A Study of Properties of Batrachotoxin Modified Sodium Channels

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Abstract. A further analysis of the effects of the steroidal alkaloid batrachotoxin (BTX) on sodium channels in frog node of Ranvier has been carried out under voltage-clamp conditions. The main properties of modified channels as compared with those of normal ones are as follows: 1) The rate of channel closing is drastically decreased, whereas that of opening is changed slightly if at all; 2) The steady-state voltage dependence of channel activation is shifted towards more negative potentials by 60—70 mV; 3) Currents through modified channels do not show a decay during maintained depolarization as it is typical for normal channels. However modified channels retain the ability to partial inactivation as shown by experiments with depolarizing prepulses; 4) Sodium against potassium selectivity beyond — 20 mV suggesting either nonhomogeneity of the modified channels as for their kinetic and selectivity properties or potential-dependence of ionic selectivity their kinetic and selectivity properties of potential-dependence of ionic selectivity for each channel; 5) The selectivity sequence determined from peak current reversal potential measurements is as follows: $H : Na : NH_4 : K = 528 : 1 : 0.47 : : 0.19$; The effective pK value of proton block is decreased by about 0.4; 7) The sensitivity of the channels to tetrodotoxin (TTX) block is practically unchanged.

Key words: Frog node — Sodium channels — Batrachotoxin — Selectivity — Gating

Introduction

Batrachotoxin (BTX) is a steroidal alkaloid, contained in the skin extract of the Colombian arrow poison frogs of the genus *Philolates* (Albuquerque et al. 1971; Daly 1982). In nerve and muscle fibres BTX induces a time and concentration dependent depolarization of cellular membranes due to a steady increase in their

sodium permeability (Albuquerque 1972; Narahashi et al. 1971). Voltage clamp experiments on frog myelinated fibres have disclosed the origin of this BTX effect. It has been shown that BTX produces a dramatic shift in the voltage dependence of Na channel activation to more negative potentials accompanied by an apparent abolishing of the normal sodium inactivation and a decrease in the ion selectivity of Na channels (Khodorov et al. 1975; Khodorov 1978; Khodorov and Revenko 1979). In addition, BTX decreases the sensitivity of Na channels to the blocking action of local anaesthetics (Khodorov et al. 1975; Khodorov 1978, 1981; Zaborovskaya and Khodorov 1982) and related drugs (Dubois and Khodorov 1982). All these effects of BTX have been shown to be practically irreversible and to result from all-or-none interaction of this drug with Na channels (Khodorov et al. 1981).

The aim of the present work was a further indepth analysis of the effect of BTX on Na channels in the node of Ranvier. In most of the previous studies only a fraction of the Na channels in the nodal membrane have been modified by BTX. Other channels retained their normal properties. Such a partial modification made it possible to compare the behaviour, especially pharmacological properties of both channel populations, the normal and the modified, simultaneously. However under these conditions it was impossible to examine the kinetic characteristics of BTX-modified channels over the potential range where also normal channels become activated. To overcome this difficulty we induced a total modification of Na channels, in combining BTX application with repetitive membrane stimulation up to a practically complete elimination of ionic currents in normal channels. This procedure alone allowed to disclose some so far unknown important properties of BTX-modified channels. The present paper summarizes data obtained in this study. Short preliminary reports on this study appeared elsewhere (Mozhayeva et al. 1982 c—f).

Materials and Methods

Experiments were carried out on myelinated fibres of *Rana ridibunda* under voltage clamp conditions at 9—10 °C. Membrane potential (E) was defined as inside minus outside. In one series of experiments the leak and linear capacitive currents were subtracted using analog electronic device. In other experiments a pulse program was used which included a positive (test) pulse with an amplitude P and 4 negative (control) pulses with amplitudes $P/4$ each. All pulses started from the holding potential (E_H). Currents elicited by all pulses were summed up by a digital averager. Thus the linear components of membrane currents were subtracted. In cases when the estimation of a very small current was required the program was repeated several times.

Membrane currents were calibrated on the assumption that the resistance of the current-feeding internode was 20 MΩ.

Nerve fibres with clear signs of a large series resistance (R_s) were discarded. In many experiments the series resistance (R_s) was compensated; R_s being considered to be 300 kΩ.

Control solution contained (in $mmol \cdot l^{-1}$): 110 NaCl; $2CaCl_2$; 10 tetraethylammonium-Cl

(TEA-Cl); 10 Tris-HCl; pH 7.5—7.6. Solutions with low pH were prepared with bipthalate buffer. Also solutions containing K^+ or NH_4^+ instead of Na^+ were used. The ends of the fibres were cut in solutions with different ionic composition (in mmol . l^{-1}): 1) 120 KF, 2) 100 KF + 20 CsF, 3) 120 CsF, 4) 115 KF + 5 Tris-HCl, 5) 110 KF + 10 TEA-Cl. Cs^+ and TEA^+ were added to the internal and TEA^+ to the external solutions to inhibit the potassium currents.

BTX in ethanol solution was added to the control solution to a final BTX concentration of 0.019 mmol . l^{-1}, this concentration being 20—25 times higher than the dissociation constant for BTX determined in experiments on the node (Khodorov and Revenko 1979) and on neuroblastoma cells (Catterall 1977). To accelerate the modification of the Na channels and make it complete the application of BTX was accompanied by repetitive stimulation of the membrane by depolarizing pulses to $E_t = +60$ mV at a frequency of 10 Hz during 5—10 min, until currents through modified channels had stopped increasing. The effect of BTX was practically irreversible; after the completion of channel modification, all measurements were therefore carried out in BTX-free solutions.

After a fibre was mounted in the experimental chamber E_H was set at -100 mV. Measurements were begun after 25—30 min waiting to allow the temperature to stabilize. Before BTX application E_H was set al -120 or -130 mV.

Results were expressed as mean ± S.E. (n = number of experiments).

Results

General description

Fig. 1 shows families of Na currents, I_{Na}, through BTX-modified channels. The records were made at two sweep speeds to follow better the time course of the currents. The corresponsing $I_{Na}(E)$ curves are shown in Fig. 1*b*. It can be seen that BTX treatment resulted in a shift in the voltage dependence of Na channel activation by about 60 mV to more negative potentials, and in a decrease of the current reversal potential (E_r). On average, E_r decreased by 24 ± 2 mV ($n = 15$). Over a potential range from -100 to -50 mV the currents were characterized by a slow activation kinetics and apparent absence of inactivation. Similar current patterns were observed earlier in experiments with a partial modification of the channels (Khodorov et al. 1975; Khodorov and Revenko 1979).

At $E > -30$ mV both inward and outward I_{Na} did not remain constant during sustained depolarization. As can be seen from Fig 1*a* over a potential range from -30 to $+40$ mV the inward currents slowly decayed while the outward ones were slowly increasing. In the vicinity of E_r currents even changed their direction during the depolarizing step. Thus E_r for peak (I_p) and steady-state (I_{ss}) currents were different. Average differences in $E_r(\Delta E_r)$ were 5.0 ± 0.4 mV ($n = 40$). Only in two experiments ΔE_r was close to zero.

At $E > +60$ mV the outward currents decayed during membrane depolarization to a steady-state value. The latter raised with voltage up to about $+80$ mV, but at more positive E, it decreased which resulted in the appearence of a negative slope in the $I_{ss}(E)$ curve.

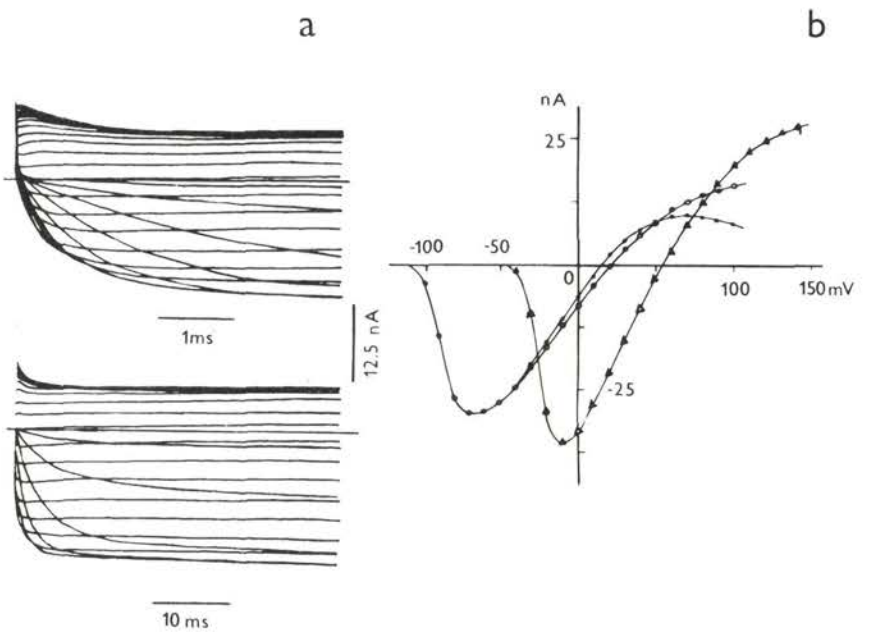

Fig. 1. Currents (*a*) and current-voltage relations (*b*) for BTX-modified Na channels. *a*) Test potentials ranging from −100 to +100 mV in 10 mV steps. Currents are shown at two sweeps. Holding potential −120 mV. *b*) Triangles — current-voltage relations before BTX-treatment; open circles and filled circles indicate peak and steady-state current-voltage relations for BTX-modified Na channels. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 27–81.

