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# Modification of Cardiac $Na^+$ Channels by Batrachotoxin: Effects on Gating, Kinetics, and Local Anesthetic Binding

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ABSTRACT The purpose of the present study was to examine the characteristics of $Na^+$ channel modification by batrachotoxin (BTX) in cardiac cells, including changes in channel gating and kinetics as well as susceptibility to block by local anesthetic agents. We used the whole cell configuration of the patch clamp technique to measure $Na^+$ current in guinea pig myocytes. Extracellular $Na^+$ concentration and temperature were lowered (5–10 mM, 17°C) in order to maintain good voltage control. Our results demonstrated that 1) BTX modifies cardiac $I_{Na}$, causing a substantial steady-state (noninactivating) component of $I_{Na}$; 2) modification of cardiac $Na^+$ channels by BTX shifts activation to more negative potentials and reduces both maximal $g_{Na}$ and selectivity for $Na^+$; 3) binding of BTX to its receptor in the cardiac $Na^+$ channel reduces the affinity of local anesthetics for their binding site; and 4) BTX-modified channels show use-dependent block by local anesthetics. The reduced blocking potency of local anesthetics for BTX-modified $Na^+$ channels probably results from an allosteric interaction between BTX and local anesthetics for their respective binding sites in the $Na^+$ channel. Our observations that use-dependent block by local anesthetics persists in BTX-modified $Na^+$ channels suggest that this form of extra block can occur in the virtual absence of the inactivated state. Thus, the development of use-dependent block appears to rely primarily on local anesthetic binding to activated $Na^+$ channels under these conditions.

## INTRODUCTION

Batrachotoxin (BTX) is a potent modifier of $Na^+$ channels in a variety of excitable cells and is thought to bind to the channel at a binding site specific for lipid-soluble alkaloids (toxin site II, see Ref. 1). Extensive electrophysiological studies in nerve have shown that BTX inhibits inactivation, shifts the voltage dependence of activation, and reduces ionic selectivity of $Na^+$ channels (Ref. 2 and see Ref. 3 for review). The effects of BTX on single nerve $Na^+$ channels have provided a basis for the observations made in intact preparations, demonstrating a reduced single channel conductance, increased open time throughout a depolarizing test step, and a pronounced increase in openings at negative potentials (4).

Additional reports have indicated that binding of BTX to nerve $Na^+$ channels decreases the binding affinity for local anesthetics (LA) such as lidocaine, benzocaine, and procaine (3). The mechanism for this drug interaction is unclear but could be the result of interference of the BTX with local anesthetic binding via an allosteric or a competitive interaction. Also, changes in state-dependent binding may result from alterations in activation and inactivation caused by BTX modification of $Na^+$ channels (5).

In contrast to the large volume of information obtained in nerve preparations, few electrophysiological studies have examined the effects of BTX on $I_{Na}$ in heart. A number of reports have described the effects of BTX to prolong the action potential, increase cardiac force, and decrease resting membrane potential in a variety of cardiac preparations (6, 7). It is assumed that these actions are the result of an increased cardiac $Na^+$ channel open time causing increased $Na^+$ influx; consequently, the positive inotropic action of BTX is thought to occur as the result of an increase in $Na_i^+$ leading to increased $Ca_i^{2+}$ via sodium-calcium exchange (8).

Few voltage clamp studies of BTX effects on cardiac $I_{Na}$ have been performed to substantiate these suppositions. Huang et al. (9) reported that there was less of a negative voltage shift and no change in slope factor of the conductance-voltage curve in neonatal rat myocytes compared to nerve and that slow inactivation of $I_{Na}$ was only partially abolished. There is little information available about the interactions between LA and BTX in heart. These contrasting results between nerve and cardiac $Na^+$ channel response to BTX modification may reflect the differences in protein structure that underlie the two channel types.

The purpose of the present study was to characterize the effects of BTX on cardiac $Na^+$ channels, including alterations in channel gating and current kinetics. In addition, we wanted to characterize the interactions of BTX and LA in cardiac $Na^+$ channels. More specifically, we wanted to determine if there is a decrease in affinity of modified channels for LA in heart and if the change in LA binding affinity for BTX-modified channels occurs as the result of drug binding interactions or of decreased LA binding in the absence of an inactivated state. A preliminary report of some of these observations has appeared in abstract form (10).

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## MATERIALS AND METHODS

### Cell isolation procedures

Guinea pig ventricular myocytes were isolated by a modification of the method described by Mitra and Morad (11). Briefly, a guinea pig was anesthetized with sodium pentobarbital (~30 mg, intraperitoneally), its chest

was opened, and the heart was removed and suspended from a Langendorff apparatus via an aortic cannula. The modified Tyrode's solution had the following composition (in millimolar): NaCl, 135; KCl, 5.4; $MgCl_2$, 1; $CaCl_2$, 1.8; glucose, 5.5; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10; $NaH_2PO_4$, 0.33; brought to pH 7.4 with NaOH. Retrograde perfusion of normal solution at 37°C was maintained at a constant hydrostatic pressure of 100 cm of water. After a period of wash (5 min), the perfusate was changed to an identical solution that was nominally $Ca^{2+}$ free. Perfusion with $Ca^{2+}$-free medium continued for 3 min, at which time perfusion with enzyme solution was initiated. This solution contained collagenase (type XXIV, 0.24 mg/ml; Sigma Chemical Co., St. Louis, MO) and protease (Sigma type A, 0.5 mg/ml). Perfusion was continued for 2.5–5 min. At this time, the perfusate was switched to a KB medium for 5 min. The composition of KB solution was as follows (in millimolar): KCl, 25; $KH_2PO_4$, 10; KOH, 116; glutamic acid, 80; taurine, 10; oxalic acid, 14; HEPES, 10; glucose, 11; brought to pH 7.0 with KOH. The heart was then removed from the apparatus and placed in KB medium where it was minced and filtered through nylon mesh (200 μm). Cells were stored for up to 8 h in KB medium at room temperature.

## Electrophysiological recordings

Recordings of $I_{Na}$ were made using a voltage clamp amplifier and whole cell patch clamp techniques. Micropipettes were pulled from borosilicate capillary tubing with filament (World Precision Instruments TW150F, New Haven, CT) on a P87 Brown-Flaming puller (Sutter Instruments, San Rafael, CA) and had a resistance of about 0.8 MΩ when filled with internal solution. In 30 cells, the time constant of the capacitative transient was 186 ± 10 μs, and the membrane capacitance was measured to be 209 ± 8 pF, yielding an average series resistance of 0.89 MΩ. The low access resistance allowed measurements of peak currents of up to about 6 nA with a voltage error of ≤5 mV in the absence of series resistance compensation. Consequently, series resistance compensation was usually not required because of the low access resistance obtained after sealing, as long as current amplitude was ≤6 nA. In some experiments, slightly larger currents were accepted (e.g., Fig. 1) if current traces showed no other signs of poor voltage control such as an "abominable notch," a delay in activation time or abrupt activation in the threshold voltage range. Series resistance compensation was usually not used, because it did not significantly improve the response time of our recordings using these procedures, assuming that the other criteria for adequate voltage control were met.

