Voltage Dependence of Intramembrane Charge Movement and Conductance Activation of Batrachotoxin-modified Sodium Channels in Frog Node of Ranvier

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ABSTRACT Sodium current and sodium channel intramembrane gating charge movement (Q) were monitored in voltage-clamped frog node of Ranvier after modification of all sodium channels by batrachotoxin (BTX). BTX caused an approximately threefold increase in steepness of the Q vs. voltage relationship and a 50-mV negative shift in its midpoint. The maximum amount of intramembrane charge was virtually identical before and after BTX treatment. BTX treatment eliminated the charge immobilization observed in untreated nodes after relatively long depolarizing pulses and slowed the rate of OFF charge movement after a pulse. After BTX treatment, the voltage dependence of charge movement was the same as the steady-state voltage dependence of sodium conductance activation. The observations are consistent with the hypothesis that BTX induces an aggregation of the charged gating particles associated with each channel and causes them to move as a unit having approximately three times the average valence of the individual particles. Movement of this single aggregated unit would open the BTX-modified sodium channel.

INTRODUCTION

The steroidal alkaloid batrachotoxin (BTX) causes a steady depolarization when applied to nerve or skeletal muscle cells (Albuquerque, 1972; Albuquerque and Daly, 1976). It is now established that the depolarization is due to a BTX-induced steady Na current flowing through Na channels that are normally closed at the resting potential in the absence of BTX (Narahashi et al., 1971; Khodorov et al., 1975). The two effects of BTX that give rise to the steady Na current are a shift of the Na conductance activation curve to more

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negative membrane potentials and the elimination of Na conductance inactivation (Khodorov et al., 1975; Khodorov and Revenko, 1979). The latter effect and the slowing of Na channel gating kinetics after BTX treatment (Khodorov and Revenko, 1979) simplify the study of gating of BTX-modified Na channels (Dubois and Khodorov, 1982).

In the present work we have determined the voltage dependence of Na conductance activation and of the movement of the intramembrane gating charge that is believed to serve as the voltage sensor for Na channel activation (Armstrong and Bezanilla, 1973), both after BTX modification of all Na channels present in the preparation. Such studies are of interest for determining the mechanism of action of BTX and for developing a model for the gating of BTX-modified Na channels. Our major findings are that BTX treatment causes about a threefold increase in the valence of the charged gating particles with no change in the maximum amount of movable charge, and that charge movement and Na channel activation have approximately the same steady-state voltage dependence after BTX treatment.

METHODS

Experiments were carried out on individual nodes of Ranvier in isolated myelinated nerve fibers from the frog *Rana esculenta*. All aspects of fiber preparation and mounting, of voltage-clamping and electrical recording, and of the pulse protocols for eliminating linear capacitive and ionic current were as previously described (Dubois and Schneider, 1982). A membrane potential of -70 mV was defined as that potential which gave 30% fast inactivation of Na current using 50-ms prepulses before BTX treatment. All experiments were carried out using a holding potential of -120 mV.

In most experiments the external solution to which the node was exposed contained 111.5 mM NaCl, 2.5 mM CsCl, 4 mM $MgCl_2$, 2.4 mM $NaHCO_3$, 10 mM tetraethylammonium (TEA) Cl, and either $\sim 5 \times 10^{-9}$ M tetrodotoxin (TTX) when monitoring Na current or 10^{-6} M TTX when monitoring charge movement. Cs^+ and Mg^{2+} were used in place of K^+ and Ca^{2+} to avoid any possible ionic current carried by the latter ions. In terms of the surface potential sensed by the Na channel, 4 mM Mg^{2+} is equivalent to the 1.8 mM Ca^{2+} present in normal Ringer's solution (Hille et al., 1975). The TTX concentration used when monitoring Na current (I_{Na}) was set to reduce I_{Na} to $\sim 20\%$ of its amplitude in the absence of TTX. Such a reduction of I_{Na} greatly decreased the relative decline of I_{Na} during long pulses after BTX treatment (Mozhayeva et al., 1982a; J. M. Dubois, unpublished observations). The partial suppression of I_{Na} should also have minimized series resistance problems. The pH of the external solution was 7.4 at room temperature.

Before cutting the fiber ends, the endpools were changed to a solution containing either 90 mM CsCl and 30 mM NaCl or 120 mM CsF. The fiber ends were then cut and the endpool solutions were left unchanged throughout the course of the experiment. Experiments were carried out at 11–15°C.

The method of BTX treatment followed that of Mozhayeva et al. (1981) for total modification of all Na channels. The flow of external solution was stopped and a few drops of Ringer's solution containing 10 μM BTX were added to the solution in the node pool. The node was then repetitively depolarized at 10 Hz for 5–6 min using 5-ms pulses to a potential close to the reversal potential for Na current (+20 to +80 mV). At the end of this period virtually all Na channels had been modified, as judged by the almost complete elimination of the decline in Na current during prolonged

depolarizing pulses. Solution flow was then resumed and the BTX-modified channels were studied. No reversal of BTX modification was noted, even after as much as ~2 h washing with BTX-free solution.

