Role of Sodium Channels in Clinical Disorders
Challenges for Rational Treatment
AET Symposium, December 4, 2010

Samuel F Berkovic
Epilepsy Research Centre
University of Melbourne
Disclosure

Bionomics Inc
(licensed SCN1A testing)

Research Support
Consultancy

UCB Pharma

Research Support
Speaker

Janssen-Cilag

Research Support

Sanofi-Aventis

Research Support

American Epilepsy Society Annual Meeting
Outline

- Sodium channels associated with neurological disorders

- Sodium channel epilepsies: The puzzle of genotypes and phenotypes

- Sodium channel blocking drugs and sodium channelopathies
Sodium Channels

- Transmembrane sodium gradient critical for neuronal function and excitability
- Sodium channels central to initiation and propagation of action potentials
- Pore formed by a single $\alpha$ subunit, nine isoforms

**Important CNS isoforms**

$Na_v1.1$ *SCN1A*, $Na_v1.2$ *SCN2A*, $Na_v1.3$ *SCN3A*, $Na_v1.6$ *SCN8A*

Others in peripheral nerve, heart, muscle

**Accessory $\beta$ subunits** ($\beta$ 1-4)
Sodium Channels & Neurological disorders

- Epilepsies - *SCN1A, SCN2A, SCN1B*  
  (*SCN3A, SCN8A, SCN9A*)

- Migraine - *SCN1A*

- Pain disorders - *SCN9A*
Migraine

**Familial Hemiplegic Migraine**

- 3 genes account for majority of families
- *CACNA1A, ATP1A2* and *SCN1A*
- Pathophysiology believed to be spreading depression
- Some cases have associated seizures

**Common and Classical Migraine**

- Complex inheritance
- Molecular basis largely unknown
- Susceptibility locus at 8q22

Sodium channel blockers used in migraine therapy

---

2. de Vries et al. Hum Genet 2009; 126: 115-3
Pain Disorders\textsuperscript{3,4}

\textit{Congenital Insensitivity to Pain}
- Loss of function of SCN9A
- Homozygous truncation mutations usual

\textit{Erythromelalgia (erythermalgia)}
- Excruciating painful attacks in limbs; “hot lava”
- Heterozygous missense mutations in SCN9A
- Gain of function - channel opens when more hyperpolarized

\textit{Paroxysmal extreme pain disorder}
- Excruciating pain in eye, jaw or viscera (rectum)
- Heterozygous missense mutations in SCN9A
- Gain of function - impaired fast inactivation

\textsuperscript{3} Lampert et al. Pflugers Arch 2010; 460: 249–263
\textsuperscript{4} Drent & Waxman J Clin Invest 2007; 117: 3603-9
Outline

• Sodium channels associated with neurological disorders

• Sodium channel epilepsies: The puzzle of genotypes and phenotypes

• Sodium channel blocking drugs and sodium channelopathies
Febrile Seizures

Febrile Seizures Plus (FS+)

FS/FS+ and Absences

FS/FS+ and Myoclonic Seizures

FS/FS+ and Atonic Seizures

FS/FS+ and Partial Epilepsy

Myoclonic-Astatic Epilepsy

Dravet syndrome

Scheffer & Berkovic Brain 1997; 120: 479-90
GEFS+ genes: Neuronal Sodium Channel

170_E74del

C121W
Recurrent Mutation

2 novel mutations R85

Multiple mutations in the four transmembrane domains

Scheffer et al. Brain 2007; 130: 100-9
GEFS+ Mutations: *In vitro* electrophysiology

- **SCN1B** mutations all cause loss of function\(^1,2\)
  Increases channel excitability (*SCN1A* not modulated)

- **SCN1A** mutations have different effects *in vitro* \(^3\)
  Gain of function (Increases channel excitability)
  Loss of function (Decreases channel excitability)
  Folding or Trafficking Defects

Do *in vitro* (oocyte, HEK cells) faithfully model effects in brain? How is phenotypic heterogeneity explained?

\(^2\) Xu et al. Neuroscience 2007; 148: 164-74
\(^3\) Catterall et al. J Physiol 2010; 588: 1849–59
Disease Mechanism of SCN1B mutations

- What is the localization of the β1 subunit?

- β1 is an axon initial segment (AIS) protein

- Mutant β1(W121) is *not* found in the AIS membrane

*Wimmer et al. JCI 2010; 120: 2661-71*
Disease Mechanism of $SCN1B$ mutations

- Neurons from mutant $\beta 1(W121)$ animals
  - altered action potential initiation: axonal hyperexcitability
  - increase membrane voltage acceleration in the first peak corresponding to AP initiation in the AIS.