Pipettes were filled with solution of the following composition (in millimolar): CsF, 145; NaF, 5; HEPES, 5; brought to pH 7.2 with CsOH. Recordings of $I_{Na}$ were made at 17°C by cooling the external solution as it flowed through a peltier device into the experimental chamber at a rate of 1–2 ml/min. External solution had the following composition (in millimolar): NaCl, 5–10; $MgCl_2$, 1.2; CsCl, 5; $CaCl_2$, 1.8; tetramethylammonium-Cl, 125; glucose, 11; HEPES, 20; brought to pH = 7.4 with tetramethylammonium-OH. The choice of solutions included internal $F^-$ which blocks $Ca^{2+}$ current and internal and external $Cs^+$ which blocks nearly all inward rectifier $K^+$ current thus leaving only tetrodotoxin-sensitive $I_{Na}$ (12).

## Experimental procedures

The general experimental protocol was as follows. An aliquot of cell suspension was placed in the experimental chamber, and the cells were allowed to settle for about 10 min before superfusion with external solution was begun. The pipette was placed in contact with the cell surface, and gentle suction was applied. A high resistance seal (0.8–1 gigaohm) formed within 2 min. Additional suction was applied to break the membrane under the pipette pore, allowing low resistance access to the intracellular space. Holding potential ($V_h$) was −140 mV in all experiments unless stated otherwise. A series of voltage clamp protocols was then performed in the absence of drug. BTX was then added to the superfusate at a predetermined concentration (100 or 200 nM) and allowed to act for about 20 min. A modest effect of BTX was detected after this period. At this time, a rapid train of pulses to −20 mV (50 ms, 10 Hz) was initiated for 20–30 s. This pulse train was repeated each min for 3–5 min until a stable effect of BTX was achieved (3) as indicated by decreased peak current and the development of a large steady-state current component. Superfusion with BTX was terminated after 25–35 min, and the effect of BTX was stable for the remainder of the experiment (up to 2 h).

After recording voltage clamp protocols designed to measure effects of BTX modification of $I_{Na}$, either lidocaine HCl (Pfaltz and Bauer, Inc., Waterbury, CT) or procaine HCl (Sigma) was then added to the superfusate in concentrations ranging from 0.01 to 30 mM. These agents were diluted from a concentrated stock (100 mM) in normal external solution. In some experiments, phenytoin (Sigma) was tested at concentrations ranging from 0.003 to 1 mM. Phenytoin was diluted into external solution from a concentrated stock prepared in dilute NaOH. Recordings were made after 7–10 min of exposure to each concentration of the LA agent. In most experiments, the cell was superfused with drug-free solution after LA in order to confirm the reversibility of drug action as well as the continued effect of BTX. In each case where recovery was attempted, BTX effects were almost fully recovered despite the absence of BTX from the external solution for long periods.

## Data acquisition and statistics

Data were filtered at 5 KHz, digitized at 10 KHz, and stored on a PDP 11/73 computer (Digital Equipment Corporation, Maynard, MA). In most experiments, capacity transients and linear leak were subtracted digitally off-line by proportional scaling of traces recorded during +10 to +50 mV steps in which no ionic current was evoked. Additional data analysis was performed off-line following completion of the experiment using locally developed algorithms. Fitting routines used a least-squares algorithm for single exponential or Boltzmann fit. Data presented here are representative of at least three experiments of each type.

Comparisons of two sample means were made using Student's $t$ test. Paired $t$ tests were used to compare two sample means when a cell served as its own control. A one-way analysis of variance of paired samples was used when two or more groups were compared, such as in experiments where multiple concentrations of one agent were tested. Differences between group means were evaluated with the Newman-Keuls test. A test was considered to be significant when $p < 0.05$. Data are presented as mean ± SE.

# RESULTS

## Effects of BTX on $I_{Na}$ in guinea pig myocytes

The characteristic effects of exposure to BTX (100 nM) are shown in Fig. 1. In control (Fig. 1 *A*), voltage clamp steps from a holding potential of −140 mV to test potentials positive to threshold show normal rapid activation until peak inward current was achieved, followed by a rapid phase of inactivation. In the presence of BTX (Fig. 1 *B*), normal activation was followed by a failure of complete inactivation, leaving a large component of steady-state (*ss*) current remaining at the end of the 100-ms test pulse.

The inset of Fig. 1 *B* shows the approach we used to distinguish between BTX-modified and unmodified channels. Since unmodified channels inactivate completely within about 100 ms, whereas modified channels do not inactivate for many seconds, we used this difference in kinetics to distinguish between the rapidly inactivating (peak − *ss*) current which represents unmodified channels and the *ss* current which represents modified channels. A similar approach was used by Khodorov (3) to distinguish between the two populations of $Na^+$ channels in nerve.

Fig. 1 *C* shows the current-voltage relationships for the *ss* and peak − *ss* (*p* − *ss*) current components. Both control and

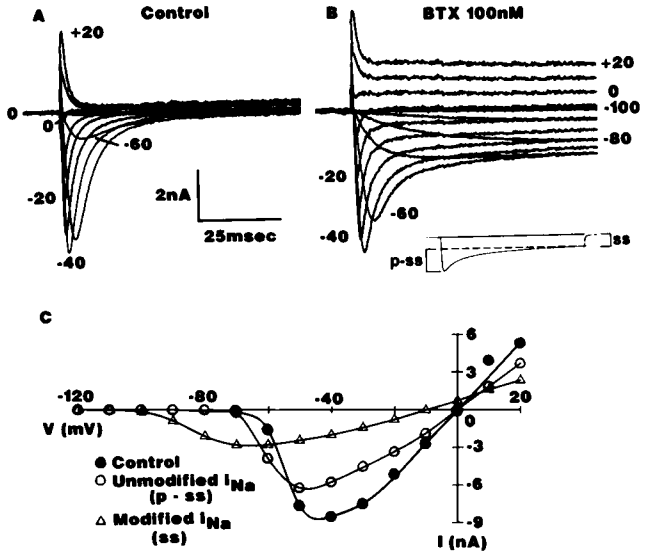


FIGURE 1 Effects of 100 nM BTX on cardiac $I_{Na}$. (*A*) Current recordings obtained from a series of voltage steps to the potentials indicated under control conditions (leak and capacity subtracted). The effects of BTX are illustrated in *B*. Following the peak current, each current trace demonstrates a steady-state (*ss*), noninactivating component. This occurs with both inward and outward currents. Between −60 and −70 mV, there is an inward current component that shows a rapid phase of inactivation prior to relaxation to the *ss* component. The inset of *B* shows the method for separating the inactivating from the noninactivating component; BTX-modified current is represented by the *ss* component, whereas the unmodified current is obtained by subtracting *ss* from the peak current ($p - ss$). *C* shows the current-voltage relationships for this experiment under control conditions (peak current, ●) and after exposure to BTX where the two current components have been separated into unmodified channels ($p - ss$, ○) and modified channels (*ss*, △).

