Kinetics of Interaction of the Lidocaine Metabolite Glycylxylidide With the Cardiac Sodium Channel

Additive Blockade With Lidocaine

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The recovery of the sodium channel from blockade by local anesthetic antiarrhythmic drugs is voltage dependent. Recovery from lidocaine-induced blockade is accelerated by hyperpolarization, whereas that from glycylxylidide (GX) blockade has been reported to be slowed by hyperpolarization. This striking difference occurs despite similarities in chemical structure. The fast recovery from GX block at depolarized potentials may lead to a partial reversal of lidocaine blockade when the two drugs are combined. We have examined the kinetics of interaction of GX with the cardiac sodium channel over a range of membrane potentials by measuring whole-cell currents in isolated rabbit myocytes under voltage clamp at 15°C. In the absence of drug, slow inactivation developed with a time constant of 10.7±5.1 seconds (n=6). During exposure to 74 μmol/l GX, block developed with a time constant of 7.0±3.2 seconds (n=6). Because of the similar time course of slow inactivation and block, we used a high concentration of GX to induce a level of block sufficient for analysis. The onset of block was slower than that induced by lidocaine and was unaffected by variation of external sodium from 20 to 75 mmol/l. Use-dependent blockade of sodium channels was greater when pulse trains were applied from a holding potential of −100 than −140 mV. This suggested that recovery from GX block might be slower at −100 than −140 mV. Direct measurements gave time constants of recovery of 10.3±4.2 seconds at −100 mV (n=6) and 4.1±0.4 seconds at −140 mV (n=4). The combination of GX with lidocaine produced only additive blocking effects when pulse trains were applied from both holding potentials. Computer simulations of the requirements for the competitive displacement of a sodium channel blocker with slow kinetics by one with fast kinetics suggest that the recovery time constant of the fast drug must be 10–100-fold smaller than that of the slow drug. Rapid association kinetics effected by a large binding rate constant or a higher concentration of the fast blocking drug is also important. The simulations suggest that, for the interaction of GX and lidocaine, only additive blocking action should be observed over the range of stimulus frequencies used in these experiments. (Circulation Research 1992;70:1254–1273)

KEY WORDS • lidocaine • glycylxylidide • sodium current

Lidocaine is extensively metabolized in the liver to its deethylated derivatives monoethylglycinexilidide and glyclyxylidide (GX). In vitro experiments suggest that GX has about 1/10 the antiarrhythmic potency of the parent drug. In principle, the accumulation of GX during chronic lidocaine therapy could result in additional or partial reversal of sodium channel blockade. If the drugs share a common receptor site, the ability of GX to add to or reverse total sodium channel blockade will be dependent on the relative kinetics of interaction with the sodium channels. Uprate velocity measurements by Broughton et al showed that the time constant for recovery from sodium channel blockade by GX was 1.3 times that of lidocaine. Channel blockade by a combination of two use-dependent drugs will recover with a time constant between those of the individual drugs. Therefore, the in vitro data of Broughton et al suggest that mixtures of GX and lidocaine should exhibit a larger recovery time constant than that of lidocaine alone and, consequently, a greater degree of steady-state block.

Bennett et al examined the sodium channel blocking actions of GX alone and in combination with lidocaine in isolated guinea pig ventricular myocytes under voltage-clamp conditions. At holding potentials of −90 to −100 mV, GX appeared to reverse the sodium channel blocking action of lidocaine, whereas greater total blockade was observed at −120 to −140 mV. They suggested that this voltage-dependent response to the drug combination resulted from the different voltage dependences of the time constants of recovery from blockade by lidocaine or GX. Depolarized membrane holding potentials slowed recovery from lidocaine block, whereas the same potentials accelerated recovery from GX block. This striking difference in voltage

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dependence of recovery from block in the absence of any net change in the charge of the metabolite or major difference in the proportion of uncharged and charged forms (pK of lidocaine and GX, 7.86 and 7.68, respectively) may provide important clues about the structural determinants of the recovery process, including its dependence on external pH. Therefore, we expanded the initial studies of Bennett et al using several protocols to assess the development and recovery from block during GX exposure over a large range of external pH. Our results demonstrate that the kinetics of GX interaction with the sodium channel has the same qualitative voltage dependence as that reported for lidocaine. Both development of and recovery from block are slower than that during lidocaine exposure at both depolarized and hyperpolarized holding potentials. Furthermore, we observed only additive blocking action during exposure to the combination of GX and lidocaine at −100 to −140 mV.

Materials and Methods

Cell Preparation

The experiments were performed on isolated rabbit atrial myocytes. The methods of heart isolation and perfusion by the Langendorff technique were similar to those previously described from our laboratory. As far as possible, a sterile technique was used, and all solutions were maintained at 37°C throughout the isolation procedure. After 40–60 minutes of perfusion with enzyme-containing Ca²⁺-free Krebs-Henseleit (K-H) solution (see below for K-H and enzyme composition), the atria were separated from the ventricular tissue. The atrial tissue chunks were placed in a preheated Petri dish containing Ca²⁺-free K-H solution, 10% calf bovine serum (which terminated the enzyme activity), penicillin G (1 unit/ml), and streptomycin (0.5 unit/ml). The atrial tissue chunks were then minced into 2–4-mm segments, and the single myocytes were separated from the undisassociated segments by filtration through a 200-μm nylon mesh. The isolated myocytes were centrifuged and washed twice with solution comprised of Ca²⁺-free K-H solution with 10% calf bovine serum, Dulbecco’s minimal essential medium (DMEM), and antibiotics (as above). The myocytes were then placed in medium containing DMEM and F-12 Ham (in a 1:1 ratio), 10% calf bovine serum, 1 unit/ml penicillin, and 0.5 unit/ml streptomycin. The cell cultures were kept at 37°C in a humidified 5% enriched CO₂ atmosphere until ready for use. The cells were used after they assumed a spherical shape (usually 1–2 days), a configuration shown to have theoretical advantages for uniform control of the membrane potential. The use of cultured cells provides a ready source of cells without daily isolation, during which the yield of viable cells is unpredictable.

Solutions

The Ca²⁺-free K-H solution had the following composition (mmol/l): NaCl 130, KCl 5.4, MgCl₂ (H₂O)₆ 1.2, NaHCO₃ 18, NaH₂PO₄ 2.1, sodium pyruvate 5, and glucose 11. The solution was gassed with 95% O₂–5% CO₂. The enzyme perfusate contained 180 units/ml collagenase (Worthington Diagnostics, Freehold, N.J.), 10 mg/100 ml hyaluronidase (Sigma Chemical Co., St. Louis, Mo.), and 88 units/100 ml Protease (type XIV, Sigma). DMEM and F-12 Ham were obtained from Gibco Scientific, Grand Island, N.Y.

The micropipettes were filled with the following solution (mmol/l): CsF 120, MgCl₂ (H₂O)₆ 0.1, K₂ (ATP) 5, KH₂PO₄ 1, EGTA 5, glucose 5, and HEPES 5. The solution was cooled to 15°C, and the pH was adjusted to 7.3 with CsOH. The solution was filtered through a 0.22-μm filter (model GS, Millipore Corp., Bedford, Mass.). The osmolality of the internal solution was ~245 mosm. In some experiments, EGTA was increased to 10 mmol/l.

External solution was comprised of the following (mmol/l): NaCl 75, CsCl 75, KCl 5, CaCl₂ 1.5, glucose 5, HEPES 10, and MgCl₂ (H₂O)₆ 1. The solution was cooled to 15°C, and the pH was adjusted to 7.2, 7.4, or 7.8 with NaOH. The osmolality of the external solution was ~295 mosm. In some experiments where low external sodium activity was desired, 20 mmol/l NaCl and 130 mmol/l CsCl were substituted for 75 mmol/l NaCl and CsCl, respectively.

