Dissecting Lidocaine Action: Diethylamide and Phenol Mimic Separate Modes of Lidocaine Block of Sodium Channels from Heart and Skeletal Muscle

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ABSTRACT   We have investigated block of sodium channels by diethylamide and phenol, which resemble the hydrophilic tertiary amine head and the hydrophobic aromatic tail of the lidocaine molecule, respectively. Diethylamide and phenol separately mimicked the fast and slow modes of block caused by lidocaine. Experiments were performed using single batrachotoxin-activated bovine cardiac and rat skeletal muscle sodium channels incorporated into neutral planar lipid bilayers. Diethylamide, only from the intracellular side, caused a voltage-dependent reduction in apparent single channel amplitude ('fast' block). Block was similar for cardiac and skeletal muscle channels, and increased in potency when extracellular sodium was replaced by N-methylglucamine, consistent with an intrapore blocking site. Thus, although occurring at 15-fold higher concentrations, block by diethylamide closely resembles the fast mode of block by lidocaine (Zamponi, G. W., D. D. Doyle, and R. J. French. 1993. Biophys. J. 65:80–90). For cardiac sodium channels, phenol bound to a closed state causing the appearance of long blocked events whose duration increased with phenol concentration. This slow block depended neither on voltage nor on the side of application, and disappeared upon treatment of the channel with trypsin. For skeletal muscle channels, slow phenol block occurred with only very low probability. Thus, phenol block resembles the slow mode of block observed for lidocaine (Zamponi, G. W., D. D. Doyle, and R. J. French. 1993. Biophys. J. 65:91–100). Our data suggest that there are separate sites for fast lidocaine block of the open channel and slow block of the "inactivated" channel. Fast block by diethylamide inhibited the long, spontaneous, trypsin-sensitive (inactivation-like) closures of cardiac channels, and hence secondarily antagonized slow block by phenol or lidocaine. This antagonism would potentiate shifts in the balance between the two modes of action of a tertiary amine drug caused by changes in the relative concentrations of the charged (fast blocking) and neutral (slow blocking) forms of the drug.

INTRODUCTION

For four decades, class I antiarrhythmic agents have been known to block sodium channels (Weidmann, 1955). The effect of the most prominent member, lidocaine, has been studied extensively on sodium channels from different excitable tissues (Strichartz, 1973; 1976; Hille, 1977a, b; Hondeghem and Katzung, 1977; Cahalan, 1978; Moczydlowski et al., 1986a, b). Lidocaine has been shown to bind both to the open and the inactivated state of unmodified sodium channels (e.g., Hille, 1977a; Bean et al., 1983; Matsubara et al.; 1987; Clarkson et al., 1988; Starmer et al., 1991; Makielski et al., 1991). We have recently reported on a study of the actions of lidocaine (Zamponi et al., 1993a, b) and procainamide (Zamponi et al., 1993c) on single, batrachotoxin (BTX)-activated sodium channels from heart and skeletal muscle. Lidocaine induced fast block (with blocked times of less than 1 ms), which was favored at depolarizing potentials, in both channel subtypes. In addition, lidocaine caused slow block (blocked times of about 1 s) of the cardiac channel subtype by binding to a long-lived closed state which showed features characteristic of inactivation. This slow mode of block appeared to be caused by the uncharged form of the drug. Procainamide, which is structurally similar to lidocaine (Ehring et al., 1988), also caused fast block, but ap-

peared to be capable of producing occasional long blocked events only at very high concentrations (>100 mM). Procainamide's most striking action, however, was the prevention of inactivation through fast block. Thus, it is of interest to determine which parts of the lidocaine and procainamide molecules are responsible for these three different actions.

Several studies (e.g., Ehring et al., 1988; Sheldon et al., 1991) have addressed the structural requirements for class 1 antiarrhythmic block of sodium channels. The study by Ehring et al. (1988) compared the actions of lidocaine and procainamide derivatives on cardiac action potentials. However, they failed to identify the parts of the lidocaine molecule which might be responsible for block of the open and the inactivated channel. Sheldon et al. (1991) studied [³H]-batrachotoxinin A 20α-benzoate ([³H]BTX-B) displacement by a range of lidocaine derivatives. This same group used a similar biochemical approach to investigate the state dependence of lidocaine action (Hill et al., 1989). Their work revealed important information about the structural requirements for drugs to displace BTX, but again, it did not provide clues about which parts of the lidocaine molecule are responsible for the state-dependent interaction with the sodium channel, or whether the interaction with both states occurs at the same binding site.

We decided to take a direct approach by essentially dissecting free the two ends from the lidocaine molecule, represented by phenol and diethylamide. The parent, lidocaine, and the two daughter compounds are displayed in Fig. 1. We
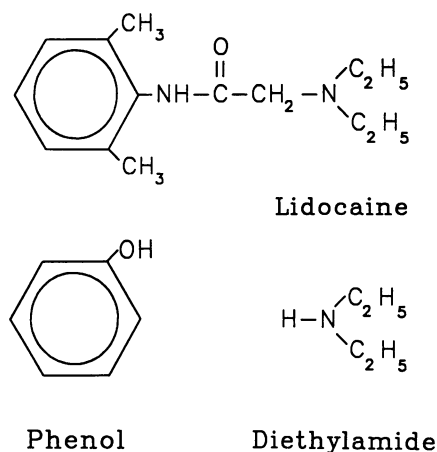
FIGURE 1   Schematic representation of the the parent compound, lido-caine, and the two daughter compounds, phenol and diethylamide. Phenol and diethylamide essentially constitute the hydrophobic and the hydrophilic ends of the lidocaine molecule. Phenol was chosen instead of dimethyl-phenol because of its greater solubilty in water. Both the tertiary amine head group of lidocaine, and diethylamide, would be protonated most of the time at physiological pH.

chose phenol instead of dimethylphenol as the aromatic moiety because of its greater solubility in water. Here, we present our observations on the interactions of these two compounds with BTX-activated cardiac and skeletal muscle sodium channels. Diethylamide mimicked the essential features of fast lidocaine block in voltage dependence, lack of tissue specificity, and sensitivity to external sodium ions. Diethylamide also mimicked procainamide's action by preventing the long-lived closed state seen with the cardiac channels. Phenol showed a dramatic effect only on the cardiac subtype by inducing long blocked events which resembled slow lidocaine block in most aspects. These data suggest the presence of two distinct binding sites for lidocaine block of the open and the inactivated state. Coapplication of the fast blocking compounds diethylamide and QX-314 with phenol or lidocaine resulted in a reduction in the number of slow blocking events, apparently because the fast blockers prevent transitions to the inactivated state required for slow block (also see Zamponi et al., 1993c).

MATERIALS AND METHODS

Membrane preparations

Membrane vesicles from bovine cardiac and rat skeletal muscle tissue were prepared and incubated with BTX as described previously (Zamponi et al., 1993a). Protein concentrations were 2–4 mg/ml for one preparation of rat skeletal muscle and for two preparations of bovine cardiac membranes used for this study.

Bilayer methods and single-channel recordings

The experimental protocols are described in the companion paper (Zamponi et al., 1993c). The duration of the recordings (10–120 min) was determined by the stability of the bilayers or, in some cases, probably by the dissociation of BTX from the channel.

Chemicals and solutions

We prepared a stock solution of 400 mM diethylamide/200 mM NaCl/20 mM MOPS (4-morpholineethanesulfonic acid) at pH 7.0. Diethylamide (purchased from Sigma Chemical Co., St. Louis, MO) was usually added to the chamber facing the intracellular side of the channel. Phenol (Sigma Chemical Co.) was dissolved in water to give a stock solution of 200 mM phenol and was applied to either side of the membrane. QX-314 was dissolved in water to a stock solution of 200 mM QX-314. Lidocaine was dissolved in MOPS to make a stock of 200 mM lidocaine/200 mM MOPS at pH 6.7. QX-314 was only applied internally, lidocaine to either side of the bilayer.

Data analysis

Generally, data were filtered at 100 Hz and sampled at 250 Hz during transcription into a PC (Compaq 386) and analyzed using pCLAMP v5.5 (Axon Instruments, Inc., Foster City, CA).

For experiments involving fast block by diethylamide, data were acquired for up to 30 s under each set of conditions. Apparent single channel amplitudes were measured using pCLAMP software at a bandwith of 50 Hz.

For experiments involving phenol, and experiments investigating the action of QX-314, diethylamide, and lidocaine on the gating behavior of the channels, data were acquired for up to 20 min giving a mean of 100 long closed events at each voltage. For analysis of open times, traces were digitally filtered at 12.5 Hz and closed events shorter than 70 ms were ignored to eliminate contributions from fast gating events and, in case of lidocaine and QX-314, fast blocked events. No events were ignored for the generation of closed time histograms, however, filtering at 12.5 Hz eliminates events shorter than 14 ms (McManus et al., 1987). We did not correct for missed events. Open times and closed times were determined from fits to dwell time histograms in pCLAMP. Preparation of figures and all other fits were performed using Sigmaplot (Jandel Scientific, Corte Madera, CA).

