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The activation gate of cardiac Na^+ channel modulates voltage- and pH-dependent unbinding of disopyramide

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Abstract

To assess the drug unbinding process from receptor sites in cardiac Na^+ channels, we examined the recovery kinetics of disopyramide-blocked Na^+ current (I_{Na}) in isolated guinea-pig ventricular myocytes using the whole-cell variation of the patch-clamp technique. In the presence of disopyramide (20 μM), the time course of I_{Na} recovery from use-dependent block (unbinding) was described by a double exponential function. Although the time constant for the fast phase (τ_f) of recovery was unchanged at different membrane voltages, the slow phase (τ_s) increased with hyperpolarizing membrane potential: 4.4 ± 0.2 s at a holding potential of -90 mV and 6.4 ± 0.3 s at -140 mV ($n = 10$, $P < 0.01$). The slow time constant of I_{Na} recovery was also increased by acidification. These findings suggest that disopyramide molecules can escape from the receptor site through the hydrophobic pathway after deprotonation, because slowing of recovery from use-dependent block by acidification is caused by a decreased deprotonation rate of receptor-bound drug molecules. In addition to the hydrophobic escape, the roles of the fast inactivation gate and activation gate (m-gate) were evaluated during the recovery process. After inhibition of the fast inactivation process of I_{Na} by pretreatment with chloramine-T (2 mM), the fast phase of recovery from use-dependent block by disopyramide was abolished. However, the slow time constant (τ), the voltage- and pH-dependent nature of the recovery time course remained almost unchanged after inhibition of the fast inactivation process (τ: 5.3–7.5 s, $n = 6$), suggesting that some population of drug molecules can escape directly from the channel when the m-gate is open. We conclude that the unbinding process of disopyramide molecules from the receptor site of Na^+ channels may be composed of both the hydrophobic pathway after deprotonation and the m-gate untrapping.

Keywords: Na^+ current; Disopyramide; Chloramine-T; Use-dependent block; Activation gate; (Fast inactivation gate)

1. Introduction

The interaction of Na^+ channels and drug molecules was extensively studied in a variety of patch-clamp experiments in cardiac myocytes (Bean et al., 1983; Sanchez-Chapula et al., 1983; Grant et al., 1984; Clarkson et al., 1988; Benz and Kohlhardt, 1991). In addition to the broad studies of drug binding to Na^+ channels, the unbinding process from Na^+ channels has recently been characterized (Grant et al., 1982; Courtney, 1988; Carmeliet, 1988; Gruber and Carmeliet, 1989; Snyders and Hondeghem, 1990; Anno and Hondeghem, 1990). Courtney (1988) reported that the rate of recovery of lidocaine-blocked Na^+ channels is slowed by both membrane depolarization and by acidification in bullfrog atrial cells. This report explained the voltage- and pH-dependent recovery kinetics during lidocaine treatment by enhancement of Na^+ current (I_{Na}) inactivation by receptor-occupied charged drug. Other previous reports suggested the important role of the activation gate during disopyramide blockade and deblockade of $\dot{V}_{max}$ in rabbit Purkinje fibers (Carmeliet, 1988; Sheets et al., 1988; Gruber and Carmeliet, 1989). However, because of the non-linear relation between $\dot{V}_{max}$ and I_{Na}, the direct assessment

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of I_{Na} may be required. The drug unbinding process appears to be critical, because drug molecules dwelling on the receptor sites directly characterize the net blocking action of Na^+ channels.

The purpose of this study was to assess the unbinding behavior of receptor-bound disopyramide molecules in cardiac Na^+ channels. We focused on the kinetics of disopyramide-bound I_{Na} recovery from use-dependent block. Disopyramide is not a typical local anesthetic agent in that it differs from smaller agents such as lidocaine in voltage-dependent recovery. To determine the role of the activation gate, we also studied I_{Na} after inhibition of the fast inactivation process by pretreatment with chloramine-T. Chloramine-T is known to inhibit the fast inactivation process of I_{Na} in a variety of preparations (Wang, 1984; Wang et al., 1985, 1987; Schmidtmayer, 1985; Schmidtmayer et al., 1983; Huang et al., 1987; Meeder and Ulbricht, 1987) and we have previously demonstrated that chloramine-T can inhibit the fast inactivation process of cardiac I_{Na} (Koumi et al., 1991, 1992a,b; Sato et al., 1991) and the hyperpolarization-induced inactivation of cardiac I_{K1} (Koumi et al., 1994a). After inhibition of the fast inactivation process, the interaction between drug molecules and channel receptor site can be evaluated directly.