GX stock solution was prepared from the crystalline form (kindly provided by Dr. Bertil Takman, Astra Pharmaceutical Products Inc., Westborough, Mass.) by dissolving 13.1 mg in 2 ml of 0.1N HCl. GX stock (200 μl) was added to each 100 ml external solution. After GX stock was added, the pH of the external solution was rechecked and adjusted if necessary.

Recording Techniques

Micropipettes were pulled from 1.5-mm-o.d. borosilicate glass (No. N-51A, Drummond Scientific Co., Broomall, Pa.) with a horizontal puller (Flaming-Brown, model P 80/PC, Sutter Instrument Co., Novato, Calif.). Micropipettes were coated with Sylgard 184 (Dow Corning, Midland, Mich.), and the tip was heat-polished (MF-83 Microforge, Narishige Scientific Instrument Laboratory, Tokyo, Japan). After filling with internal solution, the microelectrodes had resistances of 400–1,000 kΩ. A patch-clamp amplifier (Axopatch-1B, Axon Instruments, Burlingame, Calif.) was used to measure whole-cell currents. The microelectrode was coupled to the amplifier headstage by a Teflon-coated Ag/AgCl wire. The external solution was grounded via an Ag/AgCl electrode (In Vivo Metric Systems, Healdsburg, Calif.) that was coupled to the bath by an agar bridge.

Voltage-clamp command pulses were generated with an IBM-XT microcomputer (IBM, Armonk, N.Y.) using a TL-1 interface (Axon Instruments) and a custom software program. Whole-cell currents were filtered at 5 kHz, leakage-subtracted, and digitized on-line at 20 kHz using an analog-to-digital interface board (DT 2821 input-output board, Data Translation, Marlboro, Mass.) and a Compaq 386-20 microcomputer (Compaq Computer, Houston, Tex.).

A coverslip with a layer of atrial cells was placed in a perfusion chamber on the stage of an inverted microscope (BioStar, Reichert Scientific Industries, Buffalo, N.Y.). The recording chamber and perfusate were kept at a constant temperature using a Peltier-based system (TS-4 thermal microscope stage, Physitemp Instruments, Inc., Clifton, N.J.) customized to switch off during a depolarization pulse. A gigahm seal was obtained using the technique described by Hamill et al.
After polarizing (−60 to −80 mV), the membrane was ruptured, and the membrane potential was adjusted to the desired voltage. The capacity of the cell, electrode, and amplifier input were nullled, and the series resistance was simultaneously compensated. We could compensate for 50–90% of the series resistance. The voltage error resulting from residual uncompensated series resistance at peak inward sodium current (INa) was estimated. We proceeded with the experiment if this voltage error was less than 3.5 mV.8 A current–voltage relation was determined. If any threshold phenomenon was observed in the negative limb of the current–voltage curve, the experiment was abandoned.

**Voltage-Clamp Protocols**

Cells were exposed to experimental conditions for 20 minutes (i.e., after each drug change or washout), unless otherwise stated. After rupturing the membrane and achieving voltage-clamp conditions, we allowed the cell to equilibrate for at least 15 minutes before acquiring data. A time-dependent shift in the voltage dependence of inactivation is known to occur in the whole-cell voltage-clamp configuration. The 15-minute wait minimized the possible effects of the shift. External solution NaCl content was 75 mmol/l and pH 7.2 unless otherwise specified. Experiments were performed at 15°C.

**Development of Block**

We determined the onset of block by GX using two protocols: 1) a single depolarizing pulse of increasing duration and 2) pulse trains of varying frequency. For the single-pulse uptake experiments, a depolarized conditioning pulse of increasing duration from 0.15 to 14 seconds was applied from a holding potential of −120 mV to a test potential of −20 mV. After recovering at the holding potential for 500 msec, a test pulse to −20 mV was applied. This recovery interval was sufficiently long to allow drug-free channels to recover from fast inactivation. Trains of brief (50-msec) pulses that produce only fast inactivation do not produce cumulative decline of sodium current when the interpulse interval is greater than 450 msec (see Figures 2 and 3). A rest period of 90 seconds at the holding potential followed the test pulse before the next conditioning pulse was applied.

To assess uptake during pulse-train stimulation, we applied pulses to −20 mV from holding potentials of −100 and −140 mV. At pulse intervals of 0.15, 0.2, and 0.45 seconds, we applied fifty 50-msec pulses per train. As steady-state block was reached with fewer pulses at pulse intervals of 0.95, 1.95, and 2.95 seconds, we applied only forty 50-msec pulses per train at those intervals.

Since previous experiments performed with lidocaine in an external NaCl ([NaCl]o) concentration of 20 mmol/l demonstrated two components to the uptake process,9 we performed four additional experiments with GX using an [NaCl]o of 20 mmol/l. Onset of GX blockade was assessed using both the single-pulse and pulse-train protocols described above from a holding potential of −120 mV.

**Recovery From Blockade**

We determined the recovery kinetics of INa from blockade by GX. To achieve steady-state blockade, we applied a train of fifty 50-msec pulses to −20 mV at a frequency of 3 Hz from holding potentials of −100, −120, and −140 mV. After each pulse train, the membrane was returned to the holding potential, and a 15-msec test pulse to −20 mV was introduced at increasing recovery intervals (range, 50 msec to 56 seconds). After the test pulse, the membrane was returned to the holding potential and allowed to rest for a period of 90 seconds before the next pulse train. When possible, we attempted to determine INa recovery parameters at all three holding potentials in each cell.

**Interaction With Lidocaine**

To determine whether the blocking actions of GX were additive or antagonistic to those of lidocaine, we performed uptake experiments in the presence of GX and lidocaine at holding potentials of −100 to −140 mV. We applied pulse trains of varying frequencies, using protocols identical to those used to determine use-dependent uptake (as described above). After applying the pulse trains to cells bathed in external solution containing either 74 μmol/l GX or 74 μmol/l lidocaine, we exposed the cells to external solution containing a combination of 74 μmol/l lidocaine and 74 μmol/l GX and repeated the protocol. After this, the cells were returned to the initial conditions for 15 minutes, and the protocol was repeated.

**Analysis**

In single-pulse uptake experiments, INa of the test pulse was analyzed as a function of the conditioning-pulse duration. The data were normalized by expressing test INa as a fraction of the INa from the preceding conditioning pulse. In pulse-train uptake experiments, the INa of each pulse was analyzed as a function of the peak number. The data were normalized by expressing the peak current of each pulse (Ipn) as a fraction of the peak current of the first pulse (Ip1) in the train (normalized INa=Ipn/IP1). In recovery experiments, the INa of each recovery test pulse was analyzed as a function of the recovery interval duration. The data were normalized by comparing the peak current of each recovery test pulse (INa) with the peak current of the first pulse of the next pulse train (Ip1) (normalized INa=INa/IP1).

