Kinetics 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 intramembrane gating charge movement (Q) were monitored in voltage-clamped frog node of Ranvier after modification of all sodium channels by batrachotoxin (BTX). Sodium current activation followed a single-exponential time course, provided a delay was interposed between the onset of the step ON depolarization and that of the current change. The delay decreased with increased ON depolarization and, for a constant ON depolarization, increased with prehyperpolarization. ON charge movement followed a single-exponential time course with time constants \( \tau_{Q,ON} \) slightly larger than \( \tau_{Na,ON} \). For pulses between -70 and -50 mV, \( \tau_{Q,ON}/\tau_{Na,ON} = 1.14 \pm 0.08 \).

The OFF charge movement and OFF sodium current tails after a depolarizing pulse followed single-exponential time courses, with \( \tau_{Q,OFF} \) larger than \( \tau_{Na,OFF} \). \( \tau_{Q,OFF}/\tau_{Na,OFF} \) increased with OFF voltage from 1 near -100 mV to 2 near -160 mV. At a set OFF potential (-120 mV), both \( \tau_{Q,OFF} \) and \( \tau_{Na,OFF} \) increased with ON pulse duration. The delay in \( I_{Na} \) activation and the effect of ON pulse duration on \( \tau_{Q,OFF} \) and \( \tau_{Na,OFF} \) are inconsistent with a simple two-state, single-transition model for the gating of batrachotoxin-modified sodium channels.

INTRODUCTION

This paper continues the study of intramembrane charge movement and conductance activation of batrachotoxin (BTX)-modified sodium channels in frog node of Ranvier. In an earlier paper (Dubois et al., 1983), we analyzed the voltage dependence of BTX-modified Na conductance and intramembrane charge movement. The major conclusions of that work were that in BTX-modified channels: (a) the shift of the Na conductance-voltage curve toward negative voltages (Khodorov and Revenko, 1979) was accompanied by an equivalent shift of the charge movement voltage curve; (b) the suppression of Na...
inactivation (Khodorov et al., 1975; Khodorov and Revenko, 1979) was accompanied by a removal of charge immobilization (see also Dubois and Khodorov, 1982); (c) the slowing of Na current activation (Khodorov and Revenko, 1979) was accompanied by a slowing of charge movement; and (d) the particles that were supposed to gate each channel were effectively aggregated and moved within the membrane as a unit having approximately three times the average valence of the individual particles. In the present work, we investigate kinetic aspects of current activation and charge movement of BTX-modified Na channels.

METHODS

All methods were as described previously (Dubois et al., 1983). 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, voltage-clamping and electrical recording, and the pulse protocols for eliminating linear capacitative current and linear ionic and leakage currents were as previously described (Dubois and Schneider, 1982). The fiber ends were cut in a solution containing either 120 mM CsF or 90 mM CsCl and 30 mM NaCl, which was used in the end pools throughout the experiments. The external solution used in these experiments contained 111.5 mM NaCl, 2.5 mM CaCl, 4 mM MgCl₂, 2.4 mM NaHCO₃, and 10 mM tetrathylammonium (TEA) Cl. Complete BTX modification of all sodium channels in each node was achieved by repetitive pulsing in the presence of 10 µM BTX (Mozhayeva et al., 1981) following the protocol described previously (Dubois et al., 1983). To minimize changes in Na driving force during Na influx (Dubois and Coulombe, 1984), all Iₙa results were obtained after Iₙa had been reduced to ~20% of its control amplitude by 5 nM tetrodotoxin (TTX). When monitoring charge movement, Na current was completely blocked by 1 µM TTX. Any possible inward ionic current carried by K⁺ and Ca²⁺ was eliminated with external solutions containing MgCl₂ (4 mM) and CsCl (2.5 mM) in place of CaCl₂ (1.8 mM) and KCl (2.5 mM), respectively (Dubois et al., 1983). Na currents and charge movements were averaged (Dubois et al., 1983) and their kinetics were analyzed on a minicomputer (MINC 11/23, Digital Equipment Corporation, Maynard, MA). All the experiments were carried out using a holding potential of −120 mV. The temperature was 12–15°C.

