INACTIVATION OF Na^+ CURRENTS CONTRIBUTES TO THE ADAPTATION OF SPIDER MECHANOSENSORY NEURONS

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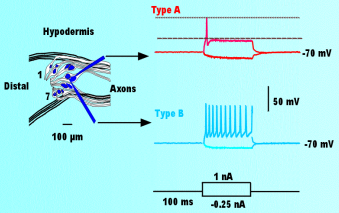
Introduction

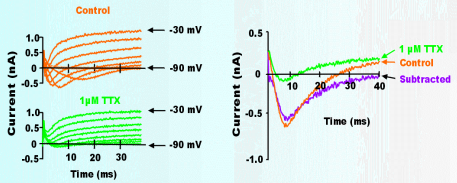
Voltage-activated sodium current (I_{Na}) is primarily responsible for the leading edge of the action potential in many neurons. While I_{Na} generally activates rapidly when a neuron is depolarized, its inactivation properties differ significantly between different neurons and even within one neuron, where I_{Na} often has slowly and rapidly inactivating components. I_{Na} inactivation has been suggested to regulate action potential firing frequency in some cells, but no clear picture of this relationship has emerged. The paired mechanosensory neurons of a spider slit-sense organ have one neuron that adapts rapidly (Type A) and the other slowly (Type B) in response to a step depolarizations. Therefore, this preparation is an excellent tool for comparing the activation and inactivation properties of I_{Na} and their contributions to the firing behavior of sensory neurons.

Preparation



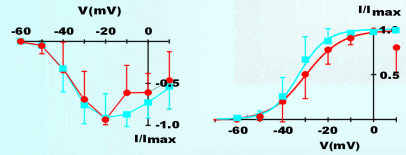
The lyriform slit-sense organ VS-3 in the patella of the spider, *Cupiennius salei* has seven to eight slits, which are each innervated by a pair of bipolar mechanosensory neurons. Both neurons in each pair respond to a sustained mechanical or electrical stimulus with a rapidly adapting burst of action potentials. However, adaptation to silence in Type A neurons occurs in ~20 ms, often producing only one or two action potentials, while Type B neurons respond with a burst of action potentials lasting several hundred milliseconds. For the electrophysiological experiments the neurons were detached from the cuticle but remained embedded in an internal membrane (hypodermis), which was spread onto a small coverslip. The axons and dendrites were crushed at ~100 µm from the somata to improve the voltage-clamp conditions. The coverslip was placed on a preparation holder where the neurons could be superfused with different solutions during recordings. The spider saline, which contained (in mM) 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, 5 sucrose, and 10 HEPES, pH 7.8 was replaced with a solution that contained blockers for K- and Ca-channels and the Na-concentration was reduced to 100 mM, because physiological Na-concentration did not allow stable voltage-clamp. Recordings were performed using the discontinuous single-electrode current- and voltage-clamp methods with an SEC-10 LAmplifier (NPI Electronic, Tamm, Germany). Electrodes were filled with 3 M CsCl and their resistances were 40-90 MΩ. All current- and voltage-clamp experiments were controlled by an IBM compatible computer with custom written software (ASF Software, Halifax, NS).

Activation of I_{Na}



I_{Na} in VS-3 neurons. *Left*: Currents elicited from a holding potential of -100 mV to potentials from -90 mV to 30 mV at 10 mV intervals. Upper panel shows the control recording and the lower panel shows identical recording after I_{Na} was blocked with 1 µM TTX. *Right*: An example of subtraction of the current trace after TTX from a trace in a control recording to eliminate the outward currents. This recording was obtained with a voltage pulse of -20 mV. There were no statistically significant differences in I_{Na} amplitudes between the two neuron types.

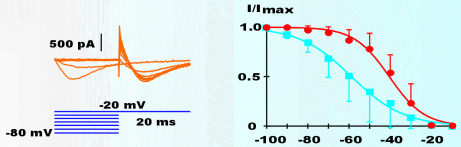
● Type A: $V_{½}$ = -30.6±8.1; s = 5.1±2.7; n = 11
■ Type B: $V_{½}$ = -32.7±6.8; s = 4.5±1.5; n = 11



Voltage dependence of activation of I_{Na}. *Left*: Points show the normalized mean (±SD) peak I_{Na} from 11 Type A and 11 Type B neurons plotted against test potentials. *Right*: Steady-state activation was determined by fitting the peak currents from each experiment with a Boltzmann distribution of the form: $I/I_{max} = 1/(1 + e^{(V - V_{½})/s})$, where I is the current at test potential V, I_{max} is the maximum current, $V_{½}$ is the potential giving the half-maximum current, and s is the slope factor. Boltzmann fits for 11 neurons of both types are shown. There were no statistically significant differences in these values between the two neuron types.

