Short communication

Reversible Blockage of Batrachotoxin-Modified Sodium Channels by Amine Compounds and Benzocaine in Frog Node of Ranvier

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In previous voltage-clamp experiments on frog nerves it has been established that modification of gating and ion selectivity of Na-channels by batrachotin (BTX) is accompanied by drastic decrease in their sensitivity to the blocking action of amine local anesthetics (Khodorov et al. 1977; Khodorov 1978) and related tertiary or quaternary amine compounds (for review see Khodorov 1981). At concentrations sufficient to cause an almost total blockage of normal sodium currents, I_{Na}, these drugs produce only relatively small suppression of modified sodium currents. Partial block of modified I_{Na} by large concentrations of amine drugs may be time- and voltage-dependent but it does not accumulate during repetitive membrane depolarization (Khodorov 1978; Zaborovskaya 1979). The aim of the present work was to examine the reversibility of this block. Experiments were carried out on single myelinated fibres of *Rana ridibunda* using the voltage clamp method (Dodge and Frankenhaeuser 1957). To block potassium currents the internodes were cut in isotonic CsCl solution. The nodes were superfused by control Ringer' solution containing (mmol/l): 112 NaCl, 2 $CaCl_2$, 2.5 KCl, 2 $NaHCO_3$, 5 Tris, pH 7.3. Application of 2×10^{-5} mmol/l BTX for 2 min to the node of Ranvier was accompanied by repetitive membrane stimulation (10 Hz, 5 ms).

Fig. 1 shows the effect of large concentrations of the quaternary-antiarrhythmic, N-propyl ajmaline (NPA, "Neo-Giluritmal") on normal I_{Na} peak and modified (steady state) I_{Na} in the node of Ranvier treated with BTX NPA concentration. 2×10^{-3} mmol/l caused in fact a complete inhibition of the peak I_{Na} (trace 2, left) and a marked (about 60%) reduction in the modified I_{Na} (trace 2, right) Washing of the node with a drug-free (control) solution for 30 min led to an almost complete recovery of the modified I_{Na} (trace 4) whereas the I_{Na} peak through the normal Na channels remained strongly blocked. Similar results have been obtained with tertiary amine ajmaline, and amine local anesthetics.

The reversibility of the block modified channels indicates that BTX and amine compounds bind to separate although strongly interacting (allosteric interaction) sites in the Na channel. Relatively fast unblocking of modified Na channels during

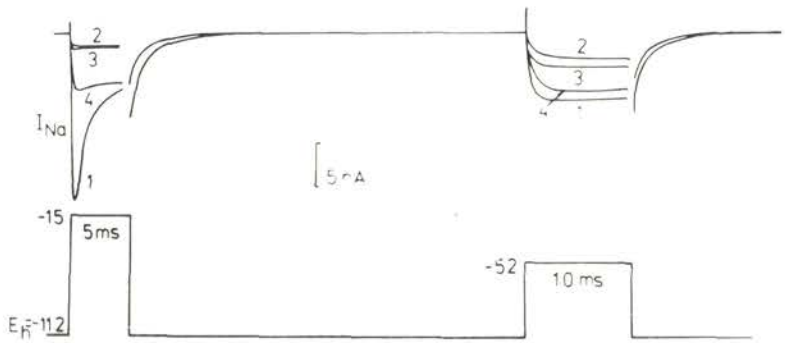

Fig. 1. Reversible block of BTX-modified Na channels by high NPA concentrations. After modification of Na channels by BTX the node was stimulated by twin pulses of different amplitude to elicite Na currents either through both modified and normal Na channels (left), or only through the modified ones (right). Current records: 1: in the control Ringer's solution; 2: during application of 2 mmol/l NPA; 3 and 4: 2 and 30 min after the beginning of wash out of the node with control solution. Temperature 15 °C, fibre 16.12.80.

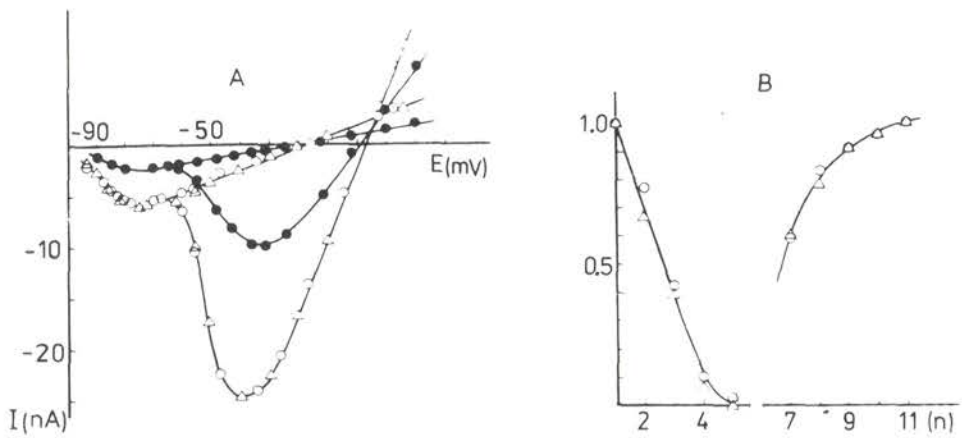

Fig. 2. Reversible block of BTX-modified and normal Na channels by benzocaine (BZ). *A*, ○ = modified (the first "hump" on the I_{Na} — E curve) and normal (the second "hump") sodium currents in BTX-treated node of Ranvier before application of BZ. ● = the same currents during the action of 0.8 mmol/l BZ; △ = modified and normal Na currents after 1 min washing of the node with control solution. Temperature 15 °C. Fibre 16.13.80. *B*, time course of Na currents inhibition through normal (○) and modified (△) channels (curve 1) by BZ (1.5 mmol/l); and their subsequent restoration (curve 2) during the washing of node. Abscissa: number of the test pulse repeated at a rate 0.2 Hz. Ordinate: relative amplitude of normal and modified I_{Na}. Expressed in percents of the pre-application values). Temperature 10 °C. Fibre 24.10.80.

washing of the node with a drug-free solution allows to conclude that in BTX-modified channels the rate constant of the drug-receptor complex dissociation is much larger than in the normal channels.

Unlike tertiary and quaternary amine compounds, the permanently uncharged (at all possible physiological pH) anesthetic benzocaine (BZ) blocks normal and BTX-modified I_{Na} almost identically (Fig. 2 A). The time course of BZ-induced current inhibition as well as the kinetics of I_{Na} restoration during washing of the node with the control Ringer's are the same for both normal and BTX-modified Na channels (Fig. 2 B).

To reconcile these data with the results of other experiments that suggest a competition between BZ and amine channels blockers for a common channel "receptor" (Schmidtmayer and Ulbricht 1980; Guselnikova et al. 1981) it is necessary to propose that BTX affects mainly the anionic site of this complex receptor with multiple binding sites (Khodorov 1981).

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