RESULTS

Delayed Activation of BTX-modified Sodium Current

The activation of BTX-modified Na current has been described as being purely single-exponential in both node of Ranvier (Khodorov and Revenko, 1979) and neuroblastoma cells (Huang et al., 1982). This would be consistent with the conclusion that the intramembrane charged particles gating each channel move as a unit after BTX treatment (Dubois et al., 1983). However, close analysis reveals that the entire time course of Iₙa activation is not single-exponential (Fig. 1). Most of the time course of Na current activation can be fitted by a single exponential, but only provided that a relatively small delay is interposed between the onset of the depolarization and of the current change (Fig. 1B). To account for this delay, the final phase of Na current activation can be described by the equation

\[ I_{Na}(t) = I_{Na}(\infty) \left[ 1 - \exp \left( -\frac{t - \delta t}{\tau_{Na}} \right) \right], \]  

(1)
where $\delta t$ represents the time delay between the onset of the depolarization and the origin of the exponential function fitting most of the current change. It should be emphasized that Eq. 1 does not describe the entire time course of $I_{Na}$ from the onset of depolarization but only describes the latter exponential turn-on of $I_{Na}$ to its final level. A complete description of the entire time course might be achieved with several exponentials. However, Eq. 1 provides a convenient way of defining the delay $\delta t$. The value of $\delta t$ corresponds to the time at which the back-extrapolation of the exponential fit to the latter part of $I_{Na}$ becomes zero. This corresponds to the time at which straight lines fit to semilog plots of $1 -$ relative $I_{Na}$ reach the value of 1. Fig. 1C clearly shows that $\delta t$ was significantly greater than zero. The value of $\delta t$ was of the order of 70 $\mu$s for pulses from $-120$ mV to either $-70$, $-60$, or $-50$ mV (Table I) and decreased with increasing depolarization (Fig. 1C). The existence of a delay has already been proposed for the activation of $I_{Na}$ through normal unmodified Na channels in the squid axon (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1976; Taylor and Bezanilla, 1983), the Myxicola axon (Schauf, 1983), and the node of Ranvier (Neumcke et al., 1976). In principle, the appearance of a delay in the BTX-modified Na current activation might be due to contamination of the inward ionic current by an outward gating current. In fact, this seems to be unlikely since (a) the gating current was much smaller than the Na current, (b) the extrapolated straight lines

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(A and B) Turn-on kinetics of sodium current obtained from a BTX-treated fiber. Traces of sodium current recorded at $-80$ (A) and $-60$ mV (B). The arrows indicate time of depolarization. (C) Representation in semilogarithmic coordinates of the sodium current activation at various voltages (numbers beside each straight line). Fiber 7-12-81.}
\end{figure}
in Fig. 1B crossed the vertical axis at a value $>1$, and (c) the delay was larger for the smaller depolarizations (Fig. 1C) that induced smaller outward gating currents. From these considerations, it seems that the activation of Na current in BTX-modified channels is really delayed.

In a variety of preparations, the delay in activation of various currents can be increased by large negative prepulses. This phenomenon, referred to as the Cole-Moore effect, was first described on the K current in squid axon (Cole and Moore, 1960). One can ask whether such a phenomenon occurs in BTX-modified Na current. Fig. 2 shows the effect of changing the holding potential from $-120$ to $-160$ mV on Na current activation at $-60$ mV. After changing the holding potential from $-120$ to $-160$ mV, the level of the steady state $I_{Na}$ at $-60$ mV was unaffected, but it clearly took more time to reach this level when the holding potential was $-160$ mV (arrow in Fig. 2A). The semilog plots of the currents from the two holding potentials indicate that in both cases the steady level of $I_{Na}$ was approached exponentially with a similar time constant. The back-extrapolations of the linear semilog plots show that the value of $\delta t$ was increased from $\sim 50$ to $\sim 250$ $\mu$s when the holding potential was changed from $-120$ to $-160$ mV (Fig. 2B). With the $-160$-mV holding potential, the current clearly deviated from the final exponential line during the first millisecond (Fig. 2B). This provides evidence for the presence of additional time-consuming steps when $I_{Na}$ is activated from $-160$ mV compared with $-120$ mV.

