Blockade of cardiac sodium channels. Competition between the permeant ion and antiarrhythmic drugs.

M J Barber, … , C F Starmer, A O Grant

J Clin Invest. 1992;90(2):368-381. https://doi.org/10.1172/JCI115871.

Research Article

A number of basic and clinical studies suggest that elevation of external sodium concentrations, [Na]o, may reverse the cardiotoxic effect of local anesthetic-class drugs. The mechanisms of reversal are uncertain. The blocking action of lidocaine and disopyramide were studied over a range of [Na]o. Both whole-cell voltage clamp and single-channel recordings were performed on isolated rabbit myocytes at 17 and 22 degrees C, respectively. In the presence of lidocaine, an inactivated channel blocker, the level of steady-state block in response to pulse train stimulation was not affected by variations in [Na]o from 20 to 150 mM. Estimates of the rate of dissociation of drug from the channel also were unaffected. In contrast, steady-state block by disopyramide, a drug that blocks open channels, was decreased as [Na]o was increased. Single-channel measurements suggest that the influence of [Na]o on channel current amplitude was small, 12% for a 25 mM increase in [Na]o. This increase in single-channel current amplitude would affect drug-free channels only, in that our studies suggest that drug-associated channels do not conduct. The association rate constant of disopyramide with open single sodium channels was decreased from 10 x 10(6) to 5 x 10(6)/M per s by an increase in [Na]o from 120 to 180 mM. Elevation of [Na]o may reverse the blocking action of local anesthetic-class drugs by an increase […]

Blockade of Cardiac Sodium Channels

Competition between the Permeant Ion and Antiarrhythmic Drugs

Michael J. Barber, David J. Wendt, C. Frank Starmer, and Augustus O. Grant
Department of Medicine, Duke University Medical Center, Durham, North Carolina 27706; and Department of Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Abstract

A number of basic and clinical studies suggest that elevation of external sodium concentrations, $[Na]_o$, may reverse the cardiotoxic effect of local anesthetic-class drugs. The mechanisms of reversal are uncertain. The blocking action of lidocaine and disopyramide were studied over a range of $[Na]_o$. Both whole-cell voltage clamp and single-channel recordings were performed on isolated rabbit myocytes at 17 and 22°C, respectively. In the presence of lidocaine, an inactivated channel blocker, the level of steady-state block in response to pulse train stimulation was not affected by variations in $[Na]_o$ from 20 to 150 mM. Estimates of the rate of dissociation of drug from the channel also were unaffected. In contrast, steady-state block by disopyramide, a drug that blocks open channels, was decreased as $[Na]_o$ was increased. Single-channel measurements suggest that the influence of $[Na]_o$ on channel current amplitude was small, 12% for a 25 mM increase in $[Na]_o$. This increase in single-channel current amplitude would affect drug-free channels only, in that our studies suggest that drug-associated channels do not conduct. The association rate constant of disopyramide with open single sodium channels was decreased from 10×10^6 to 5×10^6/M per s by an increase in $[Na]_o$ from 120 to 180 mM. Elevation of $[Na]_o$ may reverse the blocking action of local anesthetic-class drugs by an increase in single-channel current amplitude or by a decrease in drug association rate with the sodium channel. The occurrence of the latter action depends on the mode of block of the specific agent. (*J. Clin. Invest.* 1992. 90:368–381.) Key words: disopyramide • lidocaine • patch clamp • sodium channel

Introduction

The local anesthetic-class of antiarrhythmic drugs is believed to exert at least a part of its therapeutic effect by blockade of the inward sodium current, I_{Na} (1). Paradoxically, a small proportion of patients actually may show aggravation of their underlying arrhythmias with drug treatment (2). When taken in accidental or intentional overdose, arrhythmias are an important manifestation of toxicity. These arrhythmias are associated with marked QRS widening and may result from an exaggeration of the Na channel-blocking action of these drugs. Cardiac arrhythmias with marked QRS widening also are important signs of toxicity with certain psychotrophic drugs such as the tricyclic antidepressants and the phenothiazines (3, 4). A number of case reports and experimental studies suggest that these drug-induced arrhythmias may be reversed partially by the administration of Na lactate, Na bicarbonate, Na sulfate, or Na chloride (5–14). The underlying mechanisms of arrhythmia reversal by these Na salts are not clear.

Potential mechanisms by which Na salts may reverse drug-induced arrhythmias fall into three broad categories: (*a*) alteration of drug distribution or disposition with a net decrease in plasma concentration, (*b*) amelioration of associated hypotension and acidosis, or (*c*) increasing the Na current by action(s) at the level of the cell membrane. In vivo and in vitro experiments suggest a central role in elevation of $[Na]_o$ per se, rather than volume expansion or pH changes (e.g., see references 8, 12, 14). This implies an important role of the third potential mechanism of reversal. An examination of the relationship between the concentration of the permeant ion and blocking drug also may provide insight into the blocking mechanism. In nerve, block of potassium channels by internal quaternary ammonium ions is relieved by elevation of external potassium. Entry of external potassium ions into a channel changes the binding energy of receptor-bound quaternary ammonium ion leading to more rapid dissociation. Such a scheme is compatible with an ion conduction pathway which may be doubly occupied (15, 16).

Few studies have examined the relationship between $[Na]_o$ and drug-induced Na current blockade at the membrane level in cardiac muscle. Cox and West (8) showed that Na lactate, chloride, or sulfate decreased the phase zero rise time and conduction time in quinidine-treated rabbit atria. The quinidine-induced prolongation of action potential duration also was reversed. Jensen and Katzung (17) showed that a 50% or 100% increase of $[Na]_o$ reversed the increase in action potential phase zero rise time induced by diphenylhydantoin in rabbit atria. Using V_{max} as a measure of available Na conductance, Kohlhardt (18) showed that lidocaine produced greater decreases of Na current as $[Na]_o$ was reduced. He identified two components of block, phasic and tonic. Tonic block was more sensitive to variations in $[Na]_o$.

In this study, we have explored the potential mechanisms by which external sodium concentration may influence the blocking action of lidocaine, a drug which primarily blocks inactivated sodium channels, and disopyramide, a drug which blocks open sodium channels (1, 19). Macroscopic sodium current was measured in cultured atrial myocytes under voltage clamp. The small cell size and spherical shape of the cultured atrial myocyte permitted voltage clamp of the Na current at $[Na]_o$ of up to 150 mM. In freshly isolated ventricular myocytes and Purkinje cells, $[Na]_o$ must be reduced substantially below 150 mM to achieve voltage control (20, 21). The whole-

Address reprint requests to Dr. Grant, Box 3504, Duke University Medical Center, Durham, NC 27706.

Received for publication 4 November 1991 and in revised form 6 February 1992.

J. Clin. Invest.

0021-9738/92/08/0368/14 $2.00
Volume 90, August 1992, 368–381

cell voltage clamp experiments show that variation of $[Na]_o$ did not influence the level of steady-state block during lidocaine exposure whereas block was enhanced at low $[Na]_o$ during disopyramide exposure. The I_{Na} during drug exposure is given by the following relationship: $I_{Na} = iN(1 - b)P_o$, where i is the single channel current amplitude, N is the number of functioning channels, $(1 - b)$ the fraction of unblocked channels, and P_o the probability that a channel is open. It is readily apparent that elevation of $[Na]_o$ may increase i by augmenting the driving force for inward movement of Na ions across the membrane. The magnitude of this effect was evaluated with single-channel recordings. A number of studies have suggested that local anesthetics may actually block along the ion conducting pathway of the Na channel (22–24). McDonald et al. (24) have suggested that despite primary blockade of channels in the inactivated state, lidocaine-associated Na channels may still conduct, but with a reduced open time. By increasing current density, Na ions could potentially displace receptor-bound drugs, leading to their more rapid dissociation. A reexamination of this question using single-channel recordings found no evidence of conduction of lidocaine-associated channels under our recording conditions. On the other hand, we were able to show that low sodium increases the association rate constant of disopyramide with single sodium channels, suggesting competition between external sodium ions and drug for the blocking site on the channel. We conclude that the influence of the permeant ion concentration on the blocking action of antiarrhythmic drugs may depend importantly on their blocking mechanism. Some of these results have been communicated in abstract form (19, 25).

Methods

Cell preparation. The experiments were performed on isolated rabbit atrial and ventricular myocytes obtained from rabbits weighing 3–5 kg. Our methods of cell preparation have been described previously (26, 27) but will be outlined briefly. The hearts were isolated, excised from anesthetized rabbits, and transferred to a modified Langendorff perfusion system where a 5-min perfusion with Ca^{2+}-free Krebs-Henseleit buffer (K-H[1]; see below for solution composition) was performed. For the remainder of the procedure, sterile technique was utilized and all solutions were maintained at 37°C. After 5 min, the K-H Ca^{2+}-free perfusate was changed to K-H solution containing 180 U/ml collagenase (Worthington Diagnostics, Freehold, NJ) and 0.1 mg/ml hyaluronidase (Sigma Chemical Co., St. Louis, MO) and the hearts perfused for 25–45 min. Enzyme activity was terminated by perfusing the heart with K-H solution and 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan, UT). Ventricular tissue was separated from the atria, minced in K-H solution, filtered, washed three times, and suspended in medium consisting of Ham's F-12 and Dulbecco's minimal essential medium (DME) in a ratio of 1:1, 10% fetal bovine serum, penicillin G (1 U/ml), and streptomycin (0.5 U/ml). The atria were placed in a preheated petri dish containing gassed K-H solution, 10% fetal bovine serum, 1 U/ml penicillin G, and 0.5 U/ml streptomycin. The tissue was minced into small segments, transferred to K-H medium containing elastase (0.5 mg/ml; Sigma Chemical Co.) and gently agitated. Samples were incubated in a water bath at 37°C for 15–30 min until adequately dissociated. Upon completion of the dissociation procedure, single myocytes were separated from tissue chunks by filtration through a 200-μm nylon mesh. The isolated myocytes were washed twice with K-H solution and resuspended in a medium consisting of Ham's F-12/DME/antibiotics as above. Cells were then plated onto 18 × 18 mm laminin-coated coverslips and stored in a CO_2 incubator at 37°C until utilized. Under these conditions, rodlike atrial cells assume a spherical shape after 24–48 h in culture. Atrial cells were utilized after they had assumed a spherical configuration as theoretical analysis has indicated that the spherical shape is most favorable for obtaining a homogeneous potential under voltage-clamp conditions (28). Ventricular cells were utilized 2–8 h after isolation.

Solutions. The K-H solution used in the cell isolation procedure had the following composition (mM): NaCl 118.2, $CaCl_2$ 2.7, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 2, and glucose 10. Calcium-free K-H solution had no added calcium. K-H solution was gassed with a 95% oxygen-5% carbon dioxide mixture.