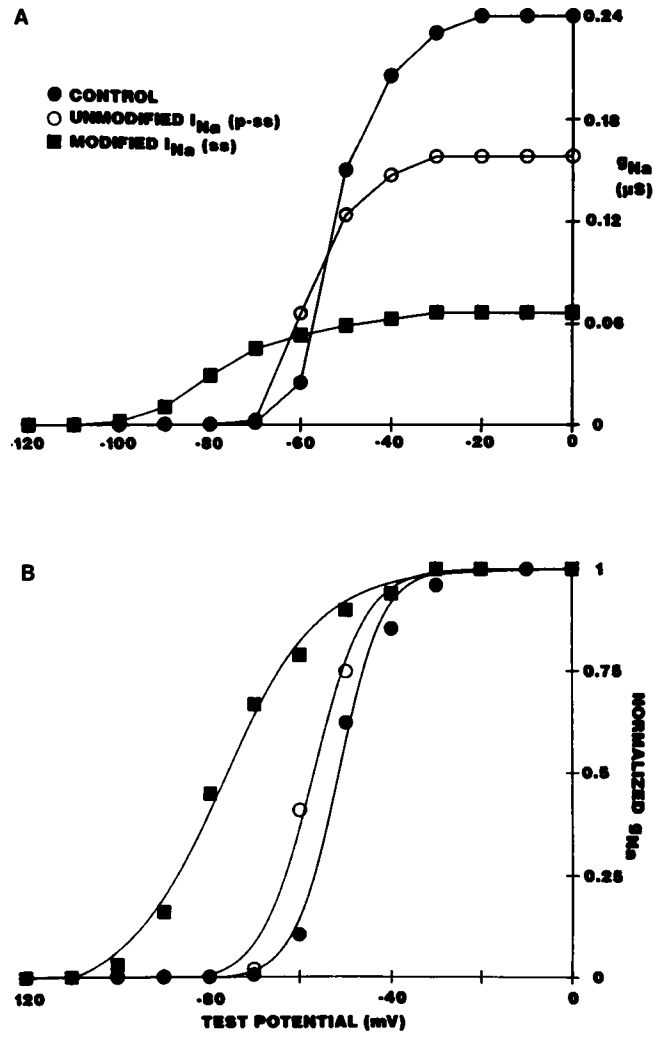


FIGURE 2 Effects of BTX on the voltage dependence of peak $Na^+$ conductance ($g_{Na}$). (*A*) Absolute values of peak $g_{Na}$ before exposure to BTX (●) and both peak − *ss* $I_{Na}$ (○) and *ss* $I_{Na}$ (■) after 30 min of exposure to BTX. (*B*) Normalized $g_{Na}$ for the same data as in *A*. The $g_{Na}$ values were calculated according to the equation $g_{Na} = I_{Na}/(V_t - V_{rev})$, where $I_{Na}$ is the absolute value of $Na^+$ current, $V_t$ is the test potential, and $V_{rev}$ is the reversal potential. The values of $V_{rev}$ were calculated from the best least-squares linear fit to the data ±20 mV to the approximate voltage where current was 0. The values of $g_{Na}$ and $V_t - V_{rev}$ were then fitted to a Boltzmann distribution from which midpoint ($V_m$) and slope ($s$) were calculated according to the equation $g = g_{max}/\{1 + \exp[(V_m - V)/s]\}$, where $V$ is the test potential and $g_{max}$ is the maximal $Na^+$ conductance.

$p - ss$ current activated between −70 and −60 mV and reached a maximum at about −40 mV. Reversal potential was nearly 0 mV, reflecting the fact that $[Na^+]$ in both external and internal solutions was the same (5 mM). In contrast, *ss* currents show the usual characteristics of BTX modification, including a negative shift of reversal potential (−10 mV) and of activation as well as a failure to inactivate during the 100-ms test pulse. These characteristics of BTX modification were also clearly evident in seven additional cells studied after exposure to 200 nM BTX, where the shift in reversal potential was −11.9 ± 3.25 mV ($p < 0.001$) and the shift in activation threshold potential was −50 ± 2.2 mV ($p < 0.001$).

The effects of BTX on $Na^+$ conductance ($g_{Na}$) as calculated from the peak current-voltage relationships are illustrated in Fig. 2. The non-normalized $g_{Na}$-V curves are shown in Fig. 2 *A*; BTX modification caused a shift in the threshold for activation of nearly −40 mV and, presumably, the loss of unmodified channels to the modified pool accounts for the decrease in maximal unmodified $g_{Na}$. When these results are normalized to compare modified and unmodified channels to control (Fig. 2 *B*), the negative shift of the modified channels is quite apparent as is the decrease in the slope factor, *s*. The midpoint, $V_m$, of the normalized $g_{Na}$-V relationship shifted from −51.6 mV in control to −57.1 mV in $p - ss$ (unmodified) and −77.2 mV in *ss* (modified) current. The slope factors for these relationships were 4.4, 5.7, and 10.8 mV, respectively. The summarized results of seven cells (top of Table 1) show that control and unmodified $I_{Na}$ had nearly the same slope but that there was a small shift in $V_m$. The negative shift in $V_m$ of unmodified $I_{Na}$ compared to control can probably be attributed to the time-dependent negative shift of activation (and possibly that of inactivation as well) that occurs as a result of the unavoidable internal dialysis with pipette solution (13, 14). For example, our measurement of a −14 mV shift in unmodified $I_{Na}$ during the 20–30-min period of exposure to BTX is very much in line with the 0.47 mV/min shift found by Hanck and Sheets (14). In a separate series of control experiments, we also found that $V_m$ shifted by about

**TABLE 1 Effects of BTX on Na+ conductance ($g_{Na}$) in guinea pig heart ($n$ = 7)**

| | $V_m$ | $s$ |
|---|---|---|
| BTX 100 nM | mV | |
| Control | −43.2 ± 1.67 | 5.7 ± 0.39 |
| Unmodified $I_{Na}$ | −57.1 ± 3.40* | 5.7 ± 0.70 |
| Modified $I_{Na}$ | −81.2 ± 3.49‡ | 9.4 ± 0.53‡ |
| BTX 200 nM | | |
| Control | −41.6 ± 1.29 | 5.8 ± 0.17 |
| BTX | −84.4 ± 1.33* | 9.3 ± 0.22* |

Data were obtained from a single Boltzmann fit to the relationship between $g_{Na}$ and test voltage as described in the legend of Fig. 2. $V_m$ represents the voltage midpoint and $s$ is the slope factor of this relationship.
* $P < 0.01$ compared to control.
‡ $P < 0.001$ compared to control and unmodified $I_{Na}$. Data are presented as mean ± SE.

−7 mV within a 20–30-min period (unpublished observations). In contrast, BTX modification produced a large and significant shift in the $V_m$ and increase in the slope factor of this relationship.

In a separate series of experiments in which cells were exposed to 200 nM BTX, very little inactivating current remained after BTX-modification (approximately 25 min). The hyperpolarizing shift in $V_m$ and the increase in slope factor were nearly the same as that which occurred during exposure to the lower concentration of BTX (bottom of Table 1). These results also confirm that the *ss* current recorded following exposure to 100 nM BTX represents modified channels.

The effects of 100 nM BTX on the voltage dependence of steady-state $I_{Na}$ availability ($h_\infty$) are shown in Fig. 3. In control, the voltage midpoint of the $h_\infty$ curve ($V_{½}$) was −81.9 mV, and the slope factor ($k$) was 4.7 mV. Following exposure to BTX for 26 min, the unmodified current showed little change in $k$ (3.7 mV), and $V_{½}$ was shifted by −16 mV. This shift in $V_{½}$ is similar to time-matched control values of about −9 mV (unpublished observations) and the value expected from the work by Hanck and Sheets (14) based on a shift of −0.41 mV/min. In contrast, modified current showed little inactivation (67% of maximal current remaining) even at conditioning potentials as low as −20 mV. For purposes of comparison, a second protocol was run which differed from the first only in test potential, $V_t$ = −80 mV, which was chosen because only modified channels open at this potential. A similar fraction of modified current remained under these conditions (80%), demonstrating the close similarity between $h_\infty$ of BTX-modified Na+ channels alone ($V_t$ = −80 mV) and of *ss* current obtained at a $V_t$ = −30 mV. Similar results were obtained in six additional cells and indicate that the rapidly inactivating current component shows normal inactivation gating, whereas the noninactivating, *ss* component demonstrates the changes in Na+ channel availability characteristic of BTX-modified channels.