Sodium currents were monitored after filtering by a Bessel filter with cutoff frequency set at 10–40 kHz. Charge movements (Q) were monitored directly as the transient nonlinear component of integrated currents (Dubois and Schneider, 1982). For both I_{Na} and Q, linear capacitative and ionic currents were approximately removed using an analog compensation circuit. Residual linear components were eliminated using either the "$-P/2$ routine" (Dubois and Schneider, 1982) for pulses larger than ~70 mV or equal numbers of the same amplitude positive and negative pulses for pulses up to ~70 mV. Generally, currents from 12 depolarizing pulses were averaged for Q, whereas 4 depolarizing pulses were sufficient for I_{Na}.

RESULTS

Charge Movement After BTX Treatment

Fig. 1 presents records of on and off charge movement for pulses to several different voltages in the same node after BTX modification of essentially all
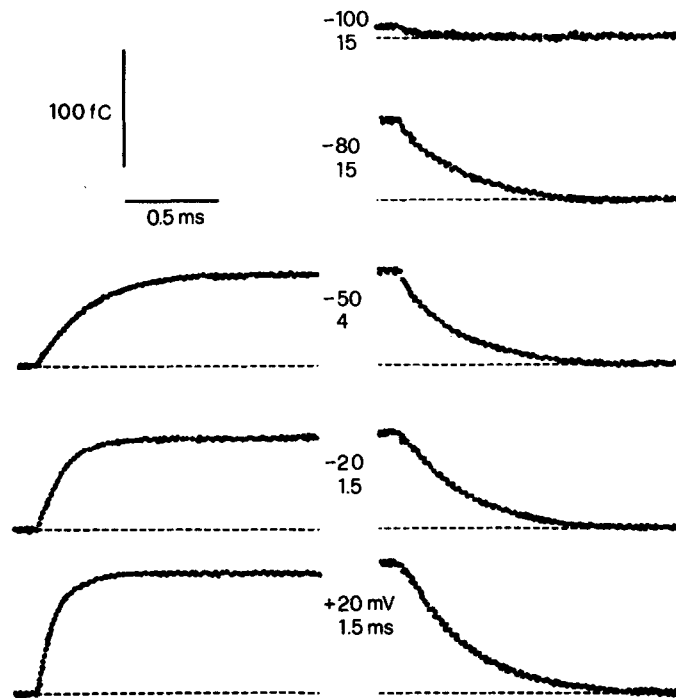

FIGURE 1.   Gating charge movement traces obtained from a fiber after BTX treatment. on (left) and off (right) gating charge movements were recorded during and after pulses to various voltages for various durations (upper and lower numbers, respectively, next to each trace). Temperature: 13°C. Fiber: 27-5-82.

Na channels. The upper and lower numbers next to each record give the membrane potential during the pulse (millivolts) and the pulse duration (milliseconds), respectively. For pulses to potentials negative to about -60 mV, the time course of ON charge movement was so slow that fluctuations in leak current introduced sufficiently large baseline uncertainties that Q_{ON} measurements by our integral method were considered to be unreliable. Thus, only Q_{OFF} records appear in Fig. 1 for the pulses to -100 and -80 mV. Comparison of the Q records in Fig. 1 with similar records from nodes not treated with BTX (cf. Dubois and Schneider, 1982) reveals two obvious differences. First, the Q_{OFF} records after BTX treatment are considerably slower than those from untreated nodes for the same OFF voltage (-120 mV), which is consistent with the slowing of I_{Na} tail currents by BTX (Khodorov and Revenko, 1979). Second, and as previously noted (Dubois and Khodorov, 1982), after BTX treatment Q_{OFF} is comparable in magnitude to Q_{ON}, whereas in untreated axons and nodes, Q_{ON} exceeds Q_{OFF} because of charge immobilization (Armstrong and Bezanilla, 1977; Nonner, 1980; Dubois and Schneider, 1982). An elimination of charge immobilization by BTX would be consistent with the elimination of Na conductance inactivation by BTX (Khodorov et al., 1975; Khodorov and Revenko, 1979) and the close association of charge immobilization with Na inactivation (Armstrong and Bezanilla, 1977).

Fig. 2 presents data from an experiment designed to test quantitatively the agreement of ON and OFF charge movements after BTX treatment. Part A of Fig. 2 presents the Q_{ON} record for a 4-ms pulse to -50 mV. The Q_{OFF} records obtained after pulses to the same potential for a variety of different durations are superimposed on the Q_{ON} record. Note that the same zero level was used for both Q_{ON} and Q_{OFF} in Fig. 2. The amounts of ON and OFF charge movement are in good agreement for all durations tested in Fig. 2A. This observation is consistent with an absence of charge immobilization and negligible ionic current contamination of measured charge movements for pulses to -50 mV.