**TABLE I**

| Fiber        | $V_{ON}$ | $\tau_Q$ | $\tau_{Na}$ | $\delta t$ | $\tau_Q/\tau_{Na}$ |
|--------------|----------|----------|-------------|-------------|---------------------|
| 8-7-82A      | -50      | 426      | 463         | 81          | 0.92                |
| 14-6-82      | -70      | 599      | 665         | 50          | 0.90                |
|              | -50      | 269      | 293         | 41          | 0.92                |
| 15-6-82      | -60      | 449      | 327         | 75          | 1.37                |
| 2-7-82A      | -60      | 537      | 379         | 78          | 1.42                |
| 5-7-82       | -60      | 611      | 543         | 64          | 1.13                |
| 6-7-82       | -60      | 588      | 510         | 80          | 1.15                |
| 7-7-82       | -60      | 638      | 485         | 87          | 1.32                |
| Mean ± SEM  |          |          |             |             | 1.14±0.08           |

The time course of ON charge movement could always be fitted by a single exponential without a delay (Dubois et al., 1983). The comparison of the time courses of Na current and charge movement in the same fibers revealed that the ON charge movement time constant ($\tau_{Q,ON}$) was slightly larger than the Na current activation time constant ($\tau_{Na,ON}$) (Fig. 3C and Table I; see also Figs. 5 and 6). Since the Na current activates after a delay, the normalized Na current and charge movement traces cross each other (Fig. 3C).
From this point of view, BTX-modified Na channels are similar to normal Na channels, for which cross-over of normalized Na activation and charge movement can be observed for large depolarizations (Neumcke et al., 1976).

**OFF Time Course of BTX-modified Sodium Current and Charge Movement**

When recorded between −110 and −160 mV, the OFF time courses of Na current and charge movement at repolarization after depolarizations of various durations to −60 or −50 mV could be always fitted by a single exponential (Fig. 4). However, a small rising phase was often observed in the Na tail current (Fig. 4A). Such a rising phase was also observed on the same preparation and under the same conditions by Mozhayeva et al. (1983, Fig. 4). The appearance of a hook in the Na tail current might be purely artifactual and could be attributed to the filtering of the current. Other possibilities, such as the existence of several open states of the channels (Mozhayeva et al., 1981), are discussed later. When determined on the same fibers, \( \tau_{Q,OFF} \) was found to be consistently larger than \( \tau_{Na,OFF} \) (Table II, Figs. 4–6).

**Voltage Dependence of Time Constants for Sodium Current and Charge Movement**

Sodium current tails and OFF charge movement were recorded at several OFF membrane potentials after depolarizations to −60 mV, and sodium current
activation and ON charge movement were recorded in the same fibers at several ON membrane potentials. The time constants $\tau_Q$ and $\tau_{Na}$ changed with voltage in a bell-shaped manner and were maximum near $-90$ mV (Fig. 5). Assuming a single type of intramembrane charged particle and a single energy barrier between two possible sites that the particles can occupy, the time constant vs. voltage relationships shown in Fig. 5 should be described by the equation

$$\tau = \frac{2\tau \bar{V}}{\exp\left(\frac{\bar{V} - V}{k}(1 - \eta)\right) + \exp\left(\frac{V - \bar{V}}{k}\eta\right)}.$$  

(Adrian, 1978; Horowicz and Schneider, 1981; Dubois and Schneider, 1982), where $\bar{V}$ is the membrane potential for 50% charge displacement, $k$ is the steepness factor for the charge movement vs. voltage relationship, $\eta$ is the fraction of the total field between sites that appears between the barrier peak and the resting site, and $\tau \bar{V}$ is the value of $\tau$ at $\bar{V}$. The data points in Fig. 5 are fairly well fit by Eq. 2. However, one can see that the values of $\tau_Q$ in Fig. 5 are larger than those of $\tau_{Na}$ at all voltages. This finding was confirmed in several fibers.
Fig. 4 presents values of the ratio $\tau_Q/\tau_{Na}$ calculated for each fiber in which $\tau_Q$ and $\tau_{Na}$ were determined at the same voltage or voltages. It appears that $\tau_Q/\tau_{Na}$ tended to increase at large negative voltages. When $V$ was close to $V_{(n)}$ (near $-100$ mV), $\tau_Q$ and $\tau_{Na}$ were almost equal. With increased depolarizations, $\tau_Q/\tau_{Na}$ tended to increase again. An increase in $\tau_{Q,OFF}/\tau_{Na,OFF}$ with hyperpolarizations has already been observed on normal nodes of Ranvier (Neumcke et al., 1976) but was not confirmed later (Dubois and Schneider, 1982). These observations may suggest

