$Na_V1.6$ Selective and $Na_V1.2$/$Na_V1.6$ Dual Inhibitors Reduce Action Potential Firing in Mouse Cortical Pyramidal Neurons While Sparing Inhibitory Interneuron Firing

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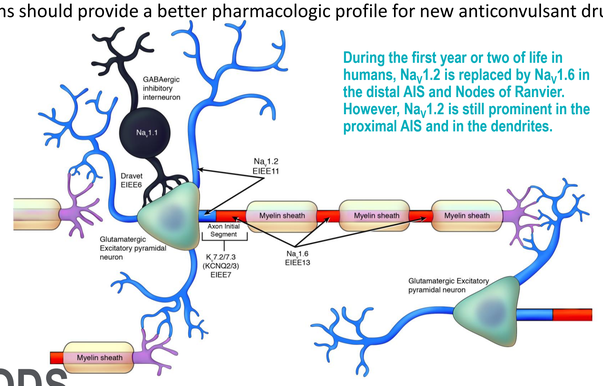
BACKGROUND

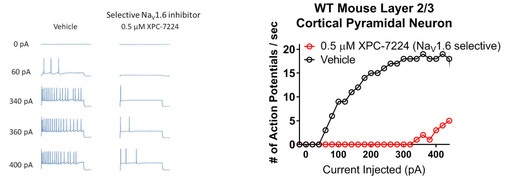
- An ideal anti-seizure medicine would inhibit excitatory circuits while sparing inhibitory circuits.
- Voltage-gated sodium channel inhibitors (e.g. carbamazepine) are effective antiseizure medicines but these drugs inhibit the sodium channels that drive inhibitory interneuron firing ($Na_V1.1$) as well as those primarily linked to excitatory neuron firing ($Na_V1.2$ & $Na_V1.6$).
- Gain-of-function mutations in both SCN8A (encoding $Na_V1.6$) and SCN2A ($Na_V1.2$) cause early infantile epileptic encephalopathy in humans (EIEE13 & EIEE11).
- Loss-of-function mutations in SCN1A (encoding $Na_V1.1$) cause Dravet Syndrome (EIEE6) and nonselective sodium channel inhibitors can exacerbate seizures in Dravet Syndrome.
- Selective inhibitors that reduce AP firing in excitatory neurons, while sparing inhibitory interneurons should provide a better pharmacologic profile for new anticonvulsant drugs.



During the first year or two of life in humans, $Na_V1.2$ is replaced by $Na_V1.6$ in the distal AIS and Nodes of Ranvier. However, $Na_V1.2$ is still prominent in the proximal AIS and in the dendrites.

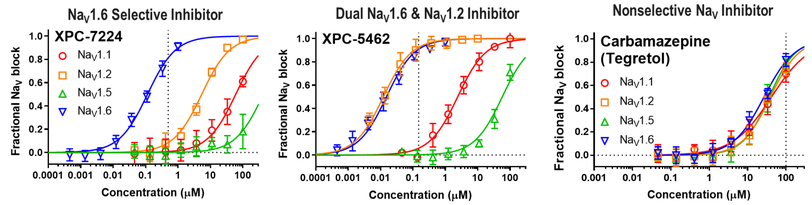
METHODS

- We created inhibitors that target $Na_V1.6$ selectively (XPC-7224) or $Na_V.12$ and $Na_V1.6$ (XPC-5462) while sparing $Na_V1.1$ and other voltage-gated sodium channels.
- Potency and selectivity were determined by automated voltage clamp (Sophion, Qube) in HEK cells expressing human $Na_V1.X$ isoforms.
- Mouse (>P21) brain slice recordings were made in cortical layer 2/3 from parasagital cortical slices. Fast-spiking interneurons were identified by their characteristic fast-spiking pattern
- N1768D$^{+/-}$ mice are heterozygous for a patient identified gain-of-function variant of $Na_V1.6$ (Wagnon et al, 2014).
- Seizure protection was assessed in N1768D$^{+/-}$ mice by application of a 12 mA 6Hz DC shock that is innocuous to wild type littermates but induces a tonic-clonic seizure with hind-limb extension in mutant mice.
- Seizure protection was assessed in wild type CF-1 mice after maximal electrical shock with a 200 ms, 50 mA, 60 Hz unidirectional square wave DC shock. This stimulus evokes a tonic-clonic seizure with hindlimb extension in ~90% of vehicle treated mice.



RESULTS

Distinct Selectivity Profiles Enable Specific Targeting of $Na_V1.6$ and $Na_V1.2$



- XPC-7224 is highly selective for $Na_V1.6$.
- XPC-5462 blocks both $Na_V1.6$ and $Na_V1.2$; spares $Na_V1.1$ and $Na_V1.5$.
- Carbamazepine is similarly potent on all Na_V isoforms.

