, 33 cardiac, 34 and muscular 35 diseases. In addition, the mutation may occur de-novo (neither parent

### QX-314 - A Local Anesthetic Blocker of Naᵥ Channels

![Dose response of QX-314 (9Q-150) on inward currents in Xenopus oocytes injected with Naᵥ 1.5 mRNA. Using two electrode voltage clamps, oocyte membrane potential was held at -100 mV and currents were elicited by 25 ms depolarization to -20 mV, delivered every 5 seconds. Traces before (blue) and during bath application of different concentrations of QX-314 indicated with arrow.](image)

Enhancement of Naᵥ 1.5 channel currents by increasing concentrations of ATX-II (A-700). Currents were recorded from Xenopus oocytes injected with Naᵥ 1.5 mRNA and were elicited every 10 seconds from holding potential of -100 mV, by 50 ms pulses to -20 mV.  

Top: Superimposed Naᵥ 1.5 current responses in control conditions (red) and during applications of increasing concentrations of ATX-II up to 1µM (lowest trace).  

Bottom: Dose response of Naᵥ currents along their typical waveform. Note that the current enhancement is greater once the channel is inactivated.

### ATX-II Potently Enhances Naᵥ Currents by Reducing Inactivation

![Enhancement of Naᵥ 1.5 channel currents by increasing concentrations of ATX-II (A-700). Currents were recorded from Xenopus oocytes injected with Naᵥ 1.5 mRNA and were elicited every 10 seconds from holding potential of -100 mV, by 50 ms pulses to -20 mV.](image)

Enhancement of Naᵥ 1.5 channel currents by increasing concentrations of ATX-II (A-700). Currents were recorded from Xenopus oocytes injected with Naᵥ 1.5 mRNA and were elicited every 10 seconds from holding potential of -100 mV, by 50 ms pulses to -20 mV.

Top: Superimposed Naᵥ current responses in control conditions (red) and during applications of increasing concentrations of ATX-II up to 1µM (lowest trace).  

Bottom: Dose response of Naᵥ currents along their typical waveform. Note that the current enhancement is greater once the channel is inactivated.

### Specific Expression of Naᵥ 1.6 and Naᵥ 1.7 in Smooth Muscle Cells

![Immunocytochemical staining of murine portal vein smooth muscle cells for Naᵥ 1.6 and Naᵥ 1.7 voltage-dependent Na⁺ channels.](image)

Fluorescence images of a single confocal plane of the cell labelled with (A) Anti-Naᵥ 1.6 antibody (#ASC-009), (B) Anti-Naᵥ 1.7 antibody (#ASC-008). (Incubation with antigen peptide eliminated staining, data not shown). Calibration bar: 10 µm. (C) Summary data on intensity of fluorescence, expressed as average pixel fluorescence. The values of all pixels in the cell’s confocal plane were added up and then divided by the number of pixels (gray). The specificity of labeling was confirmed for both antibodies by greatly reduced fluorescence after pre-incubation with respective antigenic peptide (dark gray) or by virtual lack of fluorescence in the absence of primary antibodies (white). *statistically significant.

**Acknowledgements:** Data were generated by Vladimír Pucovský in association with Sohag Saleh, S.Y.M. Yeung, Sally Prestwich and Iain Greenwood, St. George’s Hospital Medical School, London, UK.
Modulator v (A-400). Holding v v v v v v v v 1

44 kinetics, respectively. Many venoms from different animals contain peptides that are Na<sub>v</sub> channel activators. Scorpion venoms are the largest source described so far. Both α and β scorpion toxins bind to extracellular moieties on domain IV and II of the channel to slow inactivation or enhance activation kinetics, respectively. Sea anemone Na<sub>v</sub> toxins such as Anthopleurin-C, APE 1-2<sup>45</sup> or ATX II<sup>45</sup> bind to the α scorpion toxin binding site and exert similar effects on inactivation.<sup>4</sup> Two small peptide toxins found in wasp venom, α and β-Pompidotoxin, and promote Na<sub>v</sub> currents by slowing the inactivation process. Such toxins are used to mimic, or specifically up-regulate, Na<sub>v</sub> subthreshold activities such as persistent and resurgent currents. Therefore, these Na<sub>v</sub> channel toxin activators might be used as powerful tools in Na<sub>v</sub> current induction at rest or in non-excitatory cells.

**Pharmacology of Na<sub>v</sub> Channels**

Na<sub>v</sub> channels are involved in several different diseases and mediation of pain and indeed, these proteins are targets for many drugs ranging from anti-epileptic to local anesthetic agents.<sup>5</sup> In addition, many venomous organisms target their prey’s or attacker’s Na<sub>v</sub> channels.<sup>35</sup> In general, Na<sub>v</sub> pharmacophores are classified according to their receptor site on the channel. Among these, several molecules block the channel, while others support increased activity, either by enhancement of activation or by slowing inactivation.<sup>35, 40</sup> These agents are widely used to block conduction in excitable tissue in a range of experimental procedures.

Local anesthetics and related compounds, such as QX-222 and QX-314, block Na<sub>v</sub> channels by blocking the pore from outside. TTX (See Table) and STX are largely non-selective between the different Na<sub>v</sub> isoforms, while μ-Conotoxins are more specific and may differentiate between Na<sub>v</sub> current components.<sup>36, 41</sup> Local anesthetics are used to mimic, or specifically up-regulate, Na<sub>v</sub> currents by blocking the open channel.<sup>42</sup> Since these compounds can exert their blocking effect only once the channel has opened, they are called open channel blockers or activity dependent blockers.

Many venoms from different animals contain peptides that are Na<sub>v</sub> channel activators. Scorpion venoms are the largest source described so far. Both α and β scorpion toxins bind to extracellular moieties on domain IV and II of the channel to slow inactivation or enhance activation kinetics, respectively. Sea anemone Na<sub>v</sub> toxins such as Anthopleurin-C, APE 1-2<sup>45</sup> or ATX II<sup>45</sup> bind to the α scorpion toxin binding site and exert similar effects on inactivation.<sup>4</sup> Two small peptide toxins found in wasp venom, α and β-Pompidotoxin, and promote Na<sub>v</sub> currents by slowing the inactivation process. Such toxins are used to mimic, or specifically up-regulate, Na<sub>v</sub> subthreshold activities such as persistent and resurgent currents. Therefore, these Na<sub>v</sub> channel toxin activators might be used as powerful tools in Na<sub>v</sub> current induction at rest or in non-excitatory cells.

**Related Products**

**Anthopleurin-C (APE2-1) - An Enhancer of Na<sub>v</sub> Currents**

Activation of Na<sub>v</sub> channels current in mNFG 2.55 (HN-100) treated PC12 cells by 5 nM APE2-1: example current traces recorded in a typical cell before (blue) and during (black) application of Anthopleurin-C (APE2-1) (NA-400). Holding potential –120 mV, test pulse to –20 mV was delivered every 10 seconds.

**References:**