

Identification of two gating modes of Nav1.8

E.B. STEVENS[1], A.M. RUSH[1], D. SPANSWICK[1], *R.D. PINNOCK[2]

[1] NeuroSolutions Ltd., Coventry, UNITED KINGDOM, [2]Department of Pharmacology, University of Cambridge, UNITED KINGDOM.

NEUROSolutions LTD. www.nsolns.com

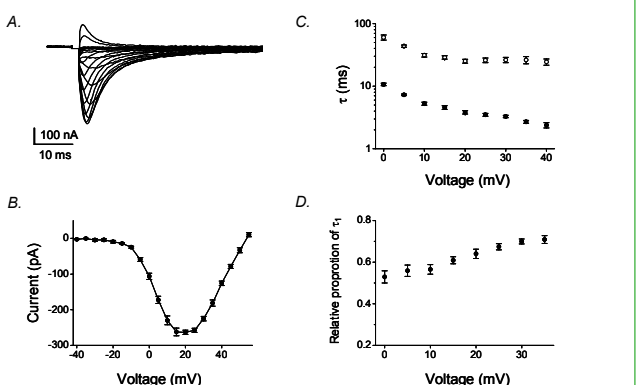
❶ INTRODUCTION

Both TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) channels are important in determining the shape of small diameter dorsal root ganglion (DRG) action potential, where TTX-S channels underlie the initial depolarization, while the TTX-R channel, Nav1.8, contributes to the majority of the upstroke of the action potential and to the inflection of the repolarizing phase (Blair & Bean, 2002, *J. Neurosci.* 22:10277–10290). Therefore, Nav1.8 activation and inactivation kinetics play a key role in shaping the action potential. Previous studies on TTX-R currents have identified both fast inactivation, occurring over several milliseconds, and slow inactivation, operating over seconds to minutes (Rush *et al.*, 1998, *J. Physiol.* 511:771-789; Blair & Bean, 2003, *J. Neurosci.* 23:10338–10350; Zhao *et al.*, 2003, *NeuroToxicology* 24:83–96). Recovery of TTX-R currents from fast inactivation has previously been shown to occur through a bi-exponential time course, suggesting the presence of multiple gating modes (Rush *et al.*, 1998). The current study further investigates the fast inactivation properties of recombinant and native Nav1.8 channels and has identified two different gating modes with properties distinct from slow inactivation. Blair & Bean (2002) have previously demonstrated that the upstroke and inflection of the action potential are associated with different phases of TTX-R availability, which could correlate with the two different Nav1.8 gating modes.

❷ METHODS

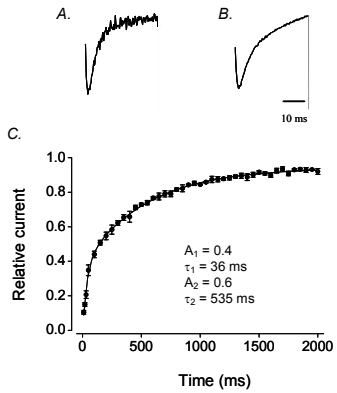
Xenopus laevis were anaesthetized by immersion in 0.3% (w/v) 3-amino benzoic acid solution before surgical removal of ovarian lobes. Oocytes were injected with rat Nav1.8 cRNA and Na+ currents were measured 4-7 days later. Currents were recorded using two-electrode voltage clamp in an external solution containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6. Dorsal root ganglion cells were isolated from neonatal Wistar rats (P4-8) and patched within 24 hours of preparation. The bathing solution for electrophysiological analysis contained: 60 mM NaCl; 5 mM KCl; 60 mM ChCl; 5 mM $MgCl_2$; 1 mM CaCl2; 2 mM TEA; 0.2 μM TTX, 100 μM $CdCl_2$; 10 HEPES pH 7.2, whilst the electrode solution contained: 130 mM CsF; 10 mM NaCl;; 1 mM EGTA; 5 mM $MgCl_2$; 10 mM HEPES pH 7.2.

1. Inactivation kinetics of Nav1.8



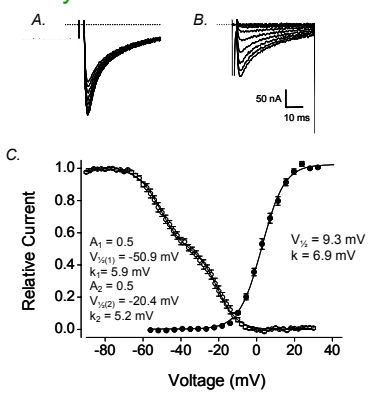
A. Family of Na^+ currents recorded from *Xenopus* oocytes injected with cRNA encoding Nav1.8. Depolarizing pulses were applied between -40 and +50 mV from a holding potential of -80 mV. ***B***. Current-voltage relationship derived from peak currents Na+ currents (n = 4). ***C***. Time constants of inactivation calculated at different voltages. Inactivation kinetics were fitted to an exponential function: $I = A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)+C$, where A_1 and A_2 are the relative amplitudes of fast and slow components, τ_1 and τ_2 are the inactivation time constants of the fast (●) and slow (○) components, while C is the steady-state asymptote (n = 4). ***D***. The relative proportion of channels inactivating by the fast exponential component τ_1 calculated from the equation $A_1/(A_1+A_2)$ (n = 4).