We fit uptake and recovery data using a Marquardt routine that estimated the parameters for the sum of a constant and an exponential of the form a+b exp(−cx) and the sum of a constant and two exponentials of the form a+b exp(−cx)+f exp(−gx).10 We compared the goodness of fit of the data to one or two exponentials with the F ratio test and accepted the two-exponential fit if it provided a significant improvement (p<0.05). We compared results in the drug-free state with those obtained in the presence of GX. Student’s paired t test was used when the same cell was exposed to both conditions. When different cells were exposed to both conditions, we used a t test for unpaired data. A value of p<0.05 was considered significant.11 Data are reported as mean±SD.
Blockade Model

We modeled the interaction between GX, lidocaine, and the sodium channel as if the drug had limited access to the channel binding site.\textsuperscript{12} At the depolarized clamp potential, we assumed that all binding sites were accessible, whereas at the rest clamp potential, we assumed that all binding sites were inaccessible. Schematically

\[
U + D_a \rightarrow B_a \quad \text{(fast)} \\
U + D_b \rightarrow B_b \quad \text{(slow)}
\]

where \(U\) represents the unbound channel, and \(B\) represents the drug-bound or blocked channel. \(D_a\) is the rapidly unbinding (subscript a) drug, and \(D_b\) is the more slowly unbinding (subscript b) "toxic" drug. \(k_a\) and \(k_b\) represent the association or binding kinetics of the drug, and \(l_a\) and \(l_b\) represent the unbinding kinetics of the drug at the depolarized potential. In the equation below, the fraction of blocked channels was determined from \(B_a\), the fraction of sites complexes with \(D_a\), and from \(B_b\), the fraction of sites complexed with \(D_b\). During the depolarized interval, the time-dependent blockade is described by

\[
\frac{dB_a}{dt} = k_a D_a [1 - (B_a + B_b)] - l_b B_b 
\]

\[
\frac{dB_b}{dt} = k_b D_b [1 - (B_a + B_b)] - l_b B_b 
\]

whereas during the rest interval, we assume that all sites are inaccessible, so the fractions of blocked channels are described by

\[
\frac{dB_a}{dt} = -l_a B_a 
\]

\[
\frac{dB_b}{dt} = -l_a B_b 
\]

where \(l_a\) and \(l_b\) represent the unbinding kinetics of the drug at the resting potential. When the four equations are solved simultaneously by matrix methods, the time-dependent (t) description for formation of drug-channel complexes \(b(t)\) during the test-clamp potential can be reduced to the sum of two exponentials:

\[
b(t) = B_a(t) + B_b(t) = C_{da} \exp(-\lambda_a t) + C_{db} \exp(-\lambda_b t) + C_s 
\]

where \(\lambda_a\) and \(\lambda_b\) are complex functions of \(k_a\), \(k_b\), \(C_{da}\), \(C_{db}\), \(D_a\) and \(D_b\). \(C\) represents the weight given to each exponent. During the rest potential interval, the time course of unblocking \(b(t)\) is described by

\[
b_i(t) = C_i \exp(-l_{ia} t) + C_i \exp(-l_{ib} t) 
\]

From this equation, it is clear that the rate of unblocking at the rest potential can neither exceed that of the most rapidly unbinding agent nor be smaller than that of the most slowly unbinding agent. In other words, if GX unbinds more rapidly than lidocaine, then the GX unbinding rate will place an upper limit on the unbinding rate of any mixture of GX and lidocaine, and GX will place a lower limit on the mixture's unbinding rate.

For our numerical experiments, we used pulse-train stimulation (50-msec pulses) to switch the channels between bindable and unbindable conformations and estimated the fraction of blocked channels at each transition of the membrane potential using Equations 5 and 6. We assumed an initial block of 0. For the rapidly unbinding competitor, we used experimentally determined values of lidocaine binding kinetics. For the test potential (-20 mV), \(k_a\) was 3.6×10\(^8\) /M/sec and \(l_b\) was 0.68/sec. At the rest potential \(l_b\) was 0.4/sec.\textsuperscript{13} For analysis purposes, we assumed that the toxic agent \(D_b\) exhibited kinetic rates at the test potential equivalent to lidocaine but exhibited slower unbinding rates at the rest potential. We probed the possibility of blockade reduction through competition by evaluating the steady-state fraction of blocked channels when the toxic agent was assigned unblocking rates ranging from 0.4 to 0.004/sec.

Since the potential for a reduction in steady-state blockade by competition for a common binding site is dominated by the unbinding kinetics at the rest potential, the results of these numerical experiments are insensitive to the details of blocking process (such as block of open or inactivated channel conformations). We further explored the role of blocking kinetics at the test potential by varying \(k_b\) for the competitive antagonist over a range from one to 64 times the basic binding rates exhibited by the toxic agent \(D_b\) while holding \(k_b\) constant.

Results

Characteristics of the Preparation and Sodium Current in the Absence of Drug

The experiments reported in this manuscript were performed on isolated cultured atrial myocytes of the rabbit. The rabbit was used because it was sufficiently large to be a suitable source of cells from all regions of the heart including the sinoatrial and atrioventricular nodes and the Purkinje fibers. The atrial myocyte was selected for a number of reasons. The cells are quite small. The measured capacitance depended on cell size. In our initial studies, capacitance averaged 28±6 and 26±1 pF.\textsuperscript{8,13} As use of the preparation has evolved, we have used smaller cells, with the capacitance in the present study averaging 16±4 pF. The small capacitance has permitted voltage clamp of \(I_{Na}\) with \([Na]_o\) of up to 150 mM, with good voltage control as judged by indirect criteria.\textsuperscript{8,9,13,26} The indirect criteria used to assess voltage control have been stated in prior publications from this laboratory,\textsuperscript{13} (see also "Materials and Methods" in this manuscript). They are similar to those used by other investigators (e.g., see Reference 9). The characteristics of the sodium current are similar to those reported in other dialyzed cardiac cells. The digitizing rates were not sufficiently rapid to calculate the time constant of the capacitive transient and, hence, the speed of the voltage clamp. However, total duration of the transient in this and previous studies from this laboratory suggest that the speed of the clamp is at least as good as that reported for other atrial and ventricular myocytes. The membrane potential for half inactivation is approximately -90 mV (-91±6 and -90±5 mV in References
8 and 13, respectively). We did observe a time-dependent shift in the inactivation curve to more negative potentials (see also Reference 14). The shift was most rapid in the first 5–10 minutes after initiation of the whole-cell configuration. For this reason, we waited 15 minutes before any data were collected. The shift may be critical when comparing the action of a single drug followed by a combination of two drugs. However, some of those experiments confirmatory data are presented showing the effect of reexposure to the initial drug only (e.g., see Figure 8). The negative shift in inactivation was associated with a slowing in the time constant of recovery. However, the time constant of recovery from inactivation in the absence of drug is at least an order of magnitude smaller than that during drug exposure. There was also time-dependent rundown of INa. Although much slower than that reported for the calcium current, it did occur in this preparation. It was our impression that rundown was much more rapid at low holding potentials compared with hyperpolarized holding potentials. Schubert et al.15 have presented data documenting the much more rapid rundown of INa at depolarized holding potentials; at a holding potential of −60 mV, INa decreased 89% in 30 minutes compared with little change at a holding potential of −120 mV. Because the holding potential and time were variables in these experiments, we make no comments about initial levels of block or inactivation.

