Short communication

Potential-Dependent Calcium Blockage of Normal and Aconitine-Modified Sodium Channels in Frog Node of Ranvier

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Hydrogen and some divalent cations block Na channels in a voltage-dependent manner (Woodhull 1973; Ulbricht and Wagner 1975; Mozhayeva et al. 1981, 1982a; Begenisich and Danko 1983). The potential-dependence of this blockage suggests that the binding site for blocking ions is situated in the pore at some fractional distance (δ) from the outside (Woodhull 1973). Alkaloid aconitine has been shown to reduce δ for H^+ blockage from about 0.4 to 0.15 (Mozhayeva et al. 1982b). The aim of the present work was to compare the potential dependence of Ca^{2+} blocks in normal and aconitine-modified Na channels.

Experiments were carried out on single myelinated fibres of *Rana ridibunda* under voltage clamp conditions (Mozhayeva et al. 1977). External solutions contained (in mmol/l): 110 NaCl, 2 or 30 $CaCl_2$, 8 tetraethylammonium chloride, 5 tris(hydroxymethyl)aminomethane-HCl; pH 7.5. The internodes were cut in isotonic KF solution. Application of 10^{-5} mol/l aconitine to the node was accompanied by repetitive stimulation (10 Hz, 2 ms). The "instantaneous" currents were determined in response to postpulses of different amplitudes following the test pulse of a constant amplitude opening nearly all Na channels, were recorded. These currents were extrapolated to the time of switching potential from test pulse tc postpulses. The setting time of the clamp membrane potential did not exceed 20 μs. Linear current components were subtracted using P/4 program; control pulses started from holding potential.

Figs. 1 and 2 show the "instantaneous" current-voltage relations of normal and aconitine-modified Na channels in solutions with 2 or 30 mmol/l Ca^{2+} ions, and the calculated $pK_{(Ca)}$, respectively. $pK_{(Ca)}$ values were calculated by equation

$$pK_{(Ca)} = \lg (I_1/I_2 - 1) - \lg (C_2 - C_1 I_1/I_2) \quad (1)$$

where I_1 and I_2 are "instantaneous" currents at 2 and 30 mmol/l Ca^{2+} ions and C_1 and C_2 are normal and increased Ca^{2+} concentrations, respectively. $pK_{(Ca)}$ for normal and aconitine-modified Na channels depended on the potential linearly and had similar slopes. The average dissociation constant at 0 mV, $K_{d(o)}$, and δ for normal Na channels were 54 ± 10 mmol/l and 0.23 ± 0.03 ($n = 8$), respectively. For aconitine-modified Na channels the analogous values were 61 ± 6 mmol/l and

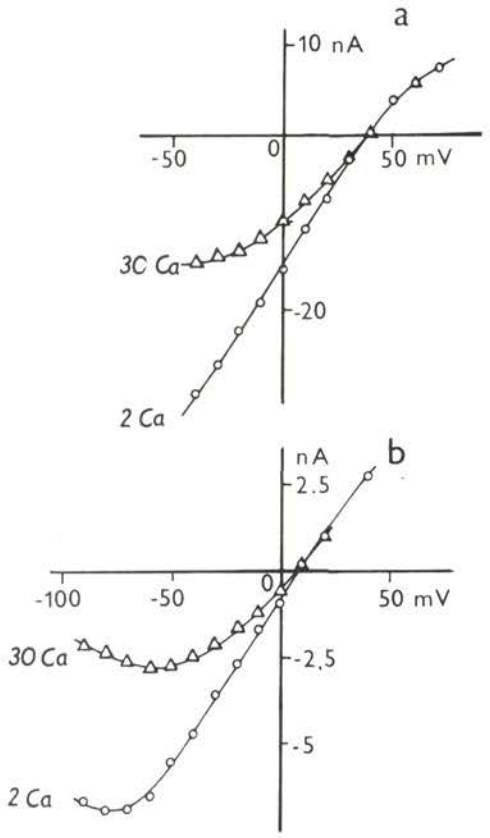

Fig. 1. "Instantaneous" current-voltage relations for normal (*a*) and aconitine-modified Na channels (*b*) in solutions with 2 and 30 mmol/l Ca^{2+} ions. "Instantaneous" currents were measured at potentials of different amplitudes after test pulses of +80 mV (*a*) and +40 mV (*b*). Holding potential was −100 mV (*a*) and −130 mV (*b*). Leakage currents were subtracted using P/4 program. Temperature 10 °C.

0.21 ± 0.02 ($n = 6$), respectively. Similar values of $K_{d(o)}$ and δ for Ca^{2+} ions were calculated for Na channels modified by batrachotoxin (Mozhayeva et al. 1985).

Thus, the susceptibility of Na channels to Ca^{2+} blockage remains, unlike to H^+ blockage, nearly unchanged after aconitine modification.

The results obtained can be explained on the assumption that in normal Na channel, the binding site for Ca^{2+} ions is situated nearer to the external pore mouth than that for H^+ ions, and that alkaloids induce alternations mostly in the internal segment of the pore.

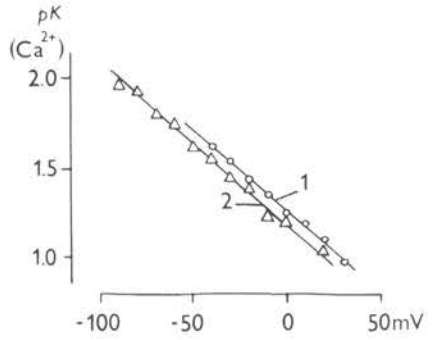

Fig. 2. $pK_{(Ca)}$ values calculated from "instantaneous" current-voltage relations presented in Fig. 1 by formula (1) (see text). 1 — normal Na channels, 2 — aconitine-modified Na channels. Dissociation constants for Ca^{2+} at 0 mV, $K_{d(o)}$, were 56 mmol/l (1) and 66 mmol/l (2). The fractional electric distance, δ, had the same value for both cases (0.25).

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