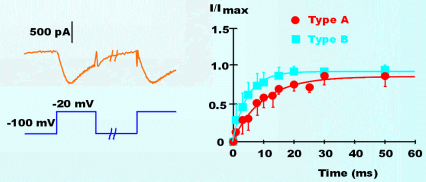
Inactivation of I_{Na}

● Type A: $V_{½}$ = -40.1±7.8; s = 6.8±4.3; n = 7
■ Type B: $V_{½}$ = -58.1±13.3; s = 9.3±4.8; n = 8



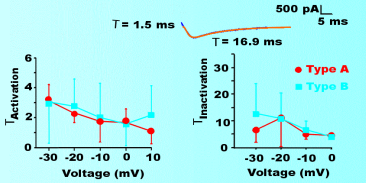
Steady-state inactivation of I_{Na} was voltage-dependent. *Left*: Recording where holding potentials from -80 mV to -20 mV for 20 ms were used before a test pulse to a constant potential of -20 mV. *Right*: Steady-state inactivation was determined by fitting the peak currents from each experiment at different voltages with a Boltzmann distribution. The fitted parameters from 7 experiments with Type A neurons and 8 experiments with Type B neurons indicate that the s values were not different for the two neuron types, the $V_{½}$ values were significantly more negative for Type B than for Type A neurons, indicating that I_{Na} inactivation occurred at more positive potentials in Type A than Type B neurons. Therefore, the ability of Type B neurons to fire a burst of action potentials, in contrast to the one or two action potentials produced by Type A neurons, does not arise from I_{Na} being more easily inactivated in Type A neurons.

Recovery from Inactivation



I_{Na} recovery from inactivation. *Left*: Recovery from inactivation was studied by activating the current from a constant holding potential of -100 mV to -20 mV for 20 ms and then applying a similar test pulse after varying periods at -100 mV. *Right*: Peak I_{Na} activated by the second test pulse was normalized to the peak I_{Na} elicited by the first test pulse and the normalized current was plotted against time between the two test pulses, and fitted by a single exponential decay. The data revealed a difference in time course of recovery from inactivation between the two neuron types. Type B neurons recovered significantly faster (τ = 6.1 ms) than Type A neurons (τ = 11.3 ms). Therefore, it seems likely that the ability of I_{Na} in Type B neurons to inactivate at more depolarized potentials allows a more rapid recovery from inactivation and contributes to the production of more action potentials.

Activation and Inactivation Time Constants



Activation and inactivation time constants of I_{Na}. *Inset*: The time courses of activation and inactivation of I_{Na} at different test potentials were fitted by the Hodgkin-Huxley equation for an inactivating current: $(I = I_{\infty}\ [1 - e^{(-t/t1)}]^m e^{(-t/t2)})$, where I_{∞} is the current level expected in the absence of inactivation, $t1$ is the time constant of activation, $t2$ is the time constant of inactivation, and m is an integer exponent. In this example the time constant was 1.5 ms for activation and 16.9 ms for inactivation. *Right*: Mean activation time constants (±SD) from 6 Type A and 11 Type B neurons are plotted against test voltage. *Left*: Mean inactivation time constants (±SD) from the same neurons at different test voltages. Both time constants were voltage dependent, being slightly faster at more depolarizing potentials, but there were no statistically significant differences in either parameter between the two neuron types.

Conclusions

This is our third investigation into the properties of voltage-activated currents in the two types of spider VS-3 neurons. We have shown that:

- LVA-I_{Ca} is not significantly different between the two neuron types, and can not explain the differences in their firing behavior.
- The normal action potentials in both types of neurons are produced by an inactivating I_{Na} and their repolarization is driven by I_K.
- I_{Na} inactivation and I_K activation both occur closer to the resting potential in Type B neurons than in Type A neurons.
- I_{Na} recovers faster from inactivation in Type B than Type A neurons.
- The time constants of activation and inactivation of I_{Na}, are similar in both neuron types.
- There are no differences in the distributions of Na or K channels in the two types of VS-3 neurons.

The slower recovery from inactivation in Type A neurons indicates that it is more difficult to reopen their inactivation gates once they have been inactivated. Therefore, a possible explanation for the different adaptation properties is that Na-channels in Type A neurons remain inactivated for a longer period than in Type B neurons. This assumption may be tested in the future by single-channel analysis.