

Ion Selectivity and Properties of the Acid Group in Na Channels Modified by Batrachotoxin in Nerve Membrane

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Application of 10^{-5} mol/l batrachotoxin (BTX) to the voltage-clamped node of Ranvier was accompanied by repetitive pulsing (10 Hz, to +60 mV) until almost all the Na channels become modified. Relative permeabilities of modified channels to Na, K and NH_4 ions were calculated according to Goldman-Hodgkin-Katz equation (Hodgkin and Katz 1949) from changes in reversal potential caused by equimolar substitution of external Na^+ with one of the test cations. Permeability ratios obtained from peak currents measurements are (mean $\pm$ S. E.): $P_{\text{NH}_4}/P_{\text{Na}} = 0.47 \pm 0.02$ and $P_{\text{K}}/P_{\text{Na}} = 0.19 \pm 0.01$. The analogous quantities obtained from steady-state current measurements are 0.57 and 0.24 for NH_4 and K ions, respectively. These values are considerably lower than those reported earlier (Revenko and Khodorov 1977). According to these new data BTX is less effective in its ability to change Na channels ion selectivity than the alkaloid aconitine. In aconitine-modified Na channels $P_{\text{NH}_4}/P_{\text{Na}}$ and $P_{\text{K}}/P_{\text{Na}}$ ratios are about 1.36 and 0.36 (Mozhayeva et al. 1977).

BTX is also less effective in lowering the sensitivity of the channels to hydrogen block. Fig. 1 demonstrates the effects of pH decrease from 7.60 to 5.12 and to 4.40 on sodium currents in BTX-modified channels. There are, at least, two acid groups at the Na channel accessible for protonation from the external solution: one in selectivity filter (Hille 1975) and another just at the external ends of the pore (Mozhayeva et al. 1982 a). But, for the sake of simplicity one can describe channel block in terms of the model assuming only one titrable acid group (Woodhull 1973). In this case calculated pK of this group should be considered as effective one (pK_{ef}), which is, generally, not constant when varying pH (Mozhayeva et al. 1982 a). Therefore pK_{ef} for different channels should be compared for experiments with close pH values. pK_{ef} of the BTX-modified channels is lower than that of intact Na channels by about 0.40—0.45. BTX does not change markedly the voltage dependence of pK_{ef} .

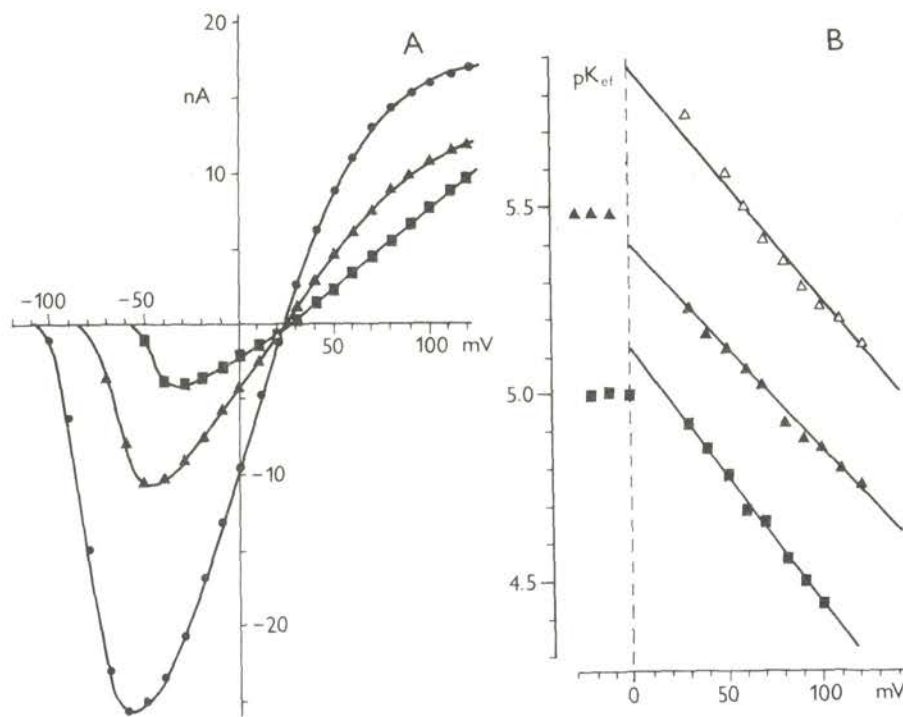


Fig. 1. Block of normal and batrachotoxin (BTX)-modified sodium channels by hydrogen ions. **A.** Peak current-voltage relations for the BTX-modified sodium channels in solutions with pH 7.60 ($\bullet$), 5.12 ($\blacktriangle$) and 4.40 ($\blacksquare$). Note the shift of voltage range of activation to the right upon lowering pH. All external solutions contain (in mmol/l): 100 Na^+ , 20 K^+ , 2 Ca^{2+} , 10 tetraethylammonium ions. pH of solutions was adjusted with Tris-HCl (pH 7.60) or biphthalate (pH 5.12 and 4.40) buffers. The fibre was cut in solution with 100 KF + 20 CsF. Holding potential was set at -130 mV. **B.** Effective pK (pK_{eff}) calculated according to single acid group model as a function of membrane potential. Δ : pK_{eff} for normal Na channels (pH 5.12). $\blacktriangle$ and $\blacksquare$ denote pK_{eff} for BTX-modified sodium channels at pH 5.12 and 4.40 respectively. Note difference in pK_{eff} for pH 5.12 and 4.40. Temperature 10°C .

Aconitine induces decrease in pK_{eff} by about 0.8 (Naumov et al. 1979). Both alkaloids seem to affect the sensitivity of the Na channels to H^+ block by lowering pK of the acid group in the selectivity filter (Mozhayeva et al. 1982 *b*). Thus, data presented imply that channel selectivity strictly depends on the properties of the acid group: the lower pK of this group, the higher relative permeability to large cations such as K^+ and NH_4^+ .

References

- Hodgkin A. L., Katz B. (1949): The effect of sodium ions on electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**, 37—77
- Hille B. (1975) An essential ionized acid group in sodium channels. *Fed. Proc.* **34**, 1318—1321
- Revenko S. V., Khodorov B. I. (1977): Effect of batrachotoxin on the selectivity of sodium channels in myelinated nerve fibre membrane. *Neirofiziologiya* **9**, 313—316 (in Russian)
- Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A., Nosyreva E. D. (1977): The permeability of aconitine-modified sodium channels to univalent cations in myelinated nerve. *Biochim. Biophys. Acta*, **466**, 461—473
- Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A. (1982 a): Interaction of H⁺ ions with acid groups in sodium channels. *Gen. Physiol. Biophys.* **1**, 5—19
- Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A. (1982 b): Interaction of H⁺ ions with acid groups in aconitine-modified sodium channels. *Gen. Physiol. Biophys.* **1**, 21—35
- Woodhull A. M. (1973): Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* **61**, 687—708

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