EFFECTS OF BENZOCAINE ON THE KINETICS OF NORMAL AND BATRACHOTOXIN-MODIFIED Na CHANNELS IN FROG NODE OF RANVIER

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ABSTRACT The effects of benzocaine (0.5–1 mM) on normal Na currents, and on Na current and gating charge movement (Q) of batrachotoxin (BTX)-modified Na channels were analyzed in voltage-clamped frog node of Ranvier. Without BTX treatment the decay of Na current during pulses to between −40 and 0 mV could be decomposed into two exponential components both in the absence and in the presence of benzocaine. Benzocaine did not significantly alter the inactivation time constant of either component, but reduced both their amplitudes. The amplitude of the slow inactivating component was more decreased by benzocaine than the amplitude of the fast one, leading to an apparently faster decline of the overall Na current. After removal of Na inactivation and charge movement immobilization by BTX, benzocaine decreased the amplitude of I_{Na} with no change in time course. I_{Na}, Q_{ON}, and Q_{OFF} were all reduced by the same factor. The results suggest that the rate of reaction of benzocaine with its receptor is slow compared to the rates of channel activation and inactivation. The differential effects of benzocaine on the two components of Na current inactivation in normal channels can be explained assuming two types of channel with different rates of inactivation and different affinities for the drug.

INTRODUCTION

The block of Na channels by local anesthetics has been interpreted as resulting from a binding reaction between the drug molecule and a receptor located within the membrane in the vicinity of the Na channel (see Hille, 1977). It is assumed that the binding of the local anesthetic molecule to its receptor is modulated by the voltage-dependent gating state of the channel according to Scheme I (Strichartz, 1973; Arhem Frankenhaeuser, 1974; Cahalan and Almers, 1979; Schmidtmayer and Ulbricht, 1980; Khodorov, 1981; Neumcke et al., 1981).

$$\begin{array}{ccccc} R & \rightleftharpoons & O & \rightleftharpoons & I \\ \downuparrows & & \downuparrows & & \downuparrows \\ R^* & \rightleftharpoons & O^* & \rightleftharpoons & I^* \end{array}$$

SCHEME I

The *upper* states in Scheme I correspond to a simplified representation of the normal states of the channel: resting (R), open (O) and inactivated (I). Binding of a drug molecule to the channel leads to the modified ("blocked") states of the channel represented on the *lower* line. Although the same transitions are indicated for drug-occupied and normal channels, drug-occupied channels may not be capable of undergoing some or all the transitions indicated in the lower part of Scheme I. In the presence of local anesthetic, a certain proportion of channels is in state R^* at a hyperpolarized holding potential. If the $R^* \rightarrow O^*$ transition were not possible, the channels in state R^* could not pass directly to the open but drug-occupied and nonconducting state O^*, and thus could not directly produce a gating charge movement upon depolarization. The rest of the channels could open directly ($R \rightarrow O$) and inactivate ($O \rightarrow I$) or be blocked along the transitions $O \rightarrow O^*$ and $I \rightarrow I^*$. The transition $I \rightarrow I^*$ would increase the apparent steady state inactivation at voltages where inactivation was incomplete, but could not alter the inactivation time course for pulse voltages where inactivation was already complete in the absence of drug. In contrast, the transition $O \rightarrow O^*$ would induce an additional block of Na channels during a maintained depolarization leading to an apparently faster inactivation of Na current (Neumcke et al., 1981). Many of the observed effects of local anesthetics on sodium currents and on sodium channel gating currents can be reasonably well interpreted on the basis of Scheme I. However, some points remain obscure and are not explained by this model (see Neumcke et al., 1981).

One reason for the limitations of Scheme I may be that it assumes only one open and one inactivated state in the absence of drug. Recently, it has been shown that the inactivation of Na current follows a double exponential

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time course in frog (Chiu, 1977; Neumcke et al., 1980; Nonner, 1980; Ochs et al., 1981) and rat myelinated fibers (Neumcke and Stämpfli, 1982). Covarience analysis of nonstationary sodium current fluctuations has indicated the presence of two kinetically distinct open states of Na channels in frog node of Ranvier (Sigworth, 1981). Finally, there are indications that the fast and slow components of the inactivatable sodium channel may correspond to different forms of the sodium channel (Benoit et al., 1985). After these observations, it seemed interesting to reinvestigate the effects of local anesthetics on Na current, taking into consideration the double exponential decay of I_{Na} and the possibility of more than one open state.

