On the Persistent Sodium Current in Squid Giant Axons

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Clay, John R. On the persistent sodium current in squid giant axons. J Neurophysiol 89: 640–644, 2003; 10.1152/jn.00652.2002—R. F. Rakowski, D. C. Gadsby, and P. DeWeer have reported a persistent, tetrodotoxin-sensitive sodium ion current (I_{NaP}) in squid giant axons having a low threshold (-90 mV) and a maximal inward amplitude of -4 μA/cm² at -50 mV. This report makes the case that most of I_{NaP} is attributable to an ion channel mechanism distinct from the classical rapidly activating and inactivating sodium ion current, I_{Na}, which is also tetrodotoxin sensitive. The analysis of the contribution of I_{Na} to I_{NaP} is critically dependent on slow inactivation of I_{Na}. The results of this gating process reported here demonstrate that inactivation of I_{Na} is complete in the steady-state for V > -40 mV, thereby making it unlikely that I_{NaP} in this potential range is attributable to I_{Na}. Moreover, -90 mV is well below I_{Na} threshold, as demonstrated by the C. A. Vandenberg and F. Bezanilla model of I_{Na} gating in squid giant axons. Their model predicts a persistent current having a threshold of -60 mV and a peak amplitude of -25 μA/cm² at -20 mV. Modulation of this component by the slow inactivation process predicts a persistent current that is finite in the -60- to -40-mV range having a peak amplitude of -1 μA/cm² at -50 mV. Subtraction of this current from the I_{NaP} measurements yields the portion of I_{NaP} that appears to be attributable to an ion channel mechanism distinct from I_{Na}.

INTRODUCTION

A step depolarization of the nerve membrane potential from its resting level (-60 mV) elicits a sodium ion current, I_{Na}, which is activated typically within a millisecond, followed by inactivation of the current on a time scale of several milliseconds (Hodgkin and Huxley 1952a). These results are well described by the Hodgkin and Huxley (1952b) model of I_{Na}. Their model also predicts a small amplitude steady-state, or persistent, current given by the overlap between activation and inactivation—the so-called “window” current (Attwell et al. 1979). Over the past 20 years, a persistent Na⁺ current (I_{NaP}) has been found in neurons from various regions of the mammalian brain that is not adequately described by the Hodgkin and Huxley (1952b) window current model (Crill 1996). In particular, the threshold of I_{NaP} is 10–15 mV below threshold of the classical Na⁺ current; this has led some investigators to suggest that it might originate from a set of ion channels within the nerve membrane that are distinct from those underlying I_{Na}, even though I_{Na} and I_{NaP} are both blocked in a similar manner by tetrodotoxin (Crill 1996). Rakowski et al. (2002) have reported careful measurements of I_{NaP} from squid giant axons, the preparation used by Hodgkin and Huxley (1952a), which they attributed to the I_{Na} channel. The analysis of their results in this report supports the alternative conclusion, namely that most of I_{NaP} originates from a subset of I_{Na} channels that lack the gating mechanism of the I_{Na} channel. In particular, the measurements of slow inactivation of I_{Na} given in the following text would appear to exclude the possibility that I_{NaP} is attributable to the I_{Na} channel, except for a small portion in the -60- to -40-mV range.

METHODS

The measurements of I_{NaP} in Fig. 2 were carried out in squid giant axons (Loligo pealei) using axial wire voltage-clamp and intracellular perfusion techniques described elsewhere (Clay and Shlesinger 1983). The extracellular solution consisted of (in mM) 425 NaCl, 10 KCl, 50 MgCl₂, 10 CaCl₂, and 10 Tris-HCl (pH 7.6). The intracellular perfusate in this experiment consisted of (in mM) 300 CsF (which effectively blocks I_{K}), 20 Na glutamate, 15 NaHPO₄, and 400 sucrose (pH 7.3). This solution is referred to as the 300 Cs perfusate. In other experiments to test for possible effects of intracellular F⁻ on Na⁺ channel gating the intracellular perfusate consisted of (in mM) 50 Na glutamate, 250 K glutamate, 25 K₂HPO₄, and 400 sucrose (pH 7.3)—0 Cs—with an extracellular solution consisting of (in MM) 300 KCl, 135 NaCl, 50 MgCl₂, 10 CaCl₂, and 10 Tris-HCl. The temperature of the extracellular solution was 5°C maintained constant to within 0.1°C by a Peltier device located within the experimental chamber. The persistent current predicted by the Vandenberg and Bezanilla (1991b) model of I_{Na} gating (APPENDIX) was calculated with the Mathematica software package (Wolfram 1999).