RESULTS

Diethylamide causes fast open channel block

At potentials more positive than −60 mV, individual BTX-activated cardiac channels usually show a constant open probability ($0.5 < p_{open} < 0.9$) in absence of blockers. In this voltage range, the skeletal muscle subtype is generally open more than 90% of the time. This difference in gating between the two channel types appears to be due to a population of long gating closures (with mean durations of several hundred milliseconds) which are characteristic of the cardiac subtype, essentially voltage-independent (French et al., 1990) and sensitive to trypsin (Zamponi et al., 1993b). Examples of records are shown in Fig. 2 A and B. Internally applied diethylamide (18 mM) caused a reduction in apparent single-channel amplitude. This reduction was favored at depolarizing potentials, increased with diethylamide concentration and appeared to affect both channel subtypes in a similar manner. These data show that diethylamide causes fast open channel block which qualitatively resembles fast block by lidocaine, QX-314 (Zamponi et al., 1993a), or procainamide (Zamponi et al., 1993c). Because we observed no increase in single-channel noise at frequencies up to 200 Hz, this type of block appeared to occur on a much more rapid time scale, than for example lidocaine block. We did not observe block when the drug was applied to the extracellular end of the channel at concentrations of up to 36 mM, which is consistent
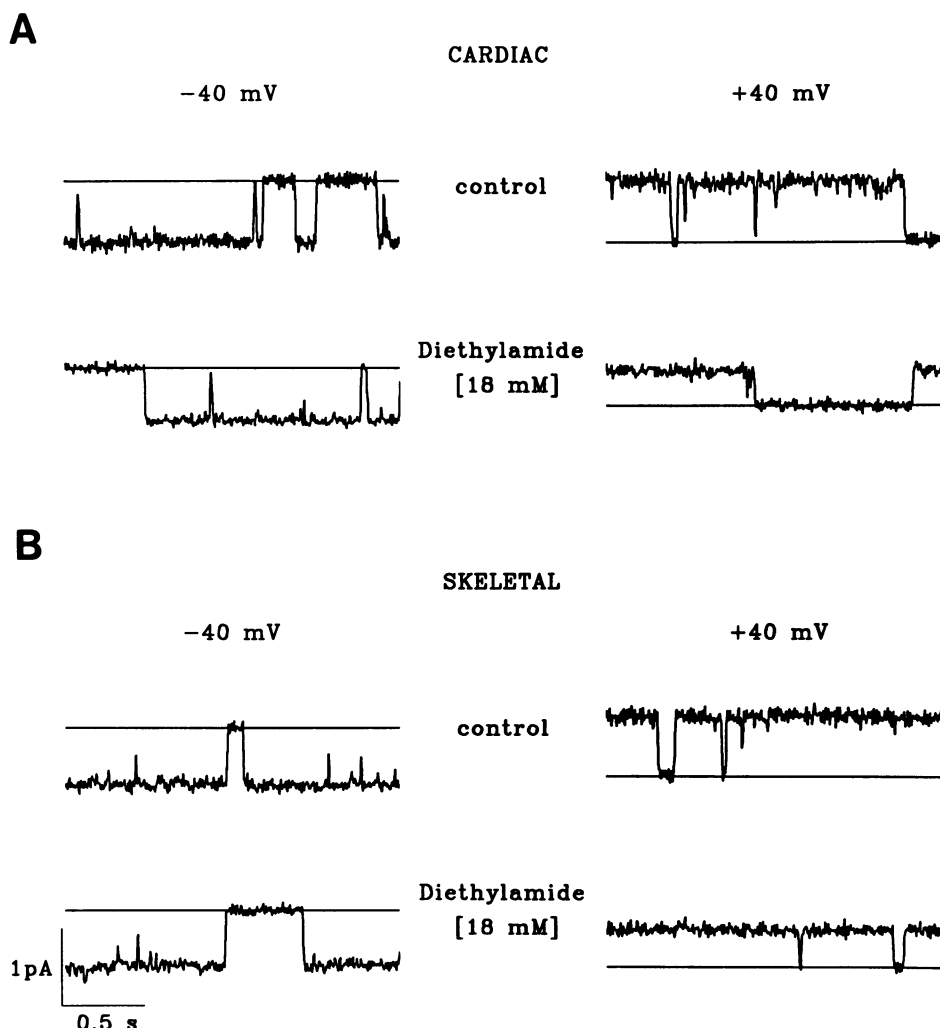
FIGURE 2   Current records obtained from BTX-activated bovine cardiac (A) and rat skeletal muscle (B) sodium channels in absence and presence of 18 mM internally applied diethylamide. In absence of the drug, both channels were open most of the time at potentials more positive than −60 mV. Internal application of diethylamide results in a reduction of the apparent single channel current amplitude. This is presumably caused by discrete blocking events which are too fast to be resolved at the illustrated bandwidth of 50 Hz. The effect is more pronounced at depolarizing potentials. Both channel types are similarly affected. The solid lines indicate the closed level.

with diethylamide being present mainly in its charged, and thus less lipid-soluble, form at a pH of 7.0, and a single blocking site accessible from the inside.

Diethylamide block is voltage-dependent and is not tissue specific

The reduction in apparent single-channel amplitude was concentration-dependent. Fig. 3 A shows dose-response curves for both channel types at +40 mV. The data were fitted with a simple hyperbola which suggests a 1:1 interaction between the drug and the channel. The concentrations at which the single-channel amplitude was reduced to 50% reflects the equilibrium dissociation constant, K_d, for fast block. The values obtained from the fits were 21.8

mM and 29.8 mM, respectively, for the cardiac and the skeletal muscle subtypes.

Fig. 3 B shows the voltage dependence of fast diethylamide block for the two channel subtypes at 18 mM internal diethylamide. The curves were fitted with a Boltzman relation. The steepness of the slope ($z\delta$ values were 0.53 and 0.41 for the cardiac and the skeletal muscle subtypes, respectively) indicates that the drugs might penetrate 53% and 41% of the way across the transmembrane voltage from the cytoplasmic end of the cardiac and the skeletal muscle channel, respectively (Woodhull, 1973).

The equilibrium dissociation constant can be calculated directly from the reduction in apparent single-channel amplitude (see legend to Fig. 4 A). Fig. 4 A includes data recorded at various diethylamide concentrations (6–54 mM).
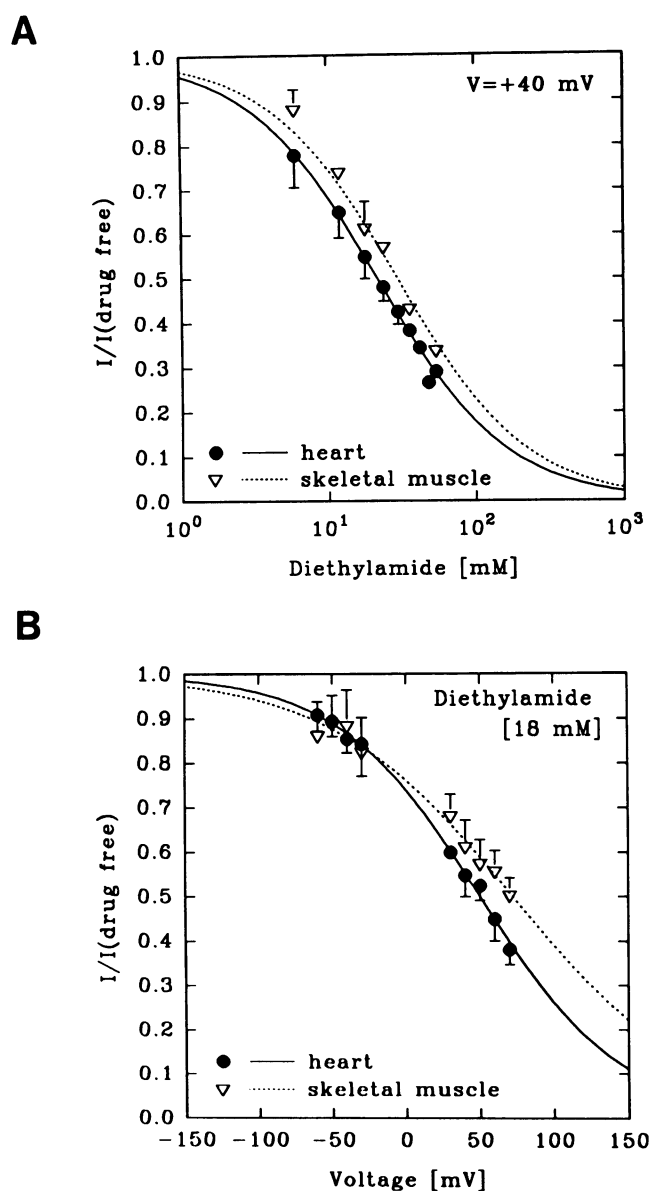
A

B

FIGURE 3 (A) Dose-response curves for diethylamide block of bovine cardiac and rat skeletal muscle sodium channels at +40 mV. Data points from five experiments each for rat skeletal muscle (upward error bars) and bovine heart (downward error bars) were fitted with a simple hyperbolic function, I/I(drug free)$=1/(1+[D]/K_d)$, where $[D]$ is the diethylamide concentration and K_d the equilibrium dissociation constant. The K_d values obtained from the fits were 21.8 mM and 29.8 mM, respectively, for the cardiac and the skeletal muscle channel. (B) Voltage dependence of block by 18 mM internal diethylamide for the cardiac (downward error bars) and the skeletal (upward error bars) muscle channels from five experiments for each channel type. The data were fitted with a Boltzman relation of the form I/I(drug free) $= 1/(1 + \exp\{z\delta(V - V_H)/25.4)\})$ where V represents the membrane potential, V_H is the potential at half-block, and $z\delta$ is proportional to the slope of the fit at V_H (e.g., Woodhull, 1973). Values for $z\delta$ were 0.53 and 0.41 for the cardiac and the skeletal muscle subtype, respectively, suggesting that the blocking site is located about halfway through the transmembrane voltage. All error bars indicate standard deviations.