For whole-cell recording, the standard intracellular solution had the following composition (mM): CsF 120, $MgCl_2$ 5, K_2ATP 5, KH_2PO_4 1, EGTA 5, glucose 5, and Hepes 10. pH of the internal solution was adjusted to 7.3 on the day of the experiment using CsOH (1 M). Internal solution was filtered with a 0.2-μm filter at time of use. Four separate external solutions were prepared and contained 20, 37.5, 75, or 150 mM NaCl with equimolar substitution by CsCl for NaCl to make the combined Na^+/Cs^+ concentrations 150 mM. All external solutions also (mM): $MgCl_2$ 1, KCl 5, $CaCl_2$ 1.5, glucose 5, and Hepes 10. The pH of the external solution was adjusted to 7.4 on the day of the experiment with CsOH.

For cell-attached patch recordings the standard micropipette solution had the following composition (mM): NaCl 180, $MgCl_2$ 5., $CaCl_2$ 0.2, CsCl 1.0, and Hepes 5. The pH was adjusted to 7.4 with NaOH. For studying the effects of $[Na]_o$ on single channels, the sodium concentration of the pipette solution was varied from 100 to 350 mM. The isolated myocytes were superfused in these experiments with a high external potassium solution of the following composition (mM): KCl 70, KAspartate 80, NaCl 5, $MgCl_2$ 3, K_2EGTA 0.05, Hepes 5, NaH_2PO_4 1.2, and glucose 10. The pH was adjusted to 7.4 with KOH. The high external potassium solution was utilized to depolarize the cells to ~ 0 mV and enable us to quote membrane potentials as absolute voltages.

In whole-cell and some of the single channel experiments, the cells were perfused with the external solution containing lidocaine (80 μM) or disopyramide (100 μM). On the day of each experiment, fresh stock solutions of lidocaine or disopyramide were added to the external solution in appropriate amounts to give the desired final concentration of drug.

Recording techniques. Micropipettes were pulled from 1.5 mm o.d. borosilicate glass (N-51A, Drummond Scientific, Broomall, PA) using a horizontal puller (Sutter P 80/PC Flaming Brown). Micropipettes were coated with Sylgard 184 (Dow Corning Corp., Midland, MI), to lower microelectrode capacitance and then fire polished on a microforge (model MF-83, Narishige USA, Inc., Greenvale, NY). For whole-cell current recordings, 400–1,000 kΩ microelectrodes were used. For single-channel recordings, we used 5–10 MΩ microelectrodes. Each microelectrode was coupled to the input of the patch-clamp amplifier (EPC 7, Adams and List Corp., Westbury, NY; or model 3900, Dagan Corp., Minneapolis, MN) by an Ag/AgCl wire-coated Teflon up to its tip (In Vivo Metric Systems, Healdsburg, CA). A similar wire was used to make a bath reference which was inserted in an agar bridge (3% agar dissolved in the same solution used in the recording microelectrode). With this bridge, voltage off-set typically 1–5 mV. These were nulled before obtaining recordings.

Whole-cell currents were filtered at a corner frequency of 5 kHz with an 8-pole Bessel filter (model 902, LPF Frequency Devices, Haverhill, MA) and digitized using a Compaq 386-20 microcomputer at 40 kHz. Single-channel currents were filtered at 2.5 kHz and digitized at 25 kHz. Data were stored on magnetic tape and analyzed off-line (see data analysis).

Experimental protocols. On the day of the study, an 18 × 18 mm coverslip containing the atrial cells was fixed to the base of the recording chamber which sat on a thermostatically controlled peltier device (TS-4 Thermal Microscope Stage, Sensortek, Clifton, NJ) on the stage

1. *Abbreviation used in this paper:* K-H, Krebs-Henseleit (buffer).

of an inverted microscope (Nikon Diaphot, Nikon Inc., Instrument Group, Melville, NY). The temperature was set at 17°C, and the bath was perfused at 1 ml/min with cooled external solution. The inflow to the bath was controlled by a series of solenoid valves which permitted the rapid switch of perfusion solutions. To record in the whole-cell configuration, a giga-ohm seal was obtained as outlined by Hamill et al. (29). The capacitance of the microelectrode and amplifier input were nulled. The membrane patch was then ruptured by a brief pulse of suction and the cell capacitance nulled. Finally, series compensation was applied to a level just below that which produced ringing (usually 70–90%). The holding potential was fixed at −130 mV and an *I-V* curve was obtained as shown in Fig. 1. Voltage error, peak current, and series resistance were determined using readings from the patch-clamp amplifier and from the equation: $\Delta V = I_p\ (1 - a)R$, where ΔV is voltage error, I_p is peak current, a is % compensation, and R is series resistance. If ΔV was > 3 mV, the experiment was abandoned.

Prior to obtaining other information, the *I-V* relation was determined using 50-ms pulses of increasing amplitude applied at 1,500-ms intervals. The pulse was incremented in 5-mV steps from a potential of −120 to +70 mV. We proceeded with the experimental protocol if the currents on the negative limb of the *I-V* curve showed a gradual increase with progressively larger depolarizations (onset to peak sodium currents spanning 30–40 mV; see Fig. 1). A steady-state inactivation curve was determined using 1 s prepulses to potentials from −170 to −50 mV followed by a 10-ms test pulse to −30 mV. The prepulse potential was incremented 5 mV after each subsequent test.

In the first series of experiments, trains of 40 pulses (50-ms duration; holding potential −130 mV; step to −30 mV) with a frequency of 4 Hz were applied from the holding potential to determine the amount of steady-state block in the absence or presence of drug(s) (see below). In these studies, current from the first pulse and last pulse of the train were measured and steady-state block expressed as a percent of the first pulse current. To determine recovery from steady-state block, pulse trains were followed by a variable interval of recovery (100–5,000 ms) to the holding potential and a subsequent 20-ms test pulse to −30 mV. Each pulse train was separated from the preceding train by 15 s. The development of frequency-dependent block was determined by the application of trains of 50 ms pulses to −30 mV from the holding potential of −130 mV with stimulus frequency ranging from 0.1 to 5 Hz. There were 40 pulses in trains with a stimulus frequency of 4 Hz. A complete set of data was obtained at one $[Na]_o$ and the level changed. At least 10 min were allowed for complete solution change. The experimental protocol was then repeated. We varied the order of solution change in each cell.

The protocol to examine the development and recovery from block during disopyramide exposure had to be modified significantly. To determine the onset kinetics and steady-state level of block, trains of 60 50-ms pulses were applied. There was little rest recovery from block. To determine the kinetics of drug dissociation from the channel, a protocol was devised to examine use-dependent unblocking. Because of the complex nature of the protocol, it is described in results along with an illustration of the pulse paradigm.

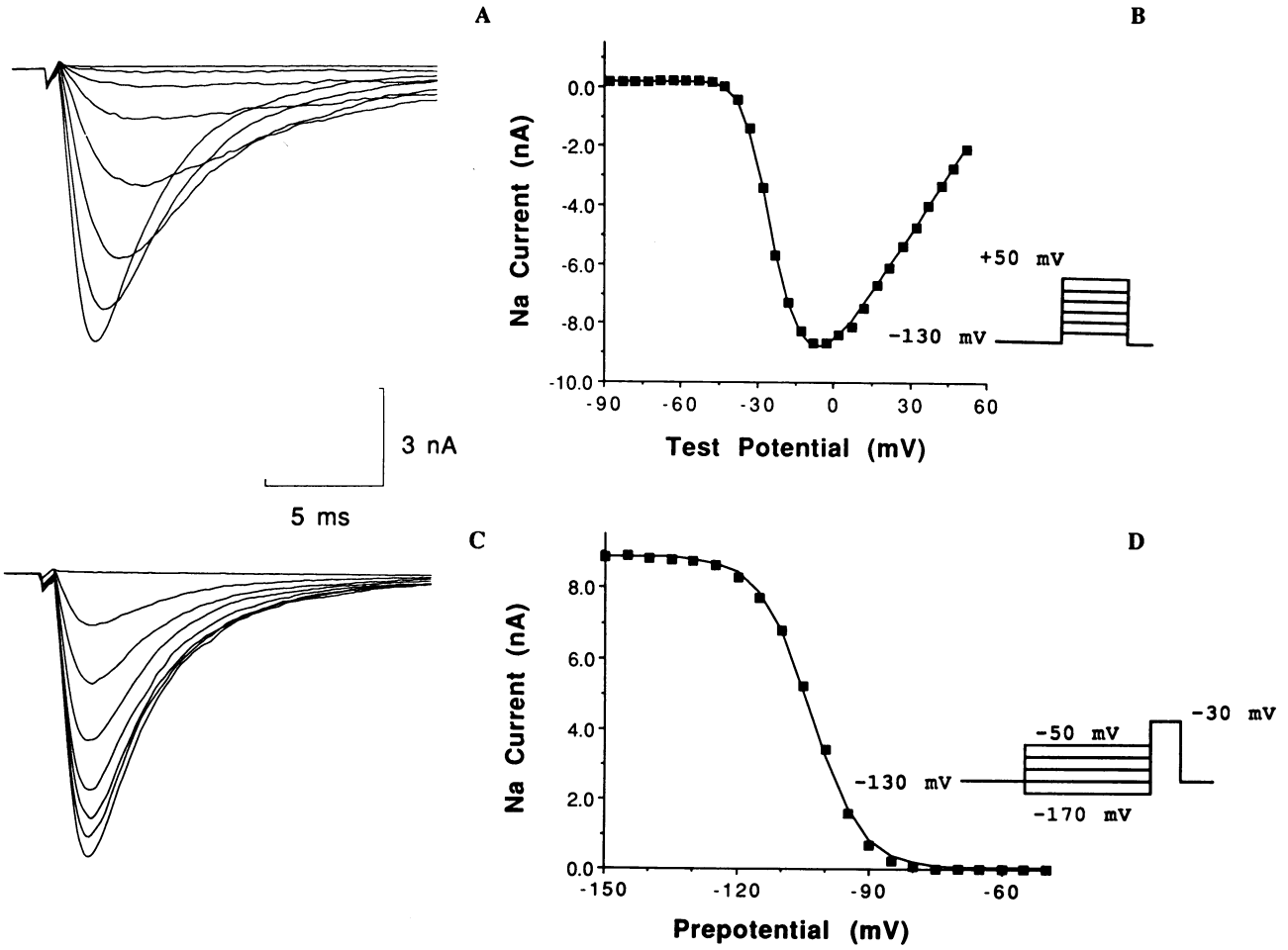

Figure 1. Sodium channel current–voltage relationship and inactivation in 150 mM $[Na]_o$. (*A*) Sodium channel currents elicited by 50-ms voltage-clamp steps from a holding potential of −130 mV to test potentials of varying amplitude incremented in 5-mV steps. (*B*) The peak value of current is plotted as a function of test potential. (*C*) Currents elicited by test pulses at −30 mV that were preceded by 1-s conditioning pulses to various potentials. (*D*) The derived inactivation curve. The time and current calibrations refer to *A* and *C*.