\begin{table}
\centering
\caption{OFF Time Constants for Sodium Current and Charge Movement at $-120$ mV after BTX Treatment}
\begin{tabular}{cccccc}
Fiber & $V_{ON}$ & $I_{ON}$ & $\tau_Q$ & $\tau_{Na}$ & $\tau_Q/\tau_{Na}$ \\
\hline
14-6-82 & $-50$ & 4 & 310 & 314 & 0.99 \\
15-6-82 & $-60$ & 6 & 423 & 396 & 1.07 \\
2-7-82A & $-60$ & 4 & 1,117 & 884 & 1.26 \\
5-7-82 & $-60$ & 4 & 500 & 384 & 1.30 \\
6-7-82 & $-60$ & 4 & 1,626 & 1,836 & 1.22 \\
7-7-82 & $-60$ & 2 & 689 & 650 & 1.06 \\
\hline
Mean $\pm$ SEM & & & 1.15$\pm$0.05 \\
\end{tabular}
\end{table}
Figure 5. Sodium current and charge movement time constants vs. voltage in a BTX-treated fiber. Sodium currents (open symbols) and charge movement (filled symbols) were recorded during pulses of various amplitudes applied either from a holding potential of $-120$ mV (squares) or after 4-ms pulses at $-60$ mV (circles). The curve was drawn according to Eq. 2 with $V = 83$ mV, $k = 5$ mV, $\eta = 0.77$, and $\tau_V = 1.3$ ms. Fiber 5-7-82.

Figure 6. Ratio of charge movement and sodium current time constants vs. voltage in BTX-treated fibers. Sodium current and charge movement were recorded in one to six fibers during pulses of various amplitudes applied either from a holding potential of $-120$ mV (open circles) or after 4-ms pulses at $-60$ mV (filled circles). The curve was drawn according to Eq. 3 with $V = -100$ mV, $\eta = 0.5$, $k_Q = 5.2$ mV, and $k_{Na} = 4.7$ mV.
a common feature of normal and BTX-modified Na channels, with the additional complexity in normal fibers being due to the inactivation process (see Discussion). The finding that \( \tau_Q \) is not equal to \( \tau_{Na} \) indicates that a two-state, single-transition model is insufficient to account fully for the gating of BTX-modified sodium channels.

**Effect of ON Pulse Duration on OFF Time Constants of Sodium Current and Charge Movement**

To further investigate possible complexities in gating of BTX-modified channels, we examined the effect of ON pulse duration \( t_{ON} \) on the decay of \( I_{Na} \) and the return of \( Q \) at a given OFF voltage after various pulses. It has been noted in normal node of Ranvier that the OFF time constants for both \( I_{Na} \) and \( Q \) change with \( t_{ON} \) (Nonner et al., 1978; Dubois and Schneider, 1982). \( \tau_{Na,OFF} \) increases continuously with increasing \( t_{ON} \) (Dubois and Schneider, 1982). In contrast, \( \tau_{Q,OFF} \) first increases with increasing, relatively short ON pulse durations but then decreases with increasing, larger ON pulse durations. The decrease in \( \tau_{Q,OFF} \) associated with long ON pulse durations was attributed to charge immobilization (Dubois and Schneider, 1982). This interpretation is consistent with the observations that the time course of the component of charge movement insensitive to immobilization is faster than that of the immobilizable charge movement (Nonner, 1980) and that the decrease in \( \tau_{Q,OFF} \) at long \( t_{ON} \) was suppressed by internal iodate (Dubois and Schneider, 1982), which removes Na inactivation (Stampfli, 1974) and charge immobilization (Dubois and Schneider, 1982).