Analysis of changes in the current reversal potential during membrane depolarization

Does the above-stated difference between E_r for peak and steady-state Na currents reflect the properties of BTX-modified channels themself, or is it due to some artifactual factors, such as incomplete Na channel modification (involvement of some nonmodified channels), incomplete K channel blockage, nonlinearity of the leakage currents, depletion (or accumulation) of Na^+ or K^+ ions in perinodal space, etc? To clear up these questions control experiments were done with Na channel blockers procaine (Pr) and tetrodotoxin (TTX).

Earlier, in experiments on frog myelinated nerves it has been shown that BTX-modified channels are by about one order less sensitive to blocking action of Pr (Khodorov et al. 1975; Khodorov 1978; Zaborovskaya and Khodorov 1982) than normal Na channels. In the present work Pr was used to reveal the contribution of normal Na channels to the observed changes in E_r. First, the

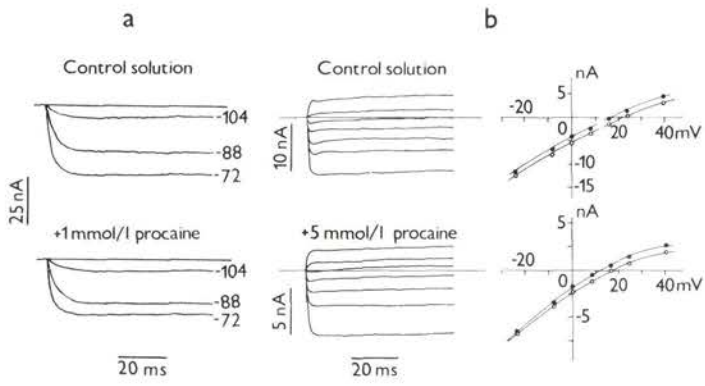

Fig. 2. Currents in BTX-modified channels in the presence of procaine. *a*) Currents at large negative potentials (−104; −88; and −72 mV). Node 108–82. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. *b*) Currents at potentials 0; +8; +16; +24; and +40 mV. Fragments of current-voltage relations are shown on the right. Filled and open circles — steady-state and peak currents, respectively. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 48–82.

sensitivity of normal Na channels in *Rana ridibunda* fibres to Pr was determined. In these experiments the membrane was held at $E_H = -120$ to -130 mV to avoid the effect of resting Na inactivation on the Pr block (Khodorov et al. 1976). The apparent dissociation constant (K_d) for Pr-induced decrease in I_{Na} proved to be 0.11 ± 0.03 mmol . l^{-1} ($n = 5$). This value is comparable with corresponding values of 0.24 and 0.27 mmol . l^{-1} reported in previous works (Århem and Frankenhaeuser 1974; Negulyaev and Nosyreva 1979).

Currents in BTX-treated nodes in the presence of Pr are shown in Fig. 2. It can be seen that over the E range from −100 to +40 mV Pr (1 and 5 mmol . l^{-1}) did not induce a significant change in current kinetics or E_r: the difference between E_r for early and steady-state currents was the same (5 mV) both before and during the action of Pr. The average (6 analogous experiments) ΔE_r without Pr application was 6.7 ± 0.8 mV and with Pr (1—5 mmol . l^{-1}) 6.3 ± 0.6 mV.

Another possible source of misinterpretation of the data is the current-dependent depletion (or accumulation) of Na^+ or K^+ ions near the membrane surface. We have therefore examined the effects of an Na channel blocker, TTX on ΔE_r. In a first series of experiments K_d for TTX blocking action (K_d^{TTX}) was determined. In some experiments the extent of I_{Na} inhibition was measured before BTX treatment and following total modification. In other experiments only a fraction of Na channels was modified by BTX. In this case K_d^{TTX} for modified channels was determined from measurements of currents at $E = -80$ mV. K_d^{TTX} for normal channels was determined from measurements of current at E where currents in

Table 1. TTX-blockage of normal and BTX-modified Na channels

Node	K_d^{TTX} ($\times 10^{-9}$ mmol . l^{-1})	
	Control	BTX
27	2.63	1.72
51		3.28
53		1.85
54		3.29
58	4.73	
60		3.92
104	2.4	
106	4.9	5.15
108		3.14
109	4.34	
110	5.72	5.15
111	5.44	4.24
mean	4.30	3.58
± S. E.	± 0.50	± 0.44
	($n = 7$)	($n = 9$)

modified channels reversed their direction. Finally, in some experiments K_d^{TTX} was estimated only for normal, and in others only for BTX-modified channels. As it is seen from Table 1, K_d^{TTX} varied considerably from experiment to experiment. However, the average values of K_d^{TTX} for normal and modified channels did not differ significantly if at all. It should be noted that TTX affected the size of the current through modified channels, and also slowed down their kinetics (Mozhayeva et al. 1982c). A special analysis showed that the latter effect of TTX was not current dependent.

Fig. 3 illustrates the effect of TTX (2×10^{-8} and 1×10^{-7} mmol . l^{-1}) on channel currents in the presence of 1 mmol . l^{-1} Pr. It may be seen that regardless of a drastic (5- and 20-fold) reduction in the current size, there were no changes in the current pattern in the vicinity of E_r. ΔE_r was practically the same for all current families (about 6 mV). The average values (11 analogous experiments) of ΔE_r were 5.7 ± 0.7 mV without TTX and 5.5 ± 0.8 mV with TTX.

Fig. 4 shows the effect of 3.9×10^{-7} mol . l^{-1} TTX: it can be seen that no currents appear under this condition. Thus, no nonlinear time-dependent currents, except for currents through Na channels, could be elicited under the above conditions.

The only way to eliminate, or even invert, ΔE_r was to replace Na^+ by K^+ or by another less permeant cation, e.g., NH_4^+; this is shown in Figs. 5 and 13.

Another possible reason for the change in E_r during a test pulse may be a drift of the clamped membrane potential, due to time dependence in attenuation artifact

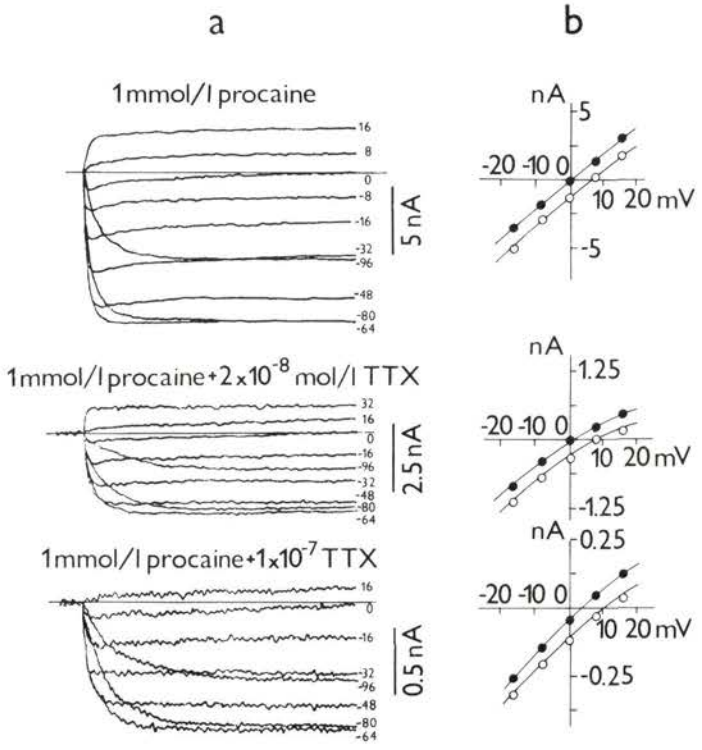

Fig. 3. Effect of tetrodotoxin on currents in BTX-modified Na channels. *a*) Current records in the presence of procaine (1 mmol/l). *Top:* 1 mmol/l procaine; *middle:* procaine + 2×10^{-8} mol . l^{-1} TTX; *Bottom:* procaine + 1×10^{-7} mol . l^{-1} TTX. Figures on the right of the records indicate potential values during test pulses. *b*) Corresponding fragments of current-voltage relations for peak (open circles) and steady-state (filled circles) currents. Node 108–82.

