A Quantitative Analysis of Use-Dependent Ventricular Conduction Slowing by Procainamid in Anesthetized Dogs

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Background. Use-dependent effects of antiarrhythmic drugs on phase 0 sodium current result in rate-dependent conduction slowing with important potential clinical consequences. The purpose of the present study was to determine whether state-dependent interactions of procainamid with sodium channels can be analyzed based on conduction changes in vivo.

Methods and Results. Procainamid infusions were used to produce stable drug concentrations causing ≥25% conduction slowing at a basic cycle length (BCL) of 300 msec in morphine/chloralose-anesthetized dogs with formalin-induced atrioventricular block. Computer-based epicardial activation mapping was applied to assess the time course and pattern of conduction over a wide range of BCLs before and after drug administration. Action potential duration was measured from recordings of monophasic action potentials. The onset and steady-state values of fractional sodium channel block estimated from conduction changes were fitted to equations obtained from a stepwise exponential analysis. The rate constant for the onset of block (β) decreased, as predicted, with decreasing cycle length. The slope of the relation between β and recovery time at each BCL averaged 0.29±0.03 sec⁻¹, resulting in a calculated recovery time constant (3.4 seconds) similar to values previously obtained by direct measurement. Estimates of binding and unbinding rate constants for the sodium channel during the action potential plateau and after repolarization were of the same order as previous results obtained using microelectrode methods in vitro.

Conclusions. Use-dependent conduction changes produced by procainamid in vivo closely follow the predictions of mathematical models of drug-channel interactions, and underlying kinetic interactions with the sodium channel inferred from conduction changes agree with previous, more direct observations. These results support the relevance of basic concepts about antiarrhythmic drug actions on sodium channels for understanding drug effects on conduction in vivo and advance analytical tools that can be used to explore the latter in humans. (Circulation 1992;85:2255–2266)

Key Words • antiarrhythmics • sodium channel • electrocardiography • arrhythmias • mathematical models

Cardiac antiarrhythmic drugs with sodium channel-blocking properties are known to exert these properties in a rate-dependent fashion.1 Molecular models have been developed to account for use-dependent actions,1–3 and these models have important potential clinical implications.1,4 In contrast to the extensive characterization of use-dependent blockade available from in vitro research,1,5 much less is known about the consequences of this blockade on cardiac conduction in vivo. A number of in vivo studies have shown that the recovery or onset of use-dependent conduction slowing by sodium6–10 or calcium11 channel blockers follows an exponential time course, with a time constant similar to that of blockade of indexes of inward current in vitro. These findings parallel results obtained with simultaneous measurements of conduction velocity and Vmax in vitro.12 Theoretical considerations, however, indicate that rate-dependent conduction changes by sodium channel blockers should not follow a simple exponential time course.13 Although the predicted kinetics of conduction changes approximate a first-order function over certain ranges of drug action, in accord with previous observations in the literature,6–12 deviations from first-order behavior are predicted and have been confirmed experimentally.13,14

A more precise analysis of the kinetics of recovery from antiarrhythmic drug-induced conduction slowing can be obtained by incorporating a squared relation between conduction velocity (θ) and phase 0 inward current.14 This relation allows for the estimation of sodium channel blockade from drug-induced changes in conduction time, provided that the conduction pathway

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is constant. Recent theoretical advances permit the detailed characterization of ion channel blockade if the onset of blockade can be studied at a variety of pacing rates. This theoretical approach has been validated by studying changes in V̇_m produced by quinidine in vitro but has not been evaluated in vivo. The purpose of the present study was to characterize in detail the use-dependent conduction slowing produced by an antarrhythmic drug in vivo. Specifically, we set out to determine whether 1) the onset of conduction block follows the predictions of basic molecular models, 2) the underlying rate constants for interactions with the sodium channel calculated from these models agree with previous in vitro observations; and 3) observed rate-dependent changes in conduction can be explained by the underlying kinetic interactions with sodium channels. A preliminary communication of these results has appeared in abstract form.

Methods

General Methods

Eleven mongrel dogs of either sex weighing 10–20 kg were anesthetized with morphine (2 mg/kg i.m.) and α-chloralose (100 mg/kg i.v.). Catheters were inserted into the left femoral artery and both femoral veins and kept patent with heparinized saline solution (0.9%). All dogs were ventilated via an endotracheal tube at a rate of 10 breaths per minute with a tidal volume obtained from a nomogram. Arterial blood gases were measured to ensure adequate oxygen saturation (Sao2 >90%) and physiological pH (7.35–7.45), and measurements were repeated during the experiment to ensure that pH remained constant. A right thoracotomy was performed in the third intercostal space. A thermistor probe was placed within the chest cavity, and a homeothermic heating blanket was used to keep body temperature between 37° and 38°C. The chest was kept covered to prevent cooling and drying of the epicardial surface.