Fig. 7A presents OFF time constants \( \tau_Q \) and \( \tau_{Na} \) recorded in a BTX-treated node of Ranvier at \(-120 \text{ mV} \), plotted as a function of the preceding ON pulse duration \( t_{ON} \) at \(-50 \text{ mV} \). Both \( \tau_{Q,OFF} \) and \( \tau_{Na,OFF} \) increased with \( t_{ON} \) and tended to reach a steady state value after 4–5-ms ON pulse durations. This observation establishes that the OFF \( \tau_Q/\tau_{Na} \) values in Fig. 6 and the \( \tau_{Q,OFF} \) and \( \tau_{Na,OFF} \) values in Fig. 5 correspond to the steady state situation established before pulse OFF. Fig. 7A shows that \( \tau_{Q,OFF} \) was larger than \( \tau_{Na,OFF} \) for short ON pulse durations but was almost equal to \( \tau_{Na,OFF} \) for long ON pulse durations. Note that the OFF voltage was \(-120 \text{ mV} \), where \( \tau_Q/\tau_{Na} \) was near unity for 4–5-ms ON pulses (Fig. 6). Since charge immobilization is removed by BTX, the results in Fig. 7A are consistent with the view that the decrease in \( \tau_{Q,OFF} \) for long \( t_{ON} \) observed previously in normal fibers is related to charge immobilization. A change of OFF time constants of \( I_{Na} \) and \( Q \) with increasing ON pulse duration is clearly inconsistent with a two-state model for gating of BTX-modified channels.

The change in OFF time constants \( \tau_Q \) and \( \tau_{Na} \) with \( t_{ON} \) in Fig. 7A follows approximately the time courses of Na activation and ON charge movement (Fig. 7B). This result on BTX-treated fibers is similar to the observation made on normal fibers of a relationship between the amount of charge movement and \( \tau_{OFF} \) (Dubois and Schneider, 1982). However, in BTX, some complications appear when comparing the build-up of \( Q_{ON} \) and \( Q_{OFF} \) as a function of increasing \( t_{ON} \). The build-up of \( Q_{OFF} \) (filled circles in Fig. 7B) is slower than the time course of \( Q_{ON} \) (dashed line in Fig. 7B), and the change in \( \tau_{Q,OFF} \) seems to be better correlated with \( Q_{OFF} \) than with \( Q_{ON} \). After long \( t_{ON} \), \( Q_{OFF} \) slightly exceeded \( Q_{ON} \).
FIGURE 7. Effect of ON pulse duration on OFF kinetics of sodium current and charge movement in a BTX-treated fiber. (A) OFF time constants for sodium current (open circles) and charge movement (filled circles) at −120 mV as a function of the duration of the ON prepulse at −50 mV. (B) Relative $I_{Na,OFF}$ (open circles) and $Q_{OFF}$ (filled circles) at −120 mV as a function of the duration of the ON prepulse at −50 mV. Curves were drawn by eye. The interrupted curve gives $Q_{ON}$ relative to the final value of $Q_{ON}$ measured during a long ON pulse. Fiber 14-6-82.

FIGURE 8. Relative OFF charge movement time constant as a function of the relative quantity of charge moved in BTX-treated fibers. $\tau_{Q,OFF}$ and $Q_{OFF}$ were measured in four fibers (different symbols) at −120 mV after pulses of various durations to either −50 or −60 mV. The straight line, described by the equation $\tau_{Q,OFF}/\tau_{Q,OFF,max} = 0.18 + 0.78 (Q_{OFF}/Q_{OFF,max})$, was obtained from a linear regression to the points ($r^2 = 0.89$). Fibers 11-6-82A (●), 14-6-82 (▽), 15-6-82 (□), and 7-7-82 (▲).
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(not illustrated in Fig. 7B since each curve is normalized; see also Dubois et al., 1983). These observations may suggest the existence of a slow component of $Q_{ON}$ that was subtracted with the $Q_{ON}$ baseline and thus is not present in Fig. 7B.