Single Na channel currents were recorded from cell-attached patches according to the method of Hammil et al. (29). For all single-channel experiments, there is a waiting period of 7–10 min after gigaohm seal formation before any data were collected. This delay covered the period during which the most marked spontaneous change in single-channel kinetics occurs. We used three sets of protocol. In one protocol, the effect of $[Na]_o$ on single-channel current amplitude, i, was measured with microelectrodes containing 100, 140, 180, 250, or 350 mM sodium. A single $[Na]_o$ was used for each patch. The test potential was set at −30 mV. We varied the holding potential between patches such that some null sweeps were obtained for leakage and capacity transient subtraction for each patch. The kinetics of block of open sodium channels by disopyramide was examined in myocytes in which inactivation and deactivation were markedly slowed by perfusing the cells with 10 μM deltamethrin (kindly provided by Wellcome Research Laboratories [Drs. R. H. Brooke and S. N. Irving] and Roussel UCLAF [Dr. P. Joubin]). In ~ 15% of depolarizing epochs, a sodium channel bursts for most of the 200-ms depolarizing step. Data were obtained during control and following exposure of the cells to 100 μM disopyramide. Micropipette solution was similar to that previously described, but $[Na]_o$ was either 120 or 180 mM. In the final series of experiments, we used a protocol designed to reexamine the question of whether lidocaine-associated sodium channels do conduct. Because the preliminary experiment by McDonald et al. (24) suggesting this effect was performed in ventricular myocytes, we also did our experiment in these cells. We selected a test potential of −30 mV. At this potential, overlapping events in multicellular patches are inescapable. Simulations show that our software can provide reliable estimates of single channel open times when events overlap. However we limited our analysis to patches that apparently contained three or fewer channels. From the holding potential, a 1-s conditioning pulse was followed by a recovery interval of 500 ms and then a 40-ms test pulse also to −30 mV. The 500-ms recovery interval permitted the recovery of drug-free channels from inactivation. The test pulse and the subsequent conditioning pulse were separated by a rest period of 5 s. We analyzed the data if a minimum of 100 such conditioning and test pulses were obtained.

Data analysis. As noted above, filtered currents from whole-cells were digitized and subsequently transferred to a SUN 4/280 microcomputer (SUN Microsystems, Inc., Mountainview, CA) where the peak values of individual current traces were determined using custom software developed in our laboratory and written in "C" programming language. These peaks were plotted and exponentials fitted to the data describing the recovery from block and the development of block and inactivation using the Marquardt routine or Gauss-Newton method (30). From the residual error an F statistic was calculated and considered to provide a good fit if a significance level of less than 0.05 was seen.

The filtered single-channel currents also were analyzed on the SUN microcomputer. To reduce the size of the files, we analyzed the first 100 ms of the conditioning pulse for the third series of experiments. At −30 mV, the averaged current relaxed toward zero in about 20 ms. On rare occasions in which prolonged bursts of opening were observed in the conditioning or test pulse, these were omitted from analysis. Residual leakage and capacity current were reduced by subtracting the average of all null sweeps from each sweep. Single-channel current amplitude was determined by plotting a histogram of all points during the ensemble of depolarizing trials, or by averaging the amplitude of all the depolarizations in the ensemble. We used an automatic detection algorithm to identify single channel events and their duration. The performance of the algorithm was checked routinely by direct comparison of the original and idealized records. Analysis of the effect of disopyramide on open sodium channel was performed on depolarizing epochs with bursts at least 5 ms in duration (greater than five times the mean duration of the unmodified channel). The association rate constant, k, of drug with the open channel was calculated according to the relationship: $k = \{1/\langle td\rangle - 1/\langle tc\rangle\}\ 1/D$, where $\langle td\rangle$ was the mean open time during disopyramide exposure, $\langle tc\rangle$ the mean open time in the absence of drug, and D the disopyramide concentration. Because $\langle td\rangle$ was much less than $\langle tc\rangle$, k largely depend $\langle td\rangle$.

In reporting results, all data are expressed as mean±standard deviation (SD). The statistical significance of the differences was determined using the appropriate Student's t test for paired or unpaired data. When multiple group comparisons were made, a one-way or two-way analysis of variance was performed. When an analysis of variance demonstrated a significance between some data group(s), a t test or Scheffe test was used to determine significance between individual means. Significance was considered to be present when a P value < 0.05 was achieved. Statistical techniques were taken from Gilbert (31) and Snedecor and Cochran (32).

Results

It is important to show that adequate voltage control could be achieved in these experiments, particularly for the highest external sodium concentration used (150 mM). Because the likelihood of simultaneous recordings with two microelectrodes in our 15–20-μm cells is extremely small, we used indirect criteria to assess voltage control. Fig. 1 shows membrane currents recorded from an isolated atrial myocyte. With increasing depolarization from −130 mV a graded increase in inward sodium current was observed. The negative limb of the complete current-voltage curve (panel *B*) spanned 35 mV. Peak Na current was 9 nA. Membrane currents obtained with 1,000-ms conditioning pulses and test pulses to −30 are shown in panel *C*, and the derived inactivation curve is shown in panel *D*. As the current amplitude was reduced by inactivation, there was no crossover of the respective current traces. The gradual rise in current and absence of crossover of current waveforms as current magnitude was varied suggest adequate voltage control. We accepted the data for further analysis if these two criteria were met (negative limb of the *I-V* curve spanned > 30 mV; no crossover of current wave forms for inactivation curve).

Block by lidocaine. Initially, we examined the phasic block of the sodium current during exposure to 80 μM lidocaine as $[Na]_o$ was varied from 20 to 150 mM. Two levels of $[Na]_o$ were examined in a given cell. The order of exposure to different $[Na]_o$ was varied. The results during exposure to 150 and 20 mM $[Na]_o$ in a cell are illustrated in Fig. 2. With $[Na]_o$ of 150 mM the sodium current declined rapidly to a steady-state level during the application of trains of 50-ms pulses. The decline could be fitted with a single exponential with a pulse constant of 1.3 pulses. The fractional steady-state block was 65%. In $[Na]_o$ of 20 mM, the decline in sodium current also was fitted by a single exponential with pulse constant of 0.85 pulses. The steady-state block was 68%. Mean values for all experiments showed uptake pulse constants of 1.3±0.3 (n = 23) and 1±0.5 (n = 11) pulses with $[Na]_o$ of 150 and 20 mM, respectively ($P < 0.05$). At both external sodium concentrations, onset of block was very rapid. There was a trend towards greater phasic block with $[Na]_o$ of 20 mM compared to 150 mM. However, this difference did not achieve statistical significance (Table I).

In a number of studies, a biexponential decline of the sodium current with pulse train stimulation was observed during lidocaine exposure (33, 34). Block of activated and inactivated sodium channels were proposed as the basis for the biexponential decline. The studies showing a biexponential decline were performed with $[Na]_o$ of 20 mM or less. Studies from our laboratory showed a single exponential decline in Na during exposure to lidocaine with $[Na]_o$ of 75 mM or greater (26). In an

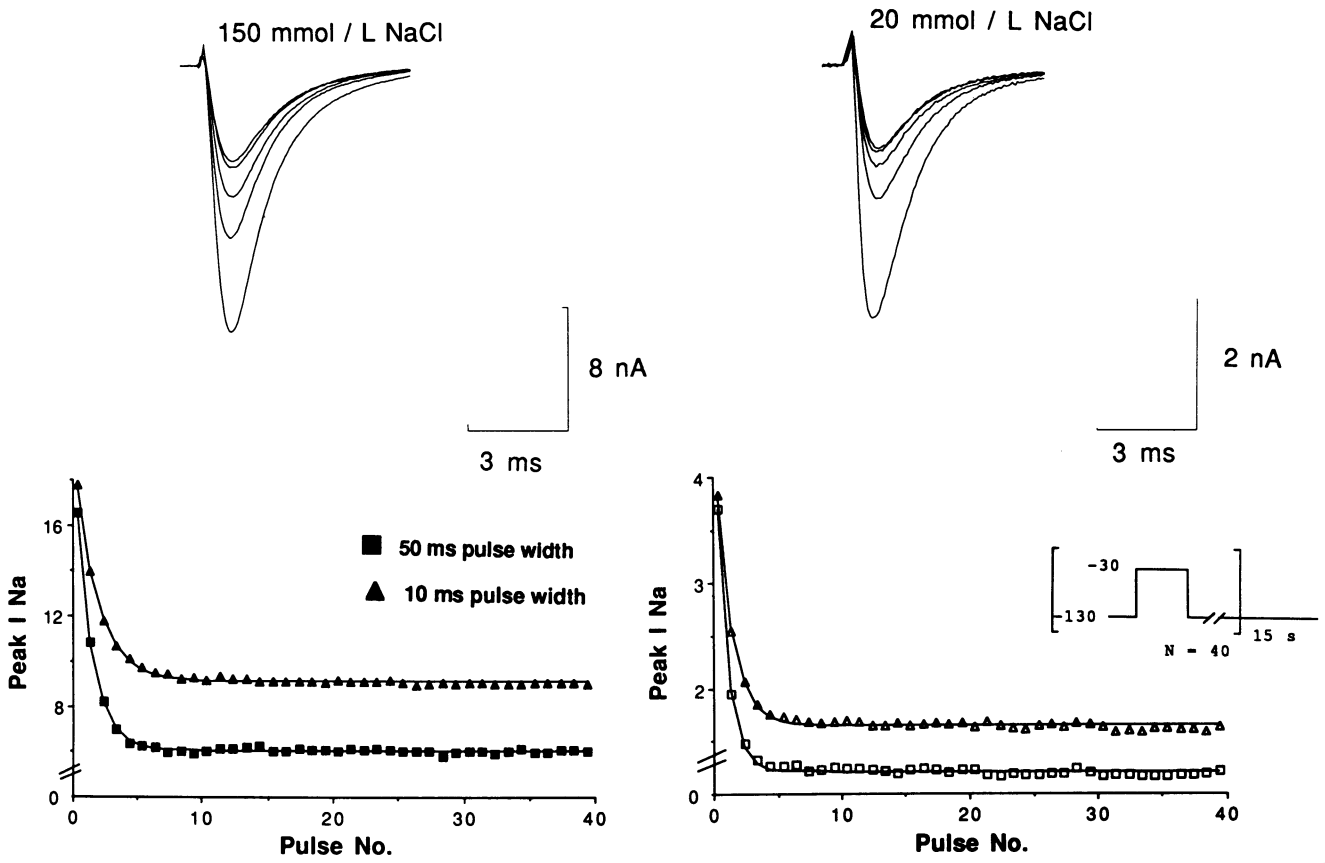

Figure 2. Kinetics of block development during stimulation with trains of 10- and 50-ms pulses during exposure to 80 μM lidocaine with $[Na]_o$ of 150 and 20 mM. The upper panel of the figure shows current in response to 50- and 10-ms pulse trains of interpulse intervals of 250 ms and holding and testing potentials of −130 and −30 mV, respectively. In the lower panel, peak current amplitude is plotted as a function of the pulse number in the train. The straight lines are single exponential fits to the declining current. For 150 mM $[Na]_o$, the time constant for the development of block was 1.8 and 1.3 pulses for pulse durations of 10 and 50 ms, respectively. The corresponding values for 20 mM were 1.03 and 0.85 pulses, respectively. L, liter.

attempt to slow the overall decline and resolve a second component of block, we used pulses of 10-ms duration in six experiments with $[Na]_o$ of 20 and 150 mM. In the experiment illustrated in Fig. 2, decreasing the duration of the pulses to 10 ms slowed the onset of block (e.g., with $[Na]_o$ of 150 mM uptake pulse constant was 0.85 and 1.03 for the 50- and 20-ms pulses, respectively). However, at both external sodium concentrations, a single exponential produced a good fit to the current decline.