The slopes of the regression lines suggest electrical distances of 0.54 ond 0.47 for the cardiac and the skeletal muscle channels. These values are consistent with the results obtained from Fig. 3 B.
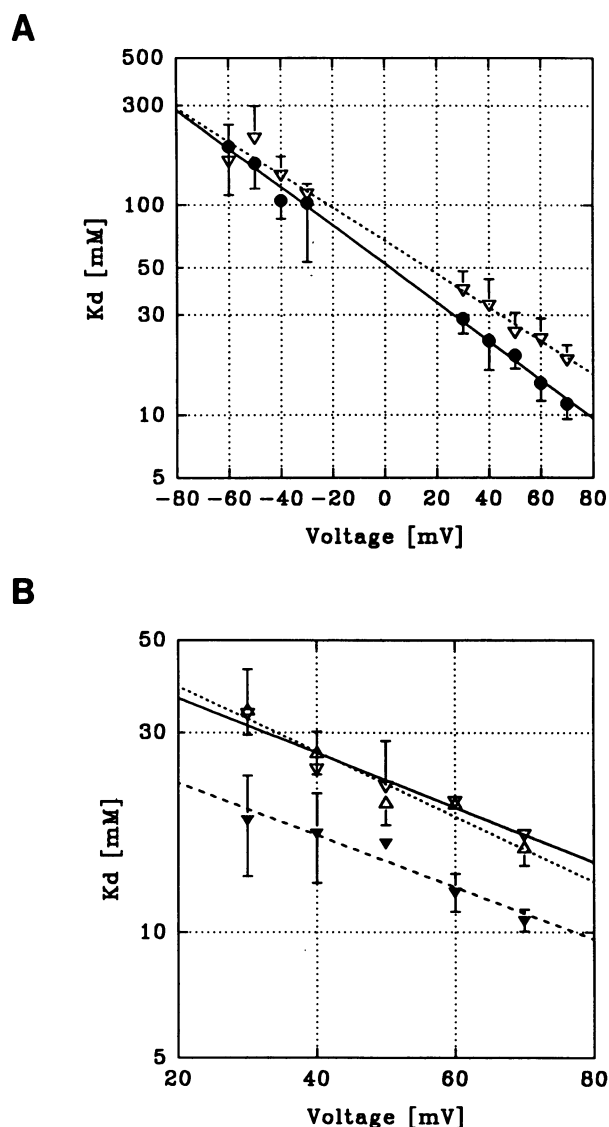
A

B

FIGURE 4 (A) Voltage dependence of the equilibrium dissociation constant for diethylamide block of cardiac (circles, solid regression line, downward error bars, five experiments) and skeletal muscle (triangles, dashed regression line, downward error bars, five experiments) sodium channels. The K_d values were calculated with the equation $K_d = [D]/(I$(drug free)$/I - 1)$ where D is the drug concentration. The voltage dependences of the K_d values suggest that the binding sites are located 54% and 47% across the transmembrane voltage from the cytoplasmic end of the channel, respectively, for the cardiac and the skeletal muscle subtypes. These data are consistent with the values obtained from Fig. 3 B. All error bars are standard deviations. (B) Effect of removal of external sodium ions on diethylamide block for the skeletal muscle subtype for two experiments. The K_d values were obtained as in part A. In each experiment, block by 18 mM internal diethylamide was determined in symmetric 200 mM NaCl, 20 mM MOPS at pH 7.0 (downward hollow triangles, upward error bars, solid regression line, semilogarithmic slope = −0.0065), under asymmetric conditions with 200 mM NMG (pH 7.0) at the extracellular side (filled triangles, medium-dashed regression line, slope = −0.0062), and again in symmetric 200 mM NaCl, 20 mM MOPS at pH 7.0 (upward hollow triangles, downward error bars, short-dashed regression line, slope = −0.0078). Error bars are standard deviations. Note that the slopes of the regression lines are consistent with the slope obtained for the skeletal muscle subtype in part A (slope = −0.0079). With the 3 M KCl-agar bridges used in our experiments, the net change in junction potential due to the replacement of sodium ions by NMG was estimated to be less than 1 mV.

The similar K_d values and electrical distances for the channels from both tissues are reminiscent of the lack of tissue specificity observed for fast block by lidocaine and QX-314 (Zamponi et al., 1993a) and procainamide (Zamponi et al., 1993c).

External sodium ions antagonize diethylamide block

We have previously shown that removal of external sodium ions (replacement of the extracellular solution by 200 mM N-methylglucamine (NMG)) resulted in an increase in the potency of QX-314 block without affecting its voltage dependence (Zamponi et al., 1993a). If diethylamide mimics QX-314 in its blocking action, one would expect to observe a similar effect of NMG on diethylamide block. Fig. 4 B shows the result of this experiment for two different channels. In each experiment, we recorded a control at five potentials in symmetric 200 mM NaCl/20 mM MOPS at pH 7.0. Diethylamide (18 mM) was added to the intracellular side and the block was determined. Then the extracellular side was perfused with 200 mM NMG at pH 7.0. After the single-channel amplitudes had been measured at the five test potentials, the drug was washed with 200 mM NaCl/20 mM MOPS (pH 7.0) from the intracellular side. This step was required to obtain a control under asymmetric ionic conditions. (Due to the altered reversal potential, single-channel amplitudes at positive potentials appear larger when external sodium ions are removed.) NMG was subsequently replaced by 200 mM NaCl/20 mM MOPS (pH 7.0) to restore the original symmetric conditions. Finally, block by 18 mM internal diethylamide was determined for a second time. As can be clearly seen from Fig. 4 B, removal of extracellular sodium ions resulted in a decrease in K_d for fast block without affecting its voltage dependence. The effect was completely reversible when the original ionic conditions were restored. These data are consistent with our previous results on QX-314, and suggest that the blocking site is located within the conducting pathway of the channel.

Phenol causes slow block of the cardiac subtype by binding to a trypsin-sensitive closed state

Phenol (1–15 mM) induced conspicuous long blocked events when applied to cardiac channels from either side of the membrane. As for slow block by lidocaine (Zamponi et al., 1993b), block from both the intracellular and the extracellular side was qualitatively similar and appeared to be correlated with the presence of trypsin-sensitive, long gating closures. Phenol block did not show any detectable voltage dependence. For a quanitative analysis we restricted the application of the drug to the extracellular side.

A typical example of phenol block from a group of 11 bilayers is shown in Fig. 5 A. In absence of the drug, this particular cardiac channel was open about 70% of the time. Application of 6 mM external phenol resulted in the appear-

ance of long blocked events which, together with most of the long gating closures, were removed upon exposure of the channel to trypsin on the intracellular side. Subsequent washout of the extracellular side revealed that phenol had no effect on the trypsin-treated channel. The effect of trypsin was observed for four channels, in all cases slow block was removed within 1 min after trypsin application. In contrast with lidocaine (Zamponi et al., 1993b), the presence of phenol did not protect the channel from proteolysis. Fig. 5 B demonstrates that phenol had only little effect on the rat skeletal muscle suptype. In three experiments we observed only sporadic longer blocked events (less than two events/100 s); however, trypsin treatment resulted in the loss of these few events (one experiment). This suggests that BTX-activated skeletal muscle channels also appear to exhibit some residual inactivation; however, the probability of this state is much lower for the skeletal muscle subtype than for cardiac channels.

Fig. 6 displays closed time histograms for the experiment shown in Fig. 5 A. In absence of blockers, the closed time distribution showed two populations of gating closures (Fig. 6 A). The longer closures (t_{c2} (drug free)) are characteristic of BTX-activated cardiac sodium channels (French et al., 1990). Application of phenol (Fig. 6 B) resulted in the appearance of a distinct third peak (t_{burst}), whereas the peak representing long gating closures (t_{c2}) was shifted to the left (as expected for the linear open-closed-blocked model presented in the Discussion). Application of trypsin removed the long blocked events and most of the long gating events (Fig. 6 C). The remaining events are probably due to voltage-dependent gating. After trypsin treatment, phenol had no effect on the closed time histogram (Fig. 6 D).

For slow lidocaine block, we have demonstrated that the duration of the long blocked events increased linearly with drug concentration, whereas the open times remained essentially unaffected (Zamponi et al., 1993b). Fig. 7 illustrates similar properties for phenol block. For this figure, data from 10 experiments and 5 different voltages were included. As can be seen from Fig. 7 A, the mean duration of the long blocked events (i.e., the C2-Bs burst duration) increased linearly with phenol concentration. According to the linear three-state open-closed-blocked model presented in the Discussion, the inverse of the slope of the regression line represents the microscopic equilibrium dissociation constant for phenol block (1.2 mM). The open times did not appear to be systematically affected by the drug (Fig. 7 B).