2. Nav1.8 recovery from inactivation



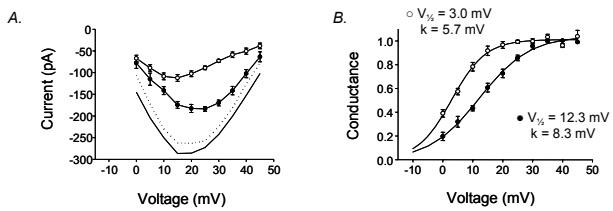
The recovery pulse protocol was a 1 s inactivating pulse to 0 mV followed by conditioning pulses to –100 mV for increasing periods of time, followed by a test pulse to 0 mV. The holding potential was –100 mV. ***A***. Current evoked following a 20 ms recovery period. ***B***. Current evoked following a 2 s recovery period. Recovery time courses were fitted with double exponentials, $I = 1-[A_1\exp(-t/\tau1)+A_2\exp(-t/\tau_2)]$, where A_1 and A_2 are the relative amplitudes of the fast and slow components, τ_1 and τ_2 are the inactivation time constants.

3. Nav1.8 steady-state inactivation



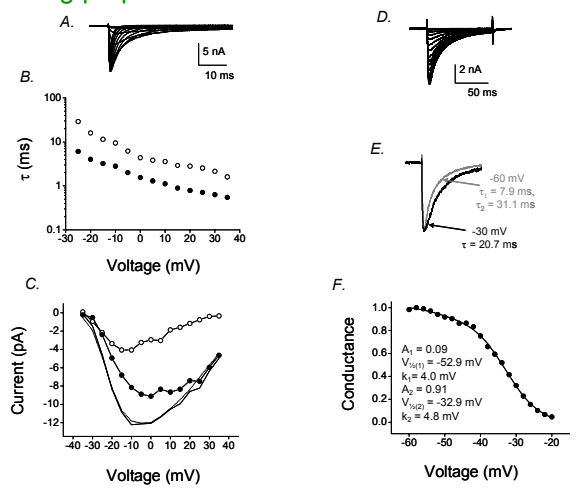
For steady-state inactivation a two-step protocol was applied with conditioning pulses of 1 s duration followed by a test pulse to 0 mV. The pulse protocol was applied from a holding potential of -100 mV. ***A***. Currents evoked by preconditioning pulses between -90mV to -40 mV. ***B***. Currents evoked by preconditioning pulses between -36 mV to +15 mV. ***C***. Conductance-voltage data, derived from peak current amplitudes, were fitted with the a single Boltzmann equation $g = 1/\{1+\exp[(V-V_{½})/k]\}$, where g is conductance, $V_{½}$ is voltage of half-maximal activation, k is the slope factor. Steady-state inactivation data were fitted with double Boltzmann equation: $g = A_1/\{1+\exp[(V-V_{½})/k]\}+A_2/\{1+\exp[(V-V_{½})/k]\}$, where g is conductance, $V_{½(1)}$ and $V_{½(2)}$ are voltages of half-maximal inactivation of fast and slow components, k_1 and k_2 are the slope factors for fast and slow components, and A_1 and A_2 are the relative amplitudes of fast and slow components.

4. Nav1.8 conductance-voltage relationship



A. Current-voltage relationships for fast (●) and slow (○) gating modes were derived from exponential fit parameters, A_1 and A_2. Dotted line is the mean sum of A_1 and A_2, while solid line is mean measured peak amplitudes (n = 4). ***B***. Conductance-voltage plots derived from data in A by correcting for sodium reversal potential. Data were fitted with a single Boltzmann function (n = 4).

5. Gating properties of TTX-R currents in rat DRGs



A. Family of Na^+ currents recorded in 200 nM TTX from a small diameter rat DRG. Depolarizing pulses were applied between -60 mV and +35 mV from a holding potential of -80 mV. ***B***. Time constants of inactivation calculated at different voltages. Inactivation kinetics were fitted to a double exponential function, (●), fast component, (○), slow component. ***C***. Current-voltage relationships for fast (●) and slow (○) gating modes were derived from exponential fit parameters, A_1 and A_2. Dotted line is the mean sum of A_1 and A_2, while solid line is mean measured peak amplitudes ***D***. For steady-state inactivation a two-step protocol was applied with conditioning pulses of 1 s duration followed by a test pulse to -20 mV. E. Inactivation kinetics of currents evoked by test pulse following conditioning pulses to -60 mV and -30 mV. F. Steady-state inactivation data were fitted with a double Boltzmann equation

❸ SUMMARY

Nav1.8 fast inactivation is composed of at least two gating modes with different activation and inactivation properties. The slow gating mode has properties distinct from previously reported slow inactivation. Preliminary data from TTX-R currents in small DRGs has confirmed the presence of the two gating modes in 3 out of 10 cells recorded.