RESULTS

The I_{NaP} results of Rakowski et al. (2002) are reproduced in Fig. 1. These measurements were carried out by holding the preparation in voltage clamp at the potentials indicated on the abscissa during application of tetrodotoxin (TTX) at a final concentration of 0.2 μM. The currents shown are the difference between the holding current prior to TTX application and after wash-in of the toxin. The difference current is present at potentials as negative as -90 mV with a maximal (inward) amplitude of approximately -4 μA/cm² at -50 mV. The curve describing the data (Fig. 1, —) is given by

\[
I_{NaP} = 4.34(V/24)(Na\ exp(V/24) - Na_0)\times(exp(V/24) - 1)(1 + exp(-(V + 65)/7))
\]  

(1)

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persistent current. Also shown in Fig. 2 are the classical, or standard, inactivation results obtained with a holding potential of −100 mV and a 50-ms prepulse to the potentials indicated (Fig. 2, bottom), followed by a step to 0 mV. These results (labeled “HH”) are described by the Hodgkin and Huxley (1952b) inactivation curve, \( \alpha_i / (\alpha_i + \beta_i) \) with

\[
\alpha_i / (\alpha_i + \beta_i) = 1/(1 + \exp(-0.1(V + 57)))
\]

and

\[
\beta_i = \exp(0.1(V + 57))
\]

FIG. 2. Slow inactivation of \( I_{NaP} \). Top panel: currents elicited by the two step protocol illustrated in the inset of the middle panel. Membrane potential was stepped from −90 to −55 mV for time \( \Delta t \) followed by a step to 0 mV. Currents elicited by the second step are shown superimposed for various \( \Delta t \) indicated alongside each record. A rest interval at −90 mV of 5 s was used between each application of the protocol. The scales represent 3 ms and 0.25 mA cm\(^{-2}\). Middle panel: semi-logarithmic plot of peak \( I_{NaP} \) from the top panel as a function of \( \Delta t \). Prepulse potential was −55 mV. The result for time [yen] was obtained after holding the potential at −55 mV for 5 min. Theoretical curve describing these results corresponds to 0.37 + 0.35 exp(−Δt/\( \tau_i \)) + 0.28 exp(−Δt/\( \tau_j \)), where \( \tau_i = 10 \) ms and \( \tau_j = 54 \) s. Bottom panel: voltage dependence of \( I_{NaP} \) inactivation. The closed circles (labeled HH) represent fractional inactivation with a 50 ms duration prepulse to the voltages indicated on the abscissa. The closed squares represent fractional inactivation obtained by holding at the potentials indicated for 5 min. The error bars represent ± SD (n = 4). The curve describing the HH results is given by \( \alpha_i / (\alpha_i + \beta_i) \) with \( \alpha_i = 0.14 \exp(- (V + 60)/20) \) and \( \beta_i = 1/(1 + \exp(-0.1(V + 30))) \). These expressions were taken from Hodgkin and Huxley (1952b) with their \( \alpha_i \) multiplied by a factor of 2. The curve describing the results labeled “true steady-state inactivation” is the product of \( \alpha_i / (\alpha_i + \beta_i) \) and \( \alpha_i / (\alpha_i + \beta_i) \) with \( \alpha_i = \exp(-0.1(V + 57)) \) and \( \beta_i = \exp(0.1(V + 57)) \).
\[ \alpha_s = 0.14 \exp[-(V + 60/20)] \] and \[ \beta_s = 1/[1 + \exp[-0.1(V + 30)]]. \]

Steady-state inactivation is described by \[ \alpha_s \alpha_m ([\alpha_s + \beta_s][\alpha_m + \beta_m]), \] where \( \alpha_s \) and \( \beta_s \) are the slow inactivation parameters, with \( \alpha_s = \exp[-0.1(V + 57)] \) and \( \beta_s = \exp[0.1(V + 57)]. \) As indicated by Fig. 2, bottom, the net effect of the slow process in the steady state is to shift inactivation leftward on the voltage axis and to steepen the slope of this relation at its midpoint. The results in Fig. 2, bottom, were not noticeably temperature dependent. Moreover, results similar to those shown in Fig. 2, top, were obtained without CsF in the perfusate (300K/300K, as described in METHODS), which suggests that Na\textsuperscript{+} gating in these experiments was not affected by intracellular F\textsuperscript{−}.

The steady-state, or persistent, current in the Vandenberg and Bezanilla (1991b) model with slow inactivation taken into account is the product of Fig. 1, ---, and \( \alpha_s / (\alpha_s + \beta_s) \), and is represented by the curve labeled a in Fig. 3. This result, in turn, was subtracted from the \( I_{NaP} \) data in Fig. 1, giving the modified \( I_{NaP} \) results in Fig. 3. The only significant changes occurred between \(-60\) and \(-40\) mV (open symbols). The other \( I_{NaP} \) results were unchanged because the predicted contribution of the sustained \( I_{Na} \) component to \( I_{NaP} \) is virtually nil outside the \(-60\) to \(-40\) mV range. The modified results in Fig. 3 represent that portion of \( I_{NaP} \)—the major part of \( I_{NaP} \) measured by Rakowski et al. (2002)—which is attributable here to a set of channels that are distinct from \( I_{Na} \).