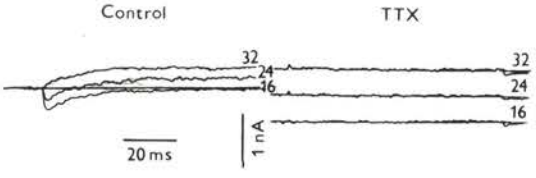

Fig. 4. Inhibition of currents through BTX-modified Na channels by TTX (3.9×10^{-7} mol . l^{-1}). Node 59—82.

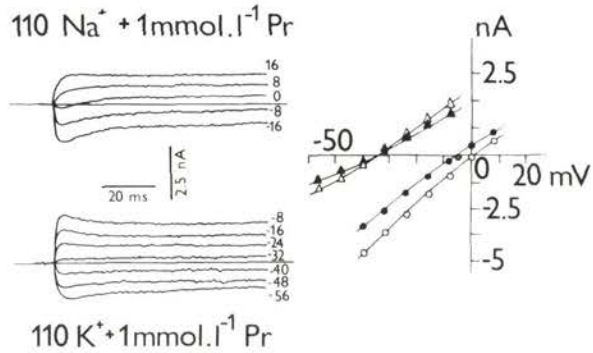

Fig. 5. Reversal potentials for peak and steady-state currents in Na^+ and K^+ external solutions. Numbers to the right of the records indicate potential values during test pulses. Fragments of current-voltage relations in Na^+ (circles) and K^+ (triangles) external solutions. Open symbols indicate peak currents, filled symbols steady state currents. The internodes were cut in a solution containing 120 mmol/l CsF. Node 63–82.

(Sigworth 1981). The constancy of E_r during prolonged depolarizing pulse in K^+ solution suggested that effects of this kind were negligible in our experiments.

Thus, changes in E_r during membrane depolarization can not be explained by artifactual factors such as incomplete Na channels modification, incomplete K channels blockage, nonlinearity of leakage currents, or some kind of accumulation-depletion phenomena. Nonequality of E_r for peak and steady-state currents in BTX-treated node seems to reflect the properties of BTX-modified channels themselves. One possible interpretation of this effect is that during membrane depolarization, the selectivity of Na channels decreases. The replacement of the natural internal solution by an artificial one through cut fiber ends is only partial, and potassium remains the major internal permeant cation (other than sodium), even if the fiber ends are cut in a potassium-free solution. Hence, the decrease in E_r during a positive voltage pulse may be due to an increase in the potassium-too-sodium permeability ratio (P_K/P_{Na}).

The above suggestion concerning the nature of E_r changes during membrane depolarization has been strongly supported by studies of the effect of a conditioning potential on "instantaneous" current-voltage relations $I^i(E)$. Sodium channels were activated by a conditioning pulse E_1 and subsequently during another pulse, E_2 of a different amplitude, currents were measured. Fig. 6 shows that parameters of $I^i(E)$ depended on E_1 . E_r^i after $E_1 = -60$ mV was constant irrespective of the duration of E_1. After a short pulse to $+80$ mV E_r^i similar as after $E_i = -60$ mV. However with increasing pulse duration E_r^i decayed by 14 mV. The decay of E_r^i after $E_1 = +10$ mV was 9 mV. Similar results were obtained in 7 analogous experiments where the current kinetics was similar to that presented in

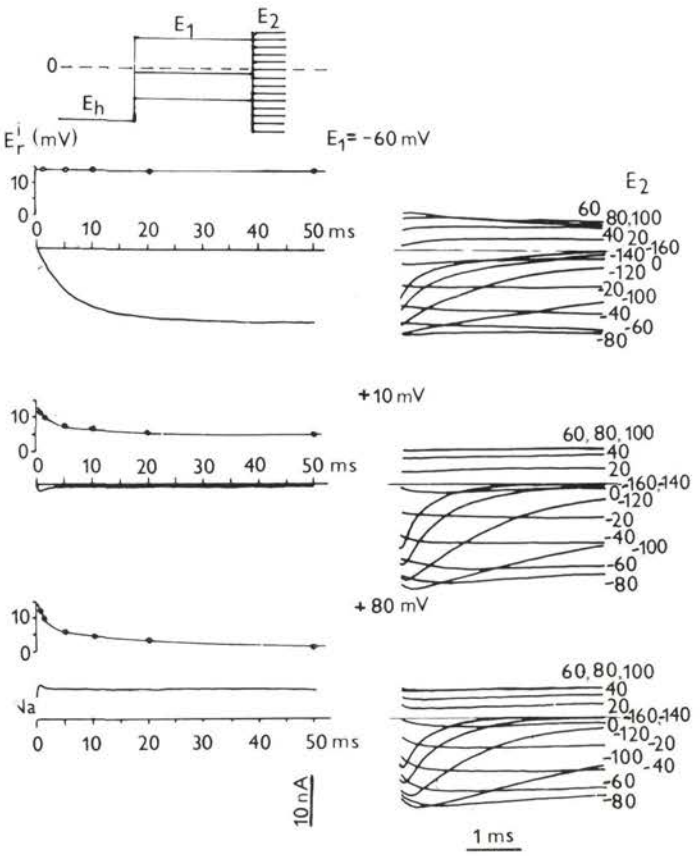

Fig. 6. Currents and reversal potentials after pulses to −60 mV (upper row), +10 mV (middle row) and +80 mV (lower row). The pulse program is shown at the top of the figure. *Left column:* currents during pulse E_1: *right column:* currents during pulse E_2 (after E_1, 50 ms in duration). Numbers to the right of the records indicate potential values during pulse E_2. Circles: reversal potentials of "instantaneous" currents in response to step E_2 as a function of pulse E_1 duration. Node 27–81.

Figs. 1—4: E_r^i did not change after $E_1 = -80 + -40$ mV, and decreased by 9.4 ± 4.7 mV after a 50 ms E_1 to +80 mV.

On repolarization E_r changed in opposite direction. Fig. 6 shows that at the end of a postpulse after $E_2 = +80$ mV currents reversed their direction at a potential more positive than did "instantaneous" currents. Thus, channel selectivity was potential dependent; it decreased at E more positive than −40 mV and returned to initial levels on repolarization of the membrane. It took some time for the changes in selectivity to complete: peak currents (first fraction of a millisecond) corresponded to a relatively high selectivity, and the steady-state ones to a lowered selectivity.

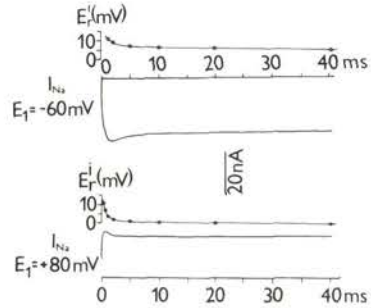

Fig. 7. Reversal potentials of "instantaneous" currents after pulses to −60 mV and +80 mV. The same pulse program as in Fig. 6. Circles denote E_r^i as a function of E_1 duration. The internodes were cut in a solution containing 120 mmol/l KF. Node 23–81.

If some accumulation-depletion of current carrying ions had occurred in these experiments E_r^i should have decreased after $E_1 = -80$ to -60 mV, producing large inward currents, and increased after $E_1 = +60 + +80$ mV, producing outward currents. Fig. 7 shows an example of an experiment where accumulation-depletion phenomena were well pronounced. They were manifested as decay of a large inward current at $E_1 = -60$ mV. This decay was accompanied by a decrease in E_r^i. Both events can be explained, e. g. by depletion of Na^+ ions in the perinodal space. It can be assumed that differences in the extent of accumulation — depletion phenomena for different fibres are due to differences in geometry of the node. After $E_1 = +80$ mV, E_r^i decreased, although following outward current accumulation-depletion is expected to shift E_r^i in the opposite direction. Hence, changes in selectivity of BTX-modified channels could be disclosed even in fibres where accumulation-depletion phenomena were well expressed.

Inhibition of steady-state currents at high positive potentials

As mentioned above, I_{Na} at $E > +80$ mV decayed from peak to steady-state values which decreased as E became more positive. The time constants of I_{Na} decay at large E were about 0.8—1.0 ms. The appearance of a transient component in the outward currents cannot be ascribed to the presence of normal inactivating channels. It can be seen from Fig. 8*a* that outward currents decay both in the presence and absence of 1 mmol . l^{-1} Pr; this concentration practically blocks normal Na channels completely. Fig. 8*b* shows that the ratio of the peak current to current at 4 ms of the depolarizing pulse remained unaffected by Pr.