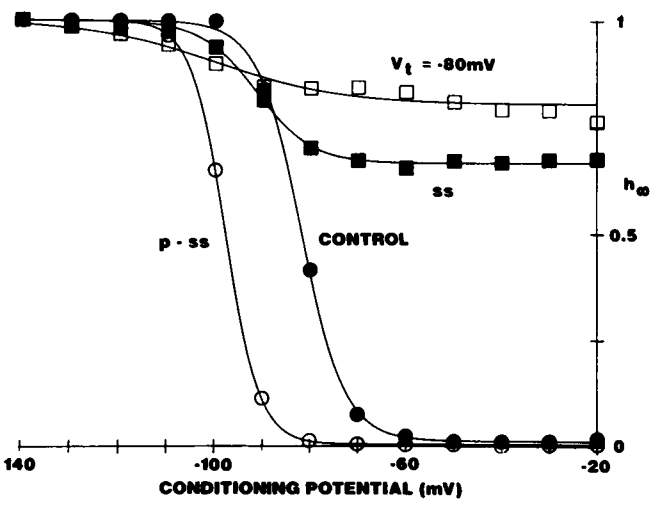


FIGURE 3 Effects of BTX on the voltage dependence of steady-state $I_{Na}$ availability ($h_\infty$). The $V$-$h_\infty$ relationship was generated using a protocol in which a 1-s voltage pulse was applied to various conditioning potentials followed by a return to a test potential ($V_t$) of either −30 or −90 mV. Each pulse pair was delivered after a 10-s interval. Closed circles indicate values of $h_\infty$ before exposure to BTX, open circles represent peak − *ss* current, and closed squares indicate *ss* current following 25 min of exposure to BTX ($V_t$ = −30 mV); open squares indicate peak inward current at $V_t$ = −80 mV. The fitted curves were obtained using the following form of the Boltzmann equation: $h_\infty = 1/\{1 + \exp[(V - V_{½})/k]\}$, where $V_{½}$ is the voltage at half inactivation, $k$ is the slope factor, and $V$ is the test voltage.

## Effects of BTX on recovery from inactivation

We also observed that the rate of recovery from inactivation was slowed by BTX modification of cardiac Na+ channels. Fig. 4 shows results obtained using a protocol in which inactivation was allowed to develop at −20 mV for 1 s, after which membrane potential was returned to −140 mV for a variable interval (1–3000 ms) after which a test pulse to −100 mV was imposed to assess how much modified current had recovered (see inset, Fig. 4 *A*). Under control conditions, recovery was extremely rapid and was completed within 250 ms. The inset of *A* shows original current recordings of both conditioning and test pulses ranging from 1–3000 ms. The earliest test pulse at 1 ms showed no signs of activation, possibly because the return to $V_h$ was too brief to allow deactivation of modified channels. Subsequent current responses occurring after longer recovery intervals showed clear evidence of reactivation but were similar in steady-state amplitude until after 7 ms (*A*, inset), at which time current amplitude began to increase (*A*). Further analysis of the unrecovered fraction of modified $I_{Na}$ revealed that the rate of recovery was biexponential (Fig. 4 *B*) with fast and slow time constants of 75 and 1064 ms, respectively, compared to 4.3 and 78 ms in control. The inset of *B* shows a graph with an expanded time scale which more clearly illustrates the delay in recovery that occurs during brief intervals (<10 ms). Nearly identical results were obtained in all three experiments of this type, with control fast and slow time constants of 4.8 ± 0.66 and 80.1 ± 4.57 ms in control compared to 58.1 ± 11.8 and 862 ± 158 ms following BTX modification. The delay in recovery was consistent in each experiment (approximately 7 ms). These results demonstrate that modified channels show a delay in recovery from inactivation followed by a biexponential recovery rate that suggests that at

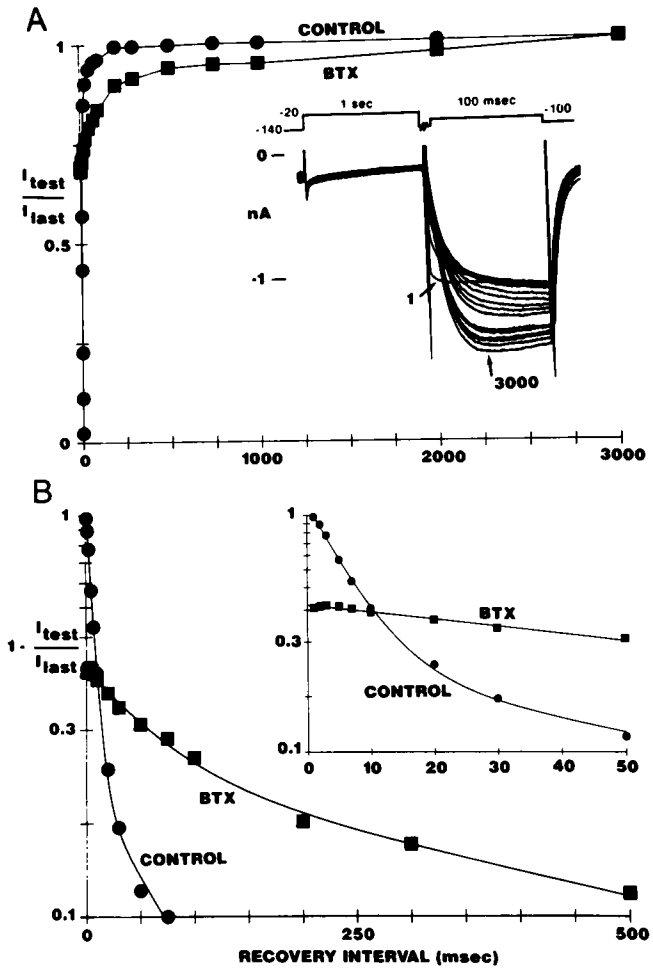


FIGURE 4 Rate of recovery of BTX-modified $I_{Na}$ from inactivation. (*A*) Rate of recovery from inactivation developed during a 1-s conditioning pulse to −20 mV as assessed after a variable recovery period at −140 mV and during a test pulse to −100 mV ($V_h$ = −140 mV). The inset shows a schematic representation of the protocol and original current recordings obtained during exposure to BTX (200 nM, 25 min) which are plotted on the graph as open circles. Closed circles indicate values obtained in control. (*B*) Semi-logarithmic transform of the unrecovered fraction of $I_{Na}$. The inset is a graph with an expanded time scale of the first 50 ms of *B*, showing the delay in the onset of recovery.

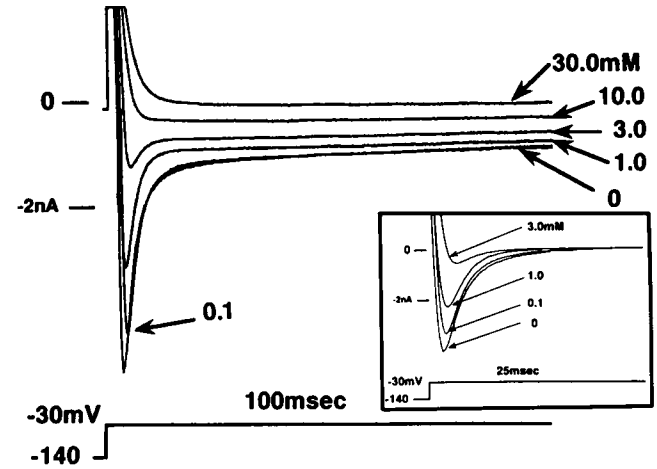


FIGURE 5 Effects of lidocaine (0.1–30 mM) on BTX-modified $I_{Na}$. This figure shows the effects of several concentrations of lidocaine on current evoked at a test potential of −30 mV in a BTX-treated myocyte (100 nM). The trace indicated by 0 was obtained prior to addition of lidocaine to the external solution. Each concentration of lidocaine was allowed to equilibrate for 7–10 min before the concentration was increased. Inset: current recordings are presented as subtracted traces (peak current − steady state) in order to indicate block of unmodified current only.

least two, and possibly three, inactivated states are prevalent in BTX-modified channels.