2. Materials and methods

2.1. Cell preparation

Single ventricular myocytes of adult guinea-pig heart were obtained by an enzymatic dissociation method similar to that described previously (Isenberg and Klockner, 1982). Briefly, guinea-pigs of either sex, weighing 300–400 g, were anesthetized with diethyl ether (1 ml/kg i.p.). The chest was opened under artificial ventilation and the aorta was cannulated in situ. The heart was excised and mounted on a Langendorff-type apparatus. Blood was washed out of the coronary arteries by retrograde perfusion with 30 ml of Tyrode's solution under a hydrostatic pressure of about 70 cm H_2O. The heart was then perfused with 50 ml of nominally Ca^{2+}-free Tyrode's solution followed by perfusion with Ca^{2+}-free Tyrode's solution containing 0.04% collagenase (Sigma, type I, St. Louis, MO, USA) for 10–20 min. The collagenase was washed out by perfusion with 60 ml of a high-K^+, low-Cl^- Kraftbrühe (KB) solution (Isenberg and Klockner, 1982). All perfusates were bubbled with 100% O_2 and warmed to 37° C. After wash-out of the collagenase, the heart was gently agitated in the KB solution, and then stored in this solution at 4° C for at least 1 h before commencing the experiments. Only those cells which were Ca^{2+}-tolerant, clearly striated, rod-shaped without any blebs on the surface were used in these experiments.

2.2. Solutions

The control Tyrode's solution contained (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 10, and Hepes-NaOH buffer 5.0 (pH = 7.4). Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ from the normal Tyrode's solution. The composition of the external solution was (in mM): NaCl 25, CsCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $CoCl_2$ 1.0, tetraethylammonium chloride 90, Hepes 20, glucose 10, and the pH was adjusted to 7.4 with tetraethylammonium hydroxide. Nifedipine (5 μM) was used instead of Co^{2+} to block L-type Ca^{2+} channels in some experiments. A low external Na^+ concentration was used to reduce the magnitude of I_{Na}, so the membrane potential could be adequately controlled. The standard pipette solution (internal solution) contained (in mM): NaF 10, CsF 125, Hepes 5, and the pH was adjusted to 7.2 by adding CsOH. Using these solutions resulted in I_{Na} being the only measurable current generated in response to the applied test potentials. Replacing K^+ with Cs^+ and adding tetraethylammonium chloride to the external solutions eliminated K^+ currents. The modified KB solution had the following composition (in mM): taurine 20, oxalic acid 10, glutamic acid 70, KCl 25, KH_2PO_4 10, $MgSO_4$ 5.0, Hepes 10, glucose 11, ethyleneglycol-bis(β-aminoethylether)N,N'-tetraacetic acid (EGTA) 0.5, and the pH was adjusted to 7.2 with KOH (Isenberg and Klockner, 1982). The desired concentrations of disopyramide were made by diluting a 1 mM stock solution. Disopyramide (disopyramide phosphate, molecular weight 339.5) and chloramine-T (*N*-chloro-*p*-toluensulfonamide Na^+ salt) were purchased from Sigma Chemical Co.

2.3. Electrical measurements

The membrane currents were studied using the whole-cell patch-clamp technique as described previously (Hamill et al., 1981; Koumi and Wasserstrom, 1994; Koumi et al., 1994b). The electrodes were pulled in two stages from microhematocrit tubes (Drummond Scientific Co., USA), using a vertical microelectrode puller (type PE-2, Narishige, Tokyo, Japan). The electrodes had diameters of 2.5–3.5 μm and resistances of 0.5–0.8 MΩ after being fire polished and filled with the internal solution. Inverted voltage-clamp pulses were applied to the bath through an Ag-AgCl pellet-KCl agar bridge. The pipette potential was maintained at ground level. The head stage of the voltage clamp circuit had an ultra-low bias current operational amplifier. An Axopatch-1C amplifier (Axon Instruments, Foster City, CA) was used for current recordings. The

patch electrode was connected to the negative input with a feedback register of 500 MΩ.

