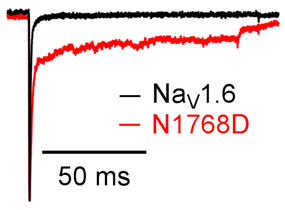
Selective antagonists of $Na_V1.6$ prevent electrically induced seizures in a mouse model of EIEE13



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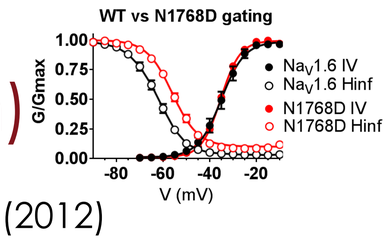
Introduction

Na_V inhibitors are useful antiepileptics, but currently available drugs are nonselective. Inhibition of $Na_V1.1$ likely limits their efficacy due to its' important role in inhibitory interneurons. Inhibition of $Na_V1.5$ introduces risk of cardiac adverse events. We set out to create new drugs that block $Na_V1.6$ but spare $Na_V1.1$ and $Na_V1.5$

Mouse model of EIEE13 ($Na_V1.6$ gain of function)



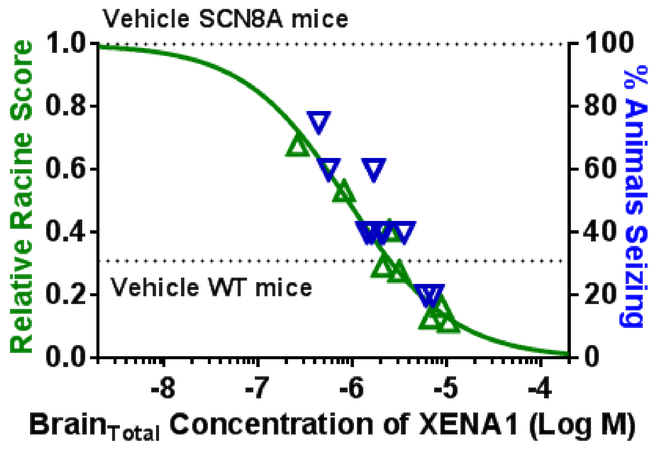
- N1768D mutation in $Na_V1.6$
  - Mutation identified by Veeramah et al. (2012)
  - Mouse model created by Wagnon et al. (2015)
- Mice in our colony behave as described by Meisler
  - Seizures begin ~ p70 to p90
  - Only about 60% become epileptic

6-Hz psychomotor seizure induction assay

- 6Hz, 12 mA, transcorneal stimulus
- WT mice are resistant to stimulus
  - ~50% show no seizure behavior,
  - ~50% have brief clonic seizure
- N1768D mice are much more sensitive
  - All vehicle treated mice have a tonic clonic seizure after stimulus, some have multiple seizures

Seizures Assessed by 2 endpoints

1. % of animals seizing
2. Modified Racine Score



Scoring

- 0 = no response
- 1 = Shaking/jerking/facial tremor, freezing, blinking
- 2 = Forelimb clonus or Straub tail
- 3 = Loss of balance, rearing, falling
- 4 = Clonic seizure
- 5 = Tonic-clonic seizure with extension of hind limbs

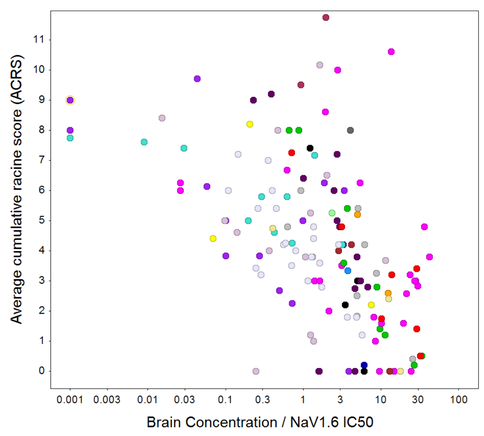
Responses of individual animals are "binary"

- An animal usually has a strong response or no response
- Improving efficacy results from recruiting more protected individuals

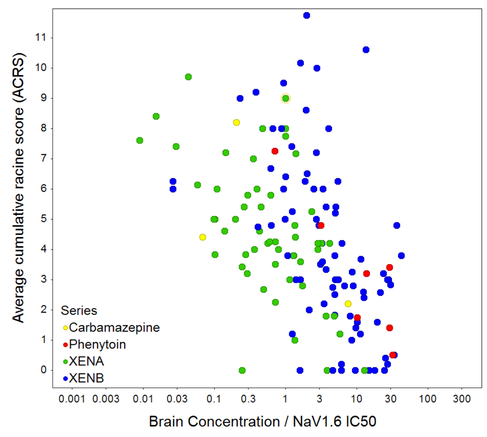
In vivo EC_{70} = 1-3 fold $Na_V1.6$ IC_{50} Blocking more Na_V subtypes does not increase efficacy