For larger pulses, the situation appeared to be more complicated. Part B of Fig. 2 presents records obtained using a protocol similar to that in part A, but now for pulses to 0 mV. In this case, Q_{OFF} consistently exceeded Q_{ON}, with the discrepancy increasing with pulse duration. One possible explanation for this observation is that an ionic current was slowly activating during the pulse (cf. Horowicz and Schneider, 1981, for an analogous situation in muscle). If conductance activation were slow and the currents were small during the pulse, the charge transferred by such a current could conceivably have been removed with the Q_{ON} baseline. However, if the inward current tails caused by this current had time courses similar to that of Q_{OFF}, they would not have been removed with the OFF baseline and could have made Q_{OFF} erroneously high. Alternatively, the Q_{OFF} records might be accurate measures of charge movement and Q_{ON} may have been underestimated because of a slow component of true ON charge movement having been subtracted with the Q_{ON} baseline.

Table I shows that the $Q_{OFF}:Q_{ON}$ agreement was consistently good for 2–10-ms pulses to -60 or -50 mV, the mean value of Q_{OFF} being just 7% less than

Q_{ON}. For 0.5–8-ms pulses to 0 or +20 mV, Q_{OFF} consistently exceeded Q_{ON}, the mean excess in Q_{OFF} being 35% of Q_{ON}.

Voltage Dependence of Charge Movement After BTX Treatment

The steady-state charge vs. voltage (Q vs. V) relationship for BTX-modified Na channels is of interest both for purposes of elucidating the mechanism of action of BTX and for developing a model for gating of BTX-modified channels. Unfortunately, several practical considerations somewhat limited the data available for determining Q vs. V. For pulses to about −60 or −50 mV, both Q_{ON} and Q_{OFF} could apparently be reliably determined (Table I),
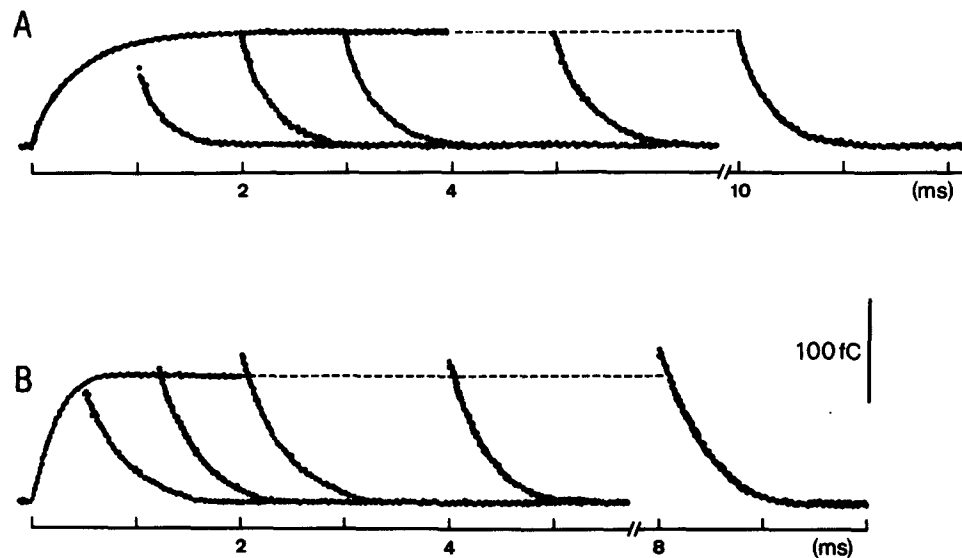

Figure 2. Absence of charge immobilization after BTX treatment. Q_{ON} traces during a 4-ms pulse to −50 mV (A), a 2-ms pulse to 0 mV (B), and superimposed Q_{OFF} traces obtained after pulses of various durations to the same voltages. Note that values of Q_{OFF} after various pulse durations are equal to Q_{ON} for the pulse to −50 mV and are slightly larger than Q_{ON} for the pulse to 0 mV. Temperature: 14°C (A), 11°C (B). Fibers: 11-6-82 (A) and 14-5-82 (B).

so that either or both could be used in analyzing Q vs. V. For pulses to potentials negative to about −60 mV, only Q_{OFF} was available because Q_{ON} was sufficiently slow that our method of direct charge measurement was considered to be unreliable for Q_{ON}. Finally, for pulses to potentials considerably positive to −50 mV, Q_{OFF} consistently exceeded Q_{ON} (Table I). Assuming Q_{OFF} to be overestimated because of contamination from a slowly activating ionic current, we have only used Q_{ON} values for voltages positive to about −50 mV. However, it should be kept in mind that if a true slow charge component were removed from Q_{ON} with baseline subtraction (see above), the Q values for voltages positive to about −50 mV would have been consistently underestimated.

Fig. 3 presents the charge vs. voltage data obtained from one BTX-treated fiber within the limitations just outlined. The solid curve in Fig. 3 gives the least-squares fit of the equation

$$Q = Q_{max}/[1 + \exp(\bar{V} - V)/k], \qquad (1)$$

for the two-state Boltzmann model (Dubois and Schneider, 1982; cf. Schneider and Chandler, 1973) to the Q values at and negative to -50 mV. This