To obtain rapid and uniform control of the membrane potential and minimize voltage errors related to the flow of I_{Na} across the series resistance, membrane current was recorded using low resistance electrodes (0.5–0.8 MΩ) and an external solution with 25 mM Na^+ at a temperature of 17° C. In addition, all other ionic currents that could have interfered with the measurement of I_{Na} were eliminated as described above. Under these conditions, leak current at the test potential (−30 mV) was less than 0.1 nA. Cell capacitance (C_m) was estimated from the current transient produced by a small (10 mV) voltage-clamp step, and determined by integrating the current transient: $C_m = 74 \pm 6$ pF. Series resistance (R_s) was determined by fitting exponentials to the current transient. The capacitative transient was well described by a single exponential. R_s was estimated from the time constant (τ) of the capacitative transient on the assumption of $\tau = R_s C_m$. The mean time constant was 98 ± 8 μs ($n = 8$), and R_s was 1.3 ± 0.4 MΩ. The series resistance attributed to the pipette tip and the cell interior was compensated by summing a fraction of the converted current signal to the command potential, and feeding it to the positive input of the operational amplifier. The capacitative transient remaining after series resistance compensation was constant throughout the experiment. The pipette potential was adjusted to give zero current when both the pipette and the bath contained normal Tyrode's solution. Series resistance compensation was done to cancel the voltage drop across the tip of the electrode. Compensation for this series resistance was performed with a maximum level of ~ 80%.

Time-dependent changes of I_{Na} consisting of shifts of inactivation in the hyperpolarizing direction (Fernandez et al., 1984; Kimitsuki et al., 1990) were observed. These shifts typically occurred during the first 15–20 min of recording similar to those in the previous report (Matsuda et al., 1992). All data were recorded after at least a 20-min stabilizing period.

2.4. Data analysis

The voltage and current signals were displayed on a storage oscilloscope (type 5113, Tektronix) and were stored on digital audio tape (R-60DM, Maxell) using a PCM data recording system (RD-100T, TEAC). Analysis of the data was performed with a computer (PC-9801, NEC) using custom software. All curve fitting was done with a nonlinear least-squares algorithm using a Marquardt routine (Marquardt, 1963). The results are expressed as means ± S.E.M. Statistical analysis was done using Student's t-test or one-way analysis of variance. The results were considered to be significant when $P < 0.05$.

3. Results

3.1. Voltage dependence of disopyramide-bound I_{Na} recovery from use-dependent block

Unbinding of disopyramide from Na^+ channels was characterized by the protocol shown in Fig. 1. The steady-state I_{Na} level of use-dependent block was first produced by a train of 30 conditioning pulses and the kinetics of recovery from use-dependent block were then defined by measuring the fraction of peak I_{Na} available with a test pulse elicited after a variable recovery interval (ΔT). In the presence of 20 μM disopyramide, there was a use-dependent block that approached the steady-state level and some extent of recovery from block was observed following the recovery time. The time course of recovery from use-dependent block reflects the unbinding process of the drug molecule from the receptor site either through the hydrophobic or the hydrophilic pathway. The recovery time course was examined at different membrane voltages and external pH levels.

Fig. 2 shows the voltage dependence of recovery from use-dependent block of I_{Na} in the presence of 20 μM disopyramide. Unbinding behavior was estimated by the time course of I_{Na} recovery from use-dependent block. The recovery time course was compared at different holding potentials. Recovery time courses were