The local anesthetic benzocaine was selected for use in the present experiments. The molecule is neutral at physiological pH and thus its binding cannot directly depend on membrane potential. Any voltage dependence of drug action must arise indirectly from voltage dependent charges in the state of the benzocaine receptor that alter its affinity for the drug. Benzocaine exhibits no after effects during repetitive pulsing as used for signal averaging. Finally, some experiments were undertaken after elimination of Na inactivation by batrachotoxin (Khodorov et al., 1975; Khodorov and Revenko, 1979) and benzocaine is the only local anesthetic known to block both normal and BTX-modified Na channels almost equally effectively (Khodorov, 1978).

METHODS

Experiments were carried out on isolated voltage-clamped myelinated nerve fibers from the frog *Rana esculenta*. All methods were as described previously (Dubois and Schneider, 1982; Dubois et al., 1983). To minimize series resistance artifacts and changes in Na driving-force during Na influx, all I_{Na} results were obtained after I_{Na} had been reduced to ~20% of its control amplitude by 5×10^{-9} M tetrodotoxin (TTX). Currents through K channels were blocked by replacement of the end pool solution with 120 mM CsF and addition of 10 mM tetraethylammonium to the external Ringer's solution. When monitoring charge movement, Na current was completely blocked by 1 μM TTX. To avoid any possible inward ionic current carried by K^+ and Ca^{2+}, most of the experiments were carried out in external solutions containing $MgCl_2$ (4 mM) and CsCl (2.5 mM) in place of $CaCl_2$ (1.8 mM) and KCl (2.5 mM), respectively (Dubois et at., 1983). No significant differences were observed between the results obtained in external solutions containing Mg^{2+} and Cs^+ and those obtained in external solutions containing Ca^{2+} and K^+. All experiments on both I_{Na} and charge movement were carried out using a holding potential of -120 mV. The temperature was 11–15°C.

For determining either I_{Na} or charge movement, linear components of capacitative and leakage currents were approximately removed with an analog compensation circuit and then completely eliminated using either the "$-P/2$ routine" (Dubois and Schneider, 1982) or equal numbers of same amplitude but opposite polarity pulses. The current records used for analysis were obtained by averaging eight to 32 applications of a given depolarizing pulse and the appropriate number of corresponding hyperpolarizing pulses (Dubois and Schneider, 1982). In most cases the averages were stored on analog tape for subsequent digitization and analysis by digital computer (MINC; Digital Equipment Corp., Maynard, MA). In a few cases average current records were photographed from an oscilloscope and subsequently analyzed from projected images.

Na current or charge movement was generally recorded in control solution, then in the presence of 0.5 or 1 mM benzocaine, and again after wash out with the benzocaine-free control solution. The magnitude and kinetics of Na current and charge movement were determined two to six times, both in control solution and in the presence of benzocaine and the values obtained were averaged. For kinetic analyses of Na current inactivation, non-inactivating components were assumed to be constant during each voltage pulse.

The decline of Na current during a step depolarization assumably follows the double exponential time course

$$I = I_{Of} \exp(-t/\tau_f) + I_{Os} \exp(-t/\tau_s) + I_\infty, \qquad (1)$$

where I_{Of} and I_{Os} are, respectively, the extrapolated initial amplitudes of the fast and slow inactivating components, τ_f and τ_s are their respective time constants, t is time after the start of the voltage step and I_∞ is the relatively small non-inactivating current assumed to be constant throughout the decay of I_{Na}. The slow exponential plus the constant in Eq. 1 were fit by computer to the latter part of each decline of I_{Na}. These two components were then subtracted from the entire I_{Na} record and the fast component in Eq. 1 was fit to the remaining current during the early part of the decline I_{Na}. Both fits were carried out using nonlinear least-squares procedures to determine the optimum values of either I_{Os}, τ_s, and I_∞ or optimum values of I_{Of} and τ_f. For a given pulse, identical analysis intervals were used for the control records and the records in benzocaine.

RESULTS

Effects of Benzocaine on the Kinetics of Na Current Inactivation

Figs. 1 *A* and *B* present Na currents recorded during depolarization to -20 mV in the absence (*A*) and in the presence (*B*) of benzocaine. The circles in Fig. 1 *A* represent I_{Na} in the presence of benzocaine, scaled by the ratio of peak inward currents without and with benzocaine. Comparison of the record and circles in Fig. 1 *A* indicates that benzocaine did not appreciably affect the time course of turn on of I_{Na}. In contrast, the decay of I_{Na} was faster in the presence of benzocaine. This result is in close agreement with that reported by Neumcke et al. (1981). The sodium current inactivated completely during the pulse in Figs. 1 *A* and *B*. Thus, if these records are interpreted according to Scheme I the speeding of I_{Na} inactivation in benzocaine would reflect the block of Na channels along the transition $O \rightarrow O^*$. If this were the case, the I_{Na} inactivation time constants should be decreased. We therefore examined the time course of decline of I_{Na} both graphically and by computer curve fitting.