## Effects of lidocaine on unmodified and BTX-modified $I_{Na}$

Fig. 5 shows the single pulse ("tonic") blocking effects of different concentrations of lidocaine on both components of $I_{Na}$. The figure shows the effects of lidocaine on $I_{Na}$ after treatment with 100 nM BTX at a test potential of −30 mV. The inset shows the same current traces presented as subtracted traces ($p$ − $ss$). The lowest concentration of lidocaine (0.1 mM) reduced peak current by 20% but had no effect on $ss$. When lidocaine concentration was increased to 1 mM, the $p$ − $ss$ current was blocked by an additional 25%, while $ss$ current showed the first evidence of block, a reduction of maximal $ss$ current by about 10%. When lidocaine concentration was increased to 3 mM, nearly all of this current was blocked (inset). However, less than 30% of the modified current was blocked. Greater block occurred when the concentration of lidocaine was further increased to 10 mM, and nearly all modified current was blocked when the lidocaine concentration was increased to 30 mM. These results demonstrate that unmodified $Na^+$ channels are more sensitive to block by lidocaine than are BTX-modified channels. This decrease in sensitivity to block by LA was also observed with procaine and phenytoin (see below).

## Concentration dependence of procaine and lidocaine block of unmodified and modified $I_{Na}$

Fig. 6 summarizes the concentration dependence of single pulse $I_{Na}$ block by lidocaine (*A*) and procaine (*B*). Block was assessed at a test potential of −20 mV from a holding potential of −140 mV. The filled circles show the effects of the local anesthetic agent alone in the absence of BTX. The number of experiments for each data point ranged from 5 to 13. The effects of increasing drug concentration on unmodified and BTX-modified channels are shown in the figure. The unmodified current had virtually the identical sensitivity to anesthetic block as control current, whereas BTX modification reduced the sensitivity to block by both local anesthetics. In *A*, the control apparent dissociation constant, $K_d$, for lidocaine was 0.54 mM which was very similar to the value obtained for unmodified current (0.69 mM). In contrast, $K_d$ for modified current was 11.12 mM, indicating a decrease in affinity for lidocaine binding by about 15-fold. In *B*, the $K_d$ for procaine in untreated myocytes was 0.81 mM compared to 0.82 mM in unmodified current of BTX-treated

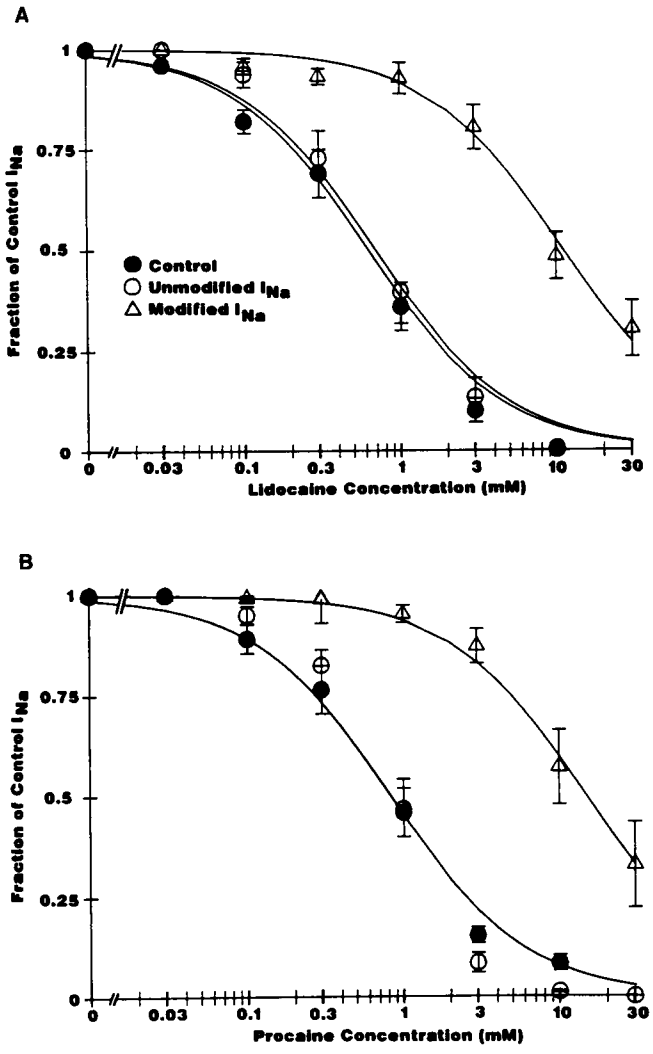


FIGURE 6 Concentration dependence for procaine and lidocaine block of unmodified and modified $I_{Na}$. (*A*) Summarizes effects of lidocaine on $I_{Na}$ in control (●) and after exposure to BTX (100 nM), giving both unmodified (○) and modified (△) current components. Curves were fitted to a single binding site model using the equation: $y = 1/[1 + (K_d/x)]$ where $x$ is drug concentration, $y$ is normalized current amplitude, $K_d$ is the apparent drug dissociation constant, and the Hill coefficient is assumed to equal 1. *B* applies the same analysis to block by procaine. Values were taken from current recordings obtained during a test voltage step to −20 mV following leak and capacity subtraction. Each point represents the mean ± SE of five to 13 experiments.

cells. The $K_d$ for modified channels was 14.66 mM, indicating a 17-fold decrease in binding affinity for modified channels compared to unmodified channels.

Although not indicated in these figures, we also tested the effects of BTX modification on binding of phenytoin to cardiac $Na^+$ channels. We found that the $K_d$ for unmodified channels was 0.33 mM, whereas that in modified channels was 1.9 mM ($n$ = 3), representing an increase of nearly 5-fold. This represents only a modest decrease in binding affinity compared to the other agents tested under identical experimental conditions.

These results demonstrate that BTX modification of cardiac $Na^+$ channels decreases the blocking potency for all three local anesthetic agents tested. Despite the similarity in the amount of decrease in potency for procaine and lidocaine, however, BTX appears to cause less of an increase in $K_d$ for phenytoin than for the other two agents.

## Use-dependent block of BTX-modified $I_{Na}$ by local anesthetics

We studied the effects of BTX modification of $Na^+$ channels on use-dependent block in cardiac cells. Since use-dependent block is usually the result of drug interactions with activated and/or inactivated channels, we expected that use-dependent block by local anesthetic agents would by altered by modification of channel inactivation (and possibly activation) by BTX. We chose to examine the development of use-dependent block in BTX-modified channels by taking advantage of the shift in activation voltage caused by BTX (200 nM). Consequently, the development of use-dependent block exclusively in modified channels was assessed by applying a train of 20 pulses from $V_h$ = −140 mV to a test potential of −90 mV, a voltage 10–20 mV negative to the activation range for unmodified channels but within the range of activation of modified channels.