The relationship between $\tau_{OFF}$ and $Q_{OFF}$ is further explored in Fig. 8, which presents $\tau_{Q,OFF}/\tau_{Q,OFF_{max}}$ as a function of $Q_{OFF}/Q_{OFF_{max}}$ for the experiment of Fig. 7 and for three other experiments in which $\tau_{Q,OFF}$ was measured at $-120$ mV after ON pulses of varying durations at $-60$ or $-50$ mV. $\tau_{Q,OFF}$ increased with $Q_{OFF}$ but, in contrast with normal fibers for which the increase in $\tau_{Q,OFF}$ was most marked at $Q_{ON}$ near $Q_{ON_{max}}$ (Dubois and Schneider, 1982), the increase in $\tau_{Q,OFF}$ in Fig. 8 was proportional to $Q_{OFF}$ over the entire range of $Q_{OFF}/Q_{OFF_{max}}$ studied. The difference between normal and BTX-treated fibers might be due to the fact that, in normal fibers, only the last part of $Q_{ON}$ opens channels (Armstrong and Gilly, 1979), whereas in BTX-treated fibers, all parts of $Q_{ON}$ open channels because of effective charge aggregation. In both normal and BTX-modified channels $\tau_{Q,OFF}$ would increase with an increased number of open channels. In any case, the results in Fig. 8 clearly show that the increase in $\tau_{Q,OFF}$ is directly correlated with the amount of charge moved during the preceding ON pulse. This result is again inconsistent with a simple two-state, single-transition model for gating of BTX-modified sodium channels.

DISCUSSION

A major finding in this report is that the kinetic properties of BTX-modified Na channels are qualitatively similar to those of normal Na channels except for the absence of Na inactivation and its directly correlated effects on gating charges. The assumption of a BTX-induced aggregation of the gating particles associated with each channel (Khodorov and Revenko, 1979; Dubois et al., 1983) is consistent with the observed simple exponential time course of Na activation. However, the existence of a delay in the Na activation and the absence of delay in $Q_{ON}$ indicate that the gating mechanism of Na channels modified by BTX cannot be described by a two-state model but requires at least a three-state model (closed-closed-open), as recently proposed by Huang et al. (1984) for BTX-modified Na channels in neuroblastoma cells.

The observation that $\tau_{Na,OFF}$ and $\tau_{Q,OFF}$ both increase with the preceding ON pulse duration in proportion to the amount of charge moved during the preceding ON pulse (Fig. 8) cannot be explained by sequential models having only a single open state. This effect might result from the interaction of different types of gating particles controlling the opening of each channel (Khodorov, 1979, 1981; Keynes, 1983), from a channel-channel interaction (Neumcke and Stämpfli, 1983), or from the interaction of gating particles with other membrane or axoplasmic constituents (Conti et al., 1984). Time-dependent interactions between gating particles in the conducting configuration and other constituents might induce conformational changes of open channels and lead to the appearance of several open states (Mozhayeva et al., 1981). Moreover, such interactions can explain the change in $\tau_{OFF}$ for sodium current and charge movement with the preceding ON pulse duration if one assumes, first, that the gating particles close more slowly when they are interacting than when they are not, and, second, that the interaction cannot occur when the gating particles are in a closed state.
With increased ON pulse duration, there would be an increase in the proportion of gating particles involved in such interactions and a consequent increase in the probability of closing via a slower transition. This explanation involves the existence of two interconvertible forms of the channel, as recently suggested for potassium (Conti et al., 1984) and sodium (Meves et al., 1984) channels. Indications for multiple states of the gating machinery have also been recently observed in single BTX-modified Na channels reincorporated into lipid bilayers (Weiss et al., 1984). The finding of drugs or experimental conditions that abolish or alter the change in \( \tau_{\text{OFF}} \) with the preceding ON pulse duration would aid in establishing this hypothesis.