A graphical semilogarithmic analysis of the I_{Na} inactivation in Fig. 1 revealed that the decay of Na current could be decomposed into two exponential phases both in the absence and in the presence of benzocaine (Figs. 1 *C* and *D*). Moreover, benzocaine produced no significant change in the time constant of either of the two phases of I_{Na} inactivation. This observation was confirmed by computer analysis of records from several fibers in which benzocaine speeded the overall decay of I_{Na} during pulses to -20, 0, or to $+20$ mV (Table I). The last three columns of Table I show that neither τ_f nor τ_s was significantly altered by benzocaine, whereas the half time for I_{Na} decline was

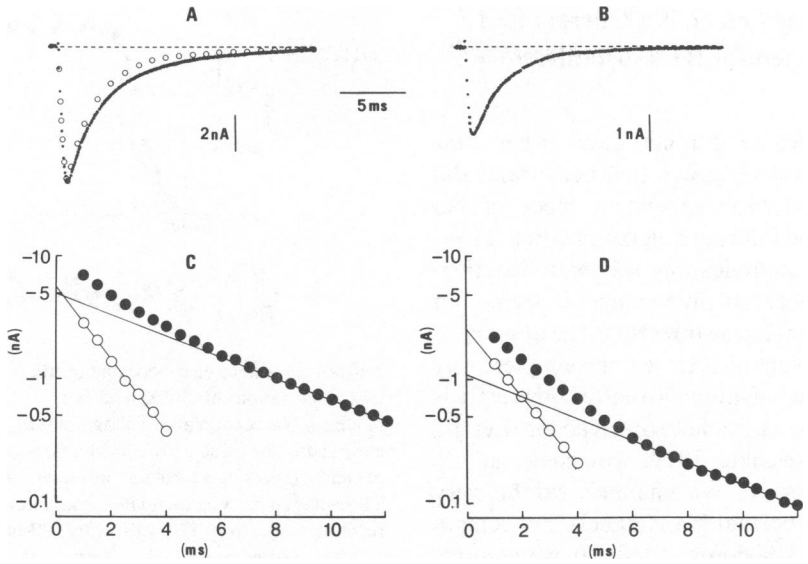

FIGURE 1 Na currents during depolarizations to −20 mV in control solution (*A* and filled symbols in *C*) and in the presence of 1 mM benzocaine (*B* and filled symbols in *D*). Records were obtained from the average of eight depolarizing pulses in the "−P/2" routine. Note difference in vertical calibration in *A* and *B*. In *A*, the circles were obtained by multiplying the values in *B* by 3.02. In *C* and *D*, filled circles give the overall current and open circles give the current remaining after subtracting the slow exponential component (straight lines through filled circles). Temperature: 12°C. Fiber: 13–10–82.

reduced in these fibers. In contrast, the zero time intercepts of the semilog plots in Figs. 1 *C* and *D* show that benzocaine did alter the relative contributions of the fast and slow inactivating phases of I_{Na} to the total current. Table I shows that the relative amplitude F_s of the slow inactivating phase extrapolated to the start of the depolarizing pulse, $F_s = I_{Os}/(I_{Os} + I_{Of})$, was significantly decreased by benzocaine ($F_{s(BZ)}/F_{s(cont)}$ column in Table I). These findings indicate that the apparently faster inactivation of I_{Na} in the presence of benzocaine was not related to a decrease in either of the inactivation time constants, but was due to a relatively larger block of the slower inactivating Na current component than of the faster inactivating component. The initial amplitudes I_{Os} and I_{Of} of the slow and fast phases, found by extrapolation to the start of the pulse, were respectively reduced to 34% ± 4% and 57% ± 6% of their control values by benzocaine (Table I).

The preceding observations concerning the effects of benzocaine on the kinetics of I_{Na} in normal nodes of Ranvier can be explained on the basis of two populations of sodium channels having different rates of inactivation (Benoit et al., 1985). If benzocaine were to react relatively slowly with each type of channel it would not alter either rate constant for decline of I_{Na}. If benzocaine were also to have a higher affinity for the resting state of the more slowly inactivating channel than for the resting state of the more rapidly inactivating channel, benzocaine would speed the overall time course of decay of I_{Na} as observed.

