Effect of Batrachotoxin (BTX) on Activation, Inactivation and Ion Selectivity of Sodium Channels in Clonal Neuroblastoma Cells

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Effects of BTX on sodium channels in the internally perfused cultural neuroblastoma cells (clones N18 A–1, its thermoresistive mutants NTR and Neuro 2a) have been studied by means of the suction-pipette voltage-clamp method (Kostyuk et al. 1975; Zubov et al. 1980). The control external solution contained (in mmol/l): 132.5 NaCl, 2.0 $CaCl_2$, 5.0 Tris-HCl, 7.5 tetraethylammonium (TEA)Cl, pH 7.5—7.6. In the test solutions NaCl was substituted equimolarly by KCl or NH_4Cl. The internal solution contained: 120 KF, 20 CsCl, 5 Tris-HCl, pH 7.5.—7.6.

External application of 2×10^{-5} mol/l BTX was accompanied by repetitive depolarisation of the membrane until all Na channels became modified. The membrane potential, E, was defined as the result of the inside/outside potential substraction. All experiments were conducted at room temperature.

Ionic currents through BTX-modified channels usually appeared already at potentials -80 mV, whereas they could be elicited only at $E = -50 + -40$ mV before BTX treatment (not illustrated). Two phases could be revealed in the current rise: the fast and the slow one. The average voltage dependence of Na activation was shifted towards more negative E by 25 -40 mV. It can be seen from Fig. 1 that currents at $-70 + -50$ mV decay after having reached peak values. This decay reflects the process of a partial inactivation of modified Na channels. The extent of this inactivation towards the end of a 40-ms pulse was voltage-dependent: at $E = -80 + -60$ mV the currents were often inactivated by about 50% of their peak value, however at $E > -40$ mV remained practically unchanged. Correspondingly, depolarizing prepulses (100 ms in duration) to $E < -60$ mV resulted in a decrease in both the peak value of the sodium current and the rate of its rise during the test pulse. At larger depolarisations the steady-state inactivation was decreased and was finally abolished at $E > 0$ mV. Such a voltage

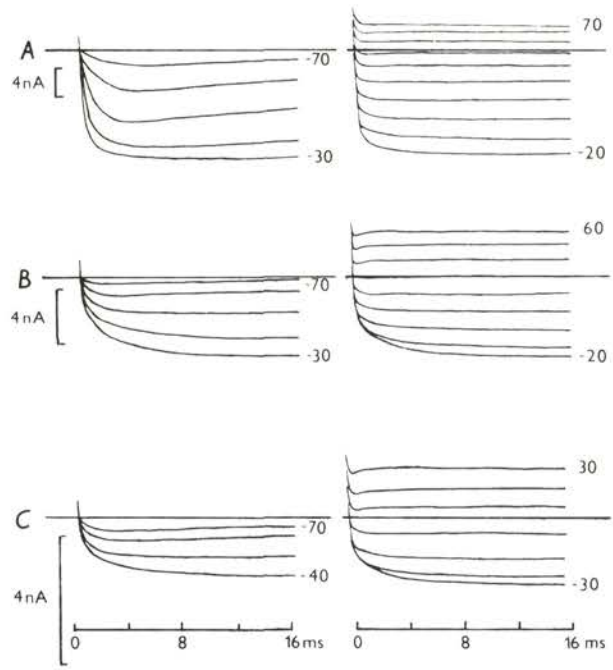

Fig. 1. Currents through BTX-modified sodium channels in neuroblastoma cells externally perfused with sodium (A), ammonium (B), and potassium (C) solutions. Test pulses ranged from −70 to +70 mV (A), to +60 mV (B), and to +30 mV (C) with the potential varying in 10 mV steps. Holding voltage −130 mV, temperature 20°C. Cell N18 A–1 26(81).

dependence of Na inactivation suggested the existence of at least two open states of the channel, the second one being analogous to the h_2 state, postulated by Chandler and Meves (1970). BTX apparently changes the parameters of these states in such a way that their probability becomes much greater than that of the inactivated state.

Replacement of external Na^+ by K^+ or NH_4^+ led to a decrease in the magnitude of the inward I_{Na} and caused a shift in the reversal potential, E_{rev}, towards less positive E. Simultaneously the kinetics of the ionic currents was changed: their decay during the depolarising step became essentially less pronounced. The relative permeabilities of Na channels before and after BTX treatment of the membrane were calculated from changes in E_{rev} (Hodgkin and Katz 1949); normal Na channels: $P_{NH_4}/P_{Na} = 0.35 \pm 0.03$ $(n = 12)$, $P_K/P_{Na} = 0.11 \pm 0.01$ $(n = 12)$; BTX-modified channels: $P_{NH_4}/P_{Na} = 0.70 \pm 0.04$ $(n = 13)$, $P_K/P_{Na} = 0.29 \pm 0.04$ $(n = 9)$.

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