Single-Pulse Uptake Results

To determine whether GX had an affinity for the inactive, open, or prepore state of the sodium channel, we examined the effect of depolarizing pulses of increasing duration on the INa of test pulses that followed the pulses by 500 msec (Figure 1). In the absence of drug, as the prepulse duration increased, INa declined very slowly. After a 14-second prepulse, INa decreased by 19±7% (n = 6). The time constant for the development of this process of slow inactivation was 10.7±5.1 seconds (n = 6). An additional component of block was evident after exposure to 74 μmol/l GX. The preliminary experiments included a number of early time points (<100-msec pulse duration), but we could discern no block with these brief pulses. In the experiment illustrated in Figure 1, fractional INa decreased by

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**FIGURE 1.** Current tracings and graphs illustrating characteristics of glycylxylidide (GX) uptake during a single depolarizing conditioning pulse. Top panel: Currents recorded from a single myocyte under voltage clamp. The tracings were obtained in the absence of drug, 20 minutes after exposure to 74 μmol/l GX, and 20 minutes after exposure to 148 μmol/l GX. The control current in each tracing is taken from the first conditioning pulse. The three test currents were recorded 500 msec after 0.7-, 5.0-, and 14.0-second conditioning pulses. The current and time calibrations are at the far right of the panel. Bottom panel: Graphs showing block of the sodium current after application of single conditioning prepulses. The normalized inward sodium current (INa) derived from the test pulse is plotted on the ordinate (peak INa was 2.6 nA under drug-free conditions, 2.3 nA after exposure to 74 μmol/l GX, and 1.9 nA after exposure to 148 μmol/l GX). The duration of the conditioning prepulse in seconds is plotted on the abscissa. The pulse paradigm is shown in the inset. From a holding potential of −120 mV, a conditioning prepulse of increasing duration is applied to −20 mV. The initial duration of the conditioning prepulse was 150 msec. The duration was incremented by 100 msec to 350 msec, by 300-msec steps from 400 to 1,000 msec, and by 3,000-msec steps from 2,000 to 14,000 msec. The open squares represent data obtained in the absence of drug; the filled triangles and squares represent data after exposure to 74 and 148 μmol/l GX, respectively. The continuous lines are single exponential fits to the data points. The time constants for the development of INa block were 5.6 seconds in the absence of drug, 6.8 seconds in 74 μmol/l GX, and 6.2 seconds in 148 μmol/l GX.
1% after a 1-second prepulse and by 39% after a 14-second prepulse. The time constant for the overall blockade of $I_{Na}$ was 6.8 seconds. The time constant for the development of slow inactivation was 5.6 seconds.

The two processes were sufficiently similar that they could not be resolved kinetically. When the concentration of GX was doubled (148 μmol/l), the fractional $I_{Na}$ at 14 seconds decreased by 52% (time constant for blockade, 4.7 seconds). The decrease in $I_{Na}$ during GX exposure was greater than that during control ($p<0.05$). This observation was noted in both unpaired experiments and in four experiments in which all observations were made in the same cell.

A similar study by Clarkson et al$^9$ showed that lidocaine block developed in two distinct phases, an initial rapid phase that paralleled channel activation and a slower phase that paralleled channel inactivation. They suggested that the rapid phase resulted from the block of activated channels. If the blocking site is along the channel-conducting pathway, this process should be sensitive to $[\text{Na}^+]$. A major difference between our study and that of Clarkson et al was that they used $[\text{Na}]_o$ of 25 mmol/l. In an attempt to identify an early rapid phase of block during GX exposure, we compared the kinetics of block development in four cells as $[\text{Na}]_o$ was varied between 20 and 75 mmol/l at holding potentials of −100 and −140 mV. In the experiments at $[\text{Na}]_o$ of 20 mM, the decrease of $I_{Na}$ plotted as a function of increasing conditioning pulse duration was well fitted by a single exponential. In two of five of the experiments with $[\text{Na}]_o$ of 75 mM, a second exponential improved the fit. However, the smaller of the two time constants was relatively large. The slow time constant for development of block during GX exposure was 6.3±1.1 and 6.8±1.2 seconds at a holding potential of −100 mV and 6.7±1.6 and 7.1±0.8 seconds at a holding potential of −140 mV in 20 and 75 mmol/l $[\text{Na}]_o$, respectively.

### Use-Dependent Uptake Results

We also examined the kinetics of the development of block by applying pulse trains of varying frequency. Trains of 40 or fifty 50-msec pulses to −20 mV were applied from holding potentials of −100 to −140 mV. In the absence of drug at a holding potential of −100 mV, $I_{Na}$ decreased when pulse trains were applied at pulse intervals of 0.15, 0.2, and 0.45 seconds (fractional current decrease, 0.28±0.04, 0.22±0.06, and 0.08±0.04, respectively). When the holding potential was increased to −140 mV, $I_{Na}$ did not decrease at any pulse interval. We believe that this reflects the changes in kinetics of recovery from sodium channel inactivation at different holding potentials.$^{17}$ To test this we performed experiments to determine recovery from sodium channel inactivation at holding potentials of −100 to −140 mV in the absence of GX. A conditioning pulse to −20 mV for 150 msec was applied; this pulse was followed by a 50-msec test pulse applied from the holding potential after increasing recovery intervals ($n=5$). The time constant for recovery from sodium channel inactivation was 0.009±0.001 second at −140 mV, 0.017±0.003 second at 120 mV, and 0.075±0.02 second at −100 mV.

The shortest pulse interval applied (0.15 second) was 15-fold greater than the time constant for recovery from inactivation at −140 mV. This compared with only a twofold difference at −100 mV. These data were consistent with the observation that $I_{Na}$ did not decrease over the course of pulses applied from −140 mV at 0.15-second intervals.

During exposure to 74 μmol/l GX, at a holding potential of −100 mV, $I_{Na}$ decreased at all pulse intervals tested. In Figure 2, the fractional $I_{Na}$ steady-state blockade was 0.29, 0.16, and 0.09 at pulse intervals of 0.45, 0.95, and 1.95 seconds, respectively. In this example, the decline in $I_{Na}$ during trains applied at each pulse interval could be fitted with a single exponential. As the pulse interval was increased, the pulse constant to steady-state blockade decreased (8.97, 5.47, and 3.47 pulses for pulse intervals of 0.45, 0.95, and 1.95 seconds, respectively). Table 1 summarizes the experimental data for pulse-train block at pH 7.2 and 7.4 when the holding potential is −100 mV. At a holding potential of −100 mV, two exponentials were required to characterize the development of block in 50% of trains with a pulse interval of 0.15 second and 20% of trains with a pulse interval of 0.2 second. This is consistent with two processes contributing to current decline at these inter-stimulus intervals in some experiments.

When the holding potential was increased to −140 mV (Figure 3), only the shortest pulse intervals (0.15 and 0.2 seconds) produced significant steady-state blockade. Table 1 also summarizes the experimental data for pulse-train block at pH 7.4 and a holding potential of −140 mV. Overall, less steady-state block was observed when the pulse trains were applied from −140 compared with −100 mV. Because the same pulse-clamp potential (−20 mV) was applied from both holding potentials, this strongly suggested that recovery from GX blockade was faster when holding at a potential of −140 mV than at −100 mV. We examined this issue with a protocol specifically designed to determine the kinetics of $I_{Na}$ recovery from GX blockade.

### Recovery Kinetics of $I_{Na}$

The kinetics of recovery from inactivation depended on the protocol used to induce inactivation. We explored two types of protocols. The simplest protocol involved the introduction of test pulses at increasing recovery intervals after a single conditioning pulse. A more complex protocol is exemplified by the paradigm used by Bennett et al$^4$ in which recovery from inactivation was determined by applying trains of pulses at rapid stimulation rates (10 Hz). Results of these experiments are shown in Figure 4. When inactivation was induced by a single 5-msec pulse, recovery was monoexponential in eight of nine experiments at a holding potential of −100 mV and in all seven experiments at −140 mV. The mean recovery time constant was 0.034±0.012 and 0.008±0.007 seconds at −100 and −140 mV, respectively. Panels C and D of Figure 4 compare recovery after single pulses and pulse trains at −100 and −140 mV in two separate cells. At −140 mV, recovery from a single pulse occurred with a time constant of 0.006 second. After the 10-Hz train, two exponentials were required to fit the recovery process. Ten percent of the current recovered slowly with a time constant of 0.214 second. Results in another cell with the holding potential at −100 mV are illustrated in panel D. In this cell, the single-pulse recovery was fitted by two exponentials.
with time constants of 0.222 and 0.023 seconds. The slow recovery time constant accounted for only 21% of the process. Most of the recovery process was complete in 0.50 second. After the 10-Hz train, the slow recovering component was more prominent, accounting for 30% of the current. We presumed that this slowly recovering component resulted from slow inactivation. Although we do not have evidence that is identical to the slow process affecting sodium availability in nerve and cardiac muscle, it seemed unnecessary to introduce an additional term to describe the process.