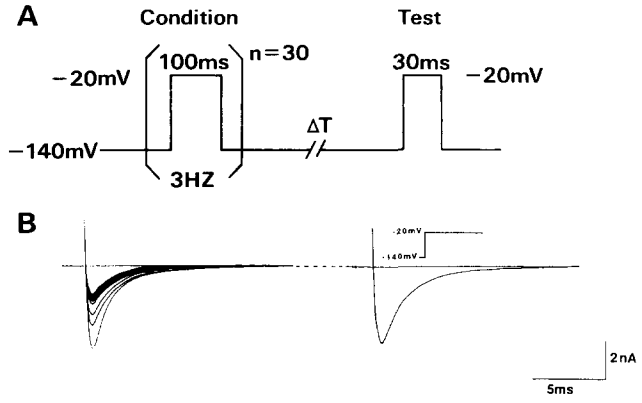

Fig. 1. Voltage-clamp pulse protocol and original current traces of time course of recovery from use-dependent block of I_{Na}. Panel A: Diagram of steady-state level of use-dependent block first produced by a train of 30 conditioning pulses of 100-ms duration to −20 mV applied at 3 Hz in the presence of disopyramide (20 μM) in the external solution. The kinetics of I_{Na} recovery from use-dependent block were then defined by measuring the fraction of peak I_{Na} available with a test pulse elicited after a variable recovery interval (ΔT). In this case, $\Delta T = 12$ s. Panel B: Original current traces obtained with the protocol displayed in panel A during exposure to 20 μM disopyramide. There was no significant decrease in peak current amplitude during repetitive pulsing under control conditions. In contrast, in the presence of disopyramide, a use-dependent block of I_{Na} was produced. I_{Na} amplitude approached a steady-state level within 20 repetitive pulses. After applying 30 beats, some recovery of I_{Na} amplitude was observed following a variable recovery time (ΔT).

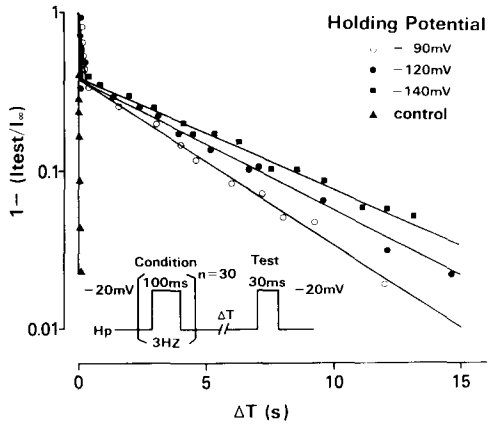

Fig. 2. Voltage dependence of recovery from use-dependent block of I_{Na} in the presence of disopyramide. Graph showing the time course of recovery of peak I_{Na} at different holding potentials: empty circles, −90 mV; filled circles, −120 mV; filled squares, −140 mV. Filled triangles indicate the control. The voltage-clamp pulse protocol shown in Fig. 1A was used. For illustration, the test currents were normalized to their steady-state rested value (I) and plotted semilogarithmically as a function of recovery time. In the absence of disopyramide, recovery of I_{Na} was well described by a single exponential function. In the presence of 20 μM disopyramide, recovery of the current was clearly biphasic and was well described by a double exponential function. −90 mV: $I = 0.64\exp(-t/59.1\ \text{ms}) + 0.31\exp(-t/4.38\ \text{s})$; −120 mV: $I = 0.62\exp(-t/48.5\ \text{ms}) + 0.32\exp(-t/5.41\ \text{s})$; −140 mV: $I = 0.62\exp(-t/31.4\ \text{ms}) + 0.33\exp(-t/6.40\ \text{s})$.