The effects of lidocaine and procaine on BTX-modified $I_{Na}$ during a train of 20 pulses from −140 to −90 mV (100-ms duration, 500-ms interpulse interval) are shown in Fig. 7. Prior to addition of local anesthetic (*A*), the slow activation rate and virtual absence of $I_{Na}$ decay of the first pulse of the train indicate that nearly all $I_{Na}$ activated under these conditions was the result of opening of BTX-modified $Na^+$

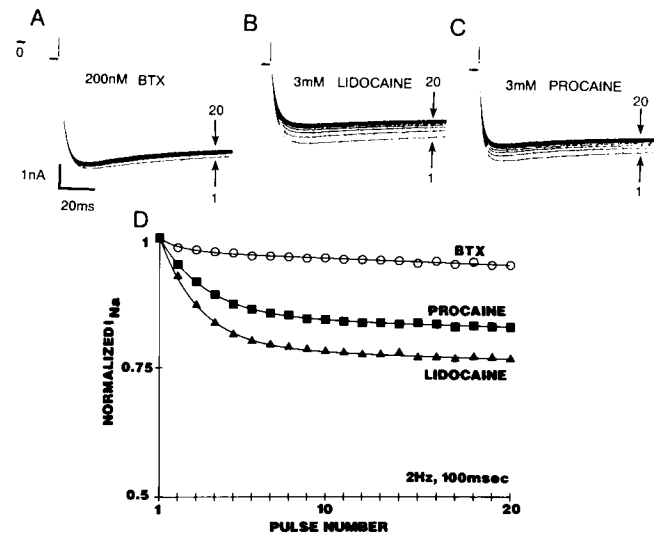


FIGURE 7 Use-dependent block of BTX-modified $I_{Na}$ by local anesthetics. Use-dependent block was tested by applying a train of 20 pulses, 100 ms in duration with an interpulse interval of 500 ms. $V_h$ = −140 mV. Test potential was −90 mV in order to activate only BTX-modified channels. The current amplitude is plotted as a function of the pulse number during the train. (*A*) Current responses following exposure to BTX (200 nM); (*B*) responses to test pulses in the presence of lidocaine (3 mM for 10 min); (*C*) responses to procaine (3 mM for 10 min). Recordings were not leak or capacity subtracted; (*D*) normalized current magnitude for each test pulse from the data in *A–C*.

channels. The maximal amplitude of the second pulse was slightly smaller than that of the first pulse but subsequent pulses were nearly identical in magnitude thereafter. During exposure to lidocaine (3 mM for 10 min (*B*)), the first pulse was markedly decreased compared to control (tonic block). There was a marked decrease in peak amplitude for each of the next seven pulses, after which a steady state was achieved.

Washout of lidocaine from and addition of procaine to the external solution (3 mM for 10 min (*C*)) caused the reappearance of use-dependent block during the subsequent pulse train. The effects of procaine were also eliminated after a 15-min recovery period in drug-free superfusate (not shown).

The development of use-dependent block is compared for each condition in *D*, which shows normalized current magnitude throughout the pulse train. Lidocaine caused a decrease of 23% and procaine reduced current by 17% compared to the first pulse, whereas *ss* current declined by only 5% following exposure to BTX alone. In a total of seven cells, BTX-modified $I_{Na}$ declined by 4.4 ± 1.0%, whereas both lidocaine and procaine caused a significant use-dependent decrease in $I_{Na}$ magnitude (20.3 ± 1.9% and 17.7 ± 5.6%, $p < 0.01$ for each). Although not studied systematically, we also observed that the amount and onset of use-dependent block were influenced by test pulse rate (interpulse intervals ranging from 300 to 1000 ms) and duration (10–100 ms).

These results demonstrate that use-dependent interactions between local anesthetic agents and BTX-modified cardiac $Na^+$ channels are maintained despite the fact that drug affinity for these channels has been diminished. More importantly, the persistence of use-dependent block under conditions where little inactivation is allowed to develop suggests that repetitive $Na^+$ channel activation plays a very important role in and is, perhaps, primarily responsible for the development of use-dependent block under these experimental conditions.

## DISCUSSION

### Characteristics of BTX modification of cardiac $I_{Na}$

Our results demonstrate that modification of $Na^+$ channels by BTX reduces $g_{Na}$ as well as the selectivity of the channel for $Na^+$, as evidenced by a negative shift in reversal potential. In addition, normal channel gating is markedly altered by BTX, as indicated by the negative shift in activation voltage and the $g_{Na}$-V relationship as well as the abolition of the normal inactivation process, just as they are in nerve (3). These observations are similar to those made in neonatal rat cardiac cells by Huang et al. (9); however, those authors reported that preincubation with 1 μM BTX for 2 h was required to produce partial loss of inactivation, whereas exposure to 8 μM was necessary to cause nearly complete failure to inactivate. In the present experiments, the voltage midpoint for activation was shifted to more negative potentials by nearly −43 mV, a value that is approximately halfway between those reported in neuroblastoma (−50 mV (15)) and in rat neonatal heart (−30 mV, and see Ref. 9 for comparisons). Given the known differences in pharmacological sensitivity and current gating between nerve and heart, it is somewhat surprising that activation in adult guinea pig heart responds so differently from neonatal rat heart. The contrast is retained in the effects of BTX on the slope factor of the $g_{Na}$-V relationship; Huang et al. (9) reported that BTX had no effect on the slope factor, whereas our results are similar to those of Tanguy and Yeh (5) in squid axon and in other nerve preparations (3) showing a marked decrease in slope factor. We found that there was a significant increase in slope factor during exposure to 200 nM BTX . However, the lack of a period of rapid stimulation even in the presence of BTX could explain the reduced shift in conductance and the apparent decreased sensitivity reported in rat neonatal heart compared to other preparations. The highly lipid-soluble, very slowly acting BTX seems to require a period of rapid repetitive activations in order to achieve full activity (5, 16). This behavior is consistent with its reported action to interact with the $Na^+$ channel in the open state (3, 5, 16, 17). Alternatively, it is possible that rat neonatal cardiac cells simply have a lower sensitivity to BTX than adult guinea pig myocytes. The basis for these differences in activation remains to be determined.

Our results also indicate that fast inactivation (<100 ms) is almost completely abolished in modified channels. In contrast, slow inactivation (i.e., that which develops over 1 s) is only partly abolished by BTX, as also reported in rat heart by Huang et al. (9). Although we did not examine the effects of BTX on very slow inactivation (>1 min), Huang et al. (9) reported that BTX also reduced this component of inactivation by 70–75% in neonatal rat heart.

Recovery from inactivation, once it has developed, was biexponential just as it is in normal cardiac $I_{Na}$. Similar results were recently reported in $GH_3$ cells (18) and taken to mean that there are at least two inactivated states for BTX-modified channels, just as there are in normal channels. However, unlike the results in $GH_3$ cells, modified cardiac cells showed a delay in recovery from inactivation suggesting the presence of a third process governing recovery rate of BTX-modified channels, one that acts as a rate-limiting or absorbing state from which modified channels must exit before recovery from inactivation can begin. The many profound differences between experimental conditions and methods might also contribute to the disparate results obtained in the two preparations, and additional study is required to describe more completely the processes underlying the recovery from inactivation of BTX-modified $I_{Na}$.

In agreement with other observations in nerve, we also found that BTX-induced modification of cardiac $I_{Na}$ was extremely stable over time once the full effect was achieved at any given BTX concentration (3, 19). This last property made it possible to stop superfusion with BTX after its full effect was realized and continue with the experiment for up to 2 h without reversal of BTX effects.