In selecting a stimulus protocol to determine recovery from block, a compromise had to be reached. On the one hand, pulse trains had to be of sufficient frequency to induce use-dependent block. On the other hand, it was desirable to avoid induction of slow inactivation with rapid trains. We used a 3-Hz train of fifty 50-msec pulses to achieve steady-state blockade in the presence of 74 μmol/l GX at three holding potentials (−100, −120, and −140 mV). Recovery from blockade was determined by measuring the peak $I_{Na}$ of test pulses applied after recovery intervals of increasing duration. Since the recovery time constant at −100 mV in the absence of drug was $0.075 \pm 0.02$ second (as mentioned above), a minimum recovery interval of 0.4 second was sufficient to permit the sodium channel to recover from inactivation. Figure 5 compares the recovery from block at −100 and −120 mV during GX exposure. The steady-state level of block was greater at −100 than at −120 mV. At each holding potential, recovery could be fitted with a single exponential. The recovery time constant was 7.1 seconds at −100 mV and 4.9 seconds at −120 mV. A similar comparison between recovery at −120 and −140 mV is shown in Figure 6. In the experiment illustrated, the recovery time constant is 7.7 seconds at −120 mV and 4.7 at −140 mV. At −140 mV, the fractional steady-state block was only 0.11 (average of 0.10 for four experiments). In three additional experiments, the steady-state block was less than 5%, and an accurate determination of the recovery time constant could not be made. We have summarized the data from 31 experiments in Table 2. The recovery from GX block
was significantly slower at -100 mV compared with -140 mV. This is internally consistent with the observation that greater fractional steady-state block was produced when pulse trains were applied from holding potentials of -100 mV than from -140 mV.

A prior study from this laboratory examined the dependence of the kinetics of recovery of maximum upstroke velocity from GX block as external pH was varied. That study showed an acceleration of recovery from block as pH was increased. Limitations of that study were as follows: maximum upstroke velocity, which is an indirect measure of \( I_{Na} \), was used; the pH-dependent changes in action potential duration could not be precisely controlled; and all studies were performed at approximately the same potential. We attempted to expand the initial observation by direct measurement of \( I_{Na} \) at three holding potentials as external pH was varied. The measurement of the recovery kinetics at three potentials and two levels of external pH required stable recording conditions for 3 hours. We were unable to do this successfully; therefore, experiments at pH 7.2 and 7.8 were performed on separate cells at each potential. There was a trend for the recovery kinetics to be faster at pH 7.8 than at pH 7.2; however, because of the intercellular variability in the recovery time constants, the changes did not meet statistical significance. The results from all of these experiments are summarized in Table 2.

**Interactions Between Lidocaine and GX**

Clinically, GX usually appears in the blood after lidocaine administration. Previous reports have indicated that either additive or antagonistic interactions between GX and lidocaine may be observed, depending on the potential at which the membrane is held. These studies reported that, at depolarized holding potentials, addition of GX reversed \( I_{Na} \) blockade by lidocaine, whereas at hyperpolarized potentials, addition of GX increased \( I_{Na} \) block by lidocaine. Therefore, we examined \( I_{Na} \) blockade at holding potentials of -100 to -140 mV during exposure to 74 \( \mu \)mol/l GX and a combination of 74 \( \mu \)mol/l lidocaine and 74 \( \mu \)mol/l GX (Figure 7). Steady-state blockade was induced by applying fifty 50-msec pulses at various interpulse intervals to -20 mV. The results of one experiment are presented in Figure 7. At a pulse interval of 0.45 seconds and holding potential of -100 mV, in the presence of GX alone \( I_{Na} \) declined from 7.6 to 5.2 nA from the first to the 50th pulse of the train. After the addition of lidocaine to GX, the \( I_{Na} \) of the first pulse of the train decreased to 5.3 nA. After 50 pulses, there was a further \( I_{Na} \) decrease to 1.9 nA. Thus, the fractional block at steady state was clearly greater during exposure to the drug combination. We use the term additive to describe this greater total block from the drug combination. When the holding potential was increased to -140 mV, the qualitative results were similar (i.e., the addition of lidocaine increased the use-dependent block). We did not observe significant use-dependent block by GX at all pulse intervals when trains were applied from a -140 mV holding potential. Since the onset of GX block is slow (uptake time constant \( \tau \), 5.8 seconds) and the recovery from block at -140 mV is more rapid, our 50-msec pulses may not have been sufficiently long to permit adequate GX uptake. The results of four experiments are summarized in histogram form in Figure 8.

After exposure to drug combinations, we attempted to return to the initial (single-drug) conditions. A decrease in the additive blocking effect of lidocaine was evident after 15 minutes of lidocaine washout and exposure to GX alone in cells previously exposed to GX plus lidocaine. Results from two separate cells are shown in the upper and lower panels of Figure 9. At a holding potential of -100 mV, 18% phasic block developed during pulse train stimulation, and the pulse constant for block development was 8.5 pulses. During exposure to the combination of GX plus lidocaine, the current during the first pulse was reduced from 5.5 to

### Table 1. Pulse-Train Data in the Presence of 74 \( \mu \)mol/l Glycylxylidide

<table>
<thead>
<tr>
<th>Pulse interval</th>
<th>0.15 sec</th>
<th>0.2 sec</th>
<th>0.45 sec</th>
<th>0.95 sec</th>
<th>1.95 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
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<td>Mean±SD n</td>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
</tr>
</tbody>
</table>

- \( \lambda_{1}^{-1} \) pulses
- \( \lambda_{2}^{-1} \) pulses

\( n \), Number of experiments; SSB, use-dependent fractional glycylxylidide blockade; pulses, uptake pulse constants for glycylxylidide blockade; \( \lambda_{1} \), pulse constant for the development of block. \( \lambda_{1} \), pulse constant derived from a single-exponential curve fit or the larger pulse constant when the curve was best described by two exponentials; \( \lambda_{2} \), the smaller pulse constant derived when the curve was best described by two experiments.

<table>
<thead>
<tr>
<th>pH 7.2 (-100 mV)</th>
<th>0.15 sec</th>
<th>0.2 sec</th>
<th>0.45 sec</th>
<th>0.95 sec</th>
<th>1.95 sec</th>
</tr>
</thead>
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<tr>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
<td>Mean±SD n</td>
</tr>
</tbody>
</table>

- \( \lambda_{1}^{-1} \) pulses
- \( \lambda_{2}^{-1} \) pulses

\( \lambda_{1}^{-1} \) pulses

\( \lambda_{2}^{-1} \) pulses

\( \lambda_{1}^{-1} \) pulses

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\( \lambda_{1}^{-1} \) pulses

\( \lambda_{2}^{-1} \) pulses
3.1 nA, suggesting a significant amount of tonic block and/or current run-down. (There was a tendency for greater run-down of $I_{Na}$ during prolonged holding at depolarized potentials.) The phasic block amounted to 52%. After washing for 15 minutes with solution containing GX only, the first pulse current was 4.2 nA, and the phasic component of block comprised only 24%, a value similar to that during the initial exposure to GX. Results from another cell in which the holding potential was $-120$ mV are shown in the lower panel. Greater phasic block (36%) developed during exposure to GX plus lidocaine than to GX alone (7% and 8% during the initial and final exposure, respectively, to GX alone).