Table I. Effects of Varying $[Na]_o$ on Steady-state Block and Recovery from Block during Exposure to 80 μM Lidocaine

$[Na]_o$	Percent steady-state block	τ_r	peak I_{Na}
mM		*ms*	*nA*
150 (n = 24)	52±3	908±75	7.0±1.0
75 (n = 8)	49±5	898±99	5.1±0.6
37 (n = 10)	52±4	920±80	3.1±0.7
20 (n = 6)	57±5	888±98	2.3±0.5

Parameters: $[Na]_o$ = Na concentration in superfusate; τ_r = time constant of recovery; peak I_{Na} = peak measured Na current.

We compared the steady-state block of inward and outward sodium currents during lidocaine exposure. For these experiments, the internal micropipette [Na] was 10 mM. By increasing the internal sodium concentration and using large changes in test potential, the driving force for sodium ions (V-V_{Na}, where V is the test potential and V_{Na} the sodium reversal potential) could be changed markedly. In three experiments, the rising phase and peak of the outward current was well resolved from the capacitive current. One of these experiments is illustrated in Fig. 3. From a holding potential of −130 mV, outward sodium currents were elicited by a depolarizing pulse to +100 mV and inward currents by a depolarizing pulse to −30 mV. At both potentials, the sodium current declined progressively during pulse train stimulation. The steady-state level of block was 60% and 62% for test potentials of +100 and −30 mV, respectively. The average fractional block for these two potentials were 0.53±0.15 and 0.54±0.1. Similar results were obtained at test potentials of +60 and +80 mV.

The steady-state block during lidocaine exposure is dependent both on the development of block during the pulses, and recovery from block in the interval between pulses. Block onset is very fast at all $[Na]_o$. However, opposing effects of variation

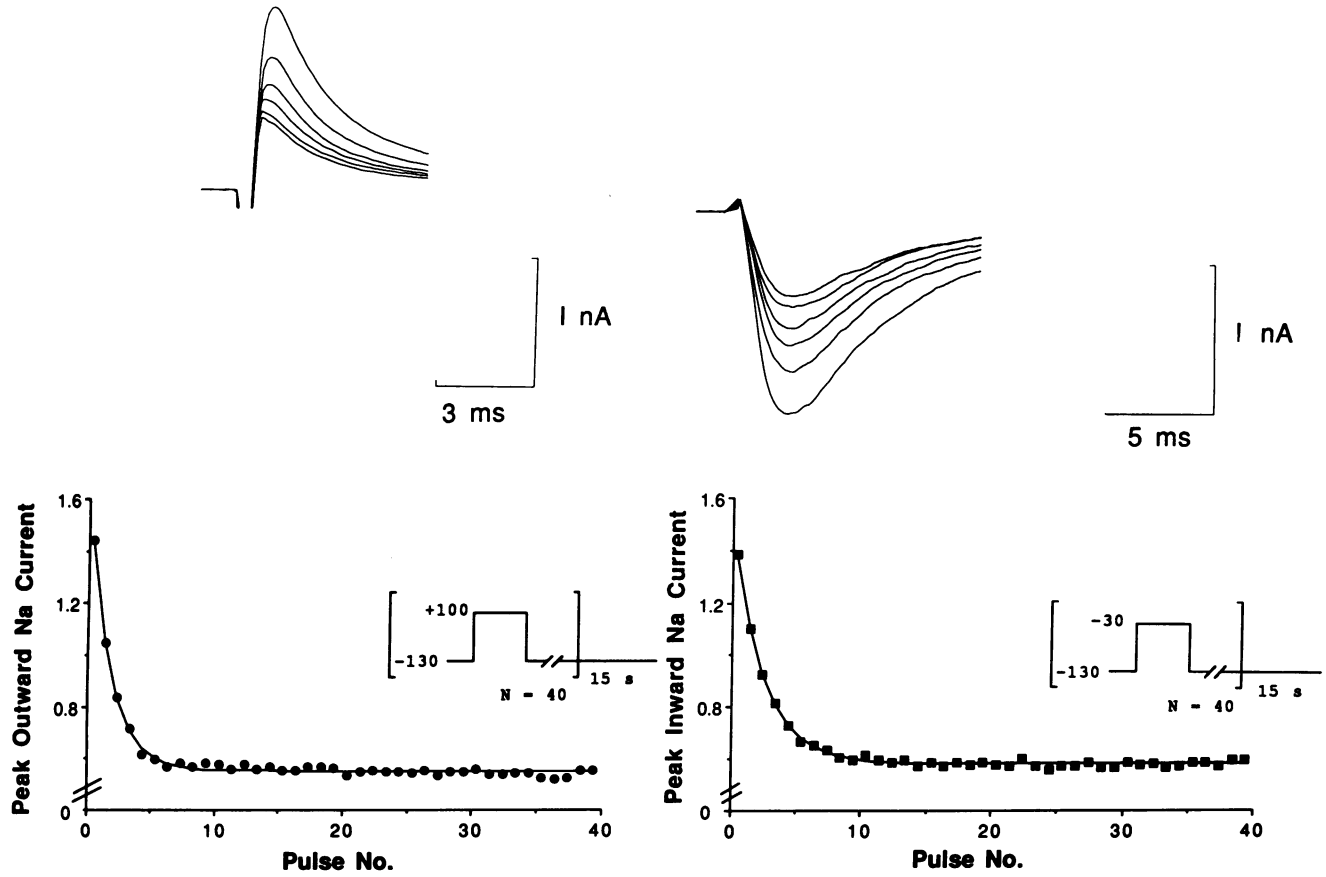

Figure 3. A comparison of block of outward and inward sodium channel currents during exposure to 80 μM lidocaine. The left panel shows current traces and peak values of outward Na current plotted as a function of pulse number. The holding potential was set at −130 mV and the test potential +100 mV. In the right panel are shown the current traces and peak inward current obtained with the test potential set at −30 mV. The fractional steady-state block was 60% and 62% at test potentials of +100 and −30 mV, respectively. In this experiment, the microelectrode filling solution [Na] was 10 mM.

of $[Na]_o$ on each of these factors may result in the apparent lack of significant changes in the level of steady-state block. We determined the recovery from block during exposure to lidocaine at $[Na]_o$ of 20, 37.5, 75, and 150 mM. The result of one experiment is illustrated in Fig. 4. Block was induced by a train of forty 50-ms pulses from a holding potential of −130 mV. Recovery from block was assessed by introducing test pulses at varying recovery intervals. In this particular experiment more steady-state block was achieved in the 20 mM $[Na]_o$ perfusate than that with the 150 mM perfusate. At both 150 and 20 mM $[Na]_o$, recovery was fitted by a single exponential with time constants of 0.77 and 0.68 s. The average results are summarized in Table I. Variation of $[Na]_o$ did not significantly affect the dissociation of lidocaine from the sodium channel. The result would argue against a blocking action of lidocaine in the ion conduction pathway. Multiple occupancy of the ion conduction pathway also appears unlikely.

Block by disopyramide. Block of the sodium current during exposure to 100 μM disopyramide was examined with $[Na]_o$ of 20 and 150 mM. For the initial experiments performed at a holding potential of −130 mV, the fraction of phasic block during pulse train stimulation was small. Therefore a holding potential of −100 mV was used for the remainder of the experiments. Fig. 5 shows sodium current in response to trains of 60 pulses from a holding potential of −100 mV to a test potential of −20 mV. At both $[Na]_o$, two exponentials were required to fit the decline in current. For [Na] of 20 mM, the uptake "pulse constants" were 0.67 and 19.8 pulses; for $[Na]_o$ of 150 mM 0.79 and 20.6 pulses. The steady-state block was 53% with $[Na]_o$ of 20 mM and 40% for 150 mM. The mean steady-state block in five cells was 55±0.03 and 38±0.1% during exposure to 20 and 150 mM $[Na]_o$, respectively.

When we attempted to determine the kinetics of dissociation of disopyramide from the sodium channel using a protocol similar to that employed for lidocaine, little unblocking was observed. Apparently rest recovery was not important as a dissociation mechanism under our recording conditions. To get a measure of the recovery process, a protocol had to be devised in which the steady-state level of block during the initial and final phases of the protocol is different, and the relaxation of the current between the two levels of block examined. Block was initially induced with a train of 60 50-ms pulses from a holding potential of −100 mV to a test potential at +20. After a 60-s rest period, the holding and test potentials were changed to −120 and −20 mV, respectively, and a second train of pulses applied. As indicated in Fig. 6, there was a progressive increase in sodium current during the second train. This is an example of use-dependent unblocking (35). A single exponential fitted

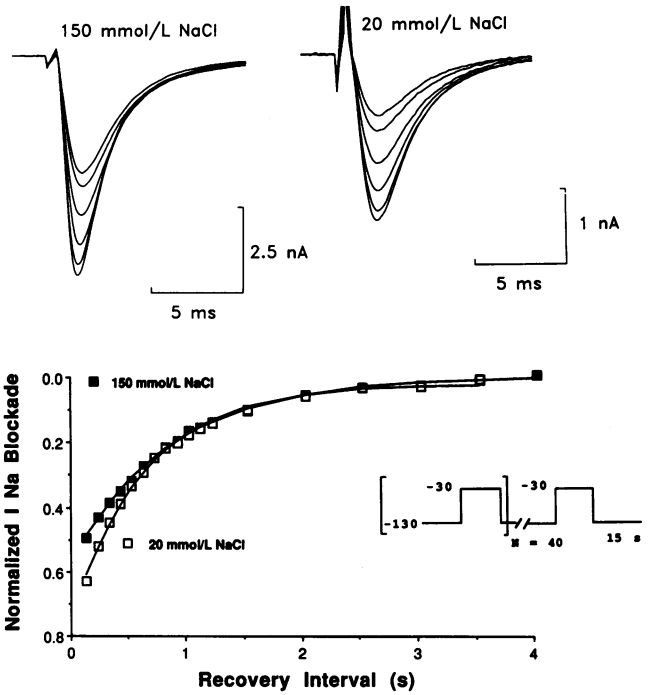

Figure 4. Recovery from lidocaine-induced block. Recovery from block by 80 μM lidocaine is compared with $[Na]_o$ of 150 and 20 mM. Block was induced by a train for 40 50-ms pulses from a holding potential of −130 mV to a test potential of −30 mV. After a variable recovery period, test pulses to −30 mV were introduced. Note the differences in amplitude calibrations between the two sets of tracings. The plot in the lower part of the figure shows the normalized test current plotted as a function of the recovery interval. The continuous lines are single exponential least squares fit to the data points. The recovery time constant was 0.77 s with $[Na]_o$ of 150 mM and .68 s with $[Na]_o$ of 20 mM. L, liter.

the data well for both sodium concentrations. The pulse constants for unblocking were 4.9 and 8.7 pulses for $[Na]_o$ of 20 and 150 mM, respectively. In four experiments the fractional unblocking was 34±14% and the pulse constant 5.2±2.4 pulses for $[Na]_o$ of 150 μM and 44±6% and 4±0.9 pulses for $[Na]_o$ of 20 μM.