**Discussion**

Rakowski et al. (2002) presumed that their \( I_{NaP} \) results in squid axons were attributable to the classical Na\textsuperscript{+} channel given that \( I_{NaP} \) reversed near the theoretical sodium ion equilibrium potential, \( E_{NaP} \); tetramethylammonium ions (TMA) and n-methylglutamate ions (NMG) were both impermeant; and \( I_{NaP} \) had a tetrodotoxin sensitivity similar to that of Na channel. The analysis provided here of the \( I_{NaP} \) results is probably attributable to a difference in experimental protocol. Rudy (1978) measured the recovery of the Na\textsuperscript{+} conductance after a long-lasting depolarization of the membrane potential, whereas the onset of this process from a negative holding potential was used in these experiments. A straightforward way of measuring slow inactivation in the steady-state is to hold the membrane potential for several minutes at various levels and then measure peak inward current with a test pulse to 0 mV, a procedure that has been used for muscle Na\textsuperscript{+} channels (Almers et al., 1983; Simonichi and Stuhmer 1987). This study appears to be the first in which this protocol has been used in squid giant axons (Fig. 2). The results suggest that inactivation of \( I_{Na} \) is complete at \(-40\) mV. A similar result was reported for rat muscle Na\textsuperscript{+} channels heterologously expressed in HEK-293 cells (Cummins and Sigworth 1996). Whole cell recordings cannot exclude the possibility that a small component of the Na\textsuperscript{+} conductance remains active in the steady-state for \( V > -40 \) mV. The single-channel recordings of Hirschberg et al. (1995) more compellingly demonstrate complete inactivation, at least for \( V > -20 \) mV. In other words, any sustained Na\textsuperscript{+} current for \( V > -20 \) mV would, in the spirit of this report, be attributable to an \( I_{NaP} \) channel distinct from \( I_{Na} \). Conversely, the results of Hirschberg et al. (1995) also demonstrate a small activation of Na\textsuperscript{+} conductance for \( V < -60 \) mV, even for potentials as negative as \(-90\) mV, which would be consistent with an identification of \( I_{NaP} \) with \( I_{Na} \) in this potential range. However, activation of \( I_{Na} \) for \( V < -60 \) mV in squid giant axons is not predicted by the Vandenberg and Bezanilla (1991b) model (Fig. 1). Moreover, Correa and Bezanilla (1994) found a very minimal activation of \( I_{Na} \) channels in steady state even at moderately depolarized potentials, such as \(-40\) mV. They did not report single Na\textsuperscript{+} channel openings at \(-90\) mV in steady-state conditions.
Persistent Na\(^+\) current appears to underlie oscillatory activity in mammalian brain neurons, such as the theta rhythm in the entorhinal cortex (Alonso and Llinas 1989; Silva et al. 1991; White et al. 1995). The role of \(I_{NaP}\) in squid giant axons under physiological conditions is unknown. It appears to cause spontaneous rhythm firing of action potentials with slightly elevated intracellular pH (pH\(_I\) > 7.7) (Clay and Shrier 2001). The axon has a small, nonselective cation conductance, sometimes referred to as a leak conductance, that is permeable to both Na\(^+\) and K\(^+\) and is activated by intracellular protons (Clay and Shrier 2001, 2002). A similar conductance was originally reported in rat dorsal root ganglion neurons by Bevan and Yeats (1991) with a proton binding site on the external rather than the intracellular side of the membrane. Intracellular perfusates in the squid giant axon with slightly alkaline pH remove this component, thereby allowing the negative slope character of \(I_{NaP}\) to destabilize the resting state of the axon (Clay and Shrier 2001, 2002).

The molecular basis of \(I_{NaP}\) has yet to be determined. It could be mediated either by a novel \(\alpha\) subunit isoform not yet cloned or by one of the genes that are known to encode \(I_{NaP}\) (Magistretti et al. 1999). Heterologous expression of cloned Na\(^+\) channel subunits will, quite likely, resolve this issue.

**APPENDIX**

The Vandenberg and Bezanilla (1991b) model of \(I_{NaP}\) gating is given by

\[
I_{NaP} = g_{NaP}V \exp(0.014(V - 10))/\cosh((V - 10)/10) \times (1 + 0.4 \exp(-0.38(V - 24))) \tag{A9}\]

with \(g_{NaP} = 130 \text{ mS/cm}^2\) at 20°C (Rosenthal and Bezanilla 2002; J.J.C. Rosenthal, personal communication) and \(E_{NaP} = 64 \text{ mV}\). (The measurements of Rakowski et al. (2002) were carried out at 17–18°C.) Steady-state current was determined from Eq. A9 with \(g_{NaP}\), the open channel probability, calculated from Eqs. A7–A8 with all time derivatives set to zero. The Mathematica software package (Wolfram 1998) was used to perform the necessary matrix inversion in this analysis.

**REFERENCES**


Vandenberg CA and Bezanilla F. A sodium channel gating model based on single channel, macroscopic ionic, and gating currents in the squid giant axon. *Biophys J* 60: 1511–1533, 1991b.