TABLE I

RATIOS OF OFF TO ON CHARGE MOVEMENT FOR
INTERMEDIATE AND LARGE PULSES AFTER BTX
TREATMENT

Fiber	V_{ON}	t_{ON}	Q_{OFF}/Q_{ON}
	mV	ms	
18-5-82A	−60	10	0.79
27-5-82	−50	4	0.84
11-6-82	−50	2	0.95
		5	1.00
		10	1.04
11-6-82A	−50	3	0.82
		5	0.85
14-6-82	−50	2	1.11
		5	1.11
15-6-82	−60	4	0.86
		6	0.82
	−50	6	0.97
Mean ± SEM			0.93±0.03
13-5-82	+20	1	1.25
		3	1.56
		6	1.58
14-5-82	0	0.5	1.12
		2	1.46
		8	1.37
27-5-82	+20	0.5	1.27
		1	1.26
		3	1.31
Mean ± SEM			1.35±0.05

restricted voltage range was chosen for fitting Q vs. V because the steady-state voltage dependence of sodium conductance activation appears to exhibit two components, one component activating completely by about -50 mV, and a second relatively smaller component activating at more positive potentials

(see below). The dashed curve in Fig. 3 was drawn by eye to indicate that there may also be a relatively small second component of charge movement that is displaced by pulses to voltages positive to −50 mV.

Values of the midpoint voltage $\bar{V}$ and steepness factor k obtained from the fit in Fig. 3 and from similar fits of Eq. 1 to Q vs. V data at and negative to −50 mV in four other fibers are presented in Table II. The mean ± SEM of the parameter values obtained from these fits were −83.2 ± 2.7 mV for $\bar{V}$ and 4.85 ± 0.48 mV for k. Although we did not determine $\bar{V}$ and k in these same
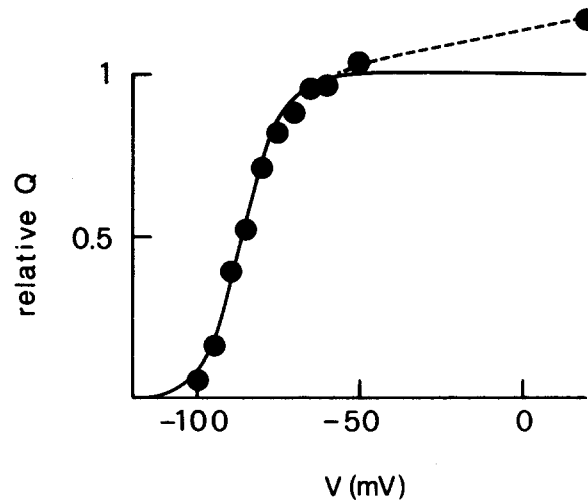

V (mV)

Figure 3.   Voltage dependence of gating charge movement after BTX treatment. For the voltage range −100 to −60 mV, each point gives the mean value of three determinations of OFF charge movement Q_{OFF} after pulses to the indicated voltage. The solid curve presents the nonlinear least-squares fit of Eq. 1 to all individual determinations at and below −60 mV. Parameter values obtained from the fit were: relative $Q_{max} = 1$, $\bar{V} = -86.3$ mV, and $k = 6.04$ mV. The value at −50 mV is the mean of three determinations of Q_{OFF} and eight determinations of Q_{ON}, and the value at +20 mV is the mean of 15 determinations of Q_{ON}. The dashed line was drawn by eye through data points beyond −60 mV. It indicates the existence of a small second component of charge movement not described by the fit of Eq. 1. Temperature: 15°C. Fiber: 15-6-82.

nodes before BTX treatment, we can compare the present values from BTX-treated nodes with our previously reported mean values of −32.1 ± 2.0 mV for $\bar{V}$ and 13.0 ± 0.9 mV for k from normal untreated nodes (Dubois and Schneider, 1982). Two points are immediately apparent: BTX treatment caused a negative shift of ~50 mV in $\bar{V}$ and a 2.7-times reduction in the value of k. The former effect underlies the well-known large negative shift in Na conductance activation that is produced by BTX (Khodorov et al., 1975; Khodorov and Revenko, 1979). The latter effect indicates an increase in valence of the charged gating particles. For the two-state model, the value of k is given by

$$k = RT/F\alpha z, \tag{2}$$

with R being the gas constant, T the absolute temperature, F the Faraday constant, z the valence of the charged intramembrane particle, and α the fraction of the membrane field that the particle traverses as it moves between its two possible locations. Thus, if α were the same in control and BTX-treated nodes, the relative change in k would indicate an approximately threefold increase in valence of the charged gating particle after BTX treatment.

Two possible alternative explanations for the approximately threefold increase in gating particle valence immediately come to mind. BTX treatment could either (*a*) cause each individual gating particle to somehow carry about

TABLE II

STEADY-STATE VOLTAGE DEPENDENCE OF CHARGE
MOVEMENT AND SODIUM CONDUCTANCE ACTIVATION
AFTER BTX TREATMENT

Fiber	For Q		For g_{Na}	
	$\bar{V}$	k	$\bar{V}$	k
	mV	mV	mV	mV
22-3-82	−80	3.73	−86	4.96
27-5-82	−92	3.72	−92	5.63
8-6-82A	−77	5.20	−82	4.54
3-12-81			−96	5.57
7-12-81			−84	5.61
6-12-81			−87	4.44
12-5-82			−79	5.89
13-5-82			−92	5.63
8-6-82			−93	4.19
11-6-82A	−80	5.55		
15-6-82	−86	6.04		
Mean ± SEM	−83±3	4.85±0.48	−87±2	5.16±0.21

