Tetrodotoxin Changes the Activation Kinetics of Batrachotoxin-Modified Sodium Channels

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Ionic currents through Na channels modified by batrachotoxin (BTX) (Khodorov 1978) were measured in myelinated fibres of frog *Rana ridibunda* by the use of voltage clamp method. Complete modification of Na channels was achieved by 5–10 min external application of 0.019 mmol/l BTX to the node combined with repetitive (10 Hz) membrane depolarization (to +60 mV) by short (2 ms) rectangular pulses.

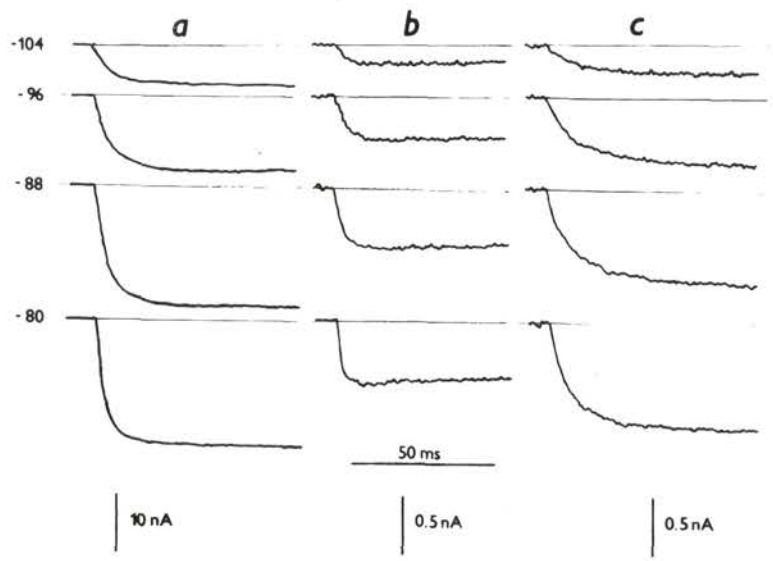

Fig. 1. Currents through BTX-modified sodium channels. Each positive test pulse was followed by four negative control pulses one-forth as large. This program was repeated 2 (a) or 4 (b, c) times and currents from all pulses were averaged. Figures on the left of the records denote membrane potential *(E)* during test pulses.
(a) – control sodium (110 mmol/l) solution; (b) – low sodium (10.2 mmol/l) solution; (c) – control solution with 10^{-7} mol/l TTX. Note difference in current scales for (a) and for (b, c). Temperature 10 °C. Holding potential −136 mV.

Control solution contained (in mmol/l): 110 Na^+, 2 Ca^{2+}, 10 tetraethylammonium$^+$, 5 $Tris^+$, 129 Cl^-; pH 7.6. In low Na solutions most part of Na^+ was replaced by $Tris^+$ so that Na concentrations were 10.2 and 5.2 mmol/l. The internodes were cut in solution containing 120 CsF or 100 KF and 20 CsF.
Modification by BTX did not change significantly the sensitivity of Na channels to the blocking action of tetrodotoxin (TTX). Dissociation constants are (±S. D.) 4.3 ± 1.3 nmol/l (n = 7) and 3.6 ± 1.3 nmol/l (n = 9) for normal and BTX-modified channels, respectively. In addition to Na conductance decrease TTX induces an essential slowing of activation process (Fig. 1). This effect cannot be explained by some current dependent artefact, since the small currents in low Na^+ solutions have the same activation kinetics as in control solution. The time course of a current rise was always approximately exponential.

Fig. 2 shows average time constant (τ_m) before and after TTX application. The effect of TTX on the time course of activation is practically irreversible. In control experiments on normal membrane TTX did not affect the activation kinetics of normal channels. It is possible, that TTX alters some properties of membrane lipids surrounding the channel and BTX makes gating mechanism of the channel sensitive to these alterations.

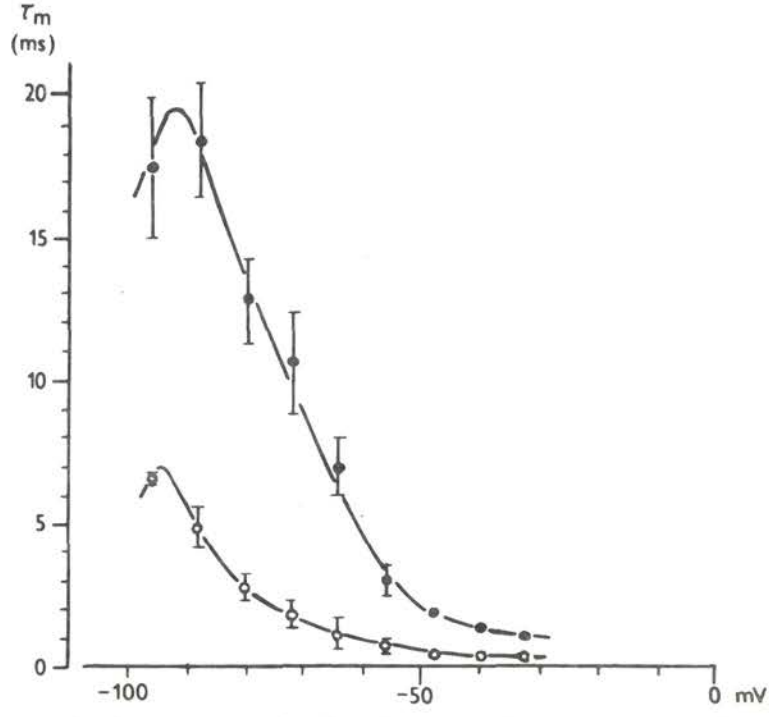

Fig. 2. Activation time constants as a function *of E.* Open circles – control solution ; filled circles – control solution with 10^{-7} mol/l TTX. Vertical bars ± S. E. ,n = 6.

References

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