Prolonged test pulses in the presence of Pr gave rise to some additional slowly developing inhibition of the current. The larger the E, the stronger the inhibition.

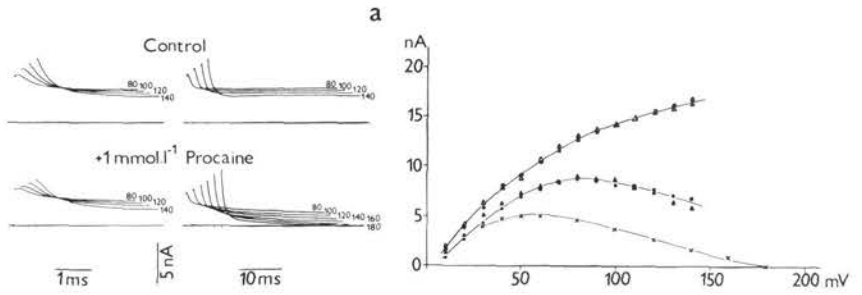

Fig. 8. Inhibition of BTX-modified Na channels by procaine. *a*) Currents at high positive potentials (shown by figures on the right of the records) during the first 4 ms of the pulse (*left*) and during long lasting pulses (*right*). The records have been shifted along the time axis relative to each other to be better distinguished. *b*) Current-voltage relations for peak (open symbols) and for the 4-th ms of the pulse (filled symbols) in the presence (triangles) or absence (circles) of procaine. Current values for the 50-th ms in the presence of Pr are represented by crosses. In the absence of Pr currents at the 50-th ms were identical with those at the 4-th ms. Currents recorded in the presence of Pr have been multiplied by the factor of 1.38 before plotting. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 40–81.

For example, in the experiment presented I_{Na} decayed to practically zero level by the end of a 50 ms pulse to +180 mV. The potential-dependent block induced by Pr can be described quantitatively by the following equation:

$$(I_{Pr}/I_C) = \frac{K^{Pr}_{d(o)} \exp(-\delta EF/RT)}{C_{pr} + K^{Pr}_{d(o)} \exp(-\delta EF/RT)} \quad (1)$$

where I_{Pr} and I_C are steady-state currents in the presence and absence of Pr, respectively; C_{Pr} is Pr concentration; $K^{Pr}_{d(o)}$ is the dissociation constant of Pr at $E = 0$ mV; δ is the steepness factor; F, R and T have their usual meanings. For the experiment presented in Fig. 8 $K^{Pr}_d = 4.17$ mmol . l^{-1} and $\delta = 0.52$. In another analogous experiment, K^{Pr}_d and δ were 10.2 mmol . l^{-1} and 0.52, respectively. According to Hodgkin and Huxley (1952) the current decay in untreated membrane is due to Na channel inactivation. To clarify whether I_{Na} decay in BTX-modified Na channels at high positive E (in the absence of Pr) resulted from inactivation or not, the *Buthus eupeus* scorpion toxin was used. This toxin has been shown to slow down and to reduce the extent of inactivation (Mozhayeva et al. 1980c).

Fig. 9 shows that the toxin slowed down the current decay and lowered its amplitude; these effects were however smaller as E increased; finally, at $E =$ +200 mV the current kinetics remained essentially unchanged. Hence, inactivation process also occurred in BTX-modified channels (see later), but at high positive E there was an additional potential-dependent process of current inhibition.

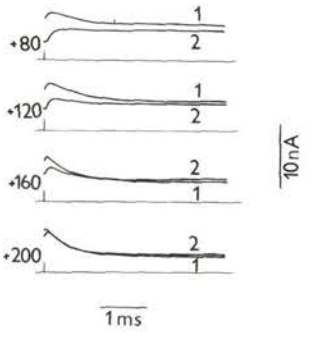

Fig. 9. Effect of scorpion toxin on the currents in BTX-modified Na channels at high positive potentials. Currents recorded in absence and presence of scorpion toxin are marked 1 and 2, respectively. Internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 34–81.

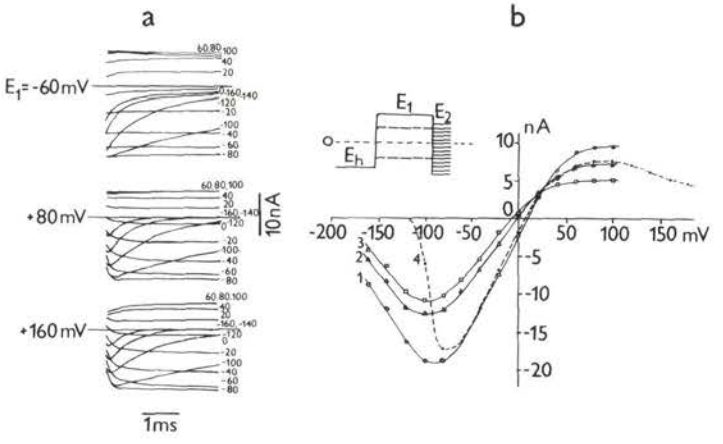

Fig. 10. Effect of preceeding depolarization on currents through BTX-modified Na channels. *a*) Currents in response to postpulse E_2 (see pulse program in set) after prepulses E_1 (50 ms in duration) to -60 mV (*top*), $+80$ mV (*middle*) and $+160$ mV (*bottom*). The value of the potential E_2 was varied over a range from -160 to $+100$ mV in 20 mV steps. *b*) "Instantaneous" current-voltage relations for prepulses to -60 mV (circles), $+80$ mV (triangles) and $+160$ mV (squares). The "instantaneous" current values were taken at the time of switching from E_1 to E_2. Filled circles and the dashed line represent peak current-voltage relationships. Internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 42–81.

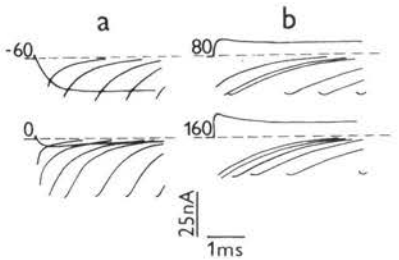

Fig. 11. Kinetics of "tail" currents depending on the amplitude and duration of the depolarizing pulse. "Tail" currents were measured on returning to holding potential −130 mV. Figures at the records show potentials during depolarizing pulses. *a*) Node 33–81; *b*) node 34–81. Internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF.

Fig. 10 shows an experiment analogous to that shown in Fig. 6. Current decrease at high E occurred at switching E from +160 mV to +40 − +100 mV; this resulted in an increase, not decrease in the current magnitude. The "instantaneous" $I^i(E)$ curve after $E_1 = +160$ mV was less steep than that after +80 or −60 mV, over the entire potential range. Hence, an increase in E induced, in addition to a decrease in ion selectivity, a decrease in the net Na channel conductance.

Repolarization of the membrane resulted in restoration of initial levels of both the channel ion selectivity and conductance. If the repolarizing potential was close to E_r the current kinetics depended mainly on the restoration of ion selectivity. At more negative repolarizing pulses the restoration of conductance became major factor that determined current kinetics. At high negative postpulses (E_2) current increment resulting from the restoration of the conductance superposed on the slower current decay resulting from channel closing. As a result "tail" current acquires a hook-like time course (see Figs. 6 and 10). The development of the hook depended on the duration of pulse E_1, as shown in Fig. 11. After $E_1 = -60$ mV no hooks were seen, regardless of the duration of E_1. After $E_1 = 0$ mV a delay in the "tail" current appeared; it was more pronounced for longer pulses. After short (200 μs or less) $E_1 = +160$ mV the "tail" current was monotonous; however, as the pulse duration increased, first a delay and then a hook appeared in the "tail" current. It can be seen that the development of the hook in the "tail" current nearly paralleled the current decay during E_1.