In 3 of 11 experiments, phenol appeared to irreversibly affect the gating behavior by shortening (two experiments) or prolonging (one experiment) mean open times without affecting closed or blocked times. We do not know why this effect occurred. Inasmuch as phenol is a mildly oxidizing reagent, it is possible that oxidation of an amino acid residue involved in inactivation might have been the cause for these changes in gating behavior. In two of these experiments it was possible to obtain appropriate controls. Data from these
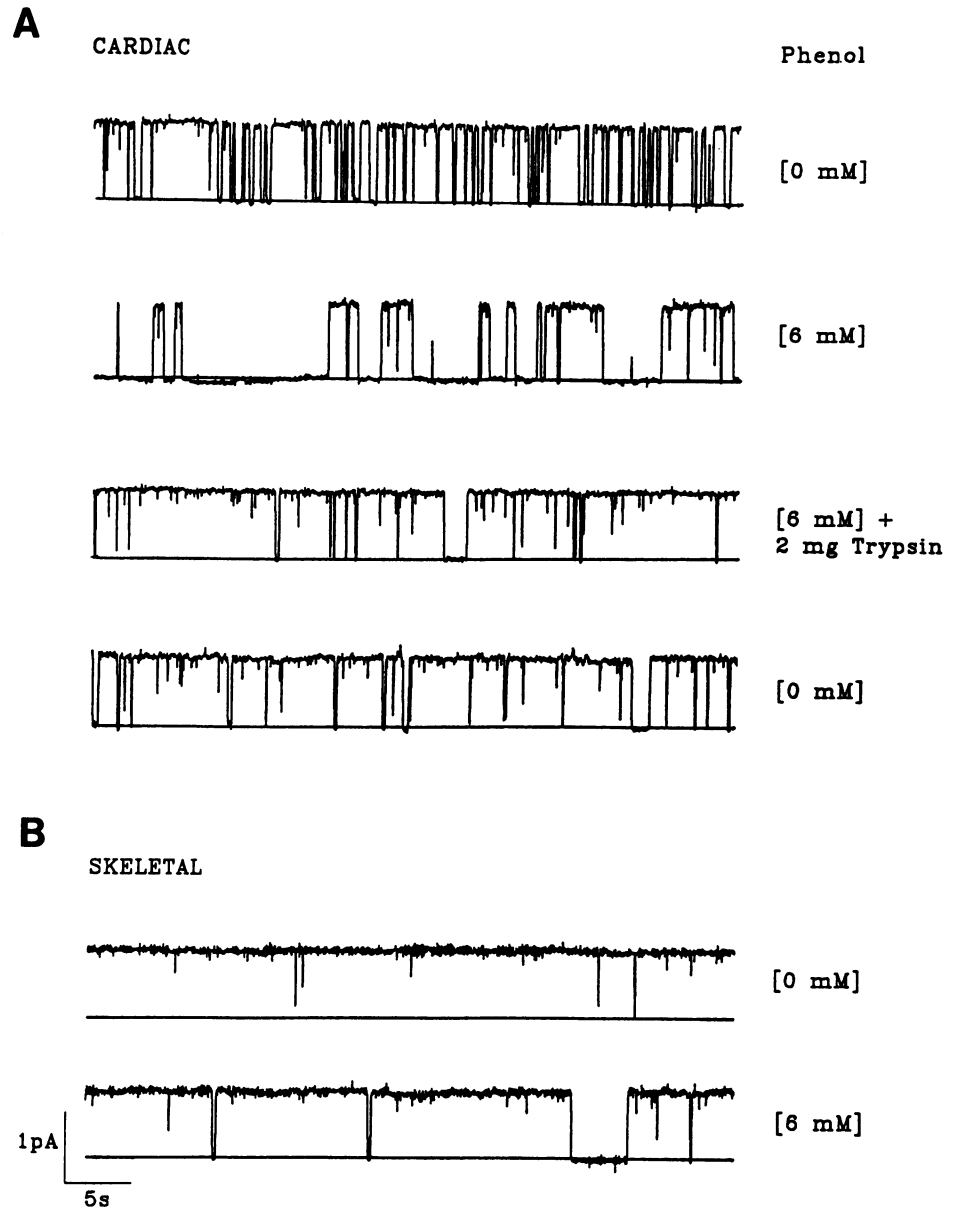
A CARDIAC Phenol

FIGURE 5 Current traces recorded from bovine cardiac (A) and rat skeletal muscle (B) sodium channels at +40 mV. Channel closures are downward deflections; solid lines indicate the closed level. The traces were filtered at 12.5 Hz. (A) Under control conditions, cardiac channels usually show a distinct population of long gating closures which are rarely seen for the skeletal muscle subtype (see B). Application of 6 mM phenol to the extracellular side of the channel resulted in the appearance of a population of long blocked events. Most of these long blocked events were removed upon intracellular application of trypsin (1.3 mg/ml). Phenol was subsequently washed out to demonstrate the effect of trypsin on the long gating closures. All traces were recorded from the same channel. (B) Skeletal muscle sodium channels were only weakly affected by phenol.

two experiments are included in Fig. 7. In both of these experiments, the time of occurrence of the irreversible change was obvious from visual inspection of the records. In one case we did not include data acquired after the change in gating; in the other case the shift occurred early in the experiment. We continued the experiment and used a control recorded after washout of phenol for data acquired after the shift had occurred.

Fig. 7 C shows that the mean duration of long gating closures (state C2) decreased with phenol concentration. The rationale for the choice of the ordinate is explained in the Discussion. Plotting the data in this form yields the rate constant for phenol binding to the long closed state (k_{on} = 0.63 M^{-1} s^{-1}). Because the microscopic equilibrium constant is determined by the ratio of unbinding to binding rate, the unbinding rate for phenol (k_{off}) is 0.76 s^{-1}. These values are of the same order as the ones obtained for slow lidocaine block (Zamponi et al., 1993b).

Fast block antagonizes long gating closures and slow block for the cardiac subtype

In the companion paper (Zamponi et al., 1993c) we have reported that procainamide and QX-314 prevented long gating closures in BTX-activated cardiac sodium channels. Our data suggested that drug binding to the fast block receptor might be responsible for this effect. Because diethylamide is a much smaller molecule than procainamide, we wanted to test whether fast block by diethylamide also prevented long gating closures in cardiac channels. If that were the case, one would expect slow block by lidocaine or phenol to be antagonized by diethylamide. Fig. 8 A shows the effect of 75 mM internal diethylamide on slow block by 6 mM internal lidocaine. Because slow block by lidocaine is best seen at hyperpolarizing potentials, a relatively high diethylamide concentration was required to cause a sufficient degree of fast diethylamide block. As can be seen from the records in Fig.
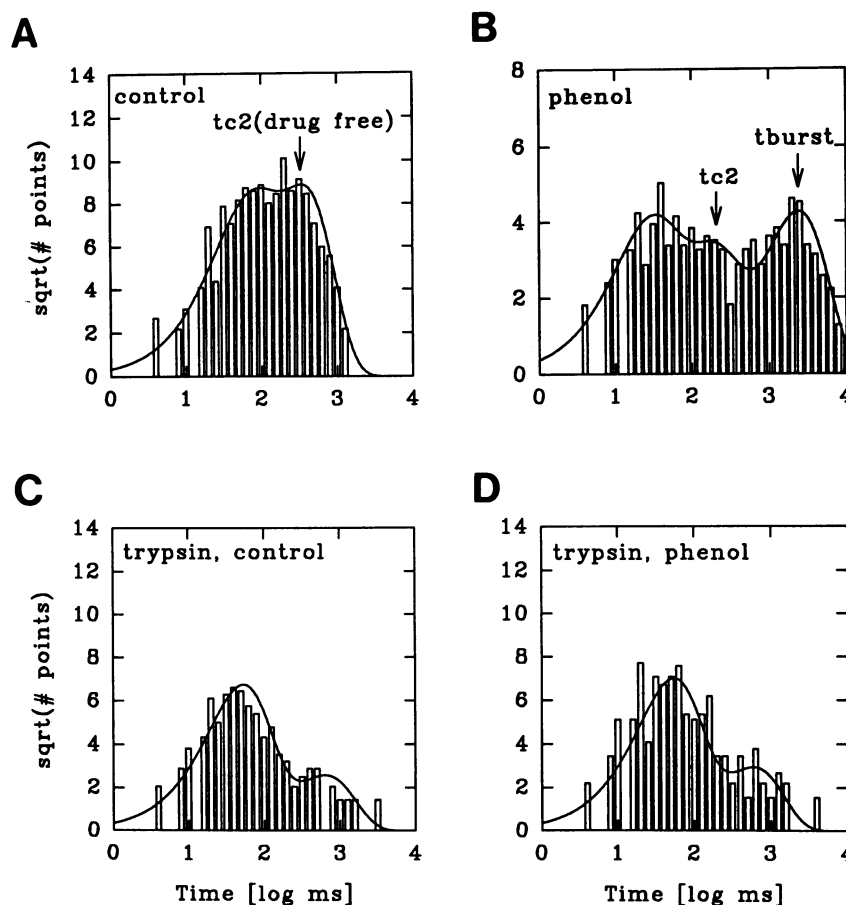
FIGURE 6   Dwell time histograms for the experiment described in Fig. 5 A in the form introduced by Sigworth and Sine (1987). Histograms and fits were constructed as described in Methods. All histograms were normalized to events per 1000 s. The durations of the records used for the individual histograms ranged from 7 to 20 min. (A) In the absence of the drug, the closed time distribution shows two distinct time constants; t_{c1} (drug free), t_{c2} (drug free). (B) Application of 6 mM phenol to the extracellular end of the channel results in the appearance of a third peak (t_{burst}), which appears to be due to a silent burst between the long closed state and a blocked state. Because phenol appears to bind to the long closed state, the mean closed time decreases and the burst time increases with concentration. Hence, the the peak representing this population of closures (t_{c2}) is shifted to the left compared with part A. (C) Internal application of trypsin (1.3 mg/ml) results in a loss of most of the long gating events. (D) Phenol does not affect the channel after it has been treated with trypsin. The time constants obtained from the fits are as follows:

	Time constants (ms)		
[Phenol] (mM)	t_{c1}	t_{c2}	t_{burst}
0 (A)	51	365	—*
6 (B)	24	146	2440
0 (C) trypsin-treated	50	640	—
6 (D) trypsin-treated	50	593	—

* Dashes indicate no parameter value.

8 A, diethylamide drastically reduced the frequency of slow lidocaine-blocked events.

Fig. 8 B shows the effect of diethylamide on slow block by phenol. In absence of the drugs, the channel showed an open probability of 0.70. Internal phenol (6 mM) resulted in pronounced slow block and the open probability dropped to about 0.30. Because the holding potential in this experiment was +40 mV, 25 mM diethylamide was expected to be sufficient to produce an effect. The application of diethylamide resulted in a reduction in frequency of long events, but their mean duration was not affected. When diethylamide was ap-

plied alone, the number of long gating closures was decreased by about 50% relative to the control trace. When applied alone, 6 mM QX-314 showed a similar effect. The inhibition of long closures by diethylamide (three experiments) was concentration-dependent, was half-maximal at concentrations near the K_d values for fast block (18 mM at +40 mV), and was weaker at negative potentials as expected from the voltage dependence of fast block.

Because QX-314 appeared to prevent long gating closures, one would expect QX-314 to antagonize slow block by lidocaine and phenol. However, coapplication of QX-

314 with lidocaine (three experiments) or with phenol (three experiments) resulted in the loss of the channels within 5 min, probably because of BTX dissociation. Even though there appeared to be an inhibitory effect of QX-314 on both the slow block by lidocaine and phenol, the relatively brief duration of the experiments did not permit a conclusive quantitative analysis. Fast block by QX-314 did not appear to be affected by the presence of phenol. At this point we do not know why BTX might dissociate under these conditions.

Because the three structurally different drugs procainamide, QX-314 (or lidocaine), and diethylamide exerted a similar action on long gating closures in cardiac channels, it

seems that any binding to the fast blocking site might prevent transitions to the long-lived closed state and, thus, slow block.

DISCUSSION

Diethylamide mimics fast block by lidocaine

We have previously described the fast blocking action of lidocaine and its permanently charged derivative, QX-314, on BTX-activated sodium channels from bovine heart and rat skeletal muscle (Zamponi et al., 1993a). This type of block did not distinguish between the two tissues, followed 1:1 blocking kinetics, was increased upon removal of external sodium ions, was favored by depolarizing potentials, and, for lidocaine, occurred from either side of the membrane.

Here, we have demonstrated similar properties for diethylamide block. Presumably because diethylamide (pK$_a$ = 10.5) was essentially permanently charged in our experiments, block occurred only when the drug was applied to the intracellular side of the channel. Its tissue specificity, voltage dependence, blocking stoichometry, and sensitivity to external sodium resembled that of block by lidocaine and QX-314. A summary of the blocking parameters of lidocaine, QX-314, procainamide, and diethylamide is given in Table 1. We compare the dissociation constants at E = 0 mV, and the apparent electrical distance from the cytoplasmic solution to the blocking site.

The potency of diethylamide block is about 15-fold lower than that of lidocaine block. It has been previously shown that quaternary compound tetramethylammonium has an even lower affinity for BTX-activated skeletal muscle sodium channels (K_d = 370 mM at E = 0 mV; Moczydlowski et al., 1986a, b) and unmodified sodium channels (K_d = 110 mM at E = 0 mV; Horn et al., 1981), than lidocaine. Tetramethylammonium comprises a central nitrogen with four
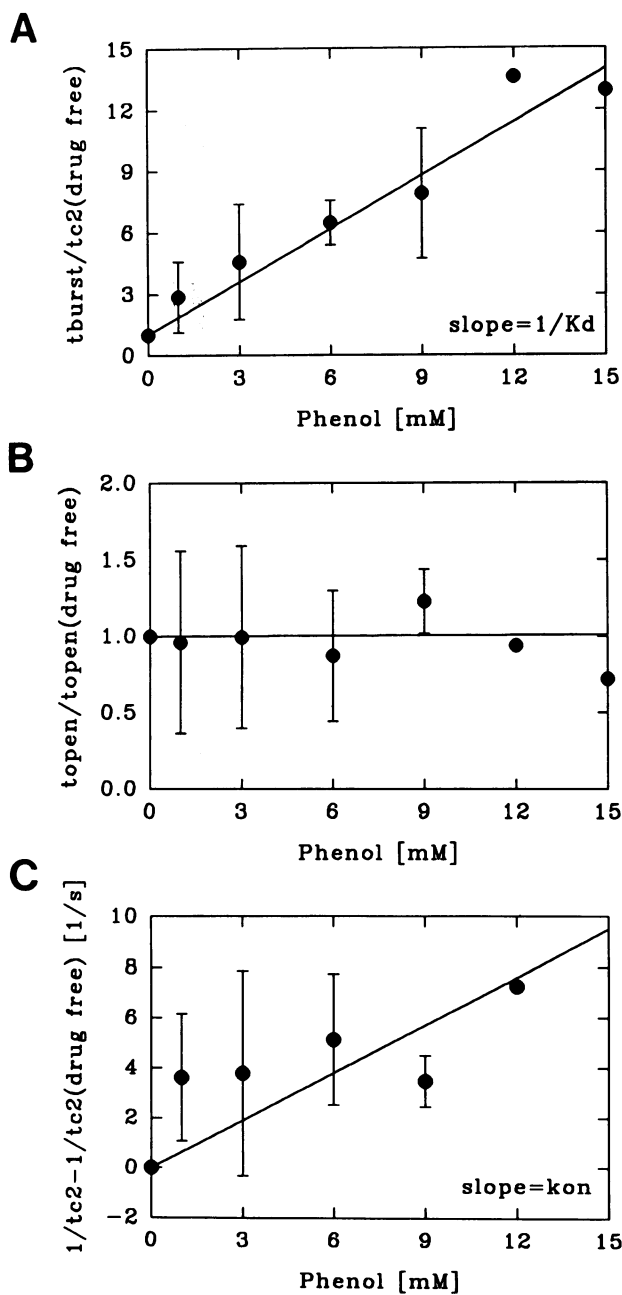

FIGURE 7   Concentration dependence of the dwell time constants for phenol block of the bovine cardiac sodium channel. Data from 10 experiments and 5 different voltages (−60 mV, −40 mV, −20 mV, +20 mV, +40 mV) are included in the figure. Closed times and burst times were obtained from fits as in Fig. 6. Solid lines are least square fits of all data points, the intercepts were not allowed to vary. Error bars are standard deviations. The data were analyzed according to the linear three-state open-closed-blocked model presented in the Discussion. The choices for the ordinates are based on this model (see Discussion). (A) The normalized closed-blocked (C2-Bs) burst durations increase linearly with concentration (slope = 0.87 mM⁻¹). According to the kinetic model described in the Discussion, the inverse of the slope reflects the equilibrium dissociation constant, K_d, for phenol block. (B) The mean time between long events does not appear to be correlated (slope = 0.0008 mM⁻¹) with concentration. (C) The mean duration of the long gating closures decreases with phenol concentration, as indicated by an increase in reciprocal closed times. For the kinetic models presented in the Discussion, the slope of the regression line (slope = 0.63 s⁻¹mM⁻¹) reflects the rate at which phenol binds to the closed state. Remark: When a linear regression is formed without holding the intercepts at a fixed value (for panel A: intercept = 1.04 s⁻¹, slope = 0.82 s⁻¹mM⁻¹, r = 0.97; for panel C: intercept = 1.83 s⁻¹, slope = 0.40 s⁻¹mM⁻¹, r = 0.78), a statistical analysis of the correlation coefficents (Larsen and Marx, 1981) indicates a probability >0.99 for a non-zero slope for panels A and C.