Potency and Selectivity Profiles of Selective Inhibitor (XPC-7224), Dual Inhibitor (XPC-5462), and Nonselective Inhibitor (Carbamazepine)

Selectivity EP Assays	$Na_V1.6$ IC_{50} (μM)	$Na_V1.1$ IC_{50} (μM)	$Na_V1.2$ IC_{50} (μM)	$Na_V1.5$ IC_{50} (μM)	Selectivity $Na_V1.1/1.6$
XPC-7224	0.11	60	5.4	430	550
XPC-5462	0.015	2.4	0.013	68	160
Carbamazepine	27	40	39	36	1.5

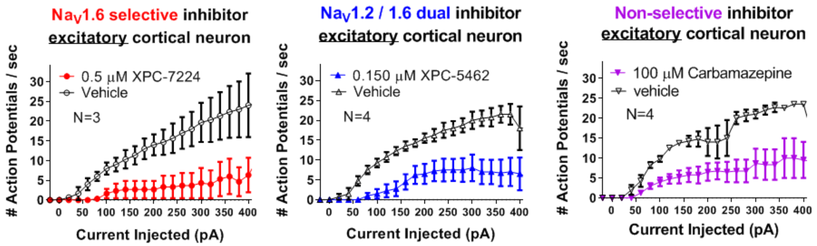
The selectivity assays were designed to best identify molecular selectivity of the compounds, independent of state- or use-dependent properties.

"Physiologic" EP assay	$Na_V1.6$ IC_{50} (μM)
XPC-7224	0.11
XPC-5462	0.015
Carbamazepine	27

The "Physiologic" assay was designed to best approximate the potency of the compounds in neurons at physiologic voltages.

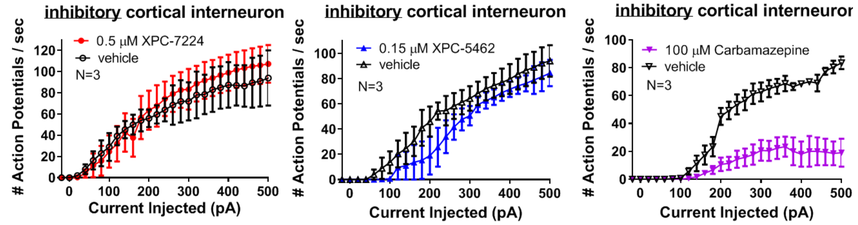
- For subsequent neuronal experiments we chose concentrations ~ 3X higher than the $Na_V1.6$ IC_{50} to target inhibition of ~ 80% of $Na_V1.6$ currents. The concentration used is indicated by the dotted vertical line on the selectivity graphs at the top:
  - XPC-7224, 0.5 μM
  - XPC-5462, 0.15 μM
  - Carbamazepine, 100 μM

Selective and Nonselective Inhibitors of Na_V's Reduced Action Potential Firing in Cortical Excitatory Pyramidal Neurons in Wild Type (WT) Mouse Brain Slices



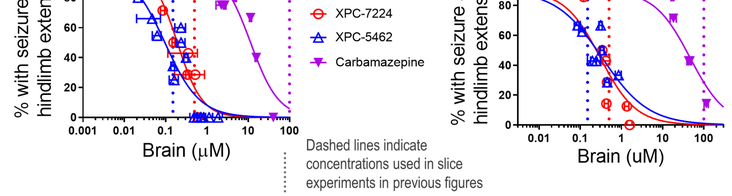
- All three test compounds, XPC-7224, XPC-5462, and Carbamazepine reduced action potential firing of excitatory glutamatergic pyramidal neurons to a significant and similar degree.

Selective Inhibitors Had Little Effect on Firing in Interneurons but Carbamazepine Blocked Inhibitory Action Potentials



- Carbamazepine reduced action potential firing of inhibitory interneurons neurons to a significant and similar degree as in pyramidal neurons.
- $Na_V1.1$ sparing compounds, XPC-7224 and XPC-5462, had little effect on interneuron firing.

Selective $Na_V1.6$ Inhibitor, Dual $Na_V1.2$/1.6 Inhibitor, and Nonselective Na_V Inhibitor All Protect Mice from Electrically Induced Seizures in $Na_V1.6$ Gain-of-Function and Wild Type (WT) Mice



Dashed lines indicate concentrations used in slice experiments in previous figures

- $Na_V1.1$ sparing compounds, XPC-7224, and XPC-5462 inhibited seizures in SCN8A N1768D$^{+/-}$ mice elicited by 6 Hz DC corneal shock at lower brain concentrations than Carbamazepine, consistent with their greater potency on $Na_V1.6$.
- Similar results were found in wild type mice where seizures were elicited with a 60 Hz DC corneal shock.

CONCLUSIONS

- Selectively targeting the specific sodium channel isoforms expressed in excitatory neurons, $Na_V1.2$ and $Na_V1.6$, enables selective reduction of action potential firing in those neurons, and prevents the simultaneous impairment of the activity of inhibitory interneurons.
- This profile provides a new, mechanistically differentiated, class of voltage-gated sodium channel inhibitors with the potential to provide improved seizure control and side effect profile for epilepsy patients.
- Xenon will soon begin Phase II trials with an $Na_V1.6$ selective inhibitor, XEN901, and is completing preclinical studies with a dual $Na_V1.2$ + $Na_V1.6$ inhibitor, XEN393.

[1]Jacy L. Wagnon, Matthew J. Korn, Rachel Parent, Taylor A. Tarpey, Julie M. Jones, Michael F. Hammer, Geoffrey G. Murphy, Jack M. Parent and Miriam H. Meisler. Convulsive seizures and SUDEP in a mouse model of SCN8A epileptic encephalopathy. Human Molecular Genetics, 2014 1–10. doi:10.1093/hmg/ddu470

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