TABLE I
EFFECT OF BENZOCAINE ON THE TWO COMPONENTS OF SODIUM CURRENT INACTIVATION

Fiber	V_{ON}	Control			Benzocaine/control					
		$F_s = \frac{I_{Os}}{I_{Of} + I_{Os}}$	τ_f	τ_s	$\frac{I_{Of(BZ)}}{I_{Of(cont)}}$	$\frac{I_{Os(BZ)}}{I_{Os(cont)}}$	$\frac{F_{s(BZ)}}{F_{s(cont)}}$	$\frac{\tau_{f(BZ)}}{\tau_{f(cont)}}$	$\frac{\tau_{s(BZ)}}{\tau_{s(cont)}}$	$\frac{t_{1/2(BZ)}}{t_{1/2(cont)}}$
	mv		*ms*	*ms*						
9-7-82	0	0.56	1.66	3.72	0.99	0.15	0.30	0.89	1.07	0.71
13-7-82	0	0.21	0.38	2.09	0.61	0.45	0.81	0.92	1.00	0.94
13-7-82A	−20	0.37	1.53	5.30	0.36	0.24	0.76	1.13	0.93	0.55
	0	0.26	0.60	1.73	0.57	0.43	0.81	1.00	0.99	0.94
	+20	0.15	0.40	1.25	0.80	0.55	0.73	0.88	0.82	0.95
15-7-82	0	0.38	0.61	2.37	0.44	0.28	0.74	1.16	0.94	0.84
	+20	0.24	0.50	1.45	0.57	0.35	0.67	0.86	0.99	0.93
6-10-82	0	0.19	1.04	4.93	0.38	0.29	0.79	1.01	1.02	0.97
13-10-82	−20	0.42	1.55	5.55	0.44	0.25	0.68	1.06	0.88	0.81
	0	0.09	1.05	4.83	0.52	0.42	0.82	0.89	0.91	0.92
Mean ± SEM					0.57 ± 0.06	0.34 ± 0.04	0.71 ± 0.05	0.98 ± 0.03	0.96 ± 0.02	0.86 ± 0.04

Effect of Benzocaine on Na Current and Charge Movement of BTX-Modified Channels

The finding that benzocaine did not alter either time constant of I_{Na} inactivation suggested that benzocaine did not induce an additional time dependent block of Na channels when they opened during a depolarization. However, because of possible complications related to inactivation and its double exponential time course, it seemed of interest to confirm this conclusion directly in the absence of Na inactivation. In the node of Ranvier, the simplest way to suppress Na current inactivation virtually completely is to modify the channels by batrachotoxin (Khodorov et al., 1975; Khodorov and Revenko, 1979; Mozhaeva et al., 1981). After BTX treatment, Na channels exhibit very different properties than normal Na channels, but remain sensitive to benzocaine (Khodorov, 1978). If we assume, for simplicity, that BTX-modified Na channels present only two configurations, resting and open (Mozhaeva et al., 1982; Dubois et al., 1983), blockade of channels in the open state along the transition $O \rightarrow O^*$ might induce an apparent inactivation of the current and might modify the steady-state conductance-voltage relationship. The presence or absence of such effects should provide information regarding the reactions of benzocaine with the resting and open states of BTX-modified channels.

Fig. 2 presents traces of Na current recorded at −80 and −60 mV after BTX treatment in the absence (*left*) and in the presence (*right*) of 1 mM benzocaine. The circles on the *left* represent I_{Na} in the presence of benzocaine scaled by the ratio of steady state currents without and with benzocaine. Benzocaine neither induced an apparent inactivation nor changed the activation kinetics of BTX-modified Na channels.

The same scaling factor was used at the two different voltages in Fig. 2, indicating that the block of BTX-modified Na channels by benzocaine was independent of pulse voltage. This is further explored in Fig. 3, which

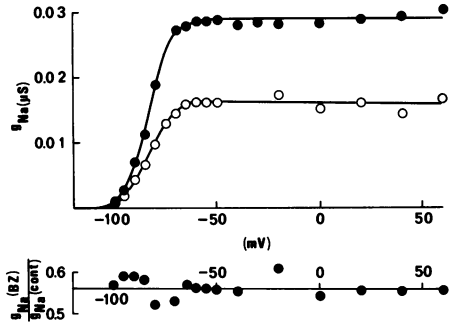

FIGURE 3 Effect of benzocaine on the voltage dependence of conductance activation of BTX-modified sodium channels. *Upper*: BTX-modified Na conductance-voltage curves in control solution (filled circles) and in the presence of 1 mM benzocaine (open circles). *Lower*: ratio of conductances in control and in benzocaine. Curves were drawn by eye. The conductance was calculated from initial tail current amplitudes upon repolarization to −120 mV after 20-ms depolarizations to various voltages. Temperature 11°C. Fiber: 8–10–82.

presents the steady state conductance of BTX-modified Na channels in the absence and in the presence of benzocaine and the ratio of these conductances as a function of voltage. Conductances were calculated from quasi-instantaneous tail currents recorded upon repolarization after 20-ms depolarizing pulses of various amplitudes (Dubois et al., 1983). It appears that the block of BTX-modified Na channels by benzocaine is independent of voltage.