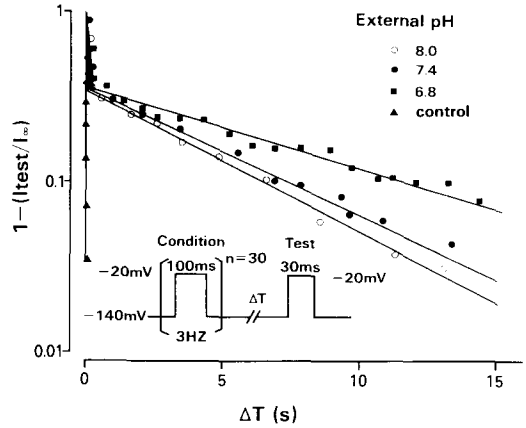

Fig. 3. pH dependence of recovery from use-dependent block of I_{Na} in the presence of disopyramide. Graph showing the time course of recovery of peak I_{Na} in different external pH: empty circles, pH = 8.0; filled circles, pH = 7.4; filled squares, pH = 6.8. Filled triangles indicate the control. The voltage-clamp pulse protocol shown in Fig. 1A was used. For illustration, the test currents were normalized to their steady-state rested value (I) and plotted semilogarithmically as a function of recovery time. In the absence of disopyramide, recovery of I_{Na} was well described by a single exponential function. In the presence of 20 μM disopyramide, recovery of current was clearly biphasic and was well described by a double exponential function. pH = 6.8: $I = 0.65\exp(-t/29.9\ \text{ms}) + 0.33\exp(-t/8.89\ \text{s})$; pH = 7.4: $I = 0.64\exp(-t/30.8\ \text{ms}) + 0.32\exp(-t/6.38\ \text{s})$; pH = 8.0: $I = 0.65\exp(-t/33.1\ \text{ms}) + 0.32\exp(-t/6.10\ \text{s})$.

biphasic and were well described by a double exponential function:

$$I = I_f\exp(-t/\tau_f) + I_s\exp(-t/\tau_s) \quad (1)$$

where I is the current amplitude, and τ_f and τ_s are time constants of recovery. At holding potential (HP) = −90 mV, $I_f = 0.64$, $I_s = 0.31$; at HP = −120 mV, $I_f = 0.62$, $I_s = 0.32$; and at HP = −140 mV, $I_f = 0.64$, $I_s = 0.33$. Time constants for the slow phase (τ_s) exhibited clear voltage dependence. τ_s became larger with hyperpolarizing holding potential. Fig. 4A shows the voltage-dependent change of τ_s. τ_s linearly increased with membrane hyperpolarization.

3.2. pH dependence of disopyramide-bound I_{Na} recovery from use-dependent block

The pH dependence of I_{Na} recovery from use-dependent block by 20 μM disopyramide was also examined by measuring the time course of recovery from use-dependent block (Fig. 3). The external pH was changed by switching the bath solution (pH = 6.8–8.0). No significant difference was seen in either the fast or slow component of the recovery time course between an external pH of 7.4 and 8.0 (not statistically significant; NS). However, under acidic conditions (pH = 6.8), τ_s was significantly greater than that at pH = 7.4 or 8.0 ($P < 0.01$). By fitting the data to Eq. (1), the following results were obtained: at pH = 6.8, $I_f = 0.65$, $I_s = 0.33$; at pH = 7.4, $I_f = 0.64$, $I_s = 0.32$; and at pH = 8.0, $I_f = 0.65$, $I_s = 0.33$. Fig. 4B shows the plot of τ_s at different external pH values. τ_s exponentially increased with lowering external pH.

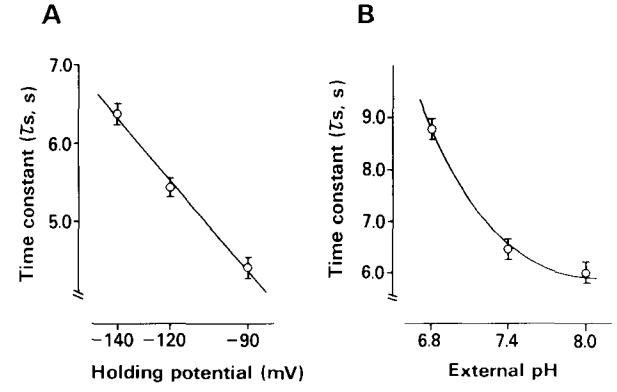

Fig. 4. The voltage and pH dependence of time constants for slow phase of recovery from use-dependent block of I_{Na} by disopyramide. Panel A: Time constants for the slow phase (τ_s) of recovery from use-dependent block of I_{Na} were plotted as a function of holding potential (−90, −120 and −140 mV). Disopyramide (20 μM) was applied to the bath solution. τ_s became larger as the holding potential was hyperpolarized. The vertical bars through each point represent the S.E.M. from seven cells. Panel B: τ_s of recovery from use-dependent block of I_{Na} were plotted as a function of external pH (6.8, 7.4 and 8.0). τ_s became larger with external acidification. The vertical bars represent the S.E.M. from six cells.