Values of $\bar{V}$ and k were obtained by nonlinear least-squares fit of Eq. 1 to data over the voltage range −100 to −60 or −50 mV after BTX treatment.

three times as much charge across the membrane as in untreated fibers or (*b*) cause several previously independent gating particles to move across the membrane as a unit having about three times the average charge of the gating particles before BTX treatment. These two alternatives make very different predictions regarding the maximum charge displaced: *a* predicts an approximately threefold increase in Q_{max}, whereas *b* predicts no change in Q_{max}. We thus attempted to determine Q_{max} both before and after BTX-treatment in several fibers. Fig. 4 presents records from such an experiment in which Q_{ON} was recorded at −40 and 0 mV in the same fiber before and after BTX treatment. Before BTX treatment, the final level of Q_{ON} at 0 mV should be just slightly less than Q_{max} (Dubois and Schneider, 1982). Its value in the control record of Fig. 4 was about equal to the final levels of Q_{ON} in the records at both −40 and 0 mV after BTX. The latter two values were about the same because of the shift of the Q vs. V relationship toward more negative voltages after BTX treatment.

Table III presents Q_{ON} values determined in each of five fibers before and after BTX treatment at voltages where Q_{ON} is close to Q_{max}. The Q_{ON} values were essentially the same before and after BTX treatment, which indicates that BTX treatment does not change Q_{max}. This result is consistent with the earlier data of Dubois and Khodorov (1982). It thus appears likely that BTX increases the gating particle valence by causing groups of individual particles present in the unmodified node to move as a unit after BTX treatment.
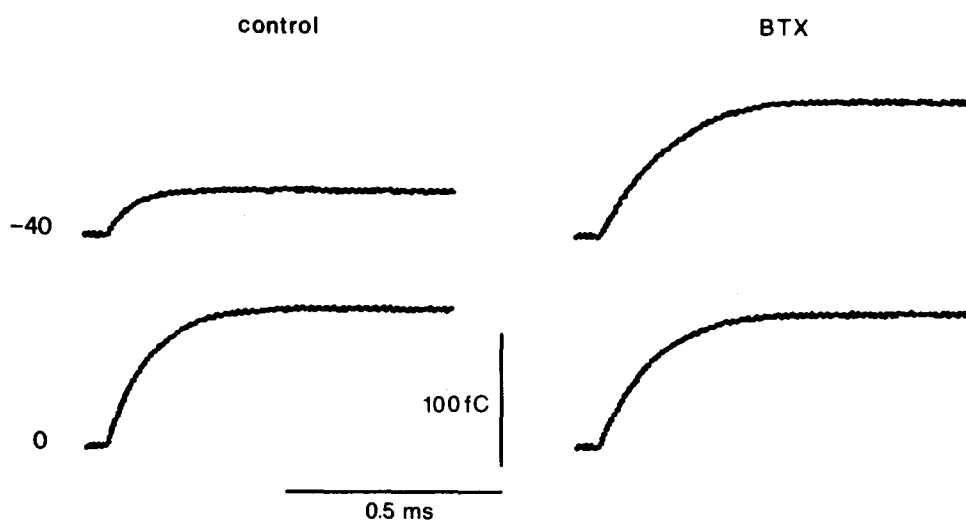

FIGURE 4.   ON gating charge movement traces during pulses to −40 and 0 mV before and after BTX treatment. Temperature: 11°C. Fiber: 18-5-82.

TABLE III

EFFECT OF BTX TREATMENT ON MAXIMUM CHARGE
MOVEMENT

Fiber	V_{ON}		Q_{ON} ratio
	Before BTX	After BTX	After:before BTX
	mV		
18-5-82	0, +20	−60	1.03
26-5-82	0, +20	0	1.10
23-6-82	0	0	1.10
24-6-82A	0	0	0.93
30-7-81	+20	+20	0.98
Mean ± SEM			1.03±0.03

Sodium Conductance Activation After BTX Treatment

BTX treatment causes both charge movement (above) and sodium conductance activation (Khodorov and Revenko, 1979) to occur at more negative voltages than in untreated axons. One possible step toward gaining an understanding of the gating mechanism of BTX-modified Na channels is to

compare quantiatively the voltage dependencies of charge movement and conductance activation.