After BTX-treatment, the charge immobilization that is normally induced by prolonged depolarization is absent (Dubois and Khodorov, 1982; Dubois et al., 1983). Under these conditions, the question arises whether and to what extent benzocaine blocks the ON and OFF charge movements. Fig. 4 presents I_{Na} and ON and OFF charge movements recorded from the same BTX-treated node of Ranvier during and after pulses to −60 mV in the absence and in the presence of benzocaine. I_{Na} was reduced by benzocaine to 64% of its control value. Both ON and OFF charge movements were also almost equally reduced by benzocaine, and the reduction of each was essentially equal

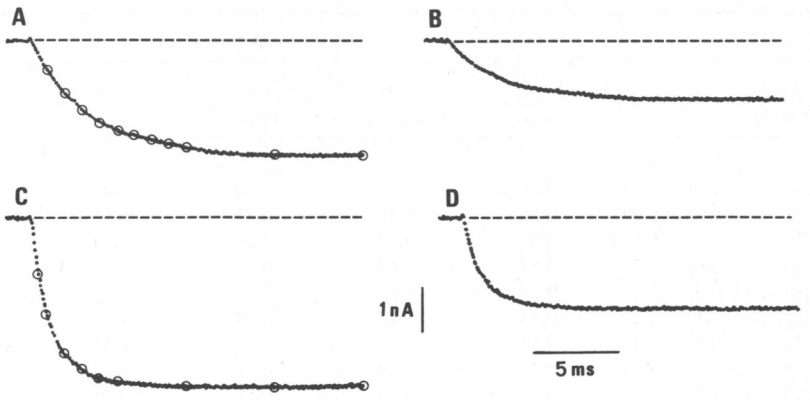

FIGURE 2 BTX-modified Na currents during depolarizations to −80 mV (*A* and *B*) and −60 mV (*C* and *D*) in control solution (*A* and *C*) and in the presence of 1 mM benzocaine. In *A* and *C*, the circles were obtained by multiplying the values of *B* and *D* by 1.82. Temperature: 11°C. Fiber: 8–10–82.

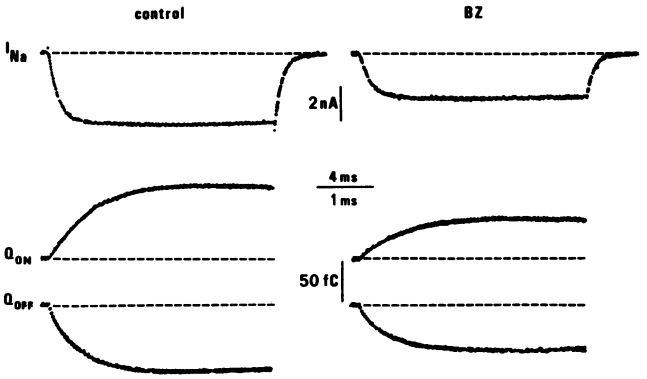

FIGURE 4 Na currents and ON and OFF charge movements recorded from a BTX-modified fiber during and after 16-ms pulses to −60 mV in control solutions (*left*) and in the presence of 1 mM benzocaine (*right*). *Upper* and *lower* horizontal scales correspond to I_{Na} and Q traces, respectively. Temperature: 13.5–14.5°C. Fiber: 12–7–82.

to the reduction of I_{Na}. In this typical experiment, four to six determinations of ON and OFF charge movements without and with benzocaine indicated that ON and OFF charge movements were respectively reduced by benzocaine to 64% ± 3% and 67% ± 1% of their control values.

According to Scheme I, the direct transition $R^* \rightarrow O^*$ from the resting benzocaine-occupied state R^* to the "open" but benzocaine-occupied, and thus nonconducting state O^*, would produce charge movement but no ionic current. In contrast, the transition $R \rightarrow O$ from the drug free resting to open states would give both charge movement and ionic current. Thus, our observation that ionic current and charge movement of BTX-treated fibers are equally suppressed by benzocaine indicates that if $R^* \rightarrow O^*$ occurs in BTX-treated fibers, the transition must be sufficiently slow so as to produce negligible charge movement on the time scale of Q in BTX-treated fibers. Similar arguments applied to the situation at repolarization indicate that $O^* \rightarrow R^*$ must be much slower than $O \rightarrow R$. Neumcke et al. (1981) have previously observed that peak I_{Na} and charge movement are also equally suppressed by benzocaine in non BTX-treated fibers, indicating that $R^* \rightarrow O^*$ is also much slower than $R \rightarrow O$ in the absence of BTX.