Single-channel studies. The results suggested that the major effect of increasing external sodium concentration during lidocaine exposure was not due to a change in the fraction of unblocked channels ($i - b$), but an increase in the single channel Na current i. In contrast, the increase in steady-state block in low $[Na]_o$ during disopyramide exposure suggest additional changes in drug binding. Assessment of the effect of variation of $[Na]_o$ on the magnitude of the sodium current during whole-cell recordings presented a number of problems. At a given $[Na]_o$, there was a marked variation of peak current between cells. During the time required for changes of solution, there may be variable run down of the current. Difficulties of voltage control became more frequent with $[Na]_o > 150$ mM. We therefore elected to determine the effect of variation of $[Na]_o$ on i by performing the direct single channel measurement. The problem of signal to noise ratio dictated the examination of a somewhat different range of $[Na]_o$ concentration. The lowest external sodium concentration used in these experiments was 100 mM; the highest 350 mM. The effect of disopyramide on block of open sodium channels was examined at a $[Na]_o$ of 120 and 180 mM. The issue of whether lidocaine-associated sodium channels do indeed conduct also was examined with single-channel measurements.

We studied the relationship between $[Na]_o$ and single-channel current amplitude by recording single sodium channel currents in cell-attached membrane patches as the micropipette filling solution (external) was varied between 100 and 350 mM. A single $[Na]_o$ was examined in each patch and the test potential was fixed at −30 mV. Considerable difficulty was encountered in obtaining seals when $[Na]_o$ was greater than 200 mM. This is consistent with the known relationship between micropipette-solution osmolality and the ease of obtaining giga-ohm seals (29). Single-channel current amplitude increased as $[Na]_o$ was increased (Fig. 7 *A*). Single-channel current amplitude as a function of $[Na]_o$ was fitted to the relationship $i = i_{max}/\{1 + k/[Na]_o\}$ where i_{max} and k are free parameters (36). From the fit, we estimated changes in i that may occur as $[Na]_o$ was varied between 150 and 175 mM. The predicted values for 150, 160, 170, and 175 mM was 1.11, 1.16, 1.22, and 1.25 pA, respectively. An increase in $[Na]_o$ of 25 mM

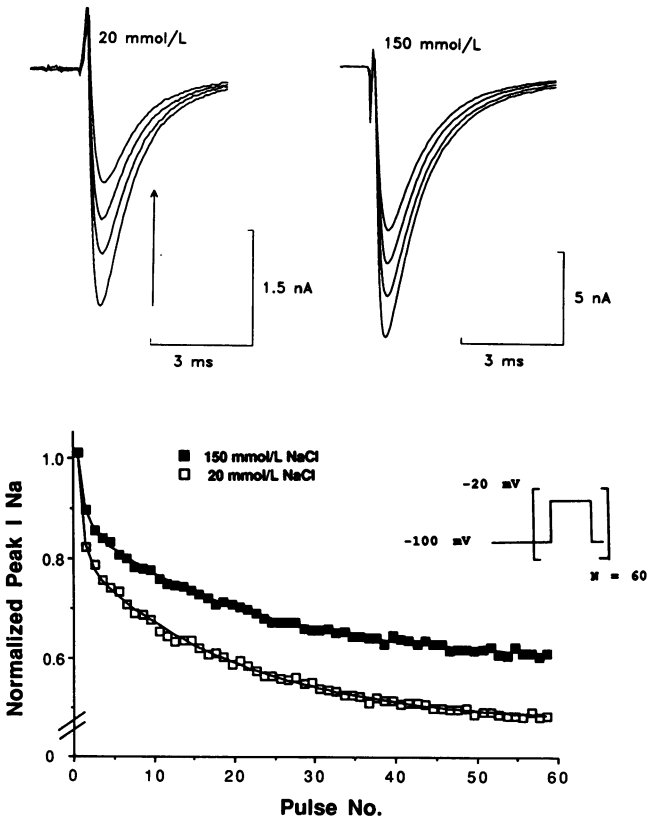

Figure 5. Use-dependent block during exposure to 100 μM disopyramide. The current traces show sodium channel current in response to a train of 50-ms pulses from a holding potential of −100 mV to a test potential of −20 mV. In the lower portion of the figure, the normalized peak Na current is plotted as a function of the order of the pulses in the train. The continuous lines represent double-exponential fits to the data points. For $[Na]_o$ of 20 mM, the time constants for block development were 0.67 and 19.8 pulses; for $[Na]_o$ of 150 mM 0.79 and 20.6 pulses. L, liter.

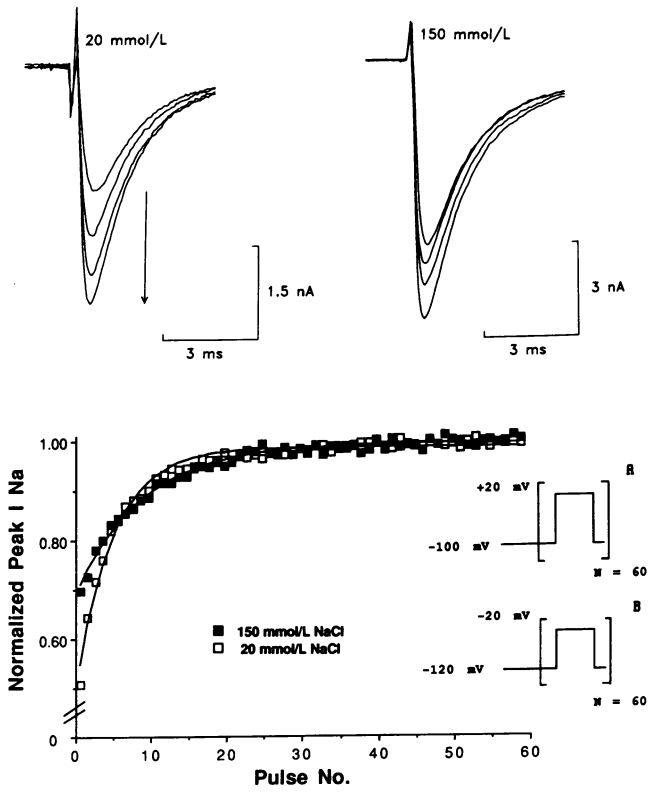

Figure 6. Use-dependent unblocking during disopyramide exposure. The pulse paradigm consisted of two components. Block was induced by a train of 60 50-ms pulses from a holding potential of −100 mV to a test potential of +20 mV (protocol A). After a 60-s rest period, the holding potential was changed to −120 mV and trains of pulses applied to a test potential of −20 mV (protocol B). The steady-state level of block was greater after protocol A than B. Therefore, the recovery process could be followed with protocol B. The currents traces are responses during protocol B (the unblocking train). Currents during the unblocking train have been normalized and are plotted in the lower panel.

in the range of physiologic interest increased i by a modest 12.0%.

Prior studies from this laboratory have shown that disopyramide blocks normal open sodium channels and those in which the inactivation process has been slowed or removed (19). The influence of 50 μM disopyramide on single sodium channels with inactivation removed by deltamethrin was examined at external sodium concentrations of 120 and 180 mM. Drug-free and disopyramide studies were performed on separate patches. Fig. 8 illustrates the results from four patches, during control and disopyramide exposure at 120 and 180 mM $[Na]_o$. In the absence of disopyramide, inactivation was slowed markedly and the sodium channels remained open for most of the 200 ms duration of the voltage-clamp step. During exposure to disopyramide the prolonged openings were interrupted by brief closures. At each sodium concentration mean open time during control and exposure to disopyramide in four patches were averaged and the association rate constant estimated. During exposure to disopyramide the association rate constant was 10×10^6/M per s with $[Na]_o$ of 120 mM and 5×10^6/M per s with $[Na]_o$ of 180 mM. Elevation of external sodium concentration slowed the rate of association of disopyramide with the sodium channel. This result is consistent with the macroscopic current experiments showing greater steady-state block during disopyramide exposure at low $[Na]_o$. Elevated $[Na]_o$ will have a dual effect on the action of open-channel blockers such as disopyramide, increasing i and decreasing b, the fraction of blocked channels.

While most models of the interaction of antiarrhythmic drugs suggest that drug-associated sodium channels do not conduct, a number of recent studies suggest that drug-associated channels may conduct with a reduced open time (24, 37). This would mean that the fraction of blocked channels, b, would make a contribution to the overall current during drug exposure. In the study of McDonald et al. (24), the patches with > 5% overlapping events were excluded from analysis. For the cardiac Na channel, at all but the potentials close to thresh-

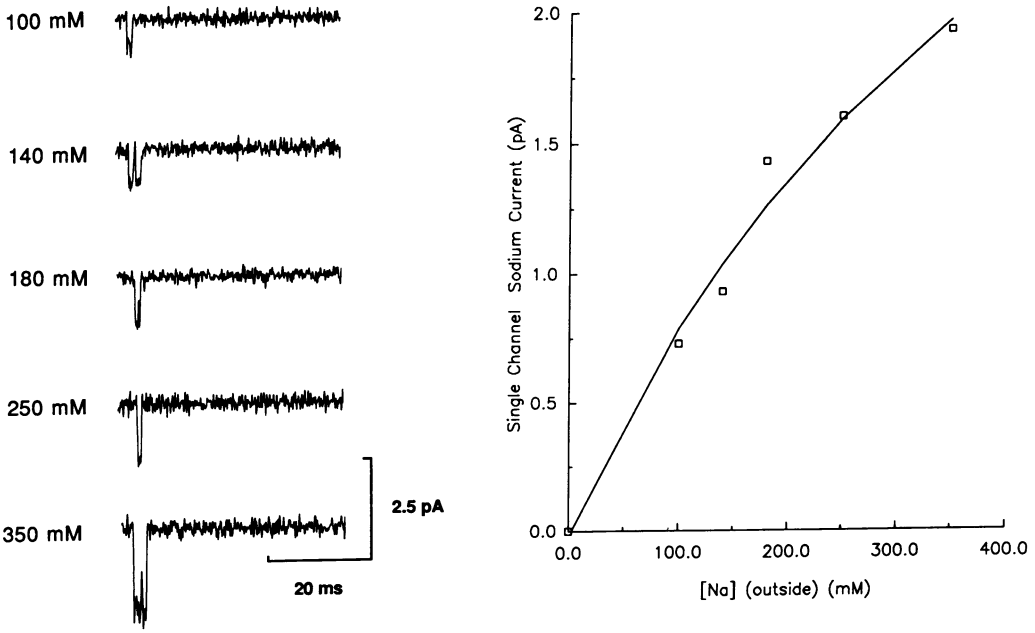

Figure 7. Relationship between external sodium concentration and single-channel current amplitude, i. The left panel shows single channels elicited at a test potential of −30 mV with external (i.e., micropipette) [Na] of 100, 140, 180, 250, and 350 mM. Each record was obtained from a different patch. In the right panel, i is plotted a function of [Na]. The data are fitted to the relationship $i = i_{max}/\{1 + k/[Na]\}$, where i is the single-channel current amplitude, k is i_{max} constants, and $[Na]_o$ is an independent variable.