Fig. 5 presents a set of sodium current records obtained during and after pulses to the indicated membrane potentials in a BTX-treated fiber. In comparison with Na current records from untreated fibers, the most obvious change in Na currents induced by BTX treatment is the almost complete elimination of Na current decay during all pulses (Khodorov and Revenko, 1979). Other quantitative differences between the records in Fig. 5 and similar records from untreated nodes are that Na currents from BTX-treated nodes appear at more negative potentials during depolarization and decline much more slowly after repolarization than is the case for untreated nodes (Kho-
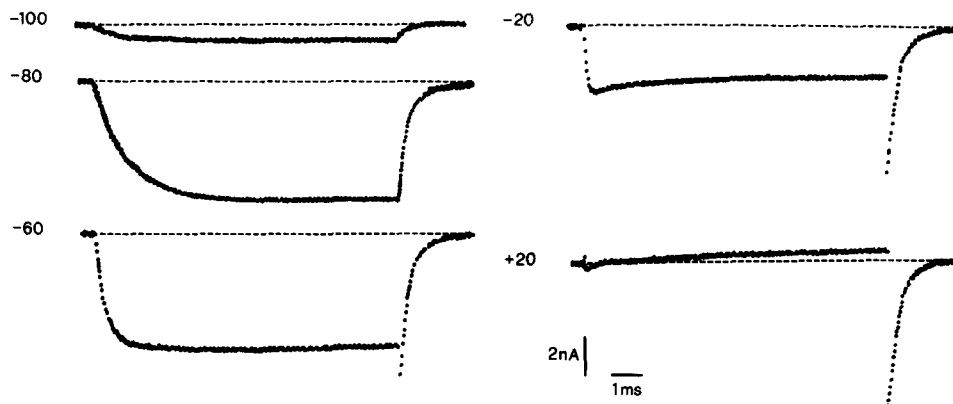

FIGURE 5.   Sodium current traces obtained from a BTX-treated fiber during and after pulses to various voltages (numbers next to each trace). Note that after activation I_{Na} did not decline during pulses to or below −60 mV, declined slightly during the pulses to −20 mV, and changed direction during the pulse to +20 mV. Temperature: 11.5°C. Fiber: 2-6-82.

dorov et al., 1975; Khodorov and Revenko, 1979). Despite the step increase in driving force at repolarization, no increase in Na current was observed at the OFF's of the pulses to −100 and −80 mV in Fig. 5. This is consistent with a nearly flat, instantaneous current-voltage relationship for BTX-modified Na channels over the voltage range −120 to −80 mV (Mozhayeva et al., 1982b).

The slowed decline of Na tail currents after BTX treatment is technically convenient because it facilitates a relatively straightforward evaluation of Na conductance activation in terms of the amplitude of the Na tail current. Such a procedure is independent of any assumptions regarding the current-voltage relationship of activated channels. It is convenient to use after BTX treatment because the tail currents become sufficiently slow compared with the capacitative transient that it is unnecessary to use the uncertain and possibly erroneous procedure of back-extrapolating an exponentially decaying tail current to the instant of command pulse turn-off.

Fig. 6 presents relative values of Na conductance activation at the end of pulses to the indicated voltages in a fiber after BTX treatment. All values

were determined from Na tail current amplitudes after repolarization to the −120-mV holding potential. The solid curve gives the fit of the equivalent of Eq. 1 to the values at and negative to −50 mV. The fit corresponds well to the data over the fit interval, but falls below the data for more positive potentials. The dashed curve was drawn by eye through values at these more positive potentials. It indicates the likely presence of a second relatively small component of Na conductance that appears to activate only at the more positive potentials. Such a second component was systematically observed in almost
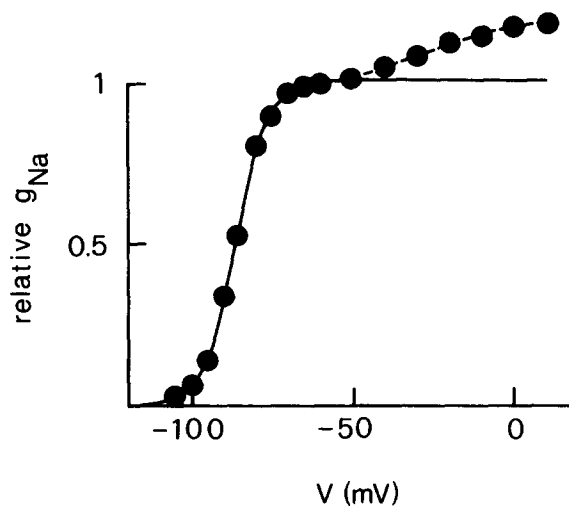
FIGURE 6.   Sodium conductance vs. voltage relationship after BTX treatment. g_{Na} was determined from quasi-instantaneous current at repolarization after depolarizations to various voltages lasting 20 ms (below −70 mV) or 10 ms (beyond −70 mV). The solid curve presents the nonlinear least-squares fit of the equivalent of Eq. 1 to the values at and negative to −60 mV. Parameter values obtained from the fit were: relative $g_{Na\,max} = 1$, $\bar{V} = -86.1$ mV, and $k = 4.96$ mV. The dashed curve was drawn by eye through data points beyond −50 mV and indicates the existence of a second g_{Na} component. Temperature: 11°C. Fiber: 22-3-82.

all experiments. In nine fibers its contribution to the tail current ranged from 2 to 20% (mean ± SEM = 13 ± 2%) of the maximum tail current estimated from the fit of Eq. 1 to tail current amplitudes after pulses to up to −50 mV in the same fiber.