Kinetic Scheme for the Action of Benzocaine on BTX-modified Na Channels

The action of benzocaine on BTX-modified sodium channels can be interpreted on the basis of Scheme II, a simplified version of Scheme I that represents the interaction of benzocaine with BTX-modified channels

$$\begin{array}{ccc} R & \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} & O \\ k_R \downuparrows l_R & & k_O \downuparrows l_O \\ R^* & & O^* \end{array}$$

SCHEME II

Since BTX treatment eliminates both inactivation of sodium current and immobilization of charge movement, inactivated states have not been included. The single transition used in Scheme II to represent activation of I_{Na} after BTX-treatment is probably a good but not perfect approximation (Dubois and Schneider, 1985). In Scheme II benzocaine-occupied channels cannot pass directly between states R^* and O^* without going through the benzocaine-free states R and O, an extreme case of the conclusion that $R^* \rightarrow O^*$ and $O^* \rightarrow R^*$ must be much slower than $R \rightarrow O$ and $O \rightarrow R$ (above). It should be noted that the two first order rate constants k_R and k_O indicated in Scheme II for the transition from benzocaine-free to benzocaine-occupied states are actually each equal to the product of a true second order rate constant and the benzocaine concentration. However, since the benzocaine concentration was constant here, we simply employ the apparent equivalent first order rate constants throughout the present treatment.

Concentrations of benzocaine that produced appreciable depression of I_{Na} in BTX-treated fibers produced little or no change in the I_{Na} time course. Based on Scheme II, this indicates that the rate constants k_R, l_R, k_O, and l_O for benzocaine reaction with BTX-modified channels cannot be large compared to $\alpha + \beta$. If k_R, l_R, k_O, and l_O were sufficiently large that R was always in equilibrium with R^* and O was always in equilibrium with O^*, Scheme II would reduce to

$$R^\dagger \underset{f_O\beta}{\overset{f_R\alpha}{\rightleftharpoons}} O^\dagger,$$

SCHEME III

where $f_R = R/(R + R^*)$, $f_O = O/(O + O^*)$, $R^\dagger = R + R^*$ and $O^\dagger = O + O^*$. An analogous treatment of rapidly equilibrating transitions but in the absence of drugs was presented previously (Dubois and Schneider, 1982). In the present case, the fractions f_R and f_O of drug free resting and open channels would be unity in the absence of benzocaine. In the presence of benzocaine f_R and f_O would become less than unity, producing a slowing of I_{Na}. Such slowing of I_{Na} of BTX-treated fibers in the presence of benzocaine was clearly not observed experimentally.

The opposite extreme to Scheme III would be the case of very slow reaction of benzocaine with BTX-modified channels. If l_R and k_O were so small that there was negligible dissociation of benzocaine from R^* and negligible reaction of benzocaine with O during the pulse durations used here, then benzocaine would have produced an equal depression of both I_{Na} and Q, but no change in I_{Na} or Q kinetics. In this case the entire effect of benzocaine would be attributable to a resting block. This interpretation is consistent with all observed effects of benzocaine on BTX-modified channels.

Various cases intermediate between the two preceding extremes of very fast or very slow reaction of benzocaine with BTX-modified sodium channels might also be consis-

tent with our observations and therefore were examined by computer simulation of Scheme II. Fig. 5 presents one such simulation. The traces in panels *A–D* are simulated I_{Na} records for BTX-modified sodium channels, *A* and *C* in the absence of benzocaine, and *B* and *D* in the presence of benzocaine. For traces *A* and *B* the values of the channel opening and closing rate constants α and β were both set to 333 s^{-1}, simulating the midpoint of the conductance activation curve. For traces *C* and *D* the value of β was set to zero and α was set to 3,333 s^{-1}, simulating a voltage for maximal conductance activation. All simulations in the presence of benzocaine (*B*, *D*, and *F*) were calculated using $k_R = k_O = 67\ s^{-1}$, and $l_R = l_O = 100\ s^{-1}$. The circles in panels *A* and *C* of Fig. 5 give 1.72 times the simulated current for the same voltage in benzocaine (*B* and *D*, respectively). Clearly the scaled up records in benzocaine very closely match the benzocaine-free records for the corresponding voltage, in agreement with the experimental observations.

The long traces in Figs. 5 *E* and *F* are the simulated charge movements corresponding to the simulated I_{Na} in Figs. 5 *C* and *D*. The slow component of simulated charge movement in the presence of benzocaine reflects the time course of the slow (rate limiting) transition from R^* to R, which is followed by the more rapid transition $R \rightarrow O$ that actually moves the charge. If the traces in Fig. 5 *F* were continued in time, eventually all charge would be moved since all channels eventually pass to O and O^* at this simulated voltage. In practice such slow components would have been experimentally indistinguishable from the slow linear components present in all raw charge movement records because of integration of constant, but slightly nonlinear ionic currents (Dubois and Schneider, 1982, Fig. 1). Such slow linear components were routinely removed from each total charge record by subtracting a straight sloping baseline (Dubois and Schneider, 1982). A similar procedure applied to the long charge trace in Fig. 5 *F* gave the shorter trace. The circles in Fig. 5 *E* present the simulated benzocaine charge movement trace after subtraction of the linear component and scaling by 1.72, the same factor as used to scale up the I_{Na} records in benzocaine (Figs. 5 *A* and *C*). Again in agreement with experimental observations, the simulated scaled charge movements in benzocaine agree closely with those before benzocaine when using the same scale factor as used to scale the I_{Na} records. The simulations in Fig. 5 thus show that this set of parameter values is consistent with the observed effects of benzocaine on sodium current and charge movement in BTX-modified sodium channels.