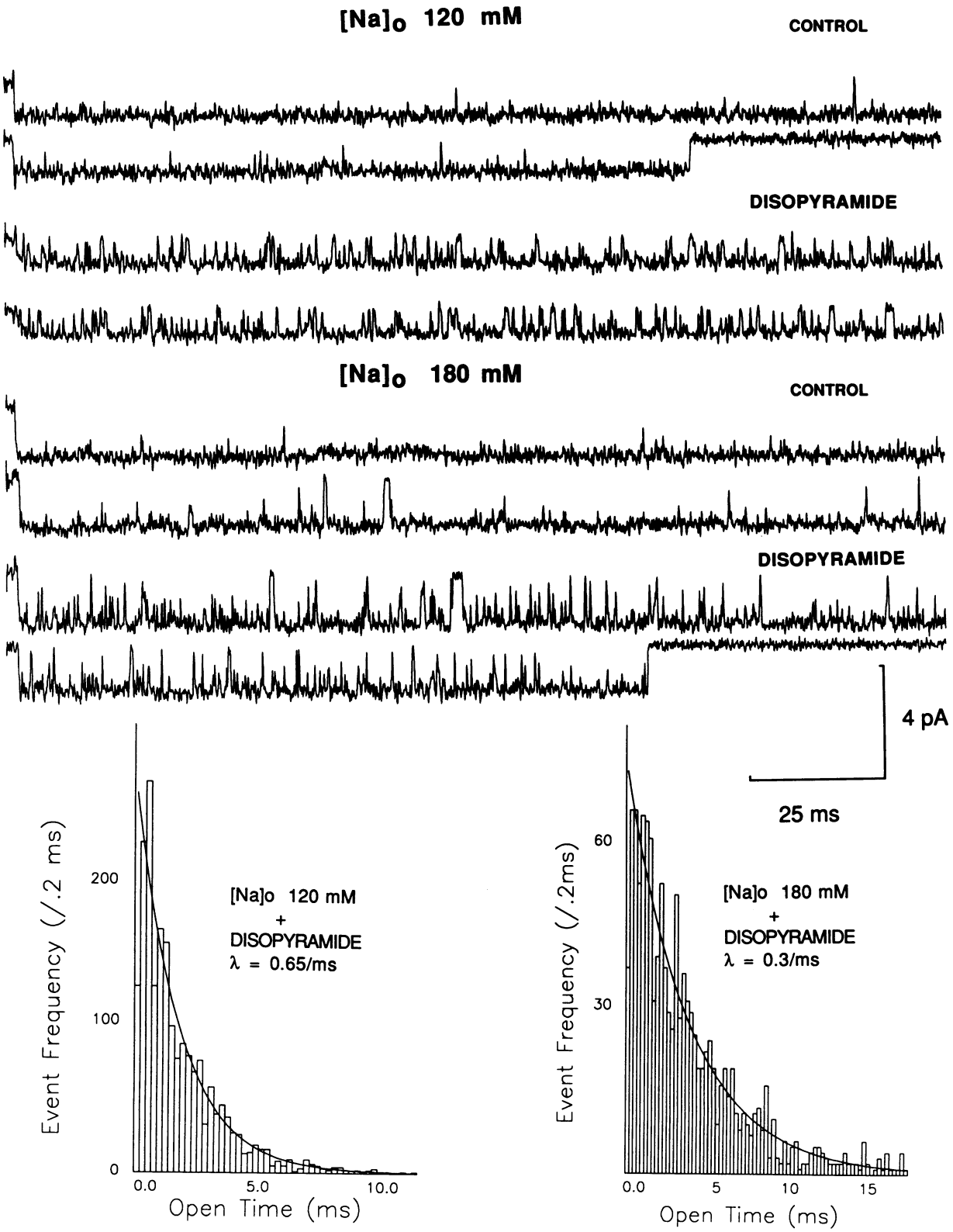

Figure 8. Block of open sodium channels during exposure to disopyramide. The upper panel shows current responses at a test potential of −30 mV during control and exposure to 100 μM disopyramide with $[Na]_o$ of 120 and 180 mM. Histograms showing the distribution of open times during exposure to disopyramide are shown in the lower panels.

old, overlapping events are inescapable except in the rare circumstance where there is a single channel in the patch. A study from our laboratory has shown that a random assignment procedure of openings and closings permits an unbiased estimate of mean open times in overlapping events (38). We have used this approach to examine the block of single Na channel currents with conditioning and test pulses during lidocaine exposure. The protocol used for these experiments are similar to those used by McDonald et al. (24).

Fig. 9 shows single sodium channel current recorded from ventricular myocytes during conditioning and test pulses in the absence of drug. The holding potential was −90 mV and the test potential was −30 mV. Conditioning and test pulses were separated by a 500-ms rest interval. Mean single-channel open time was 0.87±0.85 and 0.9±0.83 ms during the conditioning and test pulses, respectively. The average currents shown on the lowest trace were of similar amplitude in the conditioning and test pulses. This suggests that the 500-ms rest period is sufficiently long for the recovery from inactivation. Fig. 10 shows the results of a similar experiment during exposure to 80 μM lidocaine. The holding potential was −100 mV and the test potential −30 mV. Mean single channel open time was 0.75±0.8 and 0.8±0.75 ms during the conditioning and test pulses, respectively. The average current during the test pulses was significantly reduced. This resulted from an increase in the apparent probability that a channel would fail to open, 0.64 for the conditioning pulse and 0.76 during the test pulse. The results from four control patches and five lidocaine-treated patches are summarized in Table II. Lidocaine significantly decreased the average single-channel current during the test pulse compared to the conditioning pulse. However, single-channel mean open time was not affected. The decrease in test pulse current results primarily from an increase in the failure of the channel to open during lidocaine exposure. Our results do

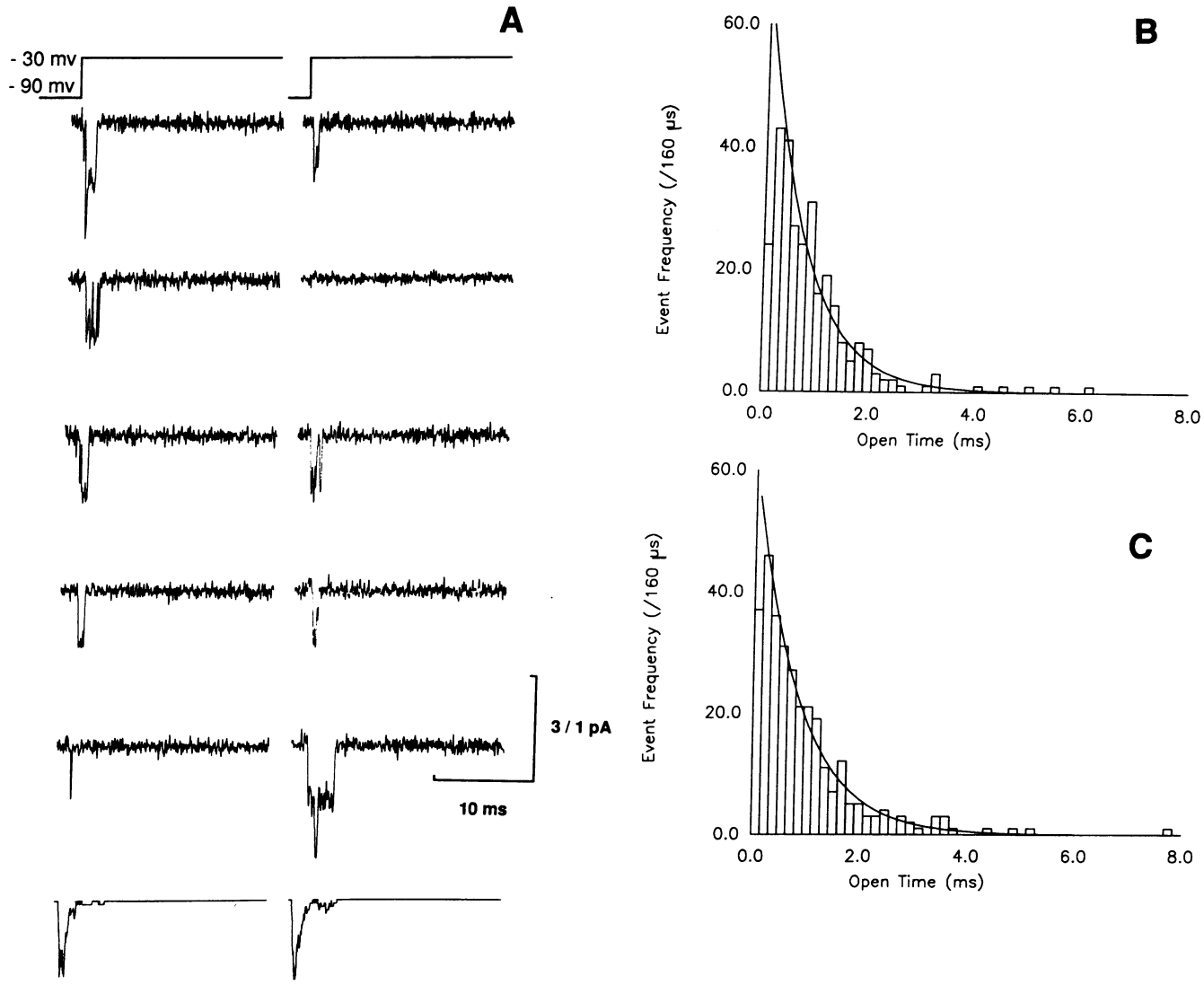

Figure 9. Single-channel currents during conditioning and test pulses in the absence of drug. Single Na channel currents elicited by depolarizing pulses from −90 to −30 mV. Current traces from five consecutive conditioning and test pulses are shown. The average currents are shown on the lowest trace. Histograms for the distribution of open times are shown on the right. The mean closing rate was 1.3/ms and 1.1/ms for the conditioning and test pulses, respectively. Current and time calibrations are shown in the insert. The current calibrations of 3 and 1 pA refer to the individual traces and the average current, respectively.