Experiments in which cells were initially exposed to lidocaine only are shown in Figure 10. At a holding potential of $-100$ mV, the phasic block at steady state increased from 54% to 63% when GX was added to the lidocaine solution. The decrease in the first pulse current during exposure to lidocaine plus GX may have resulted from increased tonic block. Some run-down of current cannot be excluded. The results of an experiment in another cell at a holding potential of $-140$ mV are shown in the right panel. Phasic block was 15% and 23% during exposure to lidocaine and lidocaine plus GX, respectively. Apparently, under our recording conditions the kinetics of interaction of GX and lidocaine with the sodium channel are such as to lead to additive fractional blockade at holding potentials of $-100$ to $-140$ mV.

**Simulation Requirements for Reversal of Blockade With Drug Combinations**

Both in vitro experiments and clinical observations suggest that the phenomenon of competition between agents binding at a single periodically accessible site may occur.\textsuperscript{19} Theoretical studies based on the guarded-receptor model have been performed using unbinding rates for lidocaine and bupivacaine.\textsuperscript{12} Use-dependent
blockade of both drugs was shown to be well described by the guarded receptor model. Moreover, predicted blockade of mixtures was in agreement with experimental results reported by Clarkson and Hondeghem.20 These numerical studies have shown that a drug with fast recovery kinetics may compete with another that has slow recovery kinetics. At some pulse intervals, when the fast recovery drug is added, the fraction of channels blocked by the slower recovery drug at steady state is reduced compared with the fraction of blocked

**FIGURE 4.** Current tracings and graphs illustrating the relation of inward sodium current recovery kinetics to the pulse protocol chosen. Panel A: Current tracings illustrating inward sodium current recovery kinetics after a single 5-msec depolarizing pulse. On the left, at a holding potential of −140 mV, test currents are illustrated after recovery intervals of 5, 8, 11, 17, and 35 msec. On the right, at a holding potential of −100 mV, test currents are illustrated after recovery intervals of 5, 20, 60, 120, and 450 msec. Panel B: The amplitude of the test current (nA) plotted as a function of the recovery interval (in seconds). The time course of inward sodium current recovery at both −140 and −100 mV was well fit with a single exponential. Recovery time constants were 6.3 and 29.6 msec, respectively. Panels C and D depict experiments in two separate cells in which inward sodium current recovery kinetics after a single 5-msec depolarizing pulse (open symbol) is compared with inward sodium current recovery kinetics after a train of fifty 5-msec pulses delivered at a frequency of 10 Hz (solid symbol). Panel C: Experiments performed while holding at a potential of −140 mV. Inward sodium current recovery after a single 5-msec depolarizing conditioning pulse was best described by a single exponential (time constant, 9.1 msec). Recovery after a train of fifty 5-msec depolarizing conditioning pulses was best described by two exponentials (time constants of 6.3 and 214 msec and relative weights of 90% and 10%, respectively). Thus, the slow recovery process accounted for 10% of inward sodium current recovery after a pulse train. Panel D: Experiments performed while holding at −100 mV. In this experiment, inward sodium current recovery after a single 5-msec depolarizing conditioning pulse was best described by two exponentials (time constants of 23.1 and 222 msec and relative weights of 79% and 21%, respectively). Recovery after a pulse train was also best described by two exponentials (time constants of 29.8 and 917 msec and relative weights of 70% and 30%, respectively).
channels at steady state in the presence of the slow recovery drug alone.

Our studies of the recovery from GX blockade of I_{Na} demonstrated a slower rate of recovery than for lidocaine blockade of I_{Na} at both -100 and -140 mV. Therefore, one would not expect a mixture of lidocaine and GX to exhibit a rate of recovery faster than that of lidocaine alone, as observed by Bennett et al. We neither would one expect less steady-state blockade in the presence of a mixture of GX and lidocaine compared with lidocaine alone. It is interesting to reverse the question in light of our results and ask under what conditions might a hypothetical agent reduce the steady-state fraction of channels blocked by a slowly unbinding drug like GX?

From Equation 6, it is clear that the rate of recovery from a mixture of two use-dependent drugs is bounded by the rates of the two components of the mixture. No general information is available that documents the magnitude of differences in rates between fast and slow unbinding drugs required for competitive reduction in the steady-state fraction of blocked channels. To explore these kinetic requirements, simulations of sodium channel blockade by fast and slow drugs were performed. For the fast unbinding drug, we selected a binding rate constant consistent with that of 20 μM lidocaine (k_d=0.72/sec and l=0.68/sec). At the rest potential, we used l=0.4/sec. For the slowly unbinding drug, we selected unbinding rate constants that ranged from equivalent to 100-fold slower (0.4/sec, 0.08/sec, 0.04/sec, 0.008/sec, and 0.004/sec) than the fast drug.

Steady-state channel blockade was determined at pulse intervals of 0.35, 0.55, 0.75, 0.95, 1.95, 2.95, 3.95, 4.95, 5.95, and 9.95 seconds for the test drug described above. This simulation was repeated under conditions of equimolar concentrations of fast and slow drug. The results are summarized in Figure 11. The derived data are represented as the difference in fractional I_{Na} blockade plotted as a function of the pulse interval. A positive difference
reflects an increase in steady-state block when the fast drug is added to the slow drug. A negative difference reflects a decrease in steady-state block (i.e., indicating relief of block) after the addition of the fast drug.

At equimolar drug concentrations, even when the unbinding kinetics of the slow drug was 100-fold slower than the fast drug, it was difficult to discern a decrease in blockade at any pulse interval; e.g., when the slow drug recovery rate constant was 0.004/sec, addition of the fast drug produced a decrease in steady-state block of only 1% (pulse interval, 9.95 seconds).

When the binding rate of the fast drug was increased 16-fold, the fraction of channels bound by the fast drug was markedly increased. In combination with a drug with very slow unbinding kinetics (1=0.004/sec), steady-state block decreased by 2% and 5% at pulse intervals of 3.95 and 9.95 seconds, respectively. When the forward binding rate was increased 64-fold (Figure 11, bottom panel), the reduction in steady-state block at the 9.95-second stimulus interval was 15%.

The simulations indicate that a large fraction of channels must be occupied by the faster drug and that the unbinding rate constant of the slow drug must be more than an order of magnitude less before a significant reduction in steady-state blockade is observed.

**TABLE 2.** Time Constants of Recovery of Inward Sodium Current From Glycylxylidide Blockade

<table>
<thead>
<tr>
<th>Study conditions</th>
<th>Time constant of recovery (sec)</th>
<th>pH 7.2</th>
<th>pH 7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>−100 mV</td>
<td>10.3±4.2</td>
<td>6</td>
<td>7.3±1.9</td>
</tr>
<tr>
<td>−120 mV</td>
<td>5.3±2.2</td>
<td>8</td>
<td>5.4±1.9</td>
</tr>
<tr>
<td>−140 mV</td>
<td>4.1±0.4*</td>
<td>4</td>
<td>3.0±1.6†</td>
</tr>
</tbody>
</table>

*n*, Number of experiments.

*p<0.05 and †p=0.06 vs. corresponding value at −100 mV.