Two bipolar Teflon-coated stainless-steel electrodes were inserted intramurally into the right ventricle. Constant-current pacing stimuli were delivered with a programmable stimulator and a stimulus isolator using 4-msec square-wave pulses at twice diastolic threshold. A Mingograf paper recorder (Siemens-Elema AB, Solna, Sweden) was used to monitor the six standard surface and V₂ ECG leads, arterial pressure, right ventricular monophasic action potential, and stimulus artifacts. A bipolar contact catheter electrode (EP Technologies, Inc.) was inserted via the right external jugular vein to record a right ventricular monophasic action potential (MAP) according to the method of Franz. MAP and ECG signals were filtered at 0.05–100 Hz and amplified by a digital amplifier system (Bloom Associates, Flying Hills, Pa.). Recordings were obtained at a paper speed of 200 mm/sec. Complete atrioventricular block was created by the injection of formalin to study a wide range of pacing cycle lengths. The right ventricle was paced at a frequency of 1 Hz, except when specific pacing protocols were used for evaluation of frequency-dependent drug action.

Activation Mapping

An array of 56 bipolar electrodes with 2-mm interpolo- lary distance, evenly spaced in a mesh sock (Bard Electrophysiology, Billerica, Mass.), was used. The sock was placed so as to cover both ventricles and fixed in position by sewing the base of the sock to the pericar dium. Each signal was filtered with a band-pass of 30–400 Hz, digitized with 12-bit resolution and a 1-kHz sampling rate, and transmitted via duplex fiberoptic cables into a microcomputer (model 286, Compaq Computer, Houston, Tex.). Software routines were used to amplify, display, and analyze each electrogram signal as well as to generate maps showing activation times at each electrode site. Each electrogram was analyzed by computer-determined peak-amplitude criteria and reviewed manually to exclude low-amplitude signals with indiscrete electrograms. The accuracy of measured activation times was ±0.5 msec. The data were downloaded on high-density (1.2 Mbyte) diskettes for subsequent off-line analysis. Isochrone maps and activation times for each test activation were recorded with an IBM inkjet printer. Hardware and software for the mapping system were obtained from Biomedical Instrumentation, Inc., Markham, Ontario, Canada.

The stimulating electrodes were positioned adjacent to a right ventricular epicardial electrode. Two electrodes were inserted so that at least one stimulation electrode would remain in place should the other become dislodged over the course of the experiment. Conduction time was calculated as the time elapsed between activation at the site adjacent to the stimulating electrode and activation at each epicardial site. Constancy of the activation pattern was evaluated by observation of the pattern of isochronal activation (qualitative) and, subsequently, by computation of the relative conduction times to each electrode site for different activations. The relative conduction time was calculated for a given complex by dividing the conduction time at each electrode site by the conduction time at the site of latest activation during that complex. This numerical index of the relative time of activation at each point on the epicardial surface should remain constant for different beats if the activation pattern is unchanged.

Drug Administration

Loading and maintenance dose infusion regimens were applied as in previous reports to produce a series of stable procainamide concentrations. As many as three loading doses of 50 mg/kg i.v. were given, followed by maintenance dosages of 12, 24, and 36 mg/kg/hr after each loading dose. The dose of procainamide was selected to produce conduction changes similar to those resulting from clinical doses of procainamide in humans. Procainamide was chosen for study because its in vitro recovery time constant is in the range of 2–3 seconds, allowing for almost complete recovery from frequency-dependent sodium channel blockade within the maximum pause range obtainable (usually 6–10 seconds). Electrophysiological studies were begun 20 minutes after the onset of each maintenance infusion. An activation map was obtained at a basic cycle length (BCL) of 300 msec, and conduction time from the first to the last site activated was compared with the corresponding value under control conditions. If conduction time was prolonged by at least 25% relative to control, the entire study was performed during that maintenance infusion. On the other hand, if drug-induced conduction
slowing was <25%, the next loading dose was administered. This procedure was repeated until either a dose was found that increased conduction time by >25% or the maximum loading dose had been given. Blood samples for procainamide concentration measurement by high-performance liquid chromatography assay were drawn before and after electrophysiological studies for confirmation of the stability of drug concentrations.