A remaining unexplained observation is that \( \tau_Q \) is larger than \( \tau_{\text{Na}} \) (Figs. 5 and 6). This finding might be explained if one considers that, in our experiments, the charge movement was recorded in the presence of 1 \( \mu \)M TTX, whereas only 5 nM TTX was present when \( I_{\text{Na}} \) was recorded. Following this consideration, one can explain the change in \( \tau_Q/\tau_{\text{Na}} \) with voltage presented in Fig. 6 if one assumes that the effective valence of gating particles is decreased by TTX. In this respect, the curve in Fig. 6 was fitted by Eq. 3, which was derived from Eq. 2, but we assumed that the steepness factor \( k \) was smaller in the absence \( (k_{\text{Na}}) \) than in the presence of TTX \( (k_Q) \).

\[
\frac{\tau_Q}{\tau_{\text{Na}}} = \frac{\exp \left( \frac{V - V}{k_{\text{Na}}} \right) + \exp \left( \frac{V - \bar{V}}{k_{\text{Na}}} \right) \eta}{\exp \left( \frac{V - V}{k_Q} \right) + \exp \left( \frac{V - \bar{V}}{k_Q} \right) \eta},
\]

(3)

The hypothesis of an interaction between gating particles and TTX was previously proposed by Cahalan and Almers (1979), who observed that the lidocaine derivative QX 314 had differential effects on the gating current recorded with and without TTX on the squid axon. The confirmation of this hypothesis on the node of Ranvier would need a comparison of the kinetics of charge movement recorded with and without TTX. However, because of the existence in the node of Ranvier of two components of Na current with different reversal potentials (Benoit et al., 1985) and the impossibility of removing all permeant ions from the axoplasm, it is impossible to record a pure charge movement without TTX. The hypothesis that \( k_Q \) is larger than \( k_{\text{Na}} \) is apparently in contradiction to our previous results (Dubois et al., 1983), where \( k_Q \) and \( k_{\text{Na}} \) were determined from steady state \( Q \) and \( g_{\text{Na}} \)-voltage curves. However, it must be noted that, in our 1983 paper, \( k_Q \) was determined in only five fibers and the values exhibited considerable scatter.

We thank Drs. J. Daly and B. Witkop for the supply of BTX used for these experiments, Dr. E. Salmeron for help with computer interfacing and programming, and Mrs. P. Richer for assistance in preparing the manuscript.

Dr. Schneider gratefully acknowledges support from Fondation Simone et Cino del Duca and Institut National de la Santé et de la Recherche Médicale (INSERM). This work was supported in part by grants from the Ministère de l’Industrie et de la Recherche (83C0529 and 83C0912) and INSERM (CRE 836010).

Original version received 28 December 1984 and accepted version received 5 April 1985.
REFERENCES

Adrian, R. H. 1978. Charge movement in the membrane of striated muscle. *Annu. Rev. Physiol. Bioeng.* 7:85–112.

Armstrong, C. M., and F. Bezanilla. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* 63:553–552.

Armstrong, C. M., and W. F. Gilly. 1979. Fast and slow steps in the activation of sodium channels. *J. Gen. Physiol.* 74:691–711.

Benoit, E., A. Corbier, and J. M. Dubois. 1985. Evidence for two transient sodium currents in the frog node of Ranvier. *J. Physiol. (Lond.)* 361:339–360.

Cahalan, M. D., and W. Almers. 1979. Interaction between quaternary lidocaine, the sodium channel gates and tetrodotoxin. *Biophys. J.* 27:39–56.

Cole, K. S., and J. W. Moore. 1960. Potassium ion current in the squid giant axons: dynamic characteristic. *Biophys. J.* 1:161–202.

Conti, F., B. Hille, and W. Nonner. 1984. Nonstationary fluctuations of the potassium conductance at the node of Ranvier of the frog. *J. Physiol. (Lond.)* 355:199–230.

Dubois, J. M., and A. Coulombe. 1984. Current-dependent inactivation induced by sodium depletion in normal and batrachotoxin-treated frog node of Ranvier. *J. Gen. Physiol.* 84:25–48.

Dubois, J. M., and B. I. Khodorov. 1982. Batrachotoxin protects sodium channels from the blocking action of oenanthotoxin. *Pflügers Arch. Eur. J. Physiol.* 395:55–58.

Dubois, J. M., and M. F. Schneider. 1982. Kinetics of intramembrane charge movement and sodium current in frog node of Ranvier. *J. Gen. Physiol.* 79:571–602.