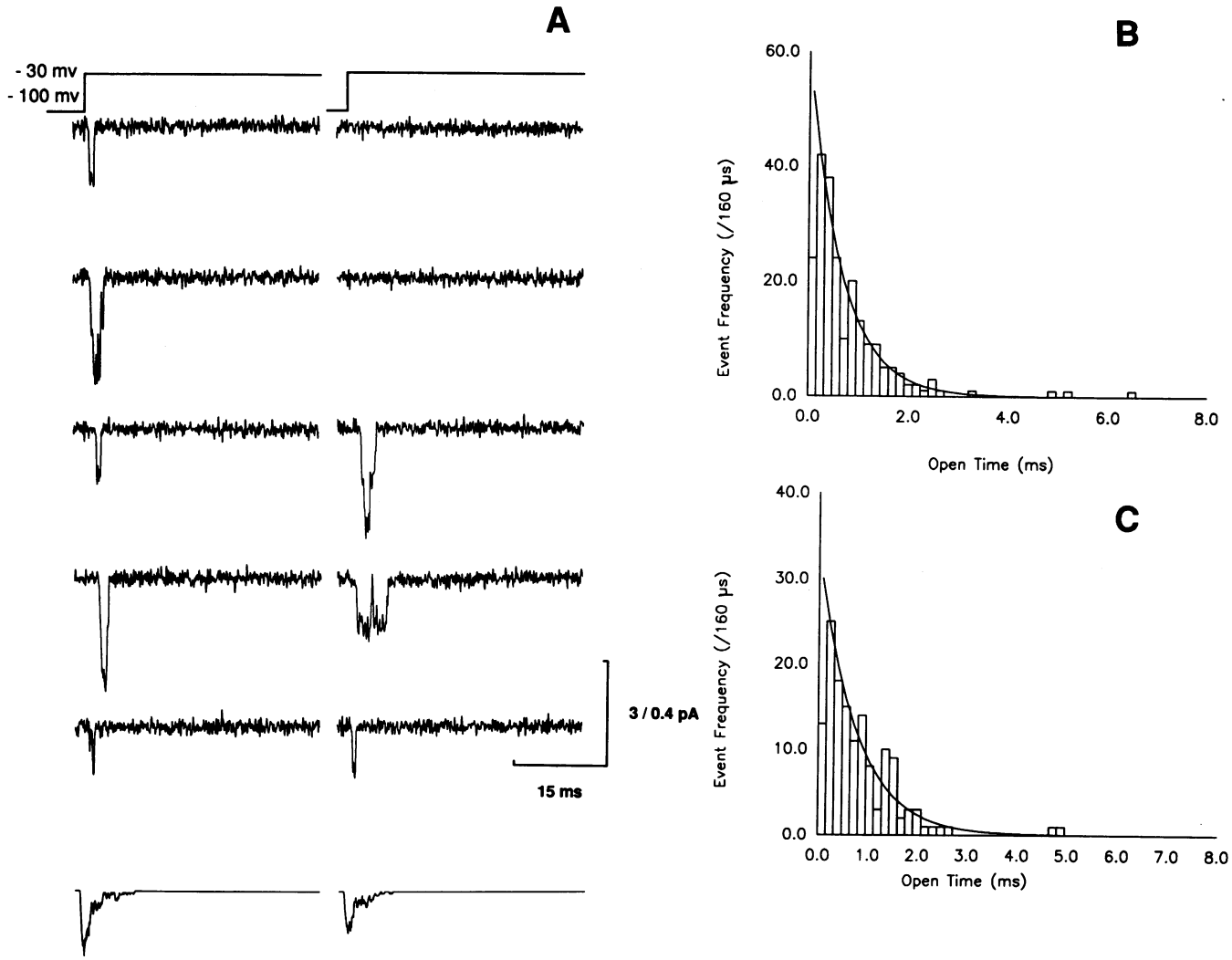

Figure 10. Single-channel current with conditioning and test pulses during exposure to 80 μM lidocaine. The holding potential was −100 mV and the test potential −30 mV. The average current, shown on the lowest trace, was decreased during the test pulse. The mean closing rate was 1.5/ms and 1.3/ms for the conditioning and test pulses, respectively. Current and time calibrations of 3 and 0.4 pA shown in the insert refer to the individual and average current traces, respectively.

not support a conclusion that lidocaine-associated sodium channels conduct. Increased ion conduction through drug-associated channels may not be an important mechanism of block relief.

Table II. Effect of Lidocaine on Single Sodium Channel Current

		$\langle t \rangle$	Pf	$\int idt$
		ms		*t pA ms*
Control (n = 4)	*Vc*	0.9±0.2	0.4±0.1	1.5±0.3
	Vt	0.85±0.2	0.4±0.1	1.5±0.3
Lidocaine (n = 5)	*Vc*	0.87±0.1	0.52±0.4	1.5±0.3
	Vt	0.9±0.1	0.74±0.1	0.9±0.3

Abbreviations: *Vc* = conditioning pulse; *Vt* = test pulse; $\langle t \rangle$ = mean open time; *Pf* = probability that a channel will fail to open; $\int idt$ = integral of the single-channel current.

Discussion

Major findings. This study has examined the relationship between the concentration of extracellular sodium and the blocking action of lidocaine and disopyramide on the sodium current under voltage clamp. The experimental technique used in each section of the study dictated what sodium concentration range could be studied. For the whole-cell voltage clamp experiments, $[Na]_o$ ranged from 20 to 150 mM. Although $[Na]_o$ of > 150 mM are of clinical interest, the large currents at those concentrations caused difficulty with voltage control. The single channel experiments were performed over a range of $[Na]_o$ of 100–350 mM. The single channel current amplitude in relationship to the baseline noise set the lower limit of concentration studied.

The whole-cell sodium current measurements showed that the level of steady-state block induced by lidocaine during pulse train stimulation was not significantly decreased by variation in $[Na]_o$. The level of steady-state block was not in-

fluenced by direction of the current as inward and outward sodium currents were blocked to a similar extent. The kinetics of dissociation of lidocaine from the sodium channel was not influenced by $[Na]_o$. Similar studies with disopyramide was more difficult to conduct because of its slowing unbinding kinetics at rest. However, less steady-state block was observed at high external sodium concentration. There was very little rest recovery. To get some measure of recovery, a protocol that induced use-dependent unblocking had to be employed. Unlike assessment of rest recovery, this protocol did not assess drug dissociation only. There was a trend for faster use-dependent unblocking low $[Na]_o$ solution.

The influence of $[Na]_o$ on single channel current amplitude was examined in cell-attached patches. Over a range of sodium concentrations that would be of pathophysiological interest, 150–175 mM, the estimated increase in single Na channel current amplitude was only 12%. Disopyramide block of sodium channels with inactivation slowed by deltamethrin was examined at external sodium concentrations of 120 and 180 mM. Prior studies in our laboratory have shown similar blocking rates of the normal and deltamethrin-modified sodium channels (19). We elected to study the deltamethrin-modified channels because in the absence of drug the mean open time was $\sim$ 10 ms or greater. The long open times, $\langle tc \rangle$, in the absence of blocking drug meant that the association rate constant was not very dependent on $\langle tc \rangle$ ($k = \{1/\langle td \rangle - 1/\langle tc \rangle\}$ $1/D$). Increasing $[Na]_o$ from 120 to 180 mM decreased the association rate constant of disopyramide with open sodium channels by 50%. The closed times provide information about drug dissociation from the channel. We did not present the data on shut times because a single exponential did not provide a good fit to the distribution of closed times. Therefore, they could not be readily interpreted in the context of a simple blocking scheme.

Single channel studies by Grant et al. (27) and McDonald et al. (24) suggest that lidocaine primarily block "inactivated" sodium channels. The study of McDonald et al. done in guinea pig myocytes also suggest an additional effect, ion conduction by lidocaine-associated sodium channels (see also Baumgarten et al. [37]). Although the authors clearly pointed out that this was not the principal effect of lidocaine, it is important for at least two reasons. Prior models of sodium channel blockade assumed that the drug-associated state was nonconducting. Secondly, in the case of drug toxicity, increasing the sodium ion concentration would increase the current through the drug-associated channels and provide an additional mechanism for reversal of toxicity. We examined this question by protocols similar to those used by McDonald et al. (24). Single sodium channel mean open time during conditioning and test pulses were compared in cell-attached patches in drug-free and lidocaine-treated myocytes. We selected a test potential −30 mV, at which overlapping events did occur and examined all epochs. A prior study from our laboratory had shown that an unbiased estimate of the mean open time of overlapping events could be made by using a random assignment of openings and closings (38). Using these approaches of experimental design and data analysis, mean open time of sodium channels in lidocaine-treated cells were no different in conditioning and test pulses. These data suggest that lidocaine-associated sodium channels do not conduct. The difference in species studied, and the more inclusive data set studied may account for the differences in results. McDonald et al. used solution with stronger buffer capacity (10 mM Hepes) compared with this study (5 mM Hepes). However, a significant difference in proton exchange kinetics of receptor bound lidocaine is unlikely. In unpublished observations from our laboratory, we have shown that proton exchange rates are not critical in determining the qualitative nature of the blocking kinetics. The interpretation is that during drug exposure, only the drug-free channels are available for ion conduction, and hence augmentation of the sodium current as $[Na]_o$ is increased.

Relationship to other studies. Recent theoretical and experimental investigations of use-dependent blockade have shown that one can reduce the fraction of steady-state block by competition with another use-dependent agent that competes for the same site with fast kinetics (39–41). The permeant ion would seem a prime candidate for such an interaction. The ion conduction process involves rapid binding and dissociation. For an agent that blocks open channels, the normal permeant ion may compete with the blocking agent. Moczydlowki (15) has reviewed models of competition between the permeant ion and blocking drugs. In the case of channels that can be singly occupied, there is simple competition between permeant ion and blockers that result in a decrease in association rate as the concentration of the permeant ion is increased. For channels that may have multiple occupancy, the permeant ion might also increase the dissociation of channel-bound drug, the so-called "knock out" effect. Thus, variation of the permeant ion concentration may provide insight into the blocking mechanism.

We selected two drugs for study, lidocaine and disopyramide. Lidocaine is known to block a state of the sodium channel occupied during prolonged depolarization. This may not necessarily correspond to the classical inactivated state of the sodium channel (26). There is some controversy whether it blocks open channels. Our own studies, those of Zilberter et al. (42) Benz and Kohlhardt (43), and Grant et al. (27) show no change in mean open time, whereas others, e.g., Baumgarten et al. (37), report a decrease in open time. There are fewer studies with disopyramide. However the evidence for open channel block with this drug is unequivocal. We observed no significant change in steady-state block with lidocaine as $[Na]_o$ was varied. Furthermore, our experiments show no difference in the level of block between inward and outward currents. This supports a model in which there is no direct competition between $[Na]_o$ and lidocaine and that the drug may not be blocking along the ion conduction pathway. In the case of disopyramide, both the whole-cell and single-channel experiments suggest direct competition between $[Na]_o$ and the blocking agent. An examination of the block of skeletal muscle sodium channels in artificial bilayers by cocaine suggest that it is external sodium ions rather than the sodium current driving force that affect block (23). Change in $[Na]_i$ over a large range had no effect on cocaine binding. There is also a parallel between the findings we report here and the recent study by Kass et al. (44) on the relationship between the nature of the permeating ion and block of the L-type calcium channel by neutral and charged dihydropyridines. Block of the calcium channel by neutral dihydropyridine is not influenced by the nature of the permeant. The neutral dihydropyridines can access their binding site throughout a maintained depolarization. On the other hand, block of the L-type calcium channel by charged dihydropyridines at external blocking sites is influenced by permeant ion. One may speculate that the permeantly charged derivative is

actually blocking along the ion conduction pathway. The present experiments on the sodium channel do not allow us to localize the site of competition to the ion conducting pathway.