Experimental Protocol

The stability of activation was first verified over a wide range of pacing rates and pause durations under control conditions. Procainamide was infused, and the appropriate dose for study was selected as described above. The longest possible pause duration without spontaneous escape beats was then defined. The onset of sodium channel blockade was estimated by observing the development of conduction slowing after the initiation of pacing following a pause at each of a series of BCLs: 300, 400, 500, 600, 800, 1,000, 1,200, 1,400, 1,600, and 2,000 msec. The longest cycle length that could be studied in each dog was determined by underlying spontaneous automaticity, and it was 1,000 msec in one dog, 1,200 msec in two dogs, 1,400 msec in two dogs, 1,600 msec in five dogs, and 2,000 msec in two dogs. A total of 11 dogs were studied, but data were incomplete for one dog (dog 2), precluding analysis. In an additional three dogs, the onset of block was studied at three cycle lengths (300, 400, and 500 msec) after both a prolonged pause and an abrupt change from a longer cycle length (1,500 msec).

Data Analysis

Theoretical framework. The fraction of sodium channels blocked during each activation was estimated by assuming that $b_n \times \theta$. Evidence to support the validity of this assumption has been provided by both in vitro and in vivo observations. If this relation holds, fractional sodium channel block can be estimated as

$$b = 1 - \left(\frac{CT_c}{CT_d}\right)^2$$

where $b$ is the fraction of sodium channels blocked during phase 0 of a given activation, $CT_c$ is the conduction time under control conditions, and $CT_d$ is the conduction time for a corresponding complex in the presence of the drug studied. The accuracy of this relation requires the conduction pattern to be constant. Theoretically, conduction time at any point could be used for this analysis, but we chose to study the time from activation at the electrode site nearest the stimulating electrode (first site activated) to the last site activated on the epicardial surface.

If sodium channel blockade can be estimated for any beat after a full unbinding pause, underlying rate constants for drug interaction with sodium channels can be estimated using a theoretical approach developed by Starmer and colleagues. They applied a piecewise exponential approach to the analysis of binding by a ligand to a receptor site, with binding during a cardiac cycle characterized by a period of apparent high affinity with binding and unbinding rate constants $k_r$ and $\ell_r$, respectively, and a period of low-affinity binding with corresponding constants $k_l$ and $\ell_l$. Changes in binding from an initial level ($b_n$) to a new steady state ($b_m$) in the presence of an altered BCL are given by

$$b_m = b_n + (b_0 - b_n) e^{-n \lambda^*}$$

where $b_m$ is the sodium channel block for the $n$th beat at the new rate and $\lambda^*$ is a constant. In turn, $\lambda^*$ can be expressed in terms of the rate constants for interaction with the high- and low-affinity states of the channel ($\lambda_h$ and $\lambda_l$, respectively), so that

$$\lambda^* = \lambda_h t_h + \lambda_l t_l$$

where $t_h$ and $t_l$ are the times at each cycle length in the high- and low-affinity states, respectively, and $\lambda_h$ and $\lambda_l$ are, respectively, functions of underlying binding and unbinding rate constants with the sodium channel, with

$$\lambda_h = k_h C + \ell_h$$

$$\lambda_l = k_l C + \ell_l$$

where $C$ is drug concentration, and $\lambda_h$ and $\lambda_l$ can be calculated from Equation 3 if $\lambda^*$, $t_h$, and $t_l$ are known for a variety of cycle lengths.

To solve for $k_h$, $\ell_h$, $k_l$, and $\ell_l$, the relation between block and cycle length must be examined in more detail. Two additional theoretical terms must be introduced: $a$, the fractional block that would be present at a given drug concentration if all channels were maintained in the high-affinity state for infinite time, and $r$, the fractional block if channels were held in the low-affinity state to infinity. Calculation of these terms would allow for determination of specific unbinding and binding rate constants, because

$$k_h C = \lambda_h a$$

$$k_l C = \lambda_l (1 - a)$$

To determine $a$, and $r$, the relation between $b_n$ and BCL must be studied. This relation is a curvilinear one, but it can be analyzed using a transformation function $\gamma$, where

$$\gamma = (1 - e^{-\lambda^* r})(1 - e^{-\lambda^*})$$

Both $\lambda_h$ and $\lambda^*$ are defined above and are determined by fitting experimental data to Equations 2 and 3. Although $\lambda_h$ is a property of the drug at a given concentration (and is independent of cycle length), $r$, and $\lambda^*$ are both functions of BCL. It can be shown that $b_n$ is a linear function of $\gamma$, as given by

$$b_n = a + \gamma (r - a)$$

Because $a$, and $r$, are constant for any given drug concentration, a graph of $b_n$ versus $\gamma$ should be linear with a slope of $r - a$, and an intercept of $a$. Because $b_n$ and $\gamma$ can be determined at each cycle length, linear regression of data obtained at a variety of cycle lengths can be used to solve for $a$, and $r$, in each experiment. Once the latter terms are known, the values of $k_h$, $\ell_h$, $k_l$, and $\ell_l$ can be calculated by substituting into the four parts of Equation 5.

