PRAX-628: A Novel Sodium Channel Blocker with Greater Potency and Activity Dependence Compared to Standard of Care

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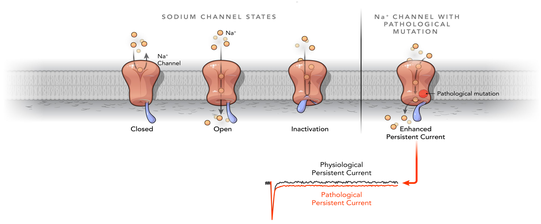
Background

- Voltage-gated sodium channels (Na_V), which contribute to action potential initiation and propagation, are important therapeutic targets for anti-seizure medications (ASMs).[1]
- Persistent I_{Na}, a small non-inactivating current, can lead to neuronal hyperexcitability and has been proposed as a novel pharmacological target for reducing pathologic neuronal hyperactivity, while sparing physiological peak sodium current (I_{Na}) is critical to ensuring normal neuronal function.[2-6]
- Na_V-targeting ASMs[1] can display severe toxicity at therapeutic doses, likely resulting from excessive inhibition of peak I_{Na}.
- Novel compounds that spare a physiological level of peak I_{Na} may therefore improve efficacy and tolerability; a hypothesis supported by previous work with persistent I_{Na} inhibitors.[7-12]
- PRAX-628 was identified as a highly differentiated, potent and activity dependent (UDB) I_{Na} blocker.
- The current study thus aimed to compare the inhibition profile of PRAX-628 on persistent and peak I_{Na} to those of standard Na_V-targeting ASMs and investigational compounds.



Methods

Persistent and Peak I_{Na} Inhibition: HEK-293 Electrophysiology

- Persistent and peak I_{Na} inhibition was studied using PatchXpress automated whole-cell patch clamp recordings of HEK-293 cell lines stably expressing human $Na_V1.6$ ($hNa_V1.6$; NP_055006).
- Voltage protocols measured I_{Na} inhibition in multiple modes:
  - Persistent I_{Na} (Vm -120mV, 200ms)
  - Tonic block (TB; Vm -120mV, 0.2Hz)
  - Voltage-dependent block (VDB; Vm inactivation $V_{1/2}$)
  - Activity/use dependent block (UDB, Vm -120mV, 10Hz)
- Data were processed using DataXpress 2.0.
- Persistent I_{Na} (200 nM ATX-II) or peak I_{Na} were measured using voltage protocols depicted as insets and percent inhibition was calculated.
- Data were fitted using a Hill equation [Max_Effect/(1+(IC_{50}/x)^Hill_Slope] to estimate IC_{50} and Hill slope using GraphPad Prism.

Binding Kinetics

- Binding kinetics were inferred from inhibition kinetics.
- The apparent binding rate (K_{ON}) was measured using the time-dependent inhibition of I_{Na} during a variable length conditioning pulse.
- The apparent unbinding rate (K_{OFF}) was measured using the time-dependent delay in the recovery of I_{Na} following an inactivating conditioning pulse.

PRAX-628 Exhibits Enhanced Activity Dependent Inhibition of $hNa_V1.6$ Peak I_{Na}

- PRAX-628 exhibited enhanced activity-dependent block which has been suggested to convey beneficial activity during periods of hyperexcitability.
- Less activity-dependent block was observed for the other tested agents, including carbamazepine and lamotrigine.