Like the Na conductance of untreated nodes, the second small g_{Na} component of the BTX-treated nodes activates between −50 and +10 mV. Thus, the second component might be thought to correspond to a small proportion of nonmodified Na channels that still activate and inactivate normally. However, this interpretation seems unlikely because the second g_{Na} component was little if at all inactivated after 10–50-ms pulses that would inactivate most normal channels. A second interpretation is that the second component corresponds to a small fraction of Na channels whose inactivation was

eliminated by BTX but whose activation remained unaltered. This would suggest that BTX induces two independent effects, which is contrary to the evidence for all-or-none effects (Khodorov et al., 1981). A third interpretation is that the second g_{Na} component corresponds to a distinct class of Na channels. In the normal frog node of Ranvier, ~2% of the total Na current fails to inactivate (Dubois and Bergman, 1975). It is possible that this "late" Na current flows through distinct Na channels insensitive to BTX. After BTX, the conductance of BTX-sensitive channels is decreased two- to fourfold and their selectivity is reduced (Khodorov et al., 1981). Assuming the conductance of the late BTX-insensitive channels to be independent of BTX, their contribution to the total current after BTX would then be ~4–8%, i.e., close to the contribution of the second g_{Na} component. Further analyses are needed to specify the significance of the second g_{Na} component. In the present investigation we have limited our study to the first component.

Voltage Dependence of Charge Movement and Sodium Conductance Activation

Fig. 7 presents relative values of steady-state sodium conductance activation and charge movement determined in the same fiber as a function of pulse membrane potential after BTX treatment. Each Q or g_{Na} value was normalized to the value of the parameter Q_{max} (or its analogue $g_{Na\,max}$) obtained by fitting Eq. 1 (or its equivalent for g_{Na}) to the combined data in the voltage range −100 to −50 mV. Fig. 7 demonstrates that the voltage dependence of Na conductance activation was similar to that of charge movement. The curve in Fig. 7, which presents the least-squares fit of Eq. 1 to all values of relative Q and g_{Na}, gives a fairly good description for both parameters.

Table II presents the values of the parameters $\bar{V}$ and k obtained by fitting Eq. 1 or its equivalent for g_{Na} to data from each fiber in which the voltage dependence of Q or g_{Na} was determined after BTX treatment. In the three (top) fibers, in which both Q and g_{Na} were studied, there was relatively good agreement of the parameters $\bar{V}$ and k. Taking parameter values from fits to data from each fiber, the mean ± SEM values obtained, respectively, from the Q and g_{Na} data were −83 ± 3 and −87 ± 2 for $\bar{V}$ and 4.8 ± 0.5 and 5.2 ± 0.2 for k. Thus, within experimental error, both $\bar{V}$ and k appear to be the same for charge movement and Na conductance activation after BTX treatment. This observation is consistent with activation of BTX-treated sodium channels being controlled by a single, charged gating particle. It is in contrast with the different voltage dependencies of Q and sodium conductance activation observed in untreated nodes (Neumcke et al., 1976).

Assuming α to equal 1, Eq. 2 can be used to calculate the gating particle valence z from the values of k. Using the k values in Table II for the fit to the g_{Na} data, a mean ± SEM value of 4.8 ± 0.2 is obtained for the valence of the single gating particle of BTX-treated Na channels. This value is somewhat larger than the previous estimates of 3.5 ± 0.5 (Khodorov and Revenko, 1979) and 3.6 (Khodorov et al., 1981; this value was obtained from a fit of Eq. 1 to the mean data in their Fig. 1). The discrepancy is probably caused by the different methods used to determine sodium channel activation. In the present

case, tail current amplitudes after each pulse were used to estimate directly activation at the end of the pulse, whereas in the earlier reports sodium currents during each pulse were converted to sodium permeabilities, P_{Na}, using the Goldman-Hodgkin-Katz equation, and Eq. 1 was fit to the steady-state P_{Na} values.

In the presence of TTX ($\sim 5 \times 10^{-9}$ M; see Methods), the decline of I_{Na} during long-lasting pulses was greatly decreased but still present in most of the experiments (see Fig. 5). This decline of I_{Na} might have been related either
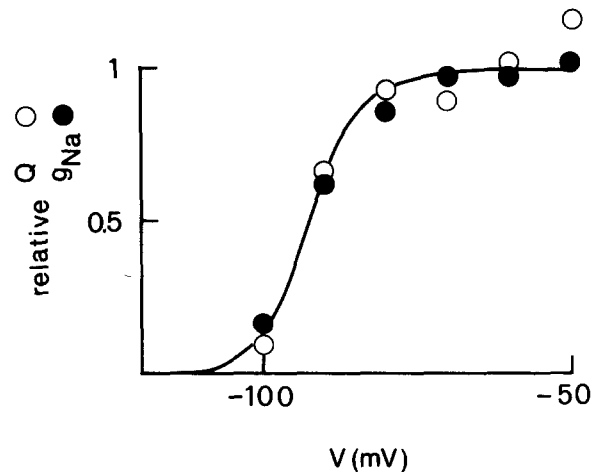