- Computational modeling: enhanced excitability caused by hyperpolarized voltage activation of AIS Na$^+$-channels
- Temperature-dependence of AIS function enhanced in pyramidal neurons of the heterozygous mouse

*Wimmer et al. JCI 2010; 120: 2661-71*
Disease Mechanism of SCN1A mutations

Two Mouse models of SCN1A R1648H mutation

- BAC transgene and knock-in
- Did not recapitulate in vitro findings
- Predominant impairment of function
- Largely affects inhibitory GABAergic neurons
- Net effect is more excitability


GEFS+ may be caused by impairment of different channels acting at different cellular sites
Dravet Syndrome

- Severe end of GEFS+ spectrum
- Normal child
- Seizure begin at 6 months
- Development plateaus ± Regression in second year
- 80% have SCN1A mutations, Half truncation

**SCN1A knockout mouse**

- Homozygous Scn1a<sup>-/-</sup> mice
  Ataxia, die P15

- Heterozygous Scn1a<sup>+/−</sup> mice
  Seizures, die > P21

Yu et al, Nat Neurosci 2006; 9: 1142-9
Dravet Mouse Models

*Inhibitory interneuron dysfunction*

- Similar findings in knockout and knock-in nonsense mutation
- Sodium current density
  - Reduced in inhibitory interneurons of mutants
  - Excitatory pyramidal neurons not changed
- $\mathrm{Na}_V1.1$ clusters at AIS of inhibitory interneurons

*Modifier Effects*

- Strain-specific penetrance
- “Rescue” by $\mathrm{SCN8A}$ mutant

Yu et al, Nat Neurosci 2006; 9: 1142-9
Ogiwara et al, J Neurosci 2007; 27: 5903-14
Phenotypic Variability in Man

- **Background/modifier effects posited but unproven**
  - Heterogeneity within GEFS+ families
  - Monozygotic twins generally similar

- **Severe seizures cause damage**
  (e.g., hippocampal sclerosis)

- **Mosaicism**
  - Severe phenotype (Dravet) in a child
  - Mild phenotype (FS+) in parent
  - Low mutation load in parent

*Scheffer et al. Brain 2007; 130: 100-9
Depienne et al. J Med Genet 2010; 47: 404-10*
**SCN2A** ($\text{Na}_\text{v} 1.2$) and Epilepsies

- Missense mutations: benign familial neonatal-infantile epilepsy
- Functional studies show gain of function
- $\text{Na}_\text{v} 1.2$ expressed at AIS of excitatory pyramidal neurons
- Age dependent expression
  - Specific effects on neonatal isoform of $\text{Na}_\text{v} 1.2$
  - $\text{Na}_\text{v} 1.2$ replaced by $\text{Na}_\text{v} 1.6$ during development

- Severe epilepsies also described with **SCN2A** mutations
  - Functional effects heterogeneous

*Xu et al. Mol Cell Neurosci 2007; 35: 100-9.*
*Liao et al. Brain 2010; 133: 1403-14*
*Kamiya et al J Neurosci 2004; 24: 2690-8*
Sodium Channel Blocking Drugs & Sodium Channelopathies

*Sodium channel blockade: main mode of action*
- Phenytoin, Carbamazepine, Oxcarbazepine, Lamotrigine, Zonisamide, Lacosamide, Rufinamide

*Sodium channel blockade: minor mode of action*
- Valproate, Topiramate, Felbamate

**Clinical Observations**
- Lamotrigine and perhaps other primary sodium channel blockers worsen Dravet syndrome
- No consistent effect on less severe sodium channelopathies

Rogawski and Loscher Nat Rev Neurosci 2004; 5: 553-64
Stafstrom Curr Opin Neurol 2010;23:157-63
Guerrini et al. Epilepsia 1998; 39: 508-12
Sodium Channel Blocking Drugs & Sodium Channelopathies

*Sodium channel blockade: mechanisms heterogeneous*

- Block high frequency firing, allow normal action potentials
- Facilitate *fast* inactivation (OXC, LTG, ZNS)
- Enhance *slow* inactivation (Lacosamide)

*Little data on sodium channel subtype selectivity*

- Cellular and subcellular distribution of sodium channels may explain disconnect between mutations effects & drug action
- Structural biology approaches to developing selective drugs

Stafstrom Curr Opin Neurol 2010;23:157-63
Conclusions

- Sodium channel mutations cause various CNS disorders
- Epilepsy syndromes varying from benign to very severe
- Axon initial segment is a critical target for channelopathies
- Phenotypic heterogeneity contributed to by
  - Cellular & age-dependent expression of subunits
  - Modifier gene effects
  - Secondary damage?
  - Mosaicism
- Deeper understanding of channel biology needed to explain effects of AEDs on channelopathies
<table>
<thead>
<tr>
<th>Acknowledgements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
</tr>
<tr>
<td>Ingrid Scheffer</td>
</tr>
<tr>
<td>Molecular Genetics</td>
</tr>
<tr>
<td>Leanne Dibbens</td>
</tr>
<tr>
<td>John Mulley</td>
</tr>
<tr>
<td>Sarah Heron</td>
</tr>
<tr>
<td>Functional Biology</td>
</tr>
<tr>
<td>Steve Petrou</td>
</tr>
<tr>
<td>Chris Reid</td>
</tr>
<tr>
<td>Verena Wimmer</td>
</tr>
</tbody>
</table>