Ca^{2+} blockage of BTX-modified Na channels

It can be seen from Fig. 10 that the inward "instantaneous" currents rised with the increase of the negative E_2 to −100 mV; however, as E_2 become more negative, I_i

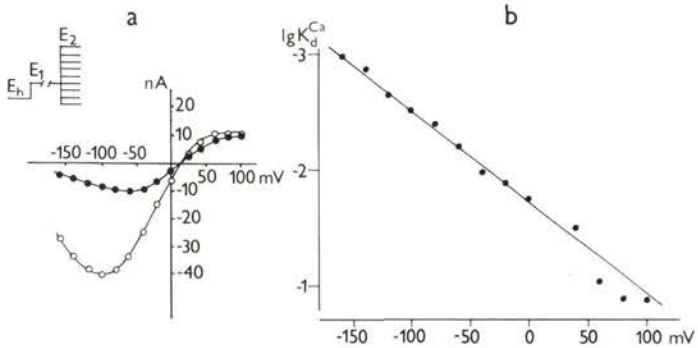

Fig. 12. Potential-dependent blockage of BTX-modified Na channels by Ca^{2+} ions. *a*) Inset: Pulse program. $E_1 = -60$ mV. "Instantaneous" currents in response to E_2 of varying amplitude (open circles) at 2 mmol/l Ca^{2+} and at 20 mmol/l Ca^{2+} (filled circles). Node 27–81. *b*) Semilogarithmic plot of the dissociation constant of calcium blockage (K_d^{Ca}) as a function of potential. K_d^{Ca} for each potential value was calculated using equation (2). The solid line has been calculated according to Woodhull's model (Woodhull 1973) with $K_d^{Ca} = 19$ mmol/l at 0 mV and $\delta = 0.21$.

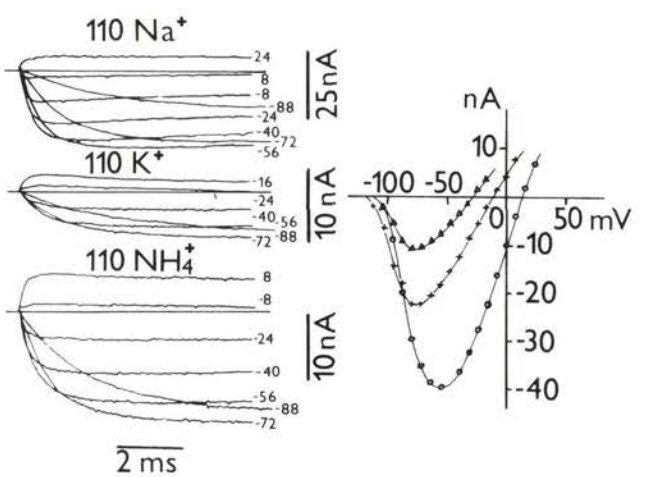

Fig. 13. Currents (*left*) and current-voltage relations (*right*) for BTX-modified Na channels in Na^+ (circles), K^+ (triangles) and NH_4^+ (crosses) external solutions. Figures at the records show potential values during test pulses. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 102–82.

decreased despite an increase of the driving force. The reason for this seems to be a fast potential-dependent blockage of open Na channels by Ca ions. This suggestion was verified in three experiments in which Ca concentration was varied. Raising the Ca concentration from 2 to 20 mmol . l^{-1} resulted in a strong inhibition of I^i at negative potentials and in a shift of the maximal I^i value toward more positive potentials. Dissociation constants (K_d^{Ca}) for any potential can be obtained from equation

$$\frac{I_{20}^i}{I_2^i} = \frac{K_d^{Ca} + 2}{K_d^{Ca} + 20} \tag{2}$$

where I_2^i and I_{20}^i are "instantaneous" currents at 2 and 20 mmol . l^{-1} Ca^{2+}, respectively. It can be seen from Fig. 12*b* that K_d^{Ca} is an exponential function of E as it would be expected from the Woodhull model (Woodhull 1973) of ionic blockage. The fit of the model to experimental data gives K_d^{Ca} at 0 mV and of the fractional electric distance in the pore for Ca ions values of 19 mmol . l^{-1} and to 0.21, respectively. With the same K_d^{Ca} a reasonably good description of results of other two analogous experiments was obtained.

The present results differ from those reported earlier (Mozhayeva et al. 1982d) for two reasons. First, calculations in the previous work were based on measurements made at a single Ca concentration of 2 mmol . l^{-1}; second, voltage-dependent Ca blokage was considered to be significant only at sufficiently negative potentials inducing a deviation of the "instantaneous" current-voltage curve from linearity. Due to this K_d^{Ca} and δ values were overestimated.

Quantitative estimation of relative permeabilities of BTX-modified Na channels

Fig. 13 shows families of ionic currents in control Na solution and in solutions with Na^+ replaced by K^+ or NH_4^+ and associated peak current-voltage relations. From the shift in E_r the permeability ratios were calculated using the Goldman-Hodgkin-Katz equation (Hodgkin and Katz 1949). For the experiment described herein the values were $P_K/P_{Na} = 0.22$ and $P_{NH_4}/P_{Na} = 0.50$. Average values were $P_K/P_{Na} = 0.19 \pm 0.01$ ($n = 9$) and $P_{NH_4}/P_{Na} = 0.47 \pm 0.02$ ($n = 13$). Analogous calculations for the steady-state current gave $P_K/P_{Na} = 0.23$ and $P_{NH_4}/P_{Na} = 0.57$, i. e. values about 1.2 times larger than those for peak currents. This has confirmed the above conclusion that the selectivity of BTX-modified Na channels slightly decreases during membrane depolarization.

Values of P_k/P_{Na} and particularly those of P_{NH_4}/P_{Na}, obtained in the present work, were considerably lower than those reported earlier (Khodorov and Revenko 1979). Since unlike of our experiments the latter work was done at room temperature, we also determined NH_4-to-Na permeability ratio at 22 °C in four

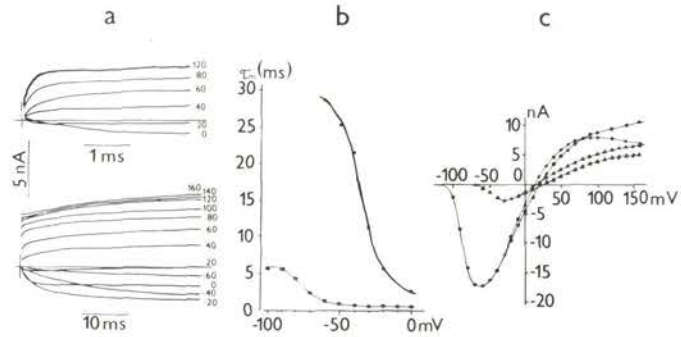

Fig. 14. Effect of low pH (4.68) on currents in BTX-modified Na channels. *a*) Time course of currents at pH 4.68. Currents are shown at two time scales. Figures at current records show values of E during test pulses. *b*) The time constant of current rise τ_a) as a function of E at normal (open circles) and low (filled circles) pH of external solution. *c*) Current-voltage relations at normal (circles) and low (triangles) pH. Filled and open symbols represent peak and steady-state currents, respectively. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 43–81.

experiments, we obtained an average value of 0.53 ± 0.03. This value was somewhat higher than that for P_{NH_4}/P_{Na} at 10 °C, being still lower than that obtained by Khodorov and Revenko (Khodorov and Revenko 1979; Revenko and Khodorov 1977). A similar decrease in selectivity with decrease in selectivity with decreasing temperature was observed earlier for aconitine-modified sodium channels in nodal membrane (Mozhayeva et al. 1977).

Effect of H^+ ions

Figs. 14 and 16 illustrate effects of pH on BTX-modified channels. It can be seen that along with a decrease in ionic currents, low pH changed some other properties of the channels as well. 1) The voltage dependence of Na channel activation was shifted to more positive E: inward currents appeared only at about -70 mV at pH 4.68, whereas at pH 7.50, they appeared at about -100 mV. 2) The kinetics of channel activation was greatly slowed down. At first approximation, the time course of the modified channels activation can be considered as monoexponential. Though voltage-dependences for τ_a at normal and low pH are insufficient to show decreased τ_a at more negative potentials, it can be seen that both curves have quite a clear tendency to pass through the maximum. The maximal value of τ_a at low pH was at least 6-fold higher than that at normal pH, and was shifted to the right along the voltage axis. Such an effect suggests that τ_a changes mainly due to decreased forward rate constant activation, whereas the backward one seems to change slightly, if at all. 3) Instead of a monotonous decay of the outward current during

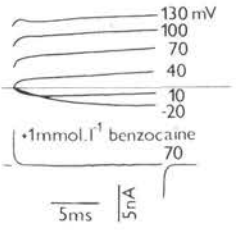

Fig. 15. Currents at low pH (4.68) and their inhibition by 1 mmol/l benzocaine. In the presence of benzocaine is shown only one record (at +70 mV). Node 42–81.

prolonged depolarization observed at normal pH (see Fig. 1) a slow secondary current appeared lasting for several tens of ms (Figs. 14*a* and 15). This effect became particularly distinctive at high positive E. Application of 1 mmol . l^{-1} of benzocaine inhibited all the current components, including the secondary current increase. This fact has suggested that this phenomena reflect the properties of BTX-modified sodium channels, and do not result from slow activation of some other ionic channels.