Starting from the set of parameter values used in Fig. 5, we altered various rate constants for the benzocaine reaction to explore the range of parameter values that might be consistent with the experimental observations. For simplicity, we will consider only simulations for the control condition of half activation ($\alpha = \beta = 333\ s^{-1}$) and consider only threefold changes in individual rate constants or various combinations of rate constants. Rather than showing records of all these simulations, we simply will describe the results. The first variation in the Fig. 5 parameter values was a threefold change of all forward and reverse rate constants, which maintained both the equality of the rate constants for the forward reaction of benzocaine with R and O and the equality of the rate constants for dissociation of R^* and O^* used in Fig. 5. A threefold increase of all benzocaine reaction rate constants resulted in a simulated benzocaine record that was clearly slower

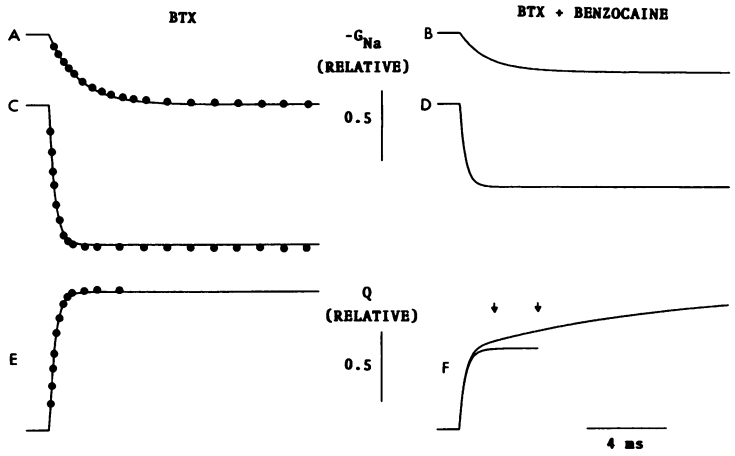

FIGURE 5 Computer simulations of the effects of benzocaine on the time course of conductance activation and gating charge movement of BTX-modified Na channels. Conductance activation, proportional to the probability of channels being in state *O*, and ON charge movement, proportional to the probability of being in states *O* or *O**, were calculated on the basis of Scheme II. The continuous records on the left give conductance activation (*A* and *C*) and charge movement (*E*) in the absence of benzocaine and the full-length traces on the right give the corresponding records in the presence of benzocaine. For *A* and *B*, $\alpha = \beta = 333\ s^{-1}$, whereas for *C–E*, $\alpha = 3{,}333\ s^{-1}$ and $\beta = 0$. In benzocaine, $k_R = k_O = 66.7\ s^{-1}$ and $l_R = l_O = 100\ s^{-1}$ in all cases. A straight line was fit to the charge movement record in benzocaine (*F*) during the interval marked by arrows (1.8–4 ms after the pulse) and subtracted to give the shorter charge movement record. The circles on the *left* give the corresponding records in benzocaine scaled by 1.72, using the linearly corrected (short) record for charge movement.

than control, whereas a threefold decrease in all rate constants gave a simulated record that had the same time course as control. Thus the Fig. 5 values for the rates of the benzocaine reaction relative to those for channel opening are almost maximal for benzocaine to produce no change in I_{Na} time course.

The next type of variation from the Fig. 5 parameters was a change in pairs of rate constants, either k_R and l_R or k_O and l_O. This maintained the equal affinity of benzocaine for R and O, but allowed for different rates of equilibration of benzocaine with R and O. For faster benzocaine reaction with O than R, a threefold decrease in k_R and l_R gave an I_{Na} record in benzocaine that was slightly faster than control, whereas a threefold increase in k_O and l_O gave a much slower I_{Na} record having a definite slow second phase. For faster benzocaine reactions with R than O, a threefold decrease in k_O and l_O gave a benzocaine I_{Na} record hardly different from control whereas a threefold increase in k_R and l_R produced a record slightly slower than control.