Each point is mean data for 4-6 animals

Brain concentration determined immediately after assay



27 compounds at various dose levels. Each color indicates a distinct compound

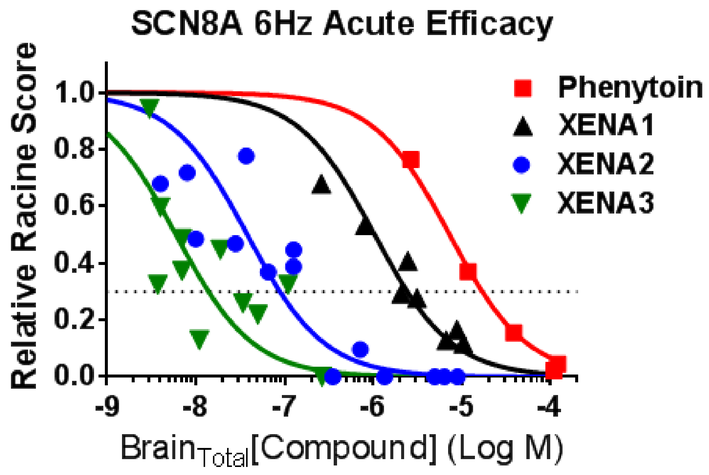


Same data colored by compound series.

XENA is green, XENB is blue

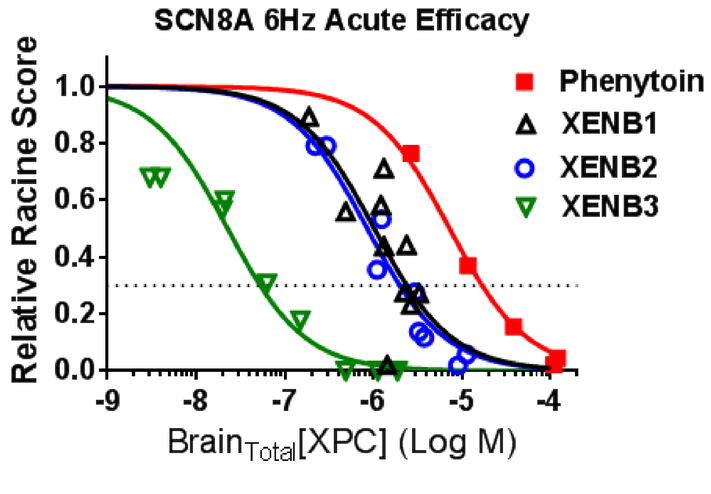
Series XENA – $Na_V1.6$ Selective

- Potency measured by Na^+ flux assays in HEK cells
- Electrophysiologic assays agree qualitatively
  - EP assays tuned to measure inactivated state potency
    - more potency for $Na_V1.6$ than Na+ flux assays
    - greater selectivity than Na+ flux assays



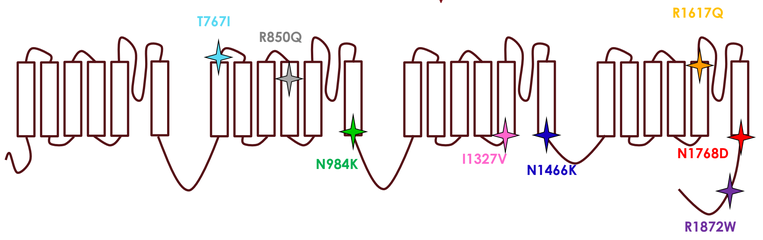
	$hNa_V1.6$ IC_{50} (µM)	$Na_V1.1$ /1.6	$Na_V1.2$ /1.6	$Na_V1.5$ /1.6
Phenytoin	4	2	3	1.5
XENA1	2	10	8	13
XENA2	0.3	50	33	89
XENA3	0.07	260	37	>400

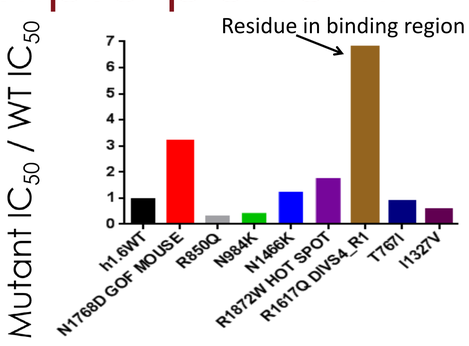
Series XENB – Dual $Na_V1.6$, $Na_V1.2$



	$hNa_V1.6$ IC_{50} (µM)	$Na_V1.1$ /1.6	$Na_V1.2$ /1.6	$Na_V1.5$ /1.6
Phenytoin	4	2	3	1.5
XENB1	0.5	10	0.2	15
XENB2	0.3	10	0.1	26
XENB3	0.08	140	2	225

XEN compounds block multiple patient identified $Na_V1.6$ variants





Summary

Novel, selective, inhibitors of $Na_V1.6$ prevented induced seizures in a modified 6Hz psychomotor assay using the N1768D $Na_V1.6$ mouse model of EIEE13 developed by Wagnon et al. at the Univ. of Michigan.

1. **Efficacy was well predicted by in vitro potency and brain exposure.**
2. **Non-selective, or less selective Na_V inhibitors had similar efficacy as selective inhibitors**
3. **The N1768D mouse 6Hz assay appears to be a good measure of on target efficacy for $Na_V1.6$ inhibitors.**

We expect that novel, selective $Na_V1.6$ inhibitors will provide the basis for new antiepileptic drugs with an improved efficacy and safety profile

XENON
from families to genes, from gene to drugs.