It has been shown previously (Woodhull 1973; Mozhayeva et al. 1982a) that at sufficiently positive potentials the number of open channels at the current peak is constant. Therefore, we estimated H^+ block from peak currents measurements. According to the current view, each Na channel has, at least, two acid groups able to interact with external H^+ ions (Spalding 1980; Mozhayeva et al. 1981, 1982 a, b). However, the estimation of the parameters of these groups requires a detailed study of H^+ block over a wide range of pH. Since data obtained in this work have not been sufficient to allow such an analysis, we have restricted our description of the results to the simple one-acid-group model (Woodhull 1973). The pK of this "averaged" group (pK_{ef}) was calculated using the relationship

$$pK_{ef} = \lg (g_{7.6}/g_{pH} - 1) + pH \qquad (3)$$

where $g_{7.6}$ and g_{pH} are Na channel conductances at normal and low pH, respectively. Fig. 16 shows the result of one of the experiments in which effects decreased pH were studied before, and after BTX treatment. It can be seen that lowering pH to 5.12 produced a smaller decrease of I_{Na} in BTX-modified channels than in the normal ones. Correspondingly, pK_{ef} for modified channels were lower than those for the normal ones over all the potential range tested. At $E = 0$ mV, pK_{ef} for normal and modified channels were 5.83 and 5.40, respectively. As E became more positive, pK_{ef} for both types of channels decreased. In the same experiment currents were also recorded in BTX-modified channels at pH 4.40. The corres-

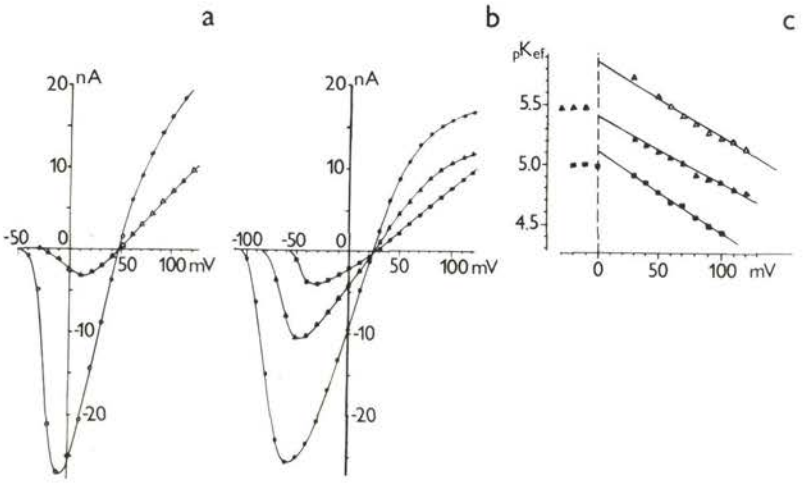

Fig. 16. Proton blockage of normal and BTX-modified Na channels. Current voltage relations for normal (*a*) and modified (*b*) channels. Circles, triangles and squares represent currents at pH 7.50; 5.12; and 4.68, respectively. *c*) Calculated pK_{ef} for normal (open triangles) and modified (filled triangles and squares) channels. Triangles represent pK_{ef} values calculated from an experiment performed at pH 5.12; squares: pH 4.68. Node 43–81.

ponding pK_{ef} proved to be lower at any E than values calculated from measurements at pH 5.12. A similar relation between pK_{ef} calculated from experiments at different pH was found earlier for normal (Mozhayeva et al. 1981, 1982a) and aconitine-modified (Mozhayeva et al. 1982b) channels. The dependence of pK_{ef} on pH suggests that the simple one-acid-group model is inadequate. To enable a comparison of pK_{ef} for normal and modified channels pK_{ef} should thus be calculated from experiments performed at similar pH values. In five experiments performed at pH 5.12, the values of $pK_{ef(o)}$ (at $E=0$ mV) for normal and BTX-modified channels were 5.61 ± 0.14 and 5.21 ± 0.13, respectively. In three other experiments performed at pH 4.83, the difference between $pK_{ef(o)}$ for normal and BTX-modified channels was 0.45.

Thus, BTX decreased $pK_{ef(o)}$ by 0.40—0.45. The voltage dependence of the H^+ block in normal and modified channels was almost identical.

Proton permeability of BTX-modified channels

In four experiments currents were recorded under conditions of Na^+ ions in the acid solutions being entirely replaced by nonpenetrating cholin ions, or by sucrose. One of these experiments is illustrated in Fig. 17. Although no Na ions were present in external solutions, small though well measurable, inward currents were observed. Under analogous conditions at normal pH, such currents were absent.

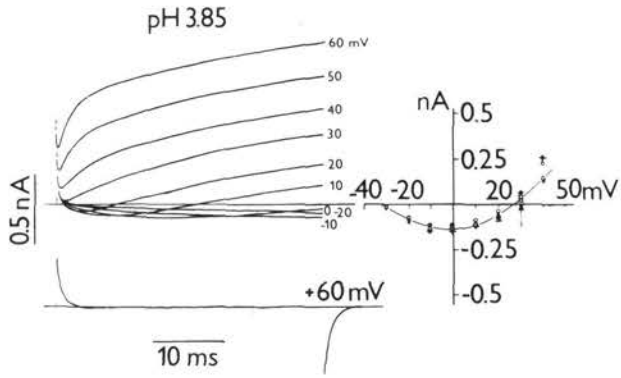

Fig. 17. Currents through BTX-modified Na channels in Na-free acid solution, and corresponding current-voltage relation. NaCl was replaced by choline, pH 3.85. Test pulses span the range from −20 to +60 mV in 10 mV steps. One record ($E = +60$ mV) in the presence of benzocaine (2.5 mmol . l^{-1}; pH 3.88) is shown. Peak current values are plotted for E from −30 to +20 mV, for E +30 mV and +40 mV current values isochronal to peak at +20 mV are plotted. Different symbols correspond to different sequential sets of measurements (two before and two after benzocaine application). Arrow indicates reversal potential in normal Na solution. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 10–81.

A detailed study of ionic currents in Na-free acid solutions before and after aconitine treatment of the membrane (Mozhayeva and Naumov 1982a, b, 1983) showed that, under the above conditions inward currents are carried through normal or modified sodium channels by protons. Based on this it can be suggested that inward currents in the present experiments were carried in BTX-modified Na channels by H^+ ions. As shown in Fig. 17, in Na-free acid solutions as well as in Na acid solutions, the currents rose very slowly and were completely blocked by 1 mmol . l^{-1} benzocaine. The change in E_r during the test pulse was very marked, and E_r was estimated from measurements of inward peak currents and from outward currents isochronal to the latter. From values of E_r measured in Na and Na-free acid solutions the relative proton permeability (P_H/P_{Na}) was calculated using the Goldman-Hodgkin-Katz equation (Hodgkin and Katz 1949). The activity coefficient for Na ions was taken to be 0.78. The mean value of the P_H/P_{Na} ratio was 528 ± 46 ($n = 4$). In several experiments choline chloride in the external Na^+-free acid solution was replaced by sucrose. This resulted in no changes in reversal potential both at the beginning and at the end of test pulses. Thus, chloride ions were not involved in the slow rise of outward currents.

Activation in BTX-modified Na channels

As mentioned above the activation kinetics of BTX-modified channels can, at first

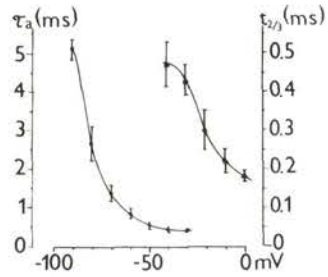

Fig. 18. Activation time constant for normal and BTX-modified Na channels. For normal channels, times to reach two thirds of maximal current values are plotted (triangles, right scale). Modified channels: circles, left scale. Symbols represent mean values. Vertical bars show S. E.

approximation, be considered as being monoexponential. Fig. 18 shows mean values of the activation time constant as a function of E. It can be seen that τ_a decreases monotonously from 5 to 0.43 ms as E is raised from -90 to -30 mV. In some cases values of τ_a at -80 and -90 mV were nearly indentical indicating that the maximum of the $\tau_a(E)$ curve lies at these potentials (see also Fig. 14*b*). Fig. 14 shows $t_{2/3}$ values (i.e. the point when I_{Na} reaches 2/3 of its maximum) for normal channels. At $E > -40$ mV τ_a and $t_{2/3}$ values are nearly identical. At E about -40 mV $t_{2/3}$ reaches the maximal value (0.47 ms). BTX induced an at least 10-fold increase in maximum activation time constant, and shifted this maximum by about 50 mV to more negative E, in good agreement with results of previous studies (Khodorov 1978; Khodorov and Revenko 1979).