A   −40 mV

6 mM Lidocaine

6 mM Lidocaine +
75 mM Diethylamide

B   +40 mV

control

6 mM Phenol

6 mM Phenol +
25 mM Diethylamide
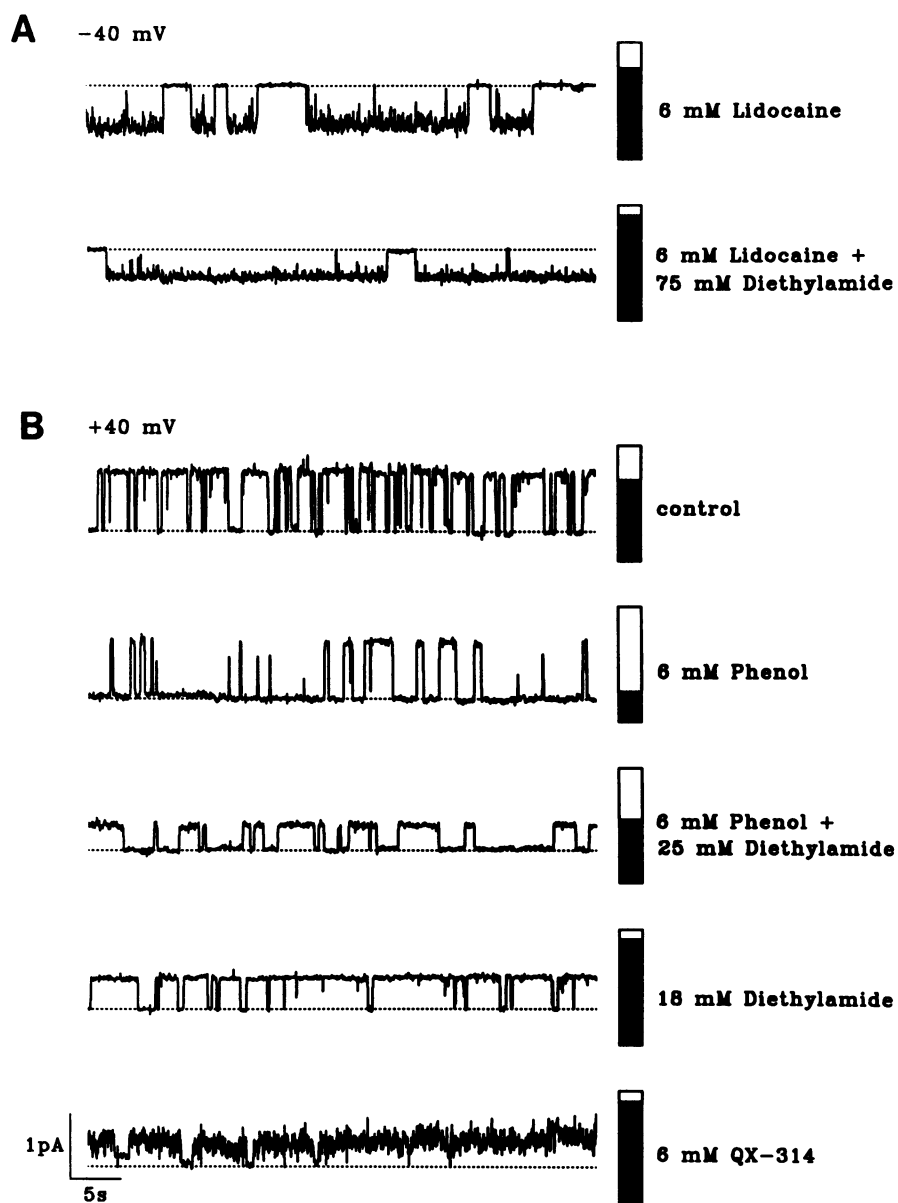
18 mM Diethylamide

1 pA

5s

6 mM QX−314

FIGURE 8   Fast block inhibits long closures (inactivation) and hence inhibits slow block by lidocaine or phenol. These current traces were recorded from two bovine cardiac sodium channels. The dashed lines indicate the closed levels, the sizes of the black bars represent the open probability, on a scale from 0 to 1, for which only nonconducting events longer than 70 ms are treated as closures. All drugs were internally applied. (A) In presence of 6 mM lidocaine, cardiac chanels show long blocked events as well as fast, flickery open channel block. Addition of 75 mM diethylamide reduces the frequency of these events (mean t_{open} increased from 5617 to 9985 ms), resulting in an increase in open probability. (B) Current traces from the same bovine cardiac channel. Here, the order of the traces does not reflect the experimental protocol, however, the open probability (0.70) in four control traces recorded after washout at different stages throughout the experiment varied by less than 3%. In absence of drugs, this channel showed pronounced long gating closures. Addition of 6 mM phenol resulted in the appearance of long blocked events. Subsequent addition of 25 mM diethylamide reduced the frequency of these long events without affecting their duration (means: t_{burst}(phenol) = 2.041 ms, t_{burst}(phenol + diethylamide) = 2.172 ms). Application of 18 mM diethylamide alone reduced the frequency of long gating events seen in absence of the drug (cf. top trace). QX-314 (6 mM) exhibited a similar effect on the gating closures. The overlying flicker is due to fast open-channel block by the drug.

attached methyl groups, and is thus structurally homologous to protonated diethylamide. Procainamide, which also causes fast block of BTX-activated sodium channels and which is structurally very similar to lidocaine (Zamponi et al., 1993c), shows an about sevenfold lower affinity for BTX-activated sodium channels. Sheldon et al. (1991) studied [³H]BTX-B displacement from cardiac sodium channels by a variety of lidocaine derivatives. Their study showed that compounds

with four or more carbons in the arylamide-amide link, and four or more amino-terminal carbons, produced maximal [³H]BTX-B displacement. Their data suggest that binding is sensitive to the structures attached to the tertiary amide head. Thus, the low potency of diethylamide is not surprising.

Second, the electrical distances obtained for the four drugs range from 0.32 to 0.54 (heart) and 0.25 to 0.47 (skeletal muscle). For all of the drugs, the values were lower for the

TABLE 1   Comparison of fast block. Equilibrium dissociation constant at 0 mV and voltage dependence.

|	Rat skeletal muscle			Bovine heart			
	K_d (mM)	$z\delta$	No. expts.	K_d (mM)	$z\delta$	No. expts.	Ref.
Lidocaine	4.2	0.33	4	4.1	0.38	3	*
QX-314	5.1	0.25	5	6.4	0.32	3	*
Procainamide	29.8	0.31	6	29.5	0.43	6	‡
Diethylamide	67.5	0.47	5	52.3	0.54	5	

* Zamponi et al. (1993a).
‡ Zamponi et al. (1993b).

skeletal muscle subtype. It has been previously shown that the electrical distances for procaine, lidocaine, QX-314, QX-222, and tetramethylammonium vary significantly, even though all of these compounds are thought to bind to the same receptor (e.g., Uehara and Moczydlowski, 1986). It has been suggested by Moczydlowski et al. (1986b) that the smaller compounds might penetrate the transmembrane voltage more deeply. However, it is likely that the charged nitrogen interacts directly with part of the receptor, because only the charged compounds cause this type of block. The variation in the apparent electrical distances may arise from differences in the size and conformation of any of the groups that are attached to the tertiary nitrogen.

Even though there are some quantitative differences among blocking actions of the four agents, diethylamide mimicks fast block by lidocaine, procainamide, or QX-314 in almost every aspect. This demonstrates that the diethylamide group from the lidocaine molecule alone is a sufficient structure to cause fast block.

Phenol mimics slow block by lidocaine

Lidocaine application to either side of the membrane of BTX-activated cardiac sodium channels results in the generation of a population of long blocked events (Zamponi et al., 1993b). These long blocked events were correlated with the presence of long gating closures which are characteristic of the cardiac sodium channel subtype. Block was less potent at depolarizing potentials. This voltage dependence was probably caused by the inhibition of long gating closures due to the overlying fast block (Zamponi et al., 1993c). Both long gating closures and slow block were eliminated upon treatment of the channel with trypsin, suggesting that slow block occurred through binding to an inactivated state of the channel. A linear three-state model of the type

$$O \underset{\beta}{\overset{\alpha}{\rightleftarrows}} C2 \underset{\delta}{\overset{[PH]\gamma}{\rightleftarrows}} Bs$$
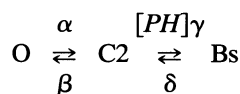
was sufficient to account for our data. By filtering heavily and ignoring closed events shorter than 70 ms, fast voltage-dependent gating closures were treated as part of the open state, O. Phenol appeared to block in a similar manner. As for lidocaine block, blocked times increased with drug concentration, whereas open times were not systematically af-

fected. Presumably because phenol is uncharged and not capable of fast block, slow block by phenol was not voltage-dependent. Block was removed by internal application of trypsin. Because the similarities to lidocaine block suggest a common blocking mechanism, we applied the same kinetic analysis to our phenol data.