The final class of variations of the Fig. 5 parameter values involved changes in the value of only a single rate constant. This altered the relative affinities of benzocaine for R and O as well as its speed of equilibration. For the case of threefold higher affinity for O than R, a threefold increase in k_O gave a simulated I_{Na} record in benzocaine that was considerably faster than control, and that had a slow second phase of I_{Na} decay. The simulated record for a threefold decrease of k_R was slightly faster than control, whereas the records for a threefold increase in l_R or decrease in l_O had essentially the same time course as control. For the case of a threefold lower affinity for O than R, simulations for appropriate threefold changes in any single rate constant gave records that were all appreciably slower than control and that all had slow second phases of increasing I_{Na} activation.

The general conclusion from all these simulations is that benzocaine reaction rate constants up to about fourfold lower than the channel transition rate constants at the half activation voltage are compatible with the same time course of I_{Na} in the presence or absence of benzocaine, providing the benzocaine affinities and rate constants are the same for resting and open channels. Various threefold changes in these benzocaine rate constants give simulated records that still have time courses essentially the same as control, whereas various other threefold changes produce time courses clearly different from control.

DISCUSSION

Kinetic schemes for the action of benzocaine on normal, non-BTX-treated sodium channels must include the transitions from the resting to open and open to inactivated states and the interaction of benzocaine with each of these states of the channel (Scheme I). It has been observed previously that inactivation occurs at more negative voltages in the presence of benzocaine than in control conditions (Hille, 1977). This phenomenon indicates that inactivated Na channels have a higher affinity for benzocaine than resting or open channels (Hille, 1977).

After decomposition of the decay of Na current during a depolarizing pulse into two exponential phases, we observed that the slow phase of Na current inactivation was more suppressed by benzocaine than the fast one when resting inactivation was removed by sufficiently negative holding potentials. Under such conditions, benzocaine produced an acceleration of the overall decay of I_{Na} with no change in the time constants of either phase. Preliminary observations not presented here indicated that when the resting inactivation was significant, benzocaine still produced no change in either time constant of inactivation. However, in partially inactivated fibers the slow phase of Na current inactivation was sometimes relatively less suppressed by benzocaine than the fast one. Consequently, the apparent overall decay of Na current could even be slowed by benzocaine when the fibers were partially inactivated.

A major question that immediately arises is how to explain the observation that the fast and slow phases of Na current inactivation are differentially affected by benzocaine. To answer this question, it is necessary to consider the origin of the two phases of Na current inactivation in the absence of benzocaine. The two phases might result either from a multiple step inactivation process in one type of Na channel or from the existence of two types of Na channels with different inactivation kinetics. Based on the hypothesis of a multiple step inactivation of one type of channel, several schemes have been proposed to account for the double exponential decay of I_{Na} during inactivation. Chiu (1977) proposed a sequential scheme consisting of 1 open and 2 inactivated states, whereas Ochs et al. (1981) proposed a sequential scheme consisting of 2 open and 1 inactivated states. To take account of both the double exponential Na inactivation and the change in time course of the probability of an open Na channel remaining open during a depolarizing pulse, Sigworth (1981) proposed two alternative schemes, each consisting of 2 open and 2 inactivated states.

Although such schemes for a single type of channel may account for the double exponential Na inactivation, they do not seem to account appropriately for the differential effects of benzocaine on the two phases of Na current inactivation. Following a similar line of reasoning to that employed in our interpretation of the action of benzocaine on BTX-treated fibers (above), the lack of effect of benzocaine on either time constant for I_{Na} inactivation indicates a relatively slow rate of reaction of benzocaine with resting and open channels. With such slow rates of reaction, the net effect of benzocaine simply would be to decrease the number of available channels without changing the time course of current through the remaining nonblocked channels. Thus, for a single type of Na channel, benzocaine would simply scale down the current without altering its time course, even if the channels had

multiple resting, open or inactivated states. This prediction is at odds with the experimental observations.

An alternative hypothesis for the action of benzocaine is that the fast and slow phases of Na inactivation correspond to two kinetically distinct types of Na channels (Corbier and Dubois, 1983; Benoit et al., 1985) and that these two types of channel have different affinities for benzocaine. Following this hypothesis, one could account for the differential effects of benzocaine on the two phases of Na current inactivation by assuming that the fast and slow inactivating channels have different affinities for benzocaine both in the resting and in the inactivated states. If the fast and slow inactivating channels had the same single channel conductance and if the gating particles of both types of channels had the same effective valence, the two types of channels hypothesis would account for the equal reduction of peak Na current and Q_{ON} in normal conditions (Neumcke et al., 1981) and of Na current, Q_{ON} and Q_{OFF} after removal of inactivation of BTX.

We thank Drs. J. Daly and B. Witkop for the supply of batrachotoxin used for these experiments.

Dr. Schneider gratefully acknowledges support from Fondation Simone et Cino del Duca and Institut National de la Santé et de la Recherche Médicale.

Received for publication 22 February 1984 and in final form 6 January 1986.

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