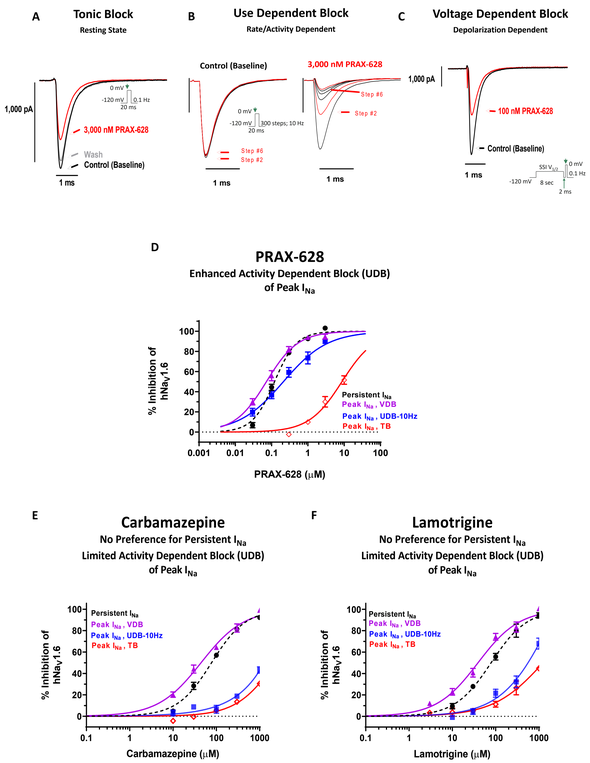


Figure 1. PRAX-628 demonstrates increased potency for I_{Na} and greater activity-dependent block of Peak I_{Na} relative to other tested Na_V targeting ASMs. Peak I_{Na} block assessed using assays for *(A)* tonic block, *(B)* use-dependent block, or *(C)* voltage-dependent block. Peak I_{Na} was measured at the beginning of the voltage step. *(D)* PRAX-628 demonstrated potent inhibition of persistent I_{Na} and enhanced activity dependent inhibition of peak I_{Na}. *(E)* Carbamazepine and *(F)* lamotrigine exhibited lower potency and preference for persistent I_{Na} (*red arrows*) and minimal activity dependent block of peak I_{Na} (*blue traces*). Voltage protocols included as *panel insets*; pharmacology measured at *green arrow*; points represent mean ± SEM.

PRAX-628 Exhibits Potent Inhibition of $hNa_V1.6$ Persistent I_{Na}

- PRAX-628 blocked $hNa_V1.6$ persistent I_{Na} with an IC_{50} of 128nM (68x preference to TB), which was at least 550x more potent than the other tested I_{Na} blocking agents.
- The inhibitory profile of PRAX-628 was different than that of carbamazepine and cenobamate.
- PRAX-628 inhibited persistent I_{Na} across Na_V isoforms and orthologs.

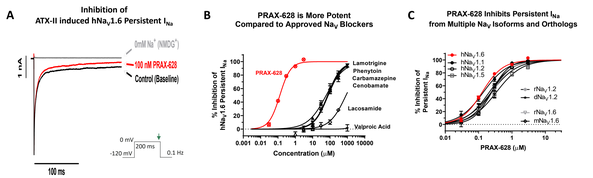


Figure 2. PRAX-628 is a potent inhibitor of persistent I_{Na}. PRAX-628 reduced *(A)* ATX-II evoked $hNa_V1.6$ persistent I_{Na}. *(B)* PRAX-628 demonstrated increased potency for persistent I_{Na} relative to standard Na_V-targeting ASMs. *(C)* PRAX-628 inhibited ATX-II induced persistent I_{Na} expressed by multiple Na_V isoforms and orthologs. Voltage protocol included as *panel inset*; pharmacology measured at *green* arrow; points represent mean ± SEM.

Table 1. PRAX-628 demonstrates greater activity dependance for peak I_{Na} compared with a panel of standard-of-care Na_V-targeting ASMs