For the linear three-state model, block occurs as a silent burst between the trypsin-sensitive closed state, C2, and the blocked state, Bs. The number of transitions between C2 and the blocked state, and hence the mean duration of the burst (t_{burst}), is linearly dependent on the drug concentration, [PH] (Colquhoun and Ogden, 1988). At the same time, the mean time spent in the closed state C2 (mean duration t_{c2}) decreases with drug concentration, because the drug promotes transitions away from this state toward the blocked state. Mathematically, this can be expressed as:

$$t_{burst}/t_{c2} \text{ (drug free)} = 1 + \gamma[PH]/\delta$$

$$(1/t_{c2}) - (1/t_{c2} \text{ (drug free)}) = \gamma[PH].$$

The expression for t_{burst}, when plotted as a function of phenol concentration, yields a straight line with a slope equivalent to the inverse of the microscopic equilibrium dissociation constant, K_d ($= \gamma/\delta$), for slow block (see Fig. 7 A). Plotting the second equation produces a straight line with a slope equivalent to the association rate constant, γ, (Fig. 7 C). In this linear three-state model the open times are not affected by the presence of the drug (Fig. 7 B) (for a more detailed description of the analysis, see Zamponi et al., 1993b).

For phenol block, we obtained an equilibrium dissociation constant of 1.2 mM. For lidocaine, the value was 1.3 mM at −40 mV. However, slow lidocaine block is caused by the uncharged form of the drug (Zamponi et al., 1993b). At pH 7.0, the concentration of uncharged form of lidocaine is roughly one-tenth of the total concentration. Thus, uncharged lidocaine is about one order of magnitude more potent than phenol in causing slow block. We do not know whether this is due to a difference in lipid solubility, or whether it is the net result of several synergistic interactions between different parts of the lidocaine molecule and the slow block receptor.

We have observed slow block for lidocaine (Zamponi et al., 1993b), phenol, procainamide (Zamponi et al., 1993c), and also aniline and flecainide (unpublished observations). These compounds contain drastically different ring substituents (lidocaine contains two methyl groups, aniline and procainamide each contain an amide group, phenol contains one hydroxyl group, flecainide contains two trifluoroethoxy groups). This suggests that the ring structure itself rather than the side groups are responsible for binding to the slow block receptor. However, it is likely that the affinity for the binding site might be modulated by particular residues on the ring.

Channel subtype specificity of phenol and some medical implications

Phenol had little effect on the rat skeletal muscle subtype; we observed only very infrequent long blocked events. These events were, however, eliminated upon trypsin treatment of

the channel. Thus, it appears as if BTX-activated rat skeletal muscle channels also exhibit some residual inactivation, but with a much lower probability of this state than for the cardiac subtype. This tissue specificity of phenol action parallels our data on slow lidocaine block.

Phenol has a considerable history of medical application, among other uses, as a focally injected neurolytic (Wood, 1978) to alleviate chronic pain or severe incontinence, and as a skin-peeling agent for treatment of skin lesions. Cardiac disrhythmias have been associated with each of these procedures (Gross, 1983; Warner and Harper, 1985; Forrest and Ramage, 1987; Morrison et al., 1991). In one study, average serum concentrations during and after phenol face peeling ranged from 0.05 to 3.4 mM (Gross, 1983). However, within phenol treatment groups, no correlation has been demonstrated between measured serum phenol concentrations and disrhythmia occurrence (Gross, 1983; Morrison et al., 1991), and there are differing reports on the ability of lidocaine administration to correct the disrhythmias (Truppman and Ellenby, 1979; Warner and Harper, 1985). In the light of our observations of phenol's heart-specific action, the cardiac complications of these therapeutic uses of phenol are hardly surprising, and detailed studies of phenol's action on native cardiac sodium channels seem long overdue.

Where are the binding sites located?

Our experiments provide direct evidence for the existence of separate sites for block of the open and inactivated states of the cardiac channel.
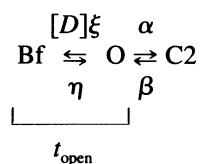
Our data provide several clues about the location of the fast blocking site. Removal of external sodium ions increases the potency of diethylamide block. This transchannel interaction suggests that the blocking site is located within the conducting pathway for sodium ions. The sodium current at $+70$ mV was almost completely blocked in the presence of high concentrations (90 mM) of diethylamide. This suggests that diethylamide is able to fully occlude the pore. Because the molecule is relatively small, the blocking site must be located at a fairly narrow region of the channel. It has been shown that ammonium ions, but no ions containing a methyl group, are able to permeate the sodium channel (Hille, 1971; 1992). Given the relatively deep penetration of the transmembrane voltage by diethylamide, it is possible that the fast blocking site might be located at one of the energy wells sensed by ammonium and other permeant ions while passing through the pore from the intracellular side.

We can only speculate about location of the phenol binding site. We have argued previously that the uncharged species of lidocaine was required for slow block (Zamponi et al., 1993b). Block was virtually identical when lidocaine was applied to either side of the membrane. Phenol, which is an uncharged molecule, also blocked the channel from either side of the membrane. The permanently charged, and thus less lipid-soluble, QX-314 did not produce any slow block. The requirement for an uncharged molecule and the lack of sidedness of action suggests that the binding site is located in a hydrophobic environment. The site could be accessed through drug partition into the bilayer and subsequent diffusion or via direct passage through a hydrophobic pocket within the channel protein. Because both phenol and lidocaine appear to stabilize an inactivated state of the channel, the site might be located in a hydrophobic environment close to the receptor for the inactivation gate. It has been shown that normal fast inactivation requires hydrophobic amino acid residues on the cytoplasmic loop connecting domains III and IV of the sodium channel (West et al., 1992). It is possible that binding of phenol to a site close to the receptor for the inactivation gate stabilizes the inactivated state by forming an additional link between the receptor and the gate.

Fast block antagonizes slow block by preventing inactivation

In the companion paper we report that procainamide reduced the frequency of long gating closures in BTX-activated cardiac sodium channels without affecting their duration. This effect was correlated with the degree of fast procainamide block and its voltage dependence. QX-314 and lidocaine also appeared to be capable of inhibiting long gating closures. Our data suggested that fast block prevented inactivation in these channels. A linear three-state model of the type

$$
\begin{array}{ccc}
& [D]\xi & \alpha \\
\text{Bf} & \underset{\eta}{\overset{}{\rightleftharpoons}} \ \text{O} & \underset{\beta}{\rightleftharpoons} \ \text{C2} \\
& \underline{\qquad\qquad} & \\
& t_{\text{open}} &
\end{array}
$$

accounted for our data. In this model, increasing the drug concentration, $[D]$, shifts the equilibrium away from the long closed state, C2, by increasing transitions to the fast blocked state, B_f. At the K_d for fast block, the number of gating transitions is reduced by 50%. Mean closed durations are not affected by fast block.

Application of diethylamide (three experiments) also prevented the long gating closures (inactivation) in these channels. The effect of diethylamide was concentration- and voltage-dependent, and a 50% reduction in long gating closures occurred near the K_d for fast block. At negative potentials, higher diethylamide concentrations were required (see Fig. 8 A). These observations are consistent with the data obtained with procainamide.
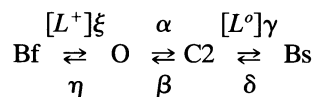
The prevention of long gating closures through fast block can account for the inhibitory effect of diethylamide on slow block by phenol and lidocaine. Because the transition to the long closed state is required for slow block, addition of a fast blocker is expected to reduce the frequency of slow blocking events without affecting their mean duration, as can be seen in Fig. 8. The observation that lidocaine also appeared to to be capable of preventing transitions to the long closed state (Zamponi et al., 1993c) is particularly interesting, since by this implies that lidocaine's fast blocking action antagonizes its own slow block.

Inhibition of inactivation by a variety of cations has been reported for native sodium channels studied in the absence of BTX. The effective ions include the impermeant blocker,

tetramethylammonium (Schauf, 1983; Oxford and Yeh, 1985), as well as Na^+, Cs^+, and K^+ (Oxford and Yeh, 1985; Goldman, 1986, 1988). Thus, our observations of inhibition, by blocking ions, of transitions into the long closed state of BTX-activated cardiac channels reveals another functional parallel between this state and the fast inactivated state of native channels. Consistent with the preceding references, these data suggest that cations in the inner mouth of the pore interact with the inactivation machinery and prevent it from closing. Blocking or permeant ions could inhibit the docking of part of the inactivation gate at its receptor by a direct steric competition, by a longer range electrostatic interaction, or by allosteric means. At present, we cannot choose between these alternatives.

A possible mechanism for lidocaine action

When we combine the two kinetic models presented earlier in this Discussion, we obtain the following scheme for lidocaine block of BTX-activated cardiac sodium channels:

$$\begin{array}{ccccc} [L^+]\xi & \alpha & [L^o]\gamma \\ Bf \rightleftarrows & O \rightleftarrows C2 \rightleftarrows & Bs \\ \eta & \beta & \delta \end{array}$$

In this model, fast voltage-dependent gating closures are ignored and treated as part of the open state, O (see Zamponi et al., 1993b). Fast block occurs only from the open state; slow block only occurs from the long-lived closed state, C2. This implies that fast block prevents slow block by shifting the equilibrium away from the long closed state. The transitions to both the fast blocked and the slow blocked states are concentration-dependent. However, it is the concentration of the charged species, L^+, of a lidocaine-like drug which is responsible for fast block, and the concentration of the uncharged species, L^o, which causes slow block. Hence, an internal drug with a high pK_a would predominantly cause fast block (e.g., procainamide), whereas a drug with a pK_a near 7 (e.g., lidocaine) would be capable of both fast and slow block. An increase in intracellular pH will shift the equilibrium toward the slow blocked state by increasing the amount of uncharged drug and reducing fast block (thus increasing the number of transitions to the long-lived closed state).

