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Pharmacology of TTX-resistant and TTX-sensitive sodium currents in non-human primate dorsal root ganglion sensory neurons

Dong Liu, Chen Tian, Adina Hazan, Mahsa Sadeghi, Christian Petroski, Robert Petroski

Neuroservices-Alliance (Neuroservice USA - in vitro cell electrophysiology)

Background

Chronic pain is poorly managed by current therapies. The opiate epidemic has highlighted the need for alternative therapies for treating pain. Genetic and functional studies have established an important role of voltage-gated sodium channels in human pain disorders. Tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels expressed in dorsal root ganglion (DRG) sensory neurons are currently being pursued as promising therapeutic targets for treating human pain. Many drug discovery programs rely on pharmacology data from rodent neurons for generating the structure-activity relationships (SAR) during lead optimization. However, the pharmacological activity of compounds on rodent target proteins sometimes differs from their human orthologs and poses a challenge for identifying the best pre-clinical candidates to advance to human clinical trials. Here we describe the development and validation of an electrophysiological assay of TTX-R sodium channels from non-human primate (NHP) and dog DRG sensory neurons and show the efficacy and potency of 14 compounds for blocking TTX-R sodium currents. These results reveal differences in compound activity on native sodium channels from different species.

Methods

DRGs were harvested from adult cynomolgus monkeys and dogs. The animals were humanely euthanized in an AAALAC accredited facility using an IACUC approved protocol to minimize pain and suffering. The tissue was dissociated using enzymatic and mechanical methods and sensory neurons were plated on poly-D-lysine and laminin coated glass coverslips in Neurobasal/B27 medium supplemented with NGF (25 ng/ml). Whole cell patch clamp recordings were conducted at 1, 2, and 3 days *in vitro*. The composition of the external recording solution was: 110 mM choline-Cl, 30 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose,10 mM HEPES pH 7.3. The composition of the internal recording solution was: 70 mM CsCl, 70 mM CsF, 3 mM $MgCl_2$, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM Na_2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3. Upon establishing the whole cell configuration, we measured the passive membrane properties for every cell (Cm, Rm, Ra). TTX-resistant sodium currents were measured in the presence of 0.5 uM TTX.

TTX-R Na currents

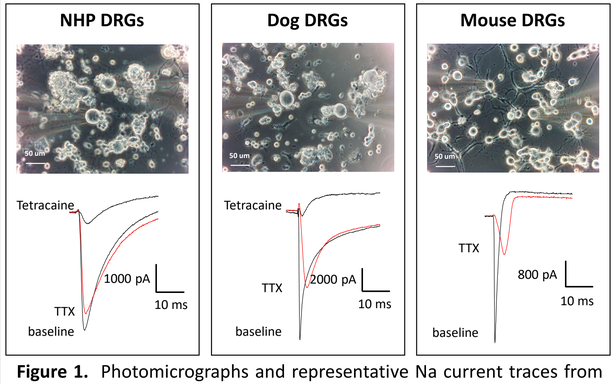
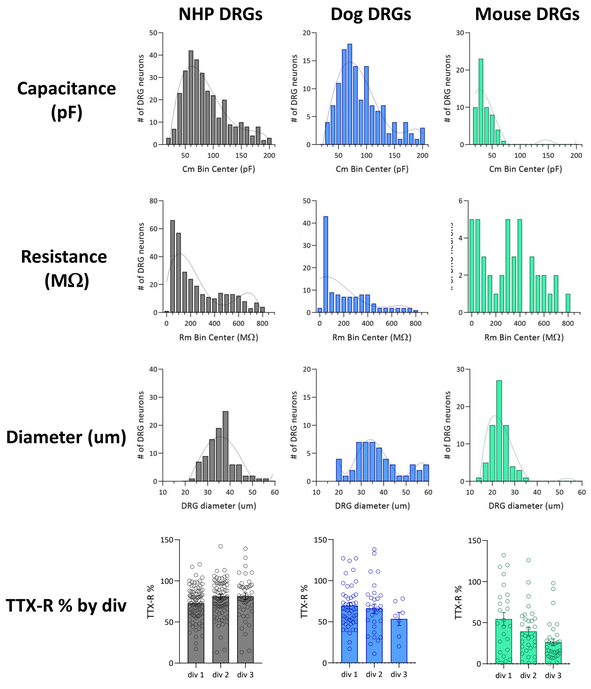


Figure 1. Photomicrographs and representative Na current traces from NHP, dog and mouse DRG neurons. TTX-resistant currents shown in red.