IC_{50}, nM (Slope)	Persistent I_{Na}	Peak I_{Na} TB	Ratio to Pers. I_{Na}	Peak I_{Na} UDB-10Hz	Ratio to Pers. I_{Na}	Peak I_{Na} VDB	Ratio to Pers. I_{Na}
PRAX-628	**128 (1.4)**	**8,707 (1.0)**	68	200 (0.7) Max 100%	1.6	**72 (1.0)**	**0.56**
Cenobamate	**71,690** (1.1)	**1,719,000** (1.1)	**24**	**749,300** (0.7)	**11**	**66,710** (0.9)	**0.9**
Phenytoin	**59,820** (0.8)	n/a**	--	**876,600** (0.6)	**15**	**47,780** (1.0)	**0.8**
Carbamazepine	**77,490** (1.1)	**2,307,000** (1.0)	**30**	**1,418,000** (0.9)	**18**	**44,370** (0.9)	**0.6**
Oxcarbazepine	**123,700** (1.0)	**1,035,000** (1.7)	**8**	**n.d.**	--	**42,000** (1.1)	**0.3**
Lamotrigine	**78,480** (1.0)	**1,249,000** (0.8)	**16**	**515,800** (1.0)	**6.6**	**39,090** (0.9)	**0.5**
Lacosamide	**832,700** (0.9)	n/a**	--	**682,200** (1.3)	**0.8**	**269,300** (1.2)	**0.3**
Valproic acid	**2% @ 1 mM**	**11 @ 1 mM**	--	**8% @ 1 mM**	--	**18% @ 1 mM**	--

Data are IC_{50} (nM) with the Hill slope in parenthesis. **could not be determined due to compound solubility limit
n.d.=not determined: Pers.=persistent; TB=tonic block; UDB=use-dependent block; VDB=voltage-dependent block

PRAX-628 Exhibits Faster Apparent Binding and Moderate Apparent Unbinding Kinetics

- Binding kinetics are a key feature for differentiating Na_V blockers.
- The combination of the rapid development and recovery from inhibition is hypothesized to selectively target pathological neuronal hyperexcitability, while sparing physiological activity.

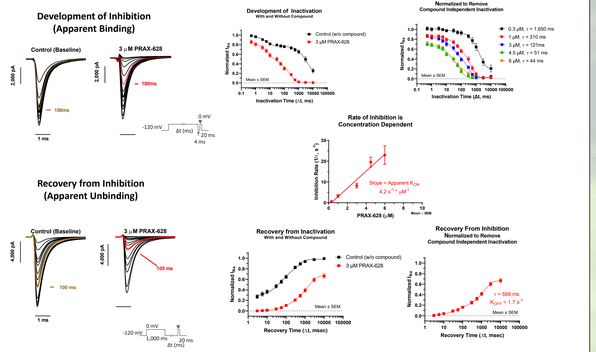


Figure 3. The development and recovery from inhibition can be used to derive the apparent binding and apparent unbinding rates of a small molecule Na_V blockers. The increased potency and activity dependence of PRAX-628 originates from the rapid development of inhibition (apparent K_{ON} = 4.2 s^{-1} * μM^{-1}) paired with a moderate dissociation rate (apparent K_{OFF} = 1.7 s^{-1}).

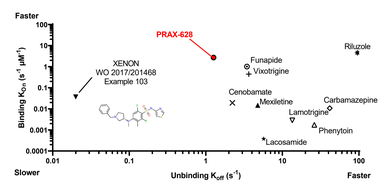


Figure 4. PRAX-628 exhibits fast apparent binding and moderate apparent unbinding compared to a panel of standard of care Na_V blockers. The K_{ON} for PRAX-628 was fast compared to the other tested compounds (>1,500x versus carbamazepine). The K_{OFF} from PRAX-628 was slower than carbamazepine (33x) suggesting a longer, but not excessive residence time. A representative hNav1.6 isoform selective inhibitor exhibits a very slow unbinding rate (2,079x slower than carbamazepine).

Conclusions

- **PRAX-628 is a next generation Na_V blocker with enhanced targeting of the I_{Na} activity contributing to neuronal hyperexcitability.**
  - **Greater potency and preference for persistent I_{Na}**
  - **Greater activity dependent inhibition (UDB) of peak I_{Na}**
- **This preferential targeting of neuronal hyperexcitability may represent a differentiated therapeutic option for diseases of hyperexcitability, where standard-of-care Na_V blockers have demonstrated efficacy but poor tolerability.**

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