Modification of Na Channels by Synthetic Dihydrobatrachotoxinin A-20α-Benzoate

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Abstract. Sodium channels in nodal membrane modified by a synthetic analog of batrachotoxin, 7,8-dihydrobatrachotoxinin A-20α-benzoate, were studied under voltage clamp conditions. The voltage dependence of channel activation was shifted by 70—80 mV towards more negative potentials. Selectivity sequence determined from peak current reversal potenial was as follows: $Na:NH_4:K = 1:0.46:0.23$. Our data suggest that 7,8-dihydrobatrachotoxinin A-20α-benzoate has qualitatively similar effects on the properties of sodium channels as does natural batrachotoxin.

Key words: Node — Voltage clamp — Sodium channel — Dihydrobatrachotoxinin

Introduction

Lipid soluble natural neurotoxins veratridine, aconitine, grayanotoxin and batrachotoxin modify both gating and selectivity of fast Na channels in excitable membranes (Mozhayeva et al. 1977; Naumov et al. 1979; Khodorov and Revenko 1979; Catterall 1980). For this reason they are valuable tools for the study of channel structure and functions. Batrachotoxin (BTX) is a promising toxin in this respect because of its profound effects on Na channel properties and low effective concentrations (Catterall 1977; Khodorov and Revenko 1979). Natural sources of BTX are limited, and efforts are therefore being made to synthesize BTX or its analogs. The aim of our work was to test the biological activity of a synthetic analog of BTX, 7,8-dihydrobatrachotoxinin A-20α-benzoate (DBTX), and to compare properties of BTX- and DBTX-modified Na channels. Synthesis and the chemical structure of DBTX have been described by Yelin et al. (1983).

Materials and Methods

Experiments were performed on myelinated nerve fibres from the frog *Rana ridibunda* under voltage clamp conditions (Mozhayeva et al. 1977). Membrane potential (E) was referred to the outside. Membrane currents were calibrated on the assumption that the resistance of the current-feeding

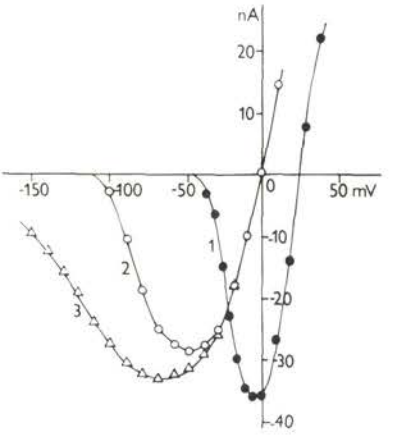

Fig. 2. Peak current-voltage relations for normal (1) and DBTX-modified Na channels (2). 3 — "Instantaneous" current-voltage relation for DBTX-modified channels. Node 165—83

(n_p) can be determined as the normalized ratio of peak (or steady-state) current to the corresponding "instantaneous" current (Chiu 1980). For normal Na channels at normal (2 mmol . l^{-1}) Ca concentration this determination is equivalent to the calculation of normalized chord conductance since, within the voltage range where normal Na conductance grows from a small value to the limiting one, the "instantaneous" current-voltage relation is essentially linear (Mozhayeva et al. 1982, Mozhayeva et al. 1984b). In the presence of 20 mmol . l^{-1} Ca, even at potentials less negative than -50 mV the "instantaneous" current-voltage relation becomes markedly nonlinear due to calcium block (Mozhayeva et al. 1985a, b). However, the error in n_p values due to not allowing for nonlinearity "instantaneous" curve did non exceed 10 percents. Partially, such a relative insensitivity of n_p to the method of estimation can be explained by the fact that high Ca concentration induces a shift of activation voltage-dependence towards more positive potentials (Frankenhaeuser and Hodgkin 1957; Hille et al. 1975), where nonlinearity due to calcium blockage is less pronounced. DBTX-modified channels activate at large negative potentials where the potential-dependent calcium block is much stronger than at the voltage range where normal channels activate. Not allowing for nonlinearity due to calcium block would have introduced a considerable error in estimated n_p values for the modified channels. Therefore, $n_p(E)$ values for the DBTX-modified channels were estimated using "instantaneous" current-voltage relations.

Fig. 3 shows n_p(E) curves for normal and DBTX-modified Na channels associated with the current-voltage relations presented in Fig. 2. It can be seen that DBTX induces a large negative shift of n_p(E) curve without any marked change in its steepness. In the given experiment the potential of half-activation changed by

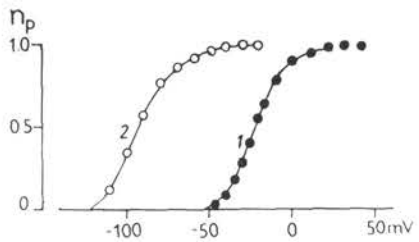

Fig. 3. Fractional number of the open channels (n_p) for normal (1) and DBTX-modified (2) Na channels. For details of calculation of n_p, see text. Node 165—83

70 mV. The average DBTX-induced shift was 78.2 ± 3.3 mV ($n = 5$). The analogous BTX-induced shift at normal Ca concentration was 67 mV (Mozhayeva et al. 1984a, 1985a). Thus, DBTX is an even more powerful modifier of the gating machinery of Na channels than its natural analog.

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