Short communication

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). Zo perform a complete modification of Na currents (I_{Na}) 4 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 Ca^{2+}, 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 programm shown in the inset: first pulse from the holding potential E_h −130 mV to E_1 −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 Ca^{2+} ions concentration from 2 to 20 mml/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 Ca^{2+} ions. Potential dependence of this block can be explained on assuption that a certain fraction δ of the applied potential affects the binding site for Ca^{2+} 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 Ca^{2+} ions, then the mean value of δ is 0.43 ± 0.02 (n = 5), the dissociation constant K_D being 200 ± 54 mmol/l.

Very close value of δ was obtained earlier for protonation of the inner acid group in the Na channel (Mozhayeva el 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 analoguous 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.

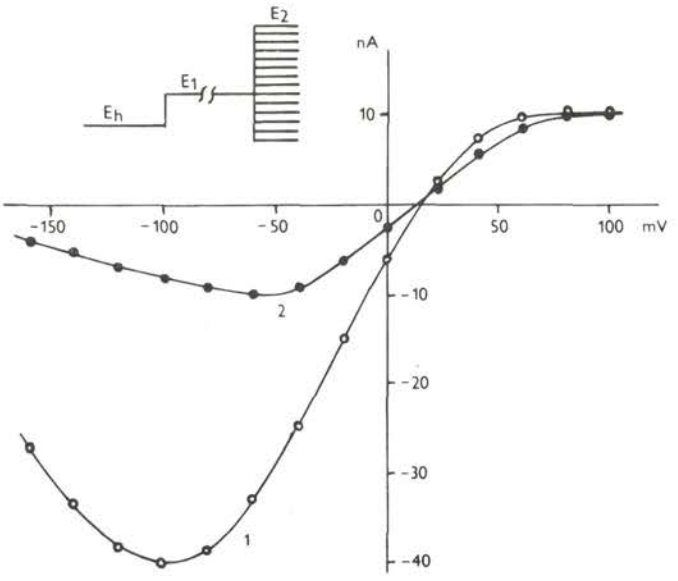

Fig. 1. Potential dependent inhibition of instantaneous Na currents (I_{Na}*) by Ca^{2+} ions. I_{Na}* were measured at E_2; $E_1 = -60$ mV. The holding potential, $E_h = -130$ mV. The leakage currents were subtracted automatically, by the use of analog circuit. Potassium currents were inhibited by 10 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^{2+} (curve 2). Temperature 10 °C.

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