The potential dependence of Na channel activation, i.e. the dependence of the fraction of open channels (n_o) on E, can be deduced from the experimental peak and "instantaneous" current-voltage relations (see Fig. 19). The "instantaneous" current-voltage relation corresponds to constant number of open channels, and its shape is identical with that for a single open channel. The peak-to-"instantaneous" current ratio is thus proportional to the fractional number of open channels at any given E; n_o, as a function of E, can be obtained by normalizing this ratio to unity (Chiu 1980). In untreated membrane the "instantaneous" current-voltage relation is nearly linear from -50 to $+40$ mV (Mozhayeva et al. 1982a). Therefore, n_o for normal channels can be determined at first approximation as a normalized peak conductance. For quantitative estimation of the activation parameters an equation based on Boltzmann's distribution of some charged particles in the electric field was used.

$$n_o = \frac{\exp a_g(E - E_g)}{1 + \exp a_g(E - E_g)} \tag{4}$$

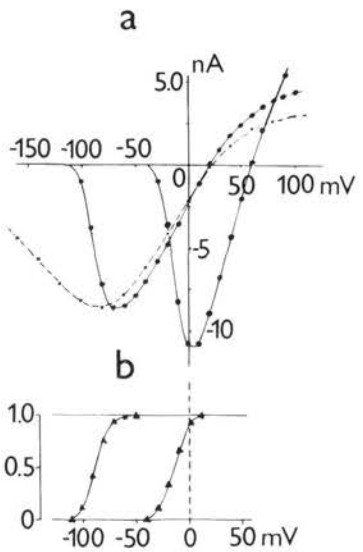

Fig. 19. Potential-dependence of BTX-modified Na channels activation. *a*) Peak current-voltage relations for normal (open circles) and modified (filled circles) channels. Crosses and dashed line represent "instantaneous" current-voltage relations for modified channels. "Instantaneous" currents were recorded after a pulse to −60 mV, 50 ms. A short interval was let to elapse after peak currents recording prior to start instantaneous current measurement and, due to usual rundown of sodium conductance, the latter currents were slightly smaller at potentials positive to about −70 mV. *b*) Fractional number of open channels before (open triangles) and after (filled triangles) modification. For details of calculation, see text. Node 43–81.

where E_g (in mV) is the potential at which $n_o = 0.5$, a_g (in mV^{-1}) is the steepness factor. For each experiment, these parameter values were calculated by the least-square method. The values derived from 9 experiments were: $a_g = 0.143 \pm 0.03\ mV^{-1}$; $E_g = -20.2 \pm 5.9$ mV; $a_g^* = 0.135 \pm 0.006\ mV^{-1}$; $E_g^* = -87.0 \pm 2.4$ mV. Asterisks denote parameters retaining to modified channels.

Thus, BTX induced a large (about 60—70 mV) shift in the voltage dependence of activation of Na channels towards more negative potentials, but it did not affect sensitivity of the gating mechanism to changes in electric field of the membrane.

Partial inactivation of modified channels

The suggestion that BTX-modified sodium channels lack inactivation has been based on the fact that currents through these channels do not decay noticeably

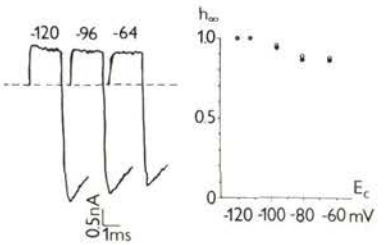

Fig. 20. Effect of depolarizing prepulse on currents through BTX-modified Na channels. *a*) Currents in response to test pulse to +88 mV and subsequent repolarization to −120 mV. Figures over each record show the respective potentials during prepulse (100 ms). Measurements were carried out in the presence of 2×10^{-8} mol . l^{-1} TTX and 1 mmol/l procaine. *b*) Amplitude of the current during the test pulse (filled circles) and on repolarization (open circles) normalized to their maximal values as a function of the prepulse potential. The internodes were cut in a solution containing 100 mmol/l KF and 20 mmol/l CsF. Node 105–82.

during maintained depolarization. In the present work, signs of the occurrence of a partial inactivation in BTX-modified Na channels were observed. Fig. 20 shows an experiment performed to examine effects of prepulses on currents through BTX-modified channels. Both the outward current at +88 mV and the inward "tail" current at $E_H = -120$ mV decreased slightly as E_c (100 ms) was made less negative. One of the possible ways by which prepulse can influence the current amplitude in response to subsequent test pulse is a change in transmembrane ionic gradients, due to the accumulation-depletion effect of currents during the prepulse. Then, the inward current is expected to raise the internal, or to decrease the external, near membrane Na concentration. This should be reflected in a decrease of inward currents and an increase of outward currents during the test pulse. Indeed, such an effect was observed in a number of experiments. However, in the present as well as in a number of other experiments, depolarizing prepulse decreased both inward and outward currents, this is inconsistent with the accumulation-depletion mechanism. It should be noted that in this experiment the current size was dramatically reduced by TTX (2×10^{-8} mol . l^{-1}), so that accumulation-depletion, if any was minimized to great extent. In two other experiments which gave the same result, accumulation-depletion was minimized owing to substitution of Na^+ in external solution by K^+. The involvement of normal Na channels to the phenomena observed was eliminated by the addition of Pr (1 mmol . l^{-1}) to the external solution. It should be noted that the effect of prepulses on currents in BTX-modified channels varied from experiment to experiment, being very weak in many of them.

Discussion

The present paper has, in principle, confirmed results of previous investigations on effects of BTX on properties of Na channels in the node of Ranvier (Khodorov et al. 1975; Khodorov 1978; Khodorov and Revenko 1979). However, total modification of Na channels allowed to study their properties over a broad range of voltages and to observe changes in the properties of these channels in time, starting from fractions of millisecond up to tens of milliseconds.

The first principal finding of the present work are voltage-dependent changes in the current reversal potential during membrane depolarization to $E > -40$ mV. Control experiments have led us to conclude that the inequality in E_r for peak and steady-state current cannot be attributed to factors such as incomplete modification of Na channels by BTX, incomplete blockage of K currents, nonlinearity of leakage currents, ion accumulation-depletion near the membrane, and that the change in E_r reflects the very properties of BTX-modified channels. Following possibilities should be discussed. 1) The ionic selectivity of each channel is not invariable and is subjected to certain time- and voltage-dependent changes during membrane depolarization. These changes could be associated with channel gating, e.g., with transitions between several open states (Chandler and Meves 1970; Armstrong and Bezanilla 1977; Mozhayeva et al. 1980a, b; Sigworth 1981). 2) The selectivity of each BTX-modified channel is constant, but there are two or more types of BTX-modified channels with different selectivities. In order to explain the data obtained it is necessary to assume further that the channels with a lower Na^+-K^+ selectivity are activated at more positive potentials and inactivated to a lesser extent than the more selective channels. According to this assumption, ΔE_r occurs, due to a decrease in the contribution of more selective channels to the total current as a result of inactivation. It should be noted that these hypothetically more selective channels should not be regarded as being normal Na channels (see Results).

The current inhibition in BTX-modified Na channels at high positive potentials can be explained in two ways: 1) At high positive potentials a fraction of Na channels became blocked by some cations present in the axoplasm; 2) the conductance of each BTX-modified Na channel at high E decreased. None of the cations in the end pool solutions seems to be able to block BTX-modified channels. Indeed, current decay was observed at high E both, when these solutions contained $Tris^+$, TEA^+ and Cs^+ and when fibres were cut in KF solution. Experiments on internally perfused cells should be done to test this assumption.

In order to make a choice between the two hypotheses, single channel measurements should be done over a wide potential range. Data on channel conductance of single BTX-modified channel obtained so far (Khodorov et al. 1981) have not been sufficient to allow definite conclusions.

Blockage or conductance of BTX-modified channels, and decrease in selectivity on the other hand seem to be interrelated. It is possible that they reflect two stages of the same process: for example, the channels pass first to a less selective state and subsequently to a less conductive, or even nonconductive, state. It should be noted, however, that potential-dependent changes in selectivity seem to occur in aconitine-modified channels, but there are no signs of potential-dependent inhibition of currents at high E (Mozhayeva et al. 1977, 1980b, 1982b).

The voltage- and time-dependent changes in selectivity and conductance may be due to some voltage-dependence of interactions of the drug molecule with the channel.