Passive properties and TTX-R fraction



		NHP	Dog	Mouse
Capacitance		95.9 ± 2.9 pF (n=326)	92.2 ± 3.8 pF (n=132)	34.7 ± 1.8 pF (n=57)
Resistance		303.3 ± 15.6 MΩ (n=326)	341.5 ± 38.6 MΩ (n=131)	557.6 ± 65.0 MΩ (n=60)
Diameter		35.9 ± 6.0 um (n=94)	39.4 ± 12.9 um (n=55)	23.4 ± 3.6 um (n=71)
TTX-R%	**1 *div***	73 ± 2 % (n=89)	70 ± 4 % (n=50)	54 ± 8% (n=25)
	2 *div*	81 ± 3 % (n=81)	66 ± 5 % (n=31)	39 ± 5% (n=32)
	3 *div*	81 ± 4 % (n=51)	53 ± 8 % (n=7)	26 ± 4% (n=32)

Figure 2. NHP and dog DRG neurons exhibited similar properties including capacitance, membrane resistance, and diameter. Conversely, mouse DRG neurons were smaller, had a lower capacitance and higher resistance. The proportion of TTX-R current did not decrease over 3 days *in vitro* in NHP or dog DRG neurons. In contrast, the proportion of TTX-R current decreased significantly in mouse DRG neurons.

Voltage-dependent activation and inactivation

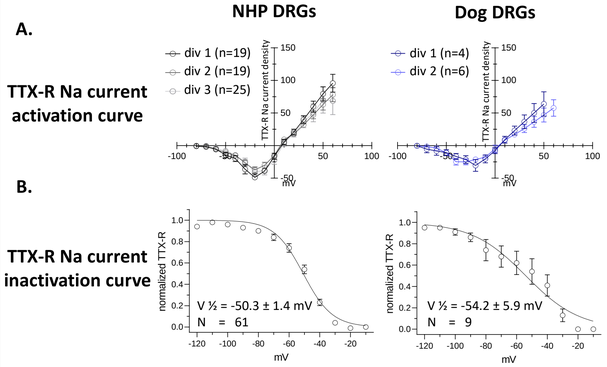
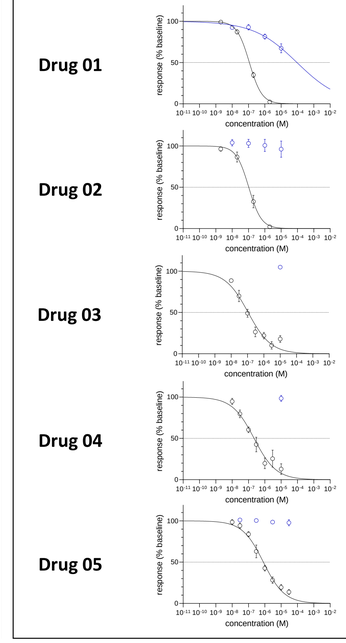


Figure 3. NHP and dog DRG neurons exhibited similar voltage-dependent activation (A) and inactivation (B) properties of TTX-R Na currents.

Concentration-response of 14 compounds on TTX-R Na currents



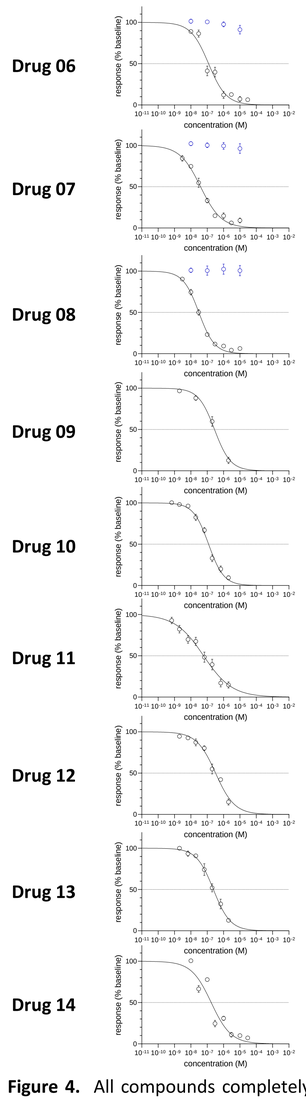
	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	114 nM	>10 uM
Slope	1.13	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	105 nM	>10 uM
Slope	1.13	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	109 nM	>10 uM
Slope	0.62	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	221 nM	>10 uM
Slope	0.65	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	820 nM	>10 uM
Slope	0.67	n.a.



	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	121 nM	>10 uM
Slope	0.80	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	39 nM	>10 uM
Slope	0.68	n.a.

	NHP	Dog
Emax (% block)	100%	<50% at 10 uM
IC_{50}	31 nM	>10 uM
Slope	0.90	n.a.

	NHP
Emax (% block)	100%
IC_{50}	281 nM
Slope	0.88

	NHP
Emax (% block)	100%
IC_{50}	121 nM
Slope	0.93

	NHP
Emax (% block)	100%
IC_{50}	60 nM
Slope	0.50

	NHP
Emax (% block)	100%
IC_{50}	326 nM
Slope	0.75

	NHP
Emax (% block)	100%
IC_{50}	239 nM
Slope	0.84

	NHP
Emax (% block)	100%
IC_{50}	176 nM
Slope	0.72

Figure 4. All compounds completely blocked TTX-R Na currents in NHP DRG neurons (black). 8 of the compounds that were also tested in dog DRG neurons did not exhibit block (blue).

Conclusions

- We developed and validated an electrophysiological assay of native TTX-resistant Na currents from sensory neurons isolated from non-human primate and dog dorsal root ganglia.
- Nav1.8 antagonists potently and completely blocked TTX-R in NHP but not dog sensory neurons.