The slow blocking action of two drugs with identical pK_a values could be modulated in three ways. First, the affinity of the drugs for the fast block receptor will determine the probability of reaching the long closed state required for slow block. Second, the lipid solubilities of the uncharged species of the drugs will determine how much of the drug reaches the slow block receptor. Third, differences between the detailed structures of the drugs might then determine the relative affinities for the two receptors.

It has been previously suggested that properties like molecular weight or lipid solubility were critical for antiarrhythmic action (Ehring et al., 1988; Courtney 1981; 1983). In addition, there are specific structural requirements for optimal interaction between the channel and antiarrhythmic drugs (Sheldon et al., 1991). In context of our kinetic model,

the structures of the blockers and their molecular weights could determine the affinity for the fast and slow block receptors, whereas their pK_a values and their ability to partition into the membrane might be critical modulators of the relative distribution of slow and fast block.

CONCLUSIONS

We have investigated the blocking actions of diethylamide and phenol on BTX-activated sodium channels from bovine heart and rat skeletal muscle. Diethylamide mimics the fast blocking action previously observed by lidocaine. In cardiac channels, phenol bound to an inactivated state and caused block similar to slow lidocaine block. Both the transitions to the inactivated state and slow block were antagonized by the fast block. Because fast and slow block require the charged and uncharged forms of lidocaine, respectively, pK_a and lipid solubility are crucial for the distribution of the activity between the two blocking modes, and thus antiarrhythmic action.

REFERENCES

Bean, B. P., C. J. Cohen, and R. W. Tsien. 1983. Lidocaine block of cardiac sodium channels. *J. Gen. Physiol.* 81:613–642.

Cahalan, M. 1978. Local anesthetic block of sodium channels in normal and pronase-treated squid giant axons. *Biophys. J.* 23:285–311.

Clarkson, C. W., C. H. Follmer, R. E. Ten Eick, L. M. Hondeghem, and J. Z. Yeh. 1988. Evidence for two components of sodium channel block by lidocaine in isolated cardiac myocytes. *Circ. Res.* 63:869–878.

Colquhoun, D., and D. C. Ogden. 1988. Activation of ion channels in the frog end-plate by high concentrations of acetylcholine. *J. Physiol.* 395:131–159.

Courtney, K. 1981. Comparative actions of mexiletine on sodium channels in nerve, skeletal muscle and cardiac muscle. *Eur. J. Pharmacol.* 74:9–18.

Courtney, K. 1983. Quantifying antiarrhythmic drug blocking during action potentials in guinea-pig papillary muscle. *J. Mol. Cell. Cardiol.* 15:749–757.

Ehring, G. R., J. W. Moyer, and L. M. Hondeghem. 1988. Quantitative structure activity studies of antiarrhythmic properties in a series of lidocaine and procainamide derivatives. *J. Pharmacol. Exp. Ther.* 244:479–492.

Forrest, T., and D. T. O. Ramage. 1987. Cardiac disrhythmias after subtrigonal phenol. *Anesthesia.* 42:777–778.

French, R. J., D. D. Doyle, L. Anscomb, M. C. Lee, K. J. Mather, and Y. Guo. 1990. Kinetic properties distinguish batrachotoxin-activated cardiac sodium channels from other subtypes in planar lipid bilayers. *Biophys. J.* 57:297a.

Goldman, L. 1986. Internal cesium and the sodium inactivation gate in *Myxicola* giant axons. *Biophys. J.* 50:231–238.

Goldman, L. 1988. Internal cations, membrane current, and sodium inactivation gate closure in *Myxicola* giant axons. *Biophys. J.* 54:1027–1038.

Gross, B. G. 1983. Cardiac arrhythmias during phenol face peeling. *Plast. Reconstr. Surg.* 73:590–594.

Hill, R. J., H. Duff, and R. S. Sheldon. 1989. Class I antiarrhythmic drug receptor. Biochemical evidence for state-dependent interactions with

quinidine and lidocaine. *Mol. Pharmacol.* 36:150–159.

Hille, B. 1971. The Permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619.

Hille, B. 1977a. The pH-dependent rate of action of local anesthetics on the node of Ranvier. *J. Gen. Physiol.* 69:475–496.

Hille, B. 1977b. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497–515.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer Associates Inc., Sunderland, MA.

Hondeghem, L. M., and B. G. Katzung. 1977. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta.* 472:373–398.

Horn, R., J. Patlak, and C. F. Stevens. 1981. The effect of tetramethylammonium on single sodium channel currents. *Biophys. J.* 36:321–327.

Larsen, R. L., and M. L. Marx. 1981. An Introduction to Mathematical Statistics and Its Applications. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Makielski, J. C., L. A. Alpert, and D. Hanck. 1991. Two components of use-dependent block of sodium current by lidocaine in voltage clamped cardiac Purkinje cells. *J. Mol. Cell. Cardiol.* 23(Suppl. I):95–102.

Matsubara, T., C. Clarkson, and L. Hondeghem. 1987. Lidocaine blocks open and inactivated cardiac sodium channels. *Naunyn-Schmiedebergs Arch. Pharmakol.* 336:224–231.

McManus, O. B., A. L. Blatz, and K. L. Magleby. 1987. Sampling, log binning, fitting, and plotting durations of open and shut intervals from single channels and the effects of noise. *Pfluegers Arch.* 410:530–553.

Moczydlowski, E., A. Uehara, and S. Hall. 1986a. Blocking pharmacology of batrachotoxin-activated sodium channels. *In* Ion Channel Reconstitution. C. Miller, editior. Plenum Publishing Corp., New York. 405–428.

Moczydlowski, E., A. Uehara, X. Guo, and J. Heiny. 1986b. Isochannels and blocking modes of voltage-dependent sodium channels. *Ann. N. Y. Acad. Sci.* 479:269–292.

Morrison, J. E., D. Matthews, R. Washington, P. V. Fennessey, and M. Harrison. 1991. Phenol motor point blocks in children: plasma concentrations and cardiac arrhythmias. *Anesthesiology.* 75:359–362.

Oxford, G. S., and J. Z. Yeh. 1985. Interactions of monovalent cations with sodium channels in squid axon. I. Modification of physiological inactivation gating. *J. Gen. Physiol.* 85:583–602.

Schauf, C. L. 1983. Tetramethylammonium ions alter sodium-channel gating in *Myxicola. Biophys. J.* 41:269–274.

Sheldon, R. S., R. J. Hill, M. Taouis, and L. Wilson. 1991. Aminoalkyl structural requirements for interaction of lidocaine with the class I an-

tiarrhythmic receptor on rat cardiac myocytes. *Mol. Pharmacol.* 39: 609–614.

Sigworth, F. J., and S. M. Sine. 1987. Data transformations for improvement and fitting of single channel dwell time histograms. *Biophys. J.* 52: 1047–1054.

Starmer, C. F., N. N. Nesterenko, A. I. Undrovinas, A. O. Grant, and L. V. Rosenshtraukh. 1991. Lidocaine blockade of continuously and transiently accessible sites in cardiac sodium channels. *J. Mol. Cell. Cardiol.* 23(Suppl. I):73–83.

Strichartz, G. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37–57.

Strichartz, G. 1976. Molecular mechanisms of nerve block by local anesthetics. *Anesthesiology.* 45:421–441.

Truppman, E. S., and J. D. Ellenby. 1979. Major electrocardiographic changes during chemical face peeling. *Plast. Reconstr. Surg.* 63:44–48.

Uehara, A., and E. Moczydlowski. 1986. Blocking mechanisms of batrachotoxin-activated Na channels in artificial bilayers. *Membr. Biochem.* 6:111–147.

Warner, M. A., and J. V. Harper. 1985. Cardiac disrhythmias associated with chemical peeling with phenol. *Anesthesiology.* 62:366–367.

Weidmann, S. 1955. The effect of calcium and local anesthetics on the electrical properties of Purkinje fibers. *J. Physiol. (Lond.).* 129:568–582.

West, J. W., D. E. Patton, T. Scheuer, Y. Wang, A. Goldin, and W. A. Catterall. 1992. A cluster of hydrophobic amino acid residues required for fast Na^+-channel inactivation. *Proc. Natl. Acad. Sci. USA.* 89: 10910–10914.

Wood, K. M. 1978. The use of phenol as a neurolytic agent: a review. *Pain.* 5:205–229.

Woodhull, A. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* 61:687–708.

Zamponi, G. W., D. D. Doyle, and R. J. French. 1993a. Fast lidocaine block of cardiac and skeletal muscle sodium channels. One site with two routes of access. *Biophys. J.* 65:80–90.

Zamponi, G. W., D. D. Doyle, and R. J. French. 1993b. State-dependent block underlies the tissue specificity of lidocaine action on BTX-activated cardiac sodium channels. *Biophys. J.* 65:91–100.

Zamponi, G. W., P. W. Codding, X. Sui, and R. J. French. 1993c. Dual actions of procainamide on batrachotoxin-activated cardiac sodium channels. Open channel block and prevention of inactivation. *Biophys J.* 65: 2324–2334.