Potential-Dependent Blockage of Batrachotoxin-Modified Sodium Channels in Frog Node of Ranvier by Calcium Ions

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Voltage clamp method has been used to study the ionic currents through sodium channels modified by steroidal alkaloid batrachotoxin (BTX). To perform a complete modification of Na currents ($I_{Na}$) to 6 min application of 0.019 mmol/l BTX to the node was accompanied by repetitive membrane depolarization (to +60 mV, 2 ms, 10 Hz). Control external solution contained (in mmol/l: 110 Na⁺, 2 Ca²⁺, 10 tetra-ethylammonium⁺, 5 tris(hydroxymethyl)aminomethane⁺, 129 Cl⁻, pH 7.6.

Fig. 1 shows the dependence of “instantaneous” $I_{Na}$ ($I_{Na}^*$) on the membrane potentials. The currents were measured by the use of two-pulse program shown in the inset: first pulse from the holding potential $E_h$—130 mV to $E_i$—60 mV (in other experiments to —40 or to —20 mV) was designed to open all (or almost all) the modified Na channels, while the second pulse $E_2$ allowed to measure the tail currents through the open channels at various membrane potentials.

It can be seen that the outward current tends to saturate at $E$ +60 mV. The inward $I_{Na}^*$ rises as $E$ approaches approximately to —100 mV, however at more negative $E$, $I_{Na}^*$ decreases (a negative slope of $I_{Na}^*-E$ curve). Increasing of external Ca²⁺ ions concentration from 2 to 20 mmol/l leads to moderate decrease of $I_{Na}^*$ over the potential range from —60 to +80 mV and to a strong depression of $I_{Na}^*$ at $E$ —60 mV. The maximum of the inward $I_{Na}^*$ is shifted by 20—30 mV to more positive $E$. Nearly the same shift was observed in two other experiments.

The results obtained allow to propose that the negative slope of $I_{Na}^*-E$ relation reflects a fast potential-dependent block of the open Na channels by external Ca²⁺ ions. Potential dependence of this block can be explained on assumption that a certain fraction $\delta$ of the applied potential affects the binding site for Ca²⁺ ion in the channel (Woodhull 1973). If one assumes that nonlinearity of $I_{Na}^*-E$ curve in the region of negative $E$ is exclusively due to blocking action of Ca²⁺ ions, then the mean value of $\delta$ is 0.43 ± 0.02 (n = 5), the dissociation constant $K_p$ being 200 ± 54 mmol/l.
Very close value of $\delta$ was obtained earlier for protonation of the inner acid group in the Na channel (Mozhayeva et al. 1981, 1982). This leads us to conclude that $Ca^{2+}$ binds directly to the acid group of the channel selectivity filter (Hille 1975). It seems to be probable that the analogous Ca-block of open Na channels at negative $E$ occurs also in normal nerve membrane, however it cannot be revealed readily due to a high rate of normal Na channels closing at these negative $E$.

Fig. 1. Potential dependent inhibition of instantaneous Na currents ($I_{Na}$) by $Ca^{2+}$ ions. $I_{Na}$ were measured at $E_2$; $E_1 = -60$ mV. The holding potential, $E_h = -130$ mV. The leakage currents were subtracted automatically, by the use of analog circuit. Potassium currents were inhibited by 10 mmol/l tetraethylammonium in external solution and 20 mmol/l CsF in the "internal solution" bathing the cut internodes. The external solution contained 110 mmol/l Na* and 2 mmol/l (curve 1) or 20 mmol/l Ca** (curve 2). Temperature 10 °C.

References


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