In summary, the results show that the slow phase of I_{Na} recovery from use-dependent block caused by disopyramide became slower with strong hyperpolarization of the holding potential or with acidification of the external solution. The slowing of channel recovery from use-dependent block by acidification may be caused by a decreased deprotonation rate of receptor-bound drug molecules, suggesting that disopyramide molecules can escape from the receptor site through the hydrophobic pathway after deprotonation. However, the recovery process could not be fully explained by the calculated lifetime of the charged form of disopyramide (see Discussion). Because of this fact, the roles of the fast inactivation gate and the activation gate (m-gate) were evaluated during recovery of I_{Na} from use-dependent block.

3.3. Voltage and pH dependence of chloramine-T-treated I_{Na} recovery from use-dependent block

Chloramine-T is known to inhibit the fast inactivation process of I_{Na} (Wang et al., 1985, 1987; Schmidtmayer, 1985; Schmidtmayer et al., 1983; Meeder and Ulbricht, 1987). We have reported that chloramine-T can inhibit the fast inactivation process of cardiac I_{Na} and disopyramide does not require a fast inactivation process to produce both tonic and use-dependent block of I_{Na} (Koumi et al., 1991, 1992a,b). Chloramine-T-pretreated I_{Na} recovery kinetics from use-dependent block in the presence of disopyramide were compared with normally inactivating (control) I_{Na}.

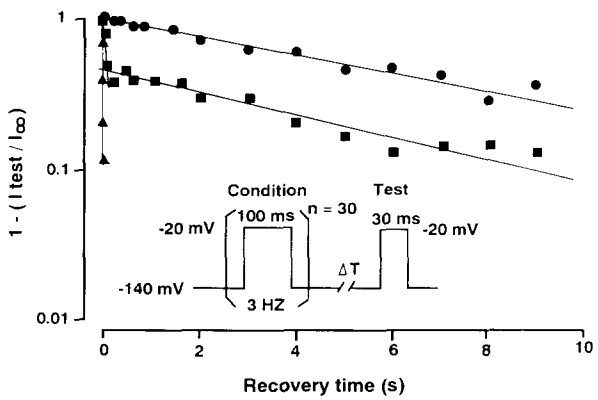

Fig. 5. Effect of disopyramide on time course of recovery from use-dependent block of I_{Na}. Voltage-clamp pulse protocol is given in the inset. The holding potential was kept at −140 mV, and a total of 30 depolarizing pulses was applied to −20 mV at 3 Hz in order to obtain the steady-state level, followed by a single test pulse at various intervals (ΔT). Disopyramide (20 μM) was present in the external solution. The recovery time course was determined by plotting normalized peak I_{Na} against the interval (ΔT) on a semilogarithmic scale. Values were obtained in the absence (squares) and in the presence (circles) of chloramine-T (2 mM). Filled triangles indicate the control. In the absence of chloramine-T, recovery from use-dependent block was well described by a double exponential function. In contrast, after treatment with chloramine-T, recovery from use-dependent block was well fitted by a single exponential function.

Table 1
Voltage dependence of time course of recovery from use-dependent block

HP (mV)	Chloramine-T (−)		Chloramine-T (+)
	τ_f (ms)	τ_s (s)	τ (s)
−90	57.8 ± 1.7 [a]	4.34 ± 0.16 [b]	5.31 ± 0.21
−120	46.1 ± 2.6 [a]	5.44 ± 0.18 [b]	5.98 ± 0.25
−140	29.5 ± 1.7 [a]	6.39 ± 0.25 [b]	7.41 ± 0.26 [c]

Values are means ± S.E.M. ($n = 10$). τ_f: time constant for fast phase; τ_s: time constant for slow phase; τ: time constant of recovery phase after treatment with chloramine-T. [a] Significantly different from the value (τ_f) at the other holding potentials ($P < 0.05$); [b] significantly different from the value (τ_s) at the other holding potentials ($P < 0.05$); and [c] significantly different from the value (τ) at the other holding potentials ($P < 0.05$) by one-way analysis of variance.