**FIGURE 6.** Current tracings and graphs comparing the recovery of inward sodium current (I Na) from use-dependent blockade during exposure to 74 μmol/l glycylxylidide at holding potentials of −120 and −140 mV. Top panel: Current tracings from a single cell. The largest current in each tracing is from the first pulse of the uptake train. The test currents (smallest to largest) were recorded after recovery intervals of 0.4, 1.2, and 8.0 seconds. When holding at a −120 mV potential, steady-state I Na was 1.7 nA. Test currents after recovery intervals of 0.4, 1.2, and 8.0 seconds were 2.2, 2.2, and 2.5 nA. When holding at a potential of −140 mV, steady-state I Na was 3.8 nA. Test currents after recovery intervals of 0.4, 1.2, and 8.0 seconds were 4.5, 4.6, and 4.8 nA. The current and time calibrations are at the far right of the top panel. Bottom panel: Graphs showing recovery of sodium current from steady-state blockade. The normalized peak I Na is plotted on the ordinate, and the recovery interval is plotted in seconds on the abscissa. The pulse paradigm is similar to that in Figure 4 except the holding potentials are −120 and −140 mV. The filled triangles represent data obtained at −120 mV, and the filled circles represent data obtained at −140 mV. The continuous lines are single-exponential fits to the data points. In this example, the time constant of recovery is 7.7 seconds at −120 mV and 4.7 seconds at −140 mV.
**Discussion**

We have studied the kinetics of interaction of GX with the cardiac sodium channel using whole-cell voltage-clamp techniques in cultured rabbit atrial myocytes. The onset of \( I_{Na} \) blockade by GX appears to be slow; a time constant for block onset of 7 seconds at the depolarized conditioning potential of \(-20\) mV was observed in these experiments. This slow block had a time course similar to that of slow inactivation of the sodium current. The time constants for the development of those two processes were so similar that they could not be separated. Whether the slow inactivated state is a prerequisite for block cannot be determined from these experiments. To demonstrate a significant increase in block, we had to use a high drug concentration. During pulse train stimulation, the phasic component of GX block also developed slowly. Long trains of 40–50 pulses were used to induce block. For protocols designed to examine the recovery from block, we used a train frequency of 3 Hz. A very rapid pulse train may lead to the accumulation of significant slow inactivation.\(^9\) During exposure to GX, greater use-dependent block was observed at a holding potential of \(-100\) mV than at \(-140\) mV. This suggested that recovery from GX block may be slower at \(-100\) mV. Direct measurements of the recovery time constants at \(-140\) and \(-100\) mV showed that the 40-mV depolarization in holding potential led to a 2.5-fold increase in the recovery time constant. Qualitatively, this trend is similar to that of lidocaine.\(^21\) At both \(-140\) and \(-100\) mV, greater steady-state block was observed in response to pulse trains applied at all pulse intervals (from 0.15 to 1.95 seconds).
during exposure to the combination of GX and lidocaine than to GX alone.

The whole-cell variation of the patch-clamp technique has simplified the measurement of the sodium current considerably. Because of the rapid activation kinetics of the sodium channel, separation of the capacitive transient and rising phase of the sodium current remains a challenge. We used cultured cells (with a total capacitance one third that of freshly isolated cells) and low resistance microelectrodes (400-1,000 kΩ). Nevertheless, it was necessary to perform the experiments at a reduced temperature of 15°C. This may have several important effects: 1) A lower temperature may shift the voltage dependence of kinetics (in particular inactivation) to more negative potentials. 2) A lower temperature may alter the PK and distribution of drug in the cell membrane. Makielski and Falleroni have shown that the fractional block of the sodium channel by lidocaine is decreased and that the kinetics are speeded up by temperature elevation. Despite these shortcomings, we believe that the qualitative interpretation of our results is correct.

Of the two determinants of use-dependent block of the sodium channel, onset and recovery from block, the bases for the latter have been studied in more detail. The work of Courtney suggests that molecular weight and lipid solubility are major factors that determine the rate of recovery from block. In general, agents with low molecular weight and high lipid solubility have the most rapid recovery kinetics. In comparing lidocaine and GX, the difference in their molecular weights (178 versus 234) is more than counterbalanced by the low lipid solubility of GX (1:100 [GX : lidocaine]).

Two previous studies have examined the interaction of GX with the cardiac sodium channel. Broughton et al performed experiments on guinea pig papillary muscle at 36°C. They measured the time constant of recovery from block during exposure to lidocaine, monoethylglycinexylidide, and GX using maximum upstroke velocity as a measure of available sodium conductance. They observed that lidocaine block dissipated 1.3 times more rapidly than GX and 1.6 times more rapidly than monoethylglycinexylidide. Furthermore, this process appeared to be independent of drug concentration. The results of Broughton et al showing
slower recovery from block with GX than with lidocaine are consistent with our results.

More recently, Bennett et al.14 reported the late recurrence of ventricular arrhythmias after their early suppression by lidocaine. The recurrences of the arrhythmias coincided with the appearance of the lidocaine metabolite GX. To explore the basis for this observation, they determined the kinetics of interaction of GX alone and GX in combination with lidocaine in guinea pig ventricular myocytes under voltage clamp. At the depolarized potentials of -80 to -100 mV, channels thought to be blocked by GX recovered faster than lidocaine-blocked channels, whereas the converse was observed at hyperpolarized potentials (from -120 to -140 mV). In one third of their studies, less block was observed during exposure to the combination of GX and lidocaine than to lidocaine alone at depolarized potentials. The results are qualitatively different from our own. Some of the possible causes of the different results shall now be reviewed.

The differences in the recording conditions are a potential source of the diametrically opposite results.
The experiments of Bennett et al. were performed on freshly isolated guinea pig ventricular myocytes, whereas our experiments were performed on cultured rabbit atrial myocytes. The difference in the species studied is a potential source of the variant results. Circumstances under which substantial species differences in mammalian sodium channel function occur are probably few. The brief period of culture may have modified sodium channel properties. Occasionally, we have recorded whole-cell sodium currents from the atrial myocytes within 4 hours of isolation and have not observed any qualitative differences when compared with the cultured cells. A time-dependent shift of the inactivation curve to a more negative potential was observed in our experiments but evidently not in the ventricular myocyte preparation used by Bennett et al (see their Figure 3). It is not apparent what difference in recording conditions obviated the shift. The smaller size of the cultured cells enabled us to routinely use higher \([Na]_o\) (75 versus 20 mmol/l). If block occurs in the ion conduction pathway, then the kinetics of onset and recovery from block may be a function of \([Na]_o\). In our experiments the rate of block onset during pulse-train stimulation was similar in \([Na]_o\) of 20 and 75 mmol/l. Barber et al. have reported that the time constants of recovery from block during lidocaine exposure were unchanged over a range of \([Na]_o\) of 20–150 mmol/l. Most of our experiments were performed at a pH of 7.2. These experiments were performed as part of a series that examined the blocking action of a number of class 1 drugs at pH 7.2, 7.4, and 7.8. Data from several of the protocols performed at pH 7.2, 7.4, and 7.8 are reported in Tables 1 and 2. Greater use-dependent block and slower recovery were observed at -100 mV compared with -140 mV during GX exposure.

Bennett et al. used a 10-Hz train of 5-msec pulses to induce block. Although the duration of pulses was quite brief, there was little time for recovery between them. Significant slow and fast inactivation would develop with such trains. Recovery from slow inactivation was also slow, with time constants of the same order of magnitude as that of GX block. Little additional decline in current was observed when the protocol was used during exposure to 47 \(\mu\)mol/l GX (Figure 2 of Reference 4); therefore, it appears that a major fraction of the process undergoing recovery is inactivation. If the amount of slow inactivation was greater at a holding potential of -90 mV than at -140 mV and slow inactivation recovered faster than block, the apparent faster recovery from block at -90 mV may be explained. The potency of GX observed in the studies of Bennett et al. is clearly different from that in other studies. In their study, 47 \(\mu\)M GX caused slightly more block than 37 \(\mu\)M lidocaine (their Figure 1). However, in vitro studies suggest that GX has 1/10 the antiarrhythmic potency of lidocaine. In the study of Broughton et al.
performed in guinea pig ventricular muscle, 120 μM GX produced 14% block at short diastolic intervals (b, in their Figure 3) compared with 54% block by 30 μM lidocaine. Similarly, in frog node of Ranvier, 800 μM GX produced 38% block of I_Na compared with 60% block by 200 μM lidocaine during pulse train stimulation (stimulation frequency, 10 Hz).26,27 These studies clearly show that GX is considerably less potent as a sodium channel blocker than lidocaine. To measure the kinetics of recovery from block, we elected to increase the drug concentration and use a slower (3-Hz) pulse train in which the amount of steady-state inactivation in the absence of drug was small at a holding potential of −100 mV and at −140 mV. Pulse-train experiments with pulse intervals of 0.15 to 1.95 seconds showed greater block at −100 mV than at −140 mV during