## Effect of BTX to alter blocking potency of local anesthetics

One of the most important pharmacological characteristics of $Na^+$ channels is their sensitivity to block by LA. In heart, this effect has important clinical applications since most of the clinically effective antiarrhythmic agents belong to this class of drugs. It is assumed that LA block of $I_{Na}$ in excitable cells is the result of a specific interaction with a binding site on the channel protein. This binding site is presumably located in the lipophilic core of the channel, since a lipophilic pathway is thought to be important to drug access to and removal from the channel (20, 21). In addition, binding of LA to their receptor is thought to be dependent on the channel state, controlled either by changes in binding affinity with channel state (modulated receptor hypothesis (20, 21)) or by changes in access to a receptor with a constant affinity (guarded receptor hypothesis (22)).

The first electrophysiological evidence indicating the ability of BTX to reduce the binding of LA to their receptor on the $Na^+$ channel was reported by Khodorov et al. (23). These investigators found that BTX "protected" modified $Na^+$ channels from block by procaine. Numerous studies have since reported that BTX modification also reduces the blocking potency of other LA in electrophysiological experiments (see Ref. 3) and using radioisotope flux measurements (24). These reports complement biochemical studies showing that different LA reduced binding affinity of a radiolabeled BTX analog in isolated nerve preparations (25). Taken together, these results strongly suggested that there is a competitive interaction between binding of BTX and LA as demonstrated directly by Huang and Ehrenstein (26) using ion flux measurements.

Previous electrophysiological results indicated that BTX increased $K_d$ for tonic (first-pulse) block and completely abolished use-dependent block, regardless of the type of LA tested; these included agents considered to have a high affinity for activated channels, such as QX-572, quinidine, and ethmozine as well as those thought to bind preferentially to inactivated channels, including lidocaine and trimecaine (16, 27, 28). The increase in values for $K_d$ was from 0.48 mM in normal channels to 5.62 mM in modified channels for procaine and from 1.21 mM in unmodified channels to 5.66 mM in modified channels for lidocaine in neuroblastoma cells (2).

Our results in cardiac cells demonstrate striking qualitative similarities to those described in nerve. The affinity for block of unmodified channels by LA was nearly identical to that of previously untreated cardiac channels. In contrast, BTX modification caused nearly the same decrease in affinity for lidocaine (15-fold decrease) and for procaine (17-fold decrease). These decreases in binding affinity are greater than corresponding changes reported in nerve (4-fold decrease for lidocaine, 11-fold for procaine (Zubov et al., 1983)). The basis for these differences is not yet clear but could be the result of differences in binding site characteristics of the $Na^+$ channel subtypes from the two tissues involved. Interestingly, phenytoin caused less of a decrease in binding affinity (about 5-fold) which is more consistent with the results in nerve.

Our values for $K_d$ were calculated using a single site model fit to the concentration-effect curve. The Hill coefficient ($n_H$) was assumed to be 1 in these calculations and the results were well fitted in general by this scheme. The only apparent exception to this was the block of unmodified $I_{Na}$ by procaine, where an $n = 1.2$ improved the fit somewhat (data not shown); however, the fit is surprisingly good when considering the fact that these data, in fact, represent subtracted values of $p - ss$ current. We suspect that the error inherent in this technique provides a more likely explanation for a deviation from $n = 1$ than cooperativity of procaine binding to unmodified channels following partial modification of $I_{Na}$ by BTX.

Although the present results do not provide a precise explanation for the reduction of blocking potency of LA for BTX-modified $I_{Na}$, it is possible that BTX binding to and modification of cardiac $Na^+$ channels prevents LA binding via a drug interaction in the channel (24, 25, 29). This could be the result either of a direct interaction at a common binding site suggesting a competitive antagonism (26) or, as appears more likely, an indirect interaction resulting from simultaneous occupation of separate binding sites that produces allosteric interactions of the ligands (29). It is also worth noting that, after complete LA block of BTX-modified channels was achieved, nearly a full recovery to the BTX-modified state was accomplished during superfusion with drug-free solution without rapid repetitive stimulation, suggesting that BTX was still bound to the channels. Thus, the interaction between agents did not result in a displacement of BTX from its receptor, a result also reported in nerve (3) that could possibly lend support to an allosteric rather than a competitive interaction between LA and BTX.

## Use-dependent block by local anesthetics of BTX-modified channels

Our results show that use-dependent block by procaine and lidocaine persists in BTX-modified cardiac $Na^+$ channels for trains of pulses with durations up to 100 ms. Our results are in distinct contrast to those reported for unmodified and BTX-modified channels in nerve (3) in which rapid repetitive pulses of durations up to 50 ms in nerve failed to induce any appreciable use-dependent decline in current amplitude. Even agents with strong affinity for activated channels such as strychnine (28) failed to produce use-dependent block in nerve, suggesting that the conventional state-dependent binding affinities no longer determined the binding interaction between LA and modified $Na^+$ channels.

If use-dependent block by certain LA is the result of a higher affinity for inactivated than for activated channels, as might be expected from the modulated receptor hypothesis (20, 21), then abolition of the inactivated state by BTX would

prevent this high affinity binding from producing use-dependent block. Consequently, we expected that the loss of inactivation (and/or alterations in activation) would in turn alter the drug affinity for state-dependent binding to the receptor on the BTX-modified $Na^+$ channels and thus result in the reduction or absence of use-dependent block. Our experimental results demonstrated quite clearly, however, that use-dependent block of BTX-modified $I_{Na}$ developed quite normally. This observation suggests that the presence of normal inactivation might be unnecessary for the development of use-dependent block in the heart.

Our original supposition arose from reports of an important role for the inactivation gate in use-dependent block of $I_{Na}$ by LA as suggested by several lines of evidence. First, voltage-dependent $Na^+$ channel availability is decreased (inactivation is increased) in the presence of LA (20, 21). Second, gating charge immobilization occurs both in inactivated nerve $Na^+$ channels and in use-dependent blocked $Na^+$ channels (30). Third, removal of fast inactivation by pronase or *N*-bromoacetamide abolishes or reduces the ability of the quaternary derivative of lidocaine, QX-314, to produce use-dependent block of $I_{Na}$ in nerve (31, 32). Finally, the time and voltage dependence of lidocaine block indicates an important contribution of drug interaction with inactivated channels to the development of use-dependent block in several cardiac preparations (12, 33, 34).

Results from several reports using other $Na^+$ channel toxins, however, suggest that the loss of *rapid* inactivation in itself is inadequate to prevent use-dependent block by LA and found similar results to ours. In nerve, LA continued to produce use-dependent block when inactivation was markedly slowed by either anthopleurin-A toxin (from *Anthopleura xanthogrammica*) or by veratridine (35). We have found that blocking fast inactivation in cardiac cells under the same experimental conditions as those employed here with anthopleurin-A does not prevent use-dependent block by either lidocaine or procaine (Wasserstrom et al., unpublished observations). In addition, longer pulses caused more block, suggesting that interaction with open channels is responsible for use-dependent block under these conditions. Since modification of inactivation with these toxins failed to prevent use-dependent block by LA, it therefore seems unlikely that an intact inactivation process alone is responsible for use-dependent block.