Dubois, J. M., M. F. Schneider, and B. I. Khodorov. 1983. Voltage dependence of intramembrane charge movement and conductance activation of batrachotoxin-modified sodium channels in frog of Ranvier. *J. Gen. Physiol.* 81:829–844.

Horowicz, P., and M. F. Schneider. 1981. Membrane charge moved at contraction thresholds in skeletal muscle fibres. *J. Physiol. (Lond.)* 314:595–633.

Huang, L. Y. M., N. Moran, and G. Ehrenstein. 1982. Batrachotoxin modifies the gating kinetics of sodium channels in internally perfused neuroblastoma cells. *Proc. Natl. Acad. Sci. USA.* 79:2082–2085.

Huang, L. Y. M., N. Moran, and G. Ehrenstein. 1984. Gating kinetics of batrachotoxin-modified sodium channels in neuroblastoma cells determined from single-channel measurements. *Biophys. J.* 45:313–322.

Keynes, R. D. 1983. Voltage-gated ion channels in the nerve membrane. *Proc. R. Soc. Lond. B Biol. Sci.* 220:1–30.

Keynes, R. D., and E. Rojas. 1976. The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid axon. *J. Physiol. (Lond.)* 255:157–189.

Khodorov, B. I. 1979. Inactivation of the sodium gating current. *Neuroscience.* 4:865–876.

Khodorov, B. I. 1981. Sodium inactivation and drug-induced immobilization of the gating charge in nerve membrane. *Prog. Biophys. Mol. Biol.* 37:49–89.

Khodorov, B. I., E. M. Peganov, S. V. Revenko, and L. D. Shishkova. 1975. Sodium currents in voltage clamped nerve fibre of frog under the combined action of batrachotoxin and procaine. *Brain Res.* 44:541–546.

Khodorov, B. I., and S. V. Revenko. 1979. Further analysis of the mechanisms of action of batrachotoxin on the membrane of myelinated nerve. *Neuroscience.* 4:1315–1330.

Meves, H., N. Rubly, and D. D. Watt. 1984. Voltage-dependent effect of a scorpion toxin on sodium current inactivation. *Pflügers Arch. Eur. J. Physiol.* 402:24–33.
Mozhayeva, G. N., A. P. Naumov, and B. I. Khodorov. 1981. Changes in properties of Na channels in the nodal membrane treated with batrachotoxin (BTX). USSR-Sweden III Symposium Physico-Chemical Biology, Abstracts. 221–222.
Mozhayeva, G. N., A. P. Naumov, and B. I. Khodorov. 1983. Ionic currents through batrachotoxin-modified sodium channels in nodal membrane at high positive and negative potentials. Neurophysiologia. 15:495–503. (In Russian.)
Neumcke, B., W. Nonner, and Stämpfli. 1976. Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve. Pflügers Arch. Eur. J. Physiol. 363:193–203.
Neumcke, B., and R. Stämpfli. 1983. Alteration of the conductance of Na+ channels in the nodal membrane of frog nerve by holding potential and tetrodotoxin. Biochim. Biophys. Acta. 727:177–184.
Nonner, W. 1980. Relations between the inactivation of Na channels and the immobilization of gating charge in frog myelinated nerve. J. Physiol. (Lond.). 299:573–603.
Nonner, W., E. Rojas, and R. Stämpfli. 1978. Asymmetrical displacement current in the membrane of frog myelinated nerve: early time courses and effects of membrane potential. Pflügers Arch. Eur. J. Physiol. 375:75–85.
Schauf, C. L. 1983. Insensitivity of activation delays in potassium and sodium channels to heavy water in Myxicola giant axons. J. Physiol. (Lond.). 337:173–182.
Stämpfli, R. 1974. Intraxonal iodate inhibits sodium inactivation. Experiments. 30:505–508.
Taylor, R. E., and F. Bezanilla. 1983. Sodium and gating current time shifts resulting from changes in initial conditions. J. Gen. Physiol. 81:773–784.
Weiss, L. B., W. N. Green, and O. S. Andersen. 1984. Single-channel studies on the gating of batrachotoxin (BTX)-modified sodium channels in lipid bilayers. Biophys. J. 45:67a. (Abstr.)