Procaine-induced inhibition of currents through BTX-modified channels at high potentials is very similar to that induced by another local anaesthetic, trimecaine (Khodorov 1978; Zaborovskaya and Khodorov 1982). From experiments with procaine it can be concluded that at least two types of channel blockage exist: potential-independent ("tonic") I_{Na} inhibition and potential-dependent inhibition. At negative and very high positive potentials, the channels become blocked by the first mechanism with the current kinetics remaining unchanged. At high positive potentials the second type of blockage becomes operative: it develops rather slowly. It is possible that different sites of procaine binding correspond to different block mechanisms. One can imagine two main mechanisms of the potential-dependent procaine block. 1) The protonated Pr molecules present in the axoplasm enter the inner mouths of the channels and block them in a potential–dependent manner; then the value of δ in Eq. (1) will represent the fractional electric distance from the internal solution to the binding site. 2) Under the influence of the electric field a structure of the Na channel undergoes some conformational changes, thus favouring Pr binding. In this case, the charge of the blocking molecules may not be important. In normal Na channels Pr binding and inactivation are interdependent. Pr binding favours transition of the channel to inactivation state(s) and vice versa (Khodorov et al. 1976; Hille 1977). It is conceivable that Pr, or another anaesthetic, facilitates potential-dependent transition of BTX-modified channels to one of the inactivation states which does not occur under normal conditions. It is difficult to decide at present conclusively between the two mentioned explanations of the Pr potential-dependent block; however, the former is poorly consistent with a high value of δ (0.53) and with the very slow development of the block. It seems unlikely that the Pr molecule travels farther than half the length of the pore. Further, for example, quaternary strychnine has been shown to induce time- and voltage-dependent block od Na channels, inserting into the pore from the axoplasm (Cahalan 1978; Shapiro 1977). However, the strychnine-induced block develops within several milliseconds or less, whereas Pr-induced block requires tens of milliseconds to be complete.

Effects of BTX on the properties of open channels are similar to those of

another alkaloid aconitine (Mozhayeva et al. 1976, 1977, 1982b; Campbell 1982). There are, however significant quantitative differences in the effect of both these alkaloids. Aconitine changes channel selectivity to a greater extent than does BTX. Thus, in BTX-modified channels the relative permeabilities are $Na^+ : NH_4^+ : K^+ : H^+ = 1 : 0.47 : 0.19 : 528$, whereas the respective relationship for aconitine-modified channels are $1 : 1.36 : 0.36 : 1182$ (Mozhayeva et al. 1977; Mozhayeva and Naumov 1983). One of the possible reasons for the difference in channel selectivities are differences in the pore size of normal, BTX- and aconitine-modified channels. Aconitine-modified Na channels seem to have the largest, normal channels the smallest, and BTX-modified channels an intermediate pore diameter. Further, according to recent studies (Mozhayeva et al. 1981, 1982a, b; Spalding 1980) there are at least two acid groups in the Na channel, accessible to protonation from the outside: one ("inner") within the pore, and another ("surface") just near the external end of the pore. Aconitine reduces pK_a of the inner group by about 0.8; according to data of the present work, BTX-induced reducing of this group pK_a is about 0.4. It can thus be concluded that the lower the pK_a of the inner group, the less the sodium vs. relatively large cations (K, NH_4) selectivity of the Na channel. This conclusion supports the notion that the selectivity of the Na channel is determined to a great extent by the properties of the acid group within the channel (Hille 1975a, b). According to the analysis of the pH block of normal Na channels the inner acid group is located approximately halfway across the membrane in the pore. The potential-dependence of the H block in BTX-modified Na channels is approximately the same as in normal channels; BTX hence does not change the fractional electric distance (δ) from the outside to the inner acid group.

Ca^{2+} block experiments suggest that Ca^{2+} ions travel in the channel to a smaller electric distance (0.2) than do H^+ ions. This difference may result from a funnel-like profile of the pore. In this case, electric distances travelled by larger ions should be shorter than those for small ones (e. g. protons). $K_{d(o)}^{Ca}$ for Ca binding in BTX-modified channel (19 mmol . l^{-1}) is similar to the respective value for normal Na channels (23 mmol . l^{-1}) (Woodhull 1973).

The decrease, or even abolishment of H^+ block of BTX-modified channels upon the prolongation of large positive test pulses seem to be of a complex nature. It may be assumed that, as the channels pass to a less selective and less conductive state (see above), they also lose (completely or partly) sensitivity to H^+ block. Alternatively (or additionally), it may be suggested that low pH impedes, in some unknown way, the channel transition to the less conductive state. Additional experiments are needed to clarify this point.

As pointed out earlier (Chizmadzhev et al. 1974; Hille 1975b; Mozhayeva et al. 1981, 1982a) the high proton permeability of the sodium channels determined from reversal potential measurements does not by any means rule out the high

blocking potency of H^+ ions in the same channels. The high proton permeability value indicated that height(s) of the rate limiting energetic barrier(s) in the channel are low for H^+ as compared with those for other cations, e.g. for Na^+. Nevertheless, proton moves slowly in the channel because of its strong binding to the acid group.

The approximate equality of the sensitivity of both BTX-modified and normal Na channels to TTX-block means that the reception of TTX and sodium selectivity are independent properties of the Na channel, and that different structures correspond to each of them. The same conclusion was drawn earlier from a comparison of the sensitivities to TTX-block of aconitine-modified and normal Na channels (Mozhayeva et al. 1976). Available data suggest that the TTX receptor is sited near the external mouth of the pore, and that it incorporates the surface acid group localized just at the end of the pore (Spalding 1980; Mozhayeva et al. 1981, 1982a, b).

The current kinetics in BTX-modified channels was considerably slower at large negative potentials, and approximately the same at $E > -40$ mV as compared with that in normal channels. This suggests that BTX reduced mainly the rate of transition of the channel from open to closed state(s), leaving the close-to-open rate essentially unchanged. A detailed estimation of both parameters was performed by Khodorov (1983). In terms of energetic description this means that in a modified channel the energy of the open state relative to that of the closed one is lower than in a normal channel. In other words, BTX made the open state energetically more favourable. This was reflected in a shift of the steady-state activation curve towards more negative potentials: more electric field energy is required to close the channel. Further, if BTX binding makes the open state of the channel more favourable, then, according to the thermodynamic rules, the open channel should bind BTX better than a closed one. Indeed, repetitive membrane depolarization sufficient to open the channels dramatically accelerated the modification of channels by BTX. BTX-induced shift of the activation voltage range was not accompanied by changes in the steepness of voltage dependence of activation (Khodorov and Revenko 1979, the present paper). This suggested that BTX does not affect the net gating charge of the Na channel. A direct confirmation of this statement has been obtained in studying the effect of BTX on the gating charge movement in the nodal membrane (Dubois et al. 1983). BTX-treatment of the node has been shown not to change the maximum charge displaced. Earlier, a similar result was obtained on aconitine-treated nodal membrane (Krutetskaya et al. 1978).

Aconitine induces qualitatively analogous, but less pronounced changes in operation of the gating machinery of Na channel. Thus, aconitine-induced shift of the voltage range of activation in the node was about 40—50 mV (Mozhayeva et al. 1976, 1980b, Schmidt and Schmitt 1974), whereas BTX-induced shift was 60—70 mV. Unlike BTX-modified channels, the aconitine-modified ones in the

node showed quite a pronounced inactivation process. Inactivation in aconitine-modified Na channels was analyzed earlier (Mozhayeva et al. 1980b) using a very simple model, according to which the channel can assume three main states: closed, open and inactivated. In the normal Na channel, the inactivated state is energetically much more favourable than the open one; due to this the steady-state probability for the channel to be open is very low. Modification with aconitine makes the open state energetically more favourable, and the steady-state probability for the channel to be open is close or even higher than that for the inactivated state. Due to this aconitine-modified channels in the node inactivate only partially and h_∞ never reaches values lower than 0.5 even at large depolarizations. According to the model, the greater the alkaloid-induced shift in the $n_o(E)$-curve, the less channels will inactivate on depolarization. Since BTX induces a greater negative shift in the $n_o(E)$ curve than does aconitine one should expect that inactivation will be less expressed in BTX-modified than in aconitine-modified channels. Indeed, this has been observed. Obviously this three-state model is too simple to be able to provide a detailed description of the channel behaviour; however, it predicts quite correctly the correlation between the steady-state parameters of activation and inactivation.

One of the factors influencing the manifestation of inactivation is the rate of activation. Let us assume that at a given E, 20% of the channels inactivate, i.e. $h_\infty = 0.8$. This is quite a realistic value for BTX-modified channels (see Fig. 20). The analysis has shown that in such a case the current time course will show a peak and a subsequent decay only if the rate of transition from closed to open state exceeds the rate of the subsequent transition from open to inactivated state by a factor no less than 8. Since at potentials more negative than -50 mV the channel activation rates are rather slow the above requirement for the activation-inactivation rate ratio may not be met. Then, inactivation in its usual form (current decay) will be invisible. Hence, for BTX-modified channels the situation is probable when inactivation occurs; however it will not be reflected in current decay.

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