Other observations in nerve shed additional light on the basis for use-dependent block in normal and modified $Na^+$ channels. Yeh and Tanguy (36) and Wang et al. (37) demonstrated that use-dependent block by QX-314 persists after the elimination of fast inactivation with chloramine-T. The basis for this maintained use-dependent block may be that QX-314 is trapped in the open channel during repolarization but that the drug itself then induces the modified channel to enter an inactivated state (38). Their conclusion was that both association with the activated state and the ability of a drug-bound channel to enter an inactivated, drug-bound state are required for the use-dependent block by LA. It is clear that use-dependent block is a complex manifestation of several processes occurring simultaneously in the $Na^+$ channel and that, in all likelihood, LA interactions with both activated and inactivated channels will ultimately determine the characteristics of use-dependent block.

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## REFERENCES

1. Catterall, W. A. 1980. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* 20: 15–43.
2. Zubov, A. N., A. P. Naumov, and B. I. Khodorov. 1983. Effect of batrachotoxin (BTX) on activation, inactivation and ion selectivity of sodium channels in clonal neuroblastoma cells. *Gen. Physiol. Biophys.* 2:75–77.
3. Khodorov, B. I. 1985. Batrachotoxin as a tool to study voltage-sensitive sodium channels of excitable membranes. *Prog. Physiol. Mol. Biophys.* 45:57–148.
4. Quandt, F. N., and T. Narahashi. 1982. Modification of single $Na^+$ channels by batrachotoxin. *Proc. Natl. Acad. Sci. USA.* 79:6732–6736.
5. Tanguy, J., and J. Z. Yeh. 1991. BTX modification of Na channels in squid axon. I. State dependence of BTX action. *J. Gen. Physiol.* 97: 499–519.
6. Hogan, P. M., and E. X. Albuquerque. 1971. The pharmacology of batrachotoxin. III. Effect on the heart Purkinje fibers. *J. Pharmacol. Exp. Ther.* 176:529–537.
7. Honerjager, P., and M. Reiter. 1977. The cardiotoxic effect of batrachotoxin. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 299:239–252.
8. Reuter, H., and N. Seitz. 1968. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol. (Lond.).* 195:451–470.
9. Huang, L.-Y. M., A. Yatani, and A. M. Brown. 1987. The properties of batrachotoxin-modified cardiac Na channels, including state-dependent block by tetrodotoxin. *J. Gen. Physiol.* 90:341–360.
10. Liberty, K., P. Santucci, J. Kelly, M. Myers, and J. A. Wasserstrom. 1991. Batrachotoxin reduces the affinity of local anesthetic agents for cardiac $Na^+$ channels. *Biophys. J.* 59:97*a*. (Abstr.)
11. Mitra, R., and M. Morad. 1985. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *Am. J. Physiol.* 249:H1056-H1060.
12. Clarkson, C. W., C. H. Follmer, R. E. TenEick, L. M. Hondeghem, and J. Z. Yeh. 1988. Evidence of two components of sodium channel block by lidocaine in isolated cardiac myocytes. *Circ. Res.* 63:869–878.
13. Fernandez, J. M., A. P. Fox, and S. Krasne. 1984. Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary ($GH_3$) cells. *J. Physiol. (Lond.).* 356:565–585.
14. Hanck, D. A, and M. F. Sheets. 1992. Time-dependent changes in kinetics of $Na^+$ channel current in single canine Purkinje cells. *Am. J. Physiol.* 262:H1197-H1207.
15. Huang, L.-Y. M., N. Moran, and G. Ehrenstein. 1982. Batrachotoxin modifies the gating kinetics of Na channels in internally perfused neuroblastoma. *Proc. Natl. Acad. Sci. USA.* 79:2082–2085.
16. Khodorov, B. I., E. M. Peganov, S. V. Revenko, and L. D. Shishkova. 1977. Gating and selectivity of sodium channels in Ranvier node treated with batrachotoxin. Effects of local anesthetics. *Proceedings of the International Union of Physiological Sciences* 12:195. (Abstr.)
17. Khodorov, B. I., and S. V. Revenko. 1979. Further analysis of the mechanisms of action of batrachotoxin on the membrane of myelinated nerve. *Neuroscience.* 4:1315–1340.
18. Wang, G. K., and S-Y. Wang. 1992. Inactivation of batrachotoxin-modified $Na^+$ channels in $GH_3$ cells. *J. Gen. Physiol.* 99:1–20.

19. Tanguy, J., and J. Z. Yeh. 1988. Batrachotoxin uncouples gating charge immobilization in squid giant axons. *Biophys. J.* 54:719–730.

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

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

22. Starmer, C. F., A. O. Grant, and H. C. Strauss. 1984. Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. *Biophys. J.* 46:15–27.

23. Khodorov, B. I., E. M. Peganov, S. V. Revenko, and L. D. Shishkova. 1975. Sodium currents in voltage-clamped nerve fiber of frog under the combined action of batrachotoxin and procaine. *Brain Res.* 84:541–546.

24. Catterall, W. A. 1981. Inhibition of voltage-sensitive sodium channels in neuroblastoma cells by antiarrhythmic drugs. *Mol. Pharmacol.* 20: 356–362.

25. Creveling, C. R., E. T. McNeal, J. W. Daly, and G. B. Brown. 1983. Batrachotoxin-induced depolarization and [$^3$H]batrachotoxinin-A 20-$\alpha$-benzoate binding in a vesicular preparation from guinea pig cerebral cortex. *Mol. Pharmacol.* 23:350–358.

26. Huang, L-Y., and G. Ehrenstein. 1981. Local anesthetics QX 572 and benzocaine act at separate sites on the batrachotoxin-activated sodium channel. *J. Gen. Physiol.* 77:137–153.

27. Khodorov, B. I. 1978. Chemicals as tools to study nerve fiber sodium channels. Effects of batrachotoxin and some local anesthetics. *In* Membrane Transport Processes. D. Tosteson, Yu. A. Ovchinnikov, and R. Latorre, editors. Raven Press, New York. 153–174.

28. Zaborovskaya, L. D., and B. I. Khodorov. 1982. Reversible blockage of batrachotoxin-modified sodium channels by amine compounds and benzocaine in frog node of Ranvier. *Gen. Physiol. Biophys.* 1:283–285.

29. Willow, M., and W. A. Catterall. 1982. Inhibition of binding of [$^3$H]-batrachotoxinin-A 20-$\alpha$-benzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine. *Mol. Pharmacol.* 22:627–635.

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

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

32. Yeh, J. Z. 1982. A pharmacological approach to the structure of the Na channel in squid axon. *In* Proteins in the Nervous System: Structure and Function. B. Haber, J. R. Perez-Polo, and J. D. Coulter, editors. Alan R. Liss, Inc., New York. 17–49.

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

34. Sanchez-Chapula, J., Y. Tsuda, and I. R. Josephson. 1983. Voltage and use-dependent effects of lidocaine on sodium current in rat single ventricular cells. *Circ. Res.* 52:557–565.

35. Khodorov B. I., and L. D. Zaborovskaya. 1983. Blockage of sodium and potassium channels in the node of Ranvier by ajmaline and N-propyl ajmaline. *Gen. Physiol. Biophys.* 2:233–268.

36. Yeh, J. Z., and J. Tanguy. 1986. The activation gate modulates use-dependent block of Na channels by QX-314. *Biophys. J.* 49:43a. (Abstr.)

37. Wang, G. K., M. S. Broderick, D. C. Eaton, and G. R. Strichartz. 1987. Inhibition of sodium currents by local anesthetics in chloramine-T–treated squid axons. *J. Gen. Physiol.* 89:645–667.

38. Tanguy, J., and J. Z. Yeh. 1989. QX-314 restores gating charge immobilization abolished by chloramine-T treatment in squid giant axons. *Biophys. J.* 56:421–427.