**FIGURE 11.** Simulations illustrating the competition between drugs with fast and slow unbinding kinetics for a single binding site on the sodium channel. Top panel: Simulations performed with equimolar concentrations of fast and slow drug. The dissociation rate constant of the slow drug was used as a model parameter. The dissociation rate constant assigned to the slow drug was 0.4/sec (solid squares), 0.08/sec (solid circles), 0.04/sec (solid triangles), 0.008/sec (open squares), or 0.004/sec (open triangles). The difference (Δ) in fractional blockade of inward sodium current at steady-state (fractional inward sodium current blockade for the drug combination minus fractional inward sodium current blockade for the slow drug) during pulse train stimulation is plotted on the ordinate. The interval between pulses in the blocking train is plotted on the abscissa. A positive difference indicates additive blockade; a negative difference indicates relief of blockade. Only additive blockade is observed for slow drugs with a dissociation rate constant of 0.4/sec, 0.08/sec, and 0.04/sec. Relief of blockade (crossover point) is observed during pulse trains applied at intervals of 3.95, 4.95, 5.95, and 9.95 seconds for slow drugs with dissociation rate constants of 0.008/sec and 0.004/sec, respectively. A maximal blockade reversal of <1% is observed under the conditions in this simulation. Bottom panel: Simulations performed with the concentration of the fast drug 64 times that of the slow drug. The data are depicted in a fashion similar to that in the top panel. The dissociation rate constants assigned to the fast and slow drugs are identical to those above. Only additive blockade is observed for slow drugs with dissociation rate constants of 0.4/sec and 0.08/sec. Relief of blockade (crossover point) is observed during pulse trains applied at intervals of 3.95–9.95 seconds for slow drugs with dissociation rate constants of 0.008/sec and 0.004/sec and 9.95 seconds for drugs with a dissociation rate constant of 0.04/sec. A maximal blockade reversal of 15% is observed under the conditions in this simulation.
exposure to GX. This result is consistent with a slower recovery from block at the more depolarized potentials.

A major motivation for performing this study was to determine the competitive interaction between the parent drug, lidocaine, and its metabolite GX. At both -100 and -140 mV, the combination of drugs produced greater block than GX alone. When measured with a single pulse of increasing duration, the rate of block onset was about one third that reported for lidocaine. This would favor some displacement of GX by lidocaine if they both interacted with the same receptor site. However, the recovery time constant for lidocaine block is only two to three times smaller than that for GX (the present study and unpublished data by D.J. Wendt, C.F. Starmer, and A.O. Grant for lidocaine). In the simulation to be discussed below, we show that such a small difference in recovery from block is not sufficient to permit observation of relief of block during exposure to a combination of agents. It is important to examine multiple trains, because relief of block may only occur at some stimulus frequencies. The analysis of competition between two drugs is best interpreted by comparing levels of use-dependent steady-state blockade. Bennett et al. reported the results of the effects of GX alone and GX and lidocaine at a single specific time after a pulse train. In order for a second drug to influence steady-state blockade in an additive or antagonistic fashion, there must be sufficient time between pulses such that a large percentage of channels blocked by the fast drug can become unblocked. Pulses applied at shorter intervals will not permit either drug to unbind, and steady-state blockade will increase. Pulses applied at longer intervals will permit unbinding of both drugs and may remove any evidence of use dependence.

A number of basic and clinical studies of sodium channel blocking agents have shown less block during exposure to a combination of drugs than to single drugs. These are summarized in Table 3. These results are counterintuitive. If two ligands bind to the same continuously accessible receptor, then an additive effect is expected. A different result may be obtained with receptors that have passively accessible binding sites or sites that transiently change their affinity. To observe a partial relief of block, the two agents must have markedly different kinetics of interaction with the receptor. If the forward binding rate of the "fast" drug is sufficiently great (e.g., a large association rate constant and/or a high drug concentration), it may displace the slow binding drug from the receptor. In the interval between pulses, the fast drug dissociates rapidly from the receptor site. The result is a decrease in steady-state blockade at some stimulation frequencies. Although lidocaine had much faster association and dissociation kinetics than GX, the combination of lidocaine and GX produced only additive blockade over a wide range of stimulus frequencies.

Despite the many clinical and basic reports of the competitive displacement of one sodium channel blocker by another, there are no data on the kinetic requirements for observation of this phenomenon. Starmer described a simplified model of the competitive interaction of two drugs with a transiently accessible receptor. The model was validated with data describing the interaction of lidocaine and bupivacaine and lidocaine and propoxyphene. In these studies, each drug was well characterized by the guarded-receptor model. Similarly, in these studies GX was also well characterized by the guarded-receptor model as determined by the linearity of the use-dependent uptake rate as a function of stimulus interval and the linearity of steady-state block as a function of \(\gamma\) (where \(\gamma = [1 - \exp(-\lambda t)]/[1 - \exp(-\lambda)]\) and \(\lambda\) is the recovery rate, \(t\) is the recovery time, and \(\lambda\) is the frequency-dependent uptake rate.)

We have used this model to determine the order of magnitude of differences in binding and unbinding kinetics that two agents must have to show competitive displacement. In the simulations, the agent with fast kinetics was assigned values similar to those reported for lidocaine. The drug dose and the recovery time

### Table 3. Clinical and Laboratory Reports of Competition Between Class 1 Local Anesthetic Agents

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### Clinical reports

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constant of the “slow” drug were model parameters. The simulation suggested that with equimolar concentration of fast and slow drugs, a difference in recovery time constant of almost 100-fold was required to observe competitive displacement. If the concentration of the fast drug was increased 64-fold (to enhance the forward binding rate), then a 10-fold difference in recovery time constant was enough to show competitive displacement. The simulations suggested that in the very specific circumstances in which a fast sodium channel blocker may be used in an attempt to reverse the blockade of a slower agent, very high concentrations of the fast agent may be required.

The simulations are consistent with some of the clinical and basic observations that suggest the importance of drug dose and binding and unbinding kinetics in effecting competitive reversal. In the clinical case reported by von Dach and Streuli, they observed QRS prolongation to 320 msec in a patient who ingested the sodium channel blocking toxin in yew needles. Despite the marked QRS prolongation, very large doses of lidocaine (300 mg/hr) led to QRS narrowing. A previous report from our laboratory showed partial reversal of propoxyphene-induced QRS duration by lidocaine. In vitro experiments suggested that the time constant for recovery from lidocaine blockade was 1/10 that of propoxyphene. In contrast, our present in vitro experiments suggested that the time constant for recovery from lidocaine blockade was only one third that of GX, and the simulation suggested no reversal would be observed.

In summary, the association and dissociation rates of GX with the cardiac sodium channel are slow. The voltage dependence of recovery from block is similar to that for lidocaine. Hyperpolarization accelerated recovery from block. Exposure to the combination of lidocaine and GX produced only additive block. Simulations suggest that marked differences in recovery time constant and or association time constant are required to observe the competitive displacement by an agent with fast recovery kinetics.

References


Kinetics of interaction of the lidocaine metabolite glycylxylidide with the cardiac sodium channel. Additive blockade with lidocaine.

D J Wendt, C F Starmer and A O Grant

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