FIGURE 7. Voltage dependence of gating charge movement and sodium conductance in a BTX-treated fiber. The sodium conductance was calculated from quasi-instantaneous current recorded at repolarization after pulses to various voltages whose durations were adjusted to give steady-state current during each voltage pulse. The charge movement values are mean values of two Q_{OFF} determinations after pulses of the same duration as used for the sodium current recordings. The curve presents the nonlinear least-squares fit of Eq. 1 to the combined Q and g_{Na} values in the voltage range -100 to -50 mV (see the text for details of the procedure). Parameter values obtained from the fit were: relative Q_{max}, $g_{Na\ max} = 1$, $\bar{V} = 92.1$ mV, and $k = 4.47$ mV. Temperature: 13°C. Fiber: 27-5-82.

to the remaining Na concentration changes in the vicinity of the membrane (J. M. Dubois, unpublished observation) or to a small g_{Na} inactivation associated with a change in reversal potential of I_{Na} during the pulse (Mozhayeva et al., 1981). Whatever the origin of this decline of I_{Na}, which we will simply term "inactivation," the question arises as to whether it is necessary to take it into account for purposes of analysis of the sodium conductance activation. In some experiments, the g_{Na} activation-voltage curve was calculated from peak Na tail currents after long pulses of various amplitudes both without and with correction for "inactivation." The "inactivation" was measured from tail currents after complete activation of g_{Na} by a 0.5-ms pulse to about $+50$ mV preceded by long-lasting prepulses of various amplitudes

(Mozhayeva et al., 1981). Under these conditions, the difference between the values of the parameters $\bar{V}$ and k in Eq. 1 calculated with and without correction for "inactivation" did not exceed 1.5%. This finding indicated that the conductance activation could be reasonably calculated from tail current amplitudes without correction for "inactivation." Thus, no correction for "inactivation" was applied to the g_{Na} data used for the fits in tables or figures presented here.

DISCUSSION

One of the three principal findings in this report is that BTX treatment produces an approximately threefold increase in the average valence of the charged gating particles without causing any appreciable change in the total amount of intramembrane charge that can be moved. A first approach to interpreting this finding can be based on the simplified model of three identical and independent gating particles for each unmodified Na channel. This corresponds exactly to the Hodgkin-Huxley (1952) m^3 formalism for Na channel activation. Under control conditions, the three gating particles would move independently of each other, whereas after BTX treatment, the three particles would move together as a unit having three times the valence of the individual particles.

The preceding interpretation of the BTX-induced increase in gating particle valence requires refinement. The model of three identical and independent particles is insufficient to account for the properties of unmodified Na channels (see the recent review by Armstrong [1981]). In unmodified nodes of Ranvier maintained near −100 mV, sodium conductance activation can be described using either m^3 or m^2 kinetics, but in both cases only after an initial arbitrary time delay (Neumcke et al., 1976). Gating charge movement kinetics in unmodified nodes also exhibit deviations from the behavior expected for the model of three identical and independent particles (Neumcke et al., 1976; Dubois and Schneider, 1982). However, models allowing a small amount of interaction between three identical gating particles in each unmodified channel can account for some of these deviations (Dubois and Schneider, 1982). Such models would also be compatible with an approximately threefold increase in measured gating particle valence if BTX treatment caused the three particles to move as a unit.

It should also be noted that the BTX-induced increase in particle valence does not necessarily correspond to the formation of an actual chemical aggregate of individual gating particles. It might correspond to an effective aggregation caused by a high degree of cooperativity in the movement of individual particles. Alternatively, the increase in valence could be produced by BTX greatly decreasing the probability of occupancy of intermediate states in a sequence of states leading from a closed to an open channel.

The second principal finding in this paper is that after BTX treatment the steady-state voltage dependencies of charge movement and sodium conductance activation are very similar. This finding is consistent with the opening of BTX-modified Na channels being controlled by movement of a single

charged gating unit. The single gating unit after BTX treatment would correspond to the gating particles of the unmodified channel aggregated together by BTX, a possibility that has previously been suggested on the basis of kinetic analysis of Na currents after BTX treatment (Khodorov and Revenko, 1979). Direct quantitative comparison of the kinetics of Na current and charge movement after BTX treatment (J. M. Dubois and M. F. Schneider, work in progress) should provide a test for the single gating unit hypothesis for the control of BTX-modified Na channels.

The third principal finding is that BTX almost completely removes the immobilization of gating charge during a long-lasting membrane depolarization. After the end of such a depolarization, all the gating particles return to their initial "resting" location, although with time constants that are several-fold larger than the normal ones at comparable repolarizing potentials. Both these effects are consistent with BTX-induced changes of Na current kinetics during and after membrane depolarization (Khodorov and Revenko, 1979; present results).

It has been previously suggested that a dramatic deceleration of the backward ("closing") charge movement caused by BTX is a major reason for the large negative shift in the voltage dependence of Na channel activation: the channels become capable of opening even at very negative potentials (less than −100 mV), where the forward (opening) charge movement is still very slow (see Khodorov, 1983). In the absence of BTX, a weak tendency of channel transition to the open state at such negative potentials is completely canceled by a stronger opposite tendency of channel closing: the deactivation rate constant (β) greatly exceeds the activation (α) one. It is tempting to speculate that it is the aggregation of the gating particles by BTX that hinders charge immobilization (inactivation) during a maintained membrane depolarization and that decreases the rate of the backward charge movement (deactivation) during membrane repolarization.

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