The time course of recovery from use-dependent block was characterized using the same protocol as shown in Fig. 1. The steady-state level of block was elicited by repetitive depolarization to −20 mV from a holding potential of −90, −120 and −140 mV, and the time course of recovery from block was measured by means of a single test pulse to −20 mV following a variable recovery time. Fig. 5 shows the time course of chloramine-T-pretreated I_{Na} recovery from use-dependent block by disopyramide. In chloramine-T-pretreated I_{Na}, the time course of recovery from use-dependent block was well described by a single exponential function. The fast phase of recovery was inhibited by pretreatment with chloramine-T. In the control, τ_f was 48.3 ± 2.5 ms and τ_s was 5.43 ± 0.18 s ($n = 6$) at HP = 120 mV. In chloramine-T-treated I_{Na}, τ was 5.95 ± 0.24 s ($n = 6$) at HP = −140 mV.

The recovery time course was assessed at different holding voltages (holding potential from −90 to −140 mV) in chloramine-T-pretreated I_{Na}. Chloramine-T-pretreated preparations were stable for more than a 30-min recording period. Table 1 summarizes the recovery time constant at different holding voltages. The time constant of recovery from use-dependent block of

Table 2
External pH dependence of time course of recovery from use-dependent block

pH	Chloramine-T (−)		Chloramine-T (+)
	τ_f (ms)	τ_s (s)	τ (s)
8.0	31.5 ± 2.3	6.09 ± 0.34	7.33 ± 0.42
7.4	31.1 ± 1.8	6.39 ± 0.27	7.51 ± 0.26
6.8	29.7 ± 2.5	8.86 ± 0.35 [a]	9.24 ± 0.38 [b]

Values are means ± S.E.M. ($n = 6$). τ_f: time constant for fast phase; τ_s: time constant for slow phase; τ: time constant of recovery phase after treatment with chloramine-T. [a] Significantly different from the value (τ_s) at other pH ($P < 0.05$); and [b] significantly different from the value (τ) at other pH ($P < 0.05$) by one-way analysis of variance.

chloramine-T-pretreated I_{Na} exhibited voltage dependence similar to that seen in the slow component of recovery in control I_{Na}. The recovery time course in different external pH was also examined using the voltage-clamp pulse protocol shown in Fig. 1. External pH was changed from 8.0 to 6.8. Table 2 summarizes the results. The time course of chloramine-T-pretreated I_{Na} exhibited pH dependence similar to that in control I_{Na}.

4. Discussion

The major findings in this study were as follows: (1) the rate of recovery from use-dependent block of I_{Na} by disopyramide was slowed by both membrane hyperpolarization and acidification; (2) after treatment with chloramine-T, the fast phase of recovery from use-dependent block of I_{Na} by disopyramide was inhibited; and (3) the time constant (τ), voltage and pH dependence of recovery remained almost unchanged.

The time course of recovery from use-dependent block reflects the unbinding process of the drug molecule from the receptor site either through the hydrophobic or the hydrophilic pathway (Hille, 1977). Since it is hard to evaluate the escape of drug molecules through the m-gate, we used chloramine-T to assess 'activation gate untrapping' without any influence of rapid channel closing. Chloramine-T is known to inhibit the fast inactivation process of the Na^+ channel (Wang, 1984; Wang et al., 1985, 1987; Schmidtmayer, 1985; Schmidtmayer et al., 1983; Huang et al., 1987; Meeder and Ulbricht, 1987). It was used to inhibit the fast I_{Na} inactivation process in nerve preparations (Ulbricht and Stole Herzog, 1984; Huang et al., 1987; Wang et al., 1987) and cardiac ventricular myocytes (Koumi et al., 1991, 1992a,b). We have previously demonstrated that chloramine-T can selectively inhibit the fast inactivation process of I_{Na} without affecting the slow inactivation process and that disopyramide can produce both tonic and use-dependent block of chloramine-T-pretreated I_{Na} (Koumi et al., 1991, 1992a,b). Because chloramine-T has no proteolytic activity, modification of a drug receptor or structural changes would not occur in chloramine-T-treated cells (Wang et al., 1987).