Clinical implication of the study. Toxicity from local anesthetic-class sodium channel blockers is a significant clinical problem. Particularly in the case of the class 1C antiarrhythmic drugs such as flecainide and encainide, the resulting arrhythmias are difficult to treat. There is little concensus on treatment strategies. Use of other drugs that have different kinetics of interaction with the sodium channel may have its place in therapy (41, 45). β-Adrenergic blockade or the use of adrenoceptive agonist have their advocates (46). The initial reports of the use of sodium salts involve the use of the lactate or bicarbonate salt (6, 7). The case for the action of sodium ions per se is now established. The use of sodium salts for example, sodium chloride, under these circumstances is not without its risks. Patients having proarrhythmic responses to antiarrhythmic drug often have advanced heart failure. At least some reports suggest that large amounts of sodium ions may be required (13). Basic studies that may more clearly define the circumstances under which treatment with sodium salts would be most beneficial.

In terms of their sodium channel blocking action, local anesthetic-class antiarrhythmic drugs interact with the sodium channel during transient state(s) associated with channel opening or over a prolonged period associated with depolarization. Agents that block the transient state may interact with the open channel or states preceding channel opening. Elevation of external sodium will have at least two effects as regards the predictable effect of increasing sodium current by an increase in the current, i, carried by individual channels. The consequence of this effect may be small. In a study of the effects of external sodium ions on conduction velocity in Purkinje fibers, Walton and Fozzard (47) show that a 20% change of external sodium concentration changes conduction velocity by $< 10\%$ (our estimates from their Fig. 2). If changes in i were the only mechanism by which $[Na]_o$ may influence conduction velocity, the potential for reversal of the blocking action of agents that interact with the sodium channel like lidocaine would be small. On the other hand, for agents that interact with open sodium channels, there may be a dual mechanism for reversal of drug action, an increase in i and a decrease in the association rate of drug with the channel. We conclude that it may be important to define the states of the sodium channel with which drugs interact and to confirm for other open channel blockers that increases in external sodium do indeed increase drug dissociation rate.

Acknowledgments

This research was supported in part by grants HL-32708, HL-32994, and HL-17670 from the National Institutes of Health and an American Heart Association Fellowship Award.

References

1. Grant, A. O., C. F. Starmer, and H. C. Strauss. 1984. Antiarrhythmic drug action: blockade of the inward sodium current. *Circ. Res.* 55:427–439.

2. Horowitz, L. N. 1988. Drugs and proarrhythmia. *In* Progress in Cardiology. D. P. Zipes and D. J. Rowlands, editors. Lea and Febiger, Philadelphia. 109–125.

3. Risch, S. C., G. P. Groom, and D. S. Jahowsky. 1981. Interfaces of psychopharmacology and cardiology, Part 1. *J. Clin. Psychiatry.* 42:23–34.

4. Risch, S. C., G. P. Groom, and D. S. Jahowsky. 1981. Interfaces of psychopharmacology and cardiology, Part 2. *J. Clin. Psychiatry.* 42:47–59.

5. Bellet, S., G. Hamdan, A. Somlyo, and R. Lara. 1959. The reversal of cardiotoxic effects of quinidine by molar sodium lactate: an experimental study. *Am. J. Med. Sci.* 237:165–176.

6. Bellet, S., G. Hamdan, A. Somlyo, and R. Lara. 1959. A reversal of cardiotoxic effects of procainamide. *Am. J. Med. Sci.* 237:177–189.

7. Wasserman, F., L. Brodsky, J. H. Kathe, P. L. Rodensky, M. M. Dick, and P. S. Danton. 1959. The effect of molar sodium lactate in quinidine intoxication. *Am. J. Cardiol.* 3:294–299.

8. Cox, A. R., and T. C. West. 1961. Sodium lactate reversal of quinidine effect studied in rabbit atria by the microelectrode technique. *J. Pharmacol. Exp. Ther.* 13:212–222.

9. Nattel, S., H. Keable, and B. I. Sasyniuk. 1984. Experimental amitriptyline intoxication: Electrophysiologic manifestations and management. *J. Cardiovasc. Pharmacol.* 6:83–89.

10. Nattel, S., and M. Mittleman. 1984. Treatment of ventricular tachyarrhythmias resulting from amitriptyline toxicity in dogs. *J. Pharmacol. Exp. Ther.* 231:430–435.

11. Sasyniuk, B. I., and Y. Jhamandas. 1984. Mechanism of amitriptyline on cardiac Purkinje fibers by sodium bicarbonate. *J. Pharmacol. Exp. Ther.* 231:387–394.

12. Pentel, P., and N. Benowitz. 1984. Efficacy and mechanism of action of sodium bicarbonate in the treatment of desipramine toxicity in rats. *J. Pharmacol. Exp. Ther.* 230:12–19.

13. Chanty, F., C. Funck-Brentano, J. M. Landan, and H. Lordoux. 1987. Efficacité de fortes doses de lactate molaire par voie veineuse lors des intoxications au flecainide. *Presse Med.* 16:808–810.

14. Bagay, A., R. L. Woosley, and D. M. Roden. 1989. Acute electrophysiologic effects of sodium administration in dogs treated with O-desmethyl encainide. *Circulation.* 80:994–1002.

15. Moczydlowski, E. 1986. Single-channel enzymology. *In* Ion Channel Reconstitution, C. Miller, editor. Plenum Publishing Corp., New York. 75–113.

16. Armstrong, C. M. 1975. Potassium pores of nerve and muscle membrane. *In* Membranes, A Series of Advances 3. G. Eisenman, editor. Marcel Dekker, Inc., New York. 325–350.

17. Jensen, R. A., and B. G. Katzung. 1970. Electrophysiological actions of diphenylhydantoin on rabbit atria. *Circ. Res.* 26:17–27.

18. Kohlhardt, M. 1982. A quantitative analysis of the Na^+-dependence of V_{max} of the fast action potential in mammalian ventricular myocardium saturation characteristics and the modulation of drug-induced I_{Na} blockade b $[Na^+]_o$. *Pflügers Arch. Eur. J. Physiol.* 392:379–387.

19. Grant, A. O., D. J. Wendt, and C. F. Starmer. 1992. Kinetics of interaction of disopyramide with cardiac sodium channels: Fast dissociation from open channels at normal rest potentials. *Biophys. J.* 61:A305. (Abstr.)

20. Brown, A. M., K. S. Lee, and T. Powell. 1981. Sodium current in single rat heart muscle cells. *J. Physiol. (Lond.).* 318:479–500.

21. Makielski, J. C., M. F. Sheets, D. A. Hanck, C. T. January, and H. A. Fozzard. 1987. Sodium current in voltage clamped internally perfused canine cardiac Purkinje cells. *Biophys. J.* 52:1–11.

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

23. Wang, G. K. 1988. Cocaine-induced closures of single-batrachotoxin-activated Na^+ channels in planar lipid bilayers. *J. Gen. Physiol.* 92:747–765.

24. McDonald, T. V., K. R. Courtney, and W. T. Clusin. 1989. Use-dependent block of single channels by lidocaine in guinea pig ventricular myocytes. *Biophys. J.* 55:1261–1266.

25. Barber, M. J., C. F. Starmer, and A. O. Grant. 1990. Changes of external sodium concentration do not affect recovery kinetics or steady-state block of rabbit atrial sodium channels during exposure to lidocaine. *Circulation.* 82:111–526. (Abstr.)

26. Gilliam, F. R. III, C. F. Starmer, and A. O. Grant. 1989. Blockade of rabbit atrial sodium channels by lidocaine: characterization of continuous and frequency-dependent blocking. *Circ. Res.* 65:723–739.

27. Grant, A. O., M. A. Dietz, F. R. Gilliam III, and C. F. Starmer. 1989. Blockade of cardiac sodium channels by lidocaine: single channel analysis. *Circ. Res.* 65:1247–1262.

28. Eisenberg, R. S., and E. A. Johnson. 1970. Three-dimensional electrical field problems in physiology. *Prog. Biophys. Mol. Biol.* 20:1–65.

29. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cell and cell-free membrane patches. *Pflügers Arch. Eur. J. Physiol.* 391:85–100.

30. Marquardt, D. W. 1963. An algorithm for least-squares estimation of non-linear parameters. *J. Soc. Ind. Appl. Math.* 11:431–441.

31. Gilbert, N. 1976: Statistics. W. B. Saunders Co., Philadelphia. 240–256.

32. Snedecor, G. W., and W. G. Cochran. 1971. Statistical Methods. Iowa State University Press, Ames, IA. 204–217.

33. 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.

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

35. Gintant, G. A., and B. F. Hoffman. 1984. Use-dependent block of cardiac sodium channels by quaternary derivatives of lidocaine. *Pflügers Arch. Eur. J. Physiol.* 400:121–129.

36. Hille, B. 1984. Ionic Channels of Excitable Membranes. Sinauer Associates, Inc., Sunderland, MA. 249–271.

37. Baumgarten, C. M., J. C. Makielski, and H. A. Fozzard. 1991. External site for local anesthetic block of cardiac Na^+ channels. *J. Mol. Cell. Cardiol.* 23(Suppl. 1):85–93.

38. Hurwitz, J. L., M. A. Dietz, C. F. Starmer, and A. O. Grant. 1991. A source of bias in the analysis of single channel data: assessing the apparent interaction between channel proteins. *Comput. Biomed. Res.* 24:584–602.

39. Starmer, C. F. 1987. Theoretical characterization of ion channel blockade: competitive binding to periodically accessible receptors. *Biophys. J.* 52:405–412.

40. Clarkson, C. W., and L. M. Hondeghem. 1985. Evidence for a specific receptor site for lidocaine, quinidine and bupivacaine, associated with cardiac sodium channels in guinea pig ventricular myocardium. *Circ. Res.* 56:496–506.

41. Whitcomb, D. C., F. R. Gilliam III, C. F. Starmer, and A. O. Grant. 1989. Marked QRS complex abnormalities and sodium channel blockade by propoxyphene reversed with lidocaine. *J. Clin. Invest.* 84:1629–1636.43.

42. Zilberter, Y., L. Motin, S. Sokolova, A. Papin, and B. Khodorov. 1991. Ca-sensitive slow inactivation and lidocaine-induced block of sodium channels in rat cardiac cells. *J. Mol. Cell. Cardiol.* 23(Suppl. 1):61–72.

43. Benz, I., and M. Kohlhardt. 1991. Responsiveness of cardiac Na^+ channels to antiarrhythmic drugs: the role of inactivation. *J. Membr. Biol.* 122:267–278.

44. Kass, R. S., J. P. Arena, and S. Chin. 1991. Block of L-type calcium channels by charged dihydropyridines: sensitivity to side of application and calcium. *J. Gen. Physiol.* 98:63–75.

45. von Dach, B., and R. A. Streuli. 1988. Lidocainbehandlung einer Vergiftung mit eibennadeln (*Taxus baccata* L). Schweiz. Med. Wochenschr. 118:1113–1116.

46. Myerburg, R., K. M. Kessler, M. M. Cox, H. Huikun, E. Terracall, A. Interian, Jr., P. Fernandez, and A. Castellanos. 1989. Reversal of proarrhythmic effects of flecainide acetate and encainide hydrochloride by propranolol. *Circulation.* 80:1571–1579.

47. Walton, M. K., and H. A. Fozzard. 1983. The conducted action potential: models and comparison to experiments. *Biophys. J.* 44:1–8.