Based on our results, the unbinding process of disopyramide molecules from the receptor site can be explained by two distinct pathways. When drug molecules dwell on the receptor site, the channel is in a non-conductive state. During recovery from use-dependent block, drug molecules can dissociate from the receptor site via two different pathways. In the first, the neutral form of the drug molecules will escape through the hydrophobic pathway (Hille, 1977). When drug molecules dwell on the receptor site in the charged form, they can convert to the neutral form after deprotonation and escape through this pathway. This process appears to be consistent with the pH-dependent unbinding behavior of disopyramide. Assuming that the deprotonation rate is constant under acidic conditions, the protonation rate can be accelerated, which causes a decrease in the probability of drug molecules being in the neutral form. As a result, the recovery time constant of I_{Na} from block increases. The voltage dependence of the recovery time constant can also be explained by this pathway. When the membrane potential is hyperpolarized, extracellular protons would be attracted to the receptor site, which would prolong the lifetime of the cationic form of the drugs and increase the dwell time on the receptor site. Snyders and Hondeghem (1990) suggested that the time constant of unbinding would increase with hyperpolarization by increasing the driving force for protons. For every 60-mV increase in driving force, the pH would drop 1 unit. This condition would prevent charged drug molecules from escaping through the hydrophobic pathway after deprotonation. The unbinding time constant of the neutral form of the drug from the receptor through the hydrophobic pathway can be calculated from the following equation:

$$k\mathrm{p}/l\mathrm{p} = 10^{\mathrm{p}K_a}$$

where $1/l\mathrm{p}$ is the lifetime of the charged form of the drug, $k\mathrm{p}$ is the protonation rate constant with a value of $5 \times 10^8/\mathrm{M/s}$ (Schwartz et al., 1977), and $\mathrm{p}K_a$ is the pH where 50% of the drug molecules are in the charged form. The $\mathrm{p}K_a$ of disopyramide is not different at its binding site. The $\mathrm{p}K_a$ is ~ 9.5 in disopyramide (Courtney, 1980). The predicted value of the lifetime of the charged form of the drug was 6.3 s, which is close to the experimental values (see Tables 1 and 2). However, the experimental time constant is faster than this predicted value at depolarizing voltages (~ 4.4 s). This result suggests that disopyramide can escape from the channel by means other than the hydrophobic pathway.

The second pathway allows the charged form of the disopyramide molecules to escape through the activation gate when the channel is open (m-gate untrapping). Because the probability of m-gate opening decreases with hyperpolarization, the time constant of recovery is expected to increase with hyperpolarization (Figs. 2 and 4). This observation is consistent with the results obtained in squid giant axon with QX-314 and QX-222 by Yeh and Tanguy (1985) in which the charged drug binds to the channel during the depolarizing phase and is trapped within the channel by the m-gate. The trapped drug molecules can only escape from the channel when the channel is open. This notion was also supported by the observation of deblockade of $\dot{V}_{max}$ by disopyramide in a conventional microelectrode study in rabbit Purkinje fibers (Gruber and Carmeliet, 1989).

After inhibition of the fast inactivation process with chloramine-T, the recovery time constant showed voltage and pH dependence similar to those in normal gating I_{Na} during disopyramide treatment. These observations, without contamination of rapid inactivation of I_{Na}, support the likelihood of the m-gate untrapping pathway. After inhibition of the fast inactivation process, there is little influence of the voltage dependence of the fast inactivation process. In this case, the m-gate can regulate the drug binding or unbinding to Na^+ channel receptors. Although drug binding can change the voltage dependence of the channel by shifting the kinetics toward more negative potentials (Anno and Hondeghem, 1990), a number of channels would still have their m-gates closed at the relatively negative holding potentials (−120 mV). Closed m-gates would also act to trap the charged form of the drug within the channel pores. The lifetime of the drug within the channels at relatively negative membrane potentials would be little affected by the low frequency of channel openings. It would be mainly controlled by the deprotonation rate of the drug to an uncharged form within the channels.

In summary, the disopyramide molecule can escape from the receptor site either through the hydrophobic pathway in its neutral form or through the m-gate in its charged form. Thus, unbinding kinetics of disopyramide from the receptor site may be composed of both the hydrophobic pathway and m-gate untrapping.

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