The above approach has been used by Packer et al to analyze data obtained by studying the effects of quinidine in vitro. Because quinidine is an open-state sodium channel blocker, Packer et al assumed $t_h$ to have a constant value of 1 msec. Procainamide, on the other
hand, blocks sodium channels predominantly when they are in the inactivated state.1,25 We therefore measured MAP duration to 90% repolarization (MAPD) as an index of the duration of the interaction with the high-affinity state (t₅) during each cycle. Time in the low-affinity state (t₁) was then calculated as the diastolic time, BCL−MAPD, at each cycle length.

Nonlinear curve fitting was performed with Marquardt's procedure on an IBM AT compatible computer. Linear regression was obtained with the standard least-squares method. Group data are presented as mean±SEM. Comparisons between paired sets of data were performed with Student's t test.

Results

General Effects of Procainamide

Procainamide produced a rate-dependent increase in conduction time (Table 1). At a cycle length of 300 msec, procainamide increased overall conduction time by an average of 34% compared with a mean prolongation of 17% at a cycle length of 1,000 msec. Conduction time was not altered by changing pacing rate or by pauses under control conditions. The mean pause duration during each study was 7±1 seconds. Procainamide concentration was kept stable by the maintenance infusion, as indicated by an average prestudy plasma concentration of 195±36 μmol·l⁻¹ compared with a mean concentration after study of 226±48 μmol·l⁻¹. Procainamide increased MAPD slightly but significantly from 261±10 to 291±13 msec (p<0.01) at a BCL of 1,000 msec. Arterial pressure averaged 136±5/86±7 mm Hg before procainamide and 131±6/86±7 mm Hg after procainamide administration (p=NS).

Kinetics of Procainamide Action

Figure 1 shows the development of block in a representative dog after the onset of pacing following a 10-second pause. The first postpause beat (defined as beat 0) was not used in the curve fitting because its action potential duration was expected to be increased by the prolonged preceding pause. Results are shown for five different cycle lengths. Data at each cycle length were well fitted (mean r, 0.992±0.002) by Equation 2, as shown by the solid lines. This curve fitting provided a determination of λ*, the onset rate constant, at each BCL. As shown, λ* increased (indicating a faster onset of block) as BCL increased.

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Top panel: Plots of onset of rate-dependent conduction block (bₙ) as a function of beat number at the five basic cycle lengths (BCLS) indicated, after a pause of 10 seconds. The curves shown indicate the best fit of the data to the equation bₙ=bₙ₀+(bₙ₋₀−bₙ₀)e⁻λ*ₙ, where bₙ₀ is steady-state block and bₙ is block of the first postpause beat. The rate constant (λ*) characterizing each curve is shown to the right of the curve. Bottom panel: Plots of changes in conduction time (CT) after a 10-second pause during pacing at the cycle length indicated under control conditions (filled symbols) or in the presence of procainamide (open symbols). Results did not change at different cycle lengths under control, so control data are shown for only one cycle length (300 msec).

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**Table 1. Characteristics of Procainamide Effect in Each Dog**

<table>
<thead>
<tr>
<th>Dog</th>
<th>BCL 300 msec</th>
<th>BCL 1,000 msec</th>
<th>Pause duration (seconds)</th>
<th>Concentration (μmol·l⁻¹)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
<td>Drug</td>
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<td>72</td>
<td>46</td>
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<td>80</td>
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<td>73</td>
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<td>11</td>
<td>96</td>
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</tr>
<tr>
<td>Mean±SEM</td>
<td>90±7</td>
<td>121±8‡</td>
<td>90±7</td>
<td>105±8§</td>
</tr>
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</table>

†Mean procainamide concentration.

‡p<0.0001 for procainamide vs. control.

§p<0.01 for procainamide vs. control.
FIGURE 2. Scatterplot of rate constants (λ*) for onset of block at each cycle length plotted as a function of recovery time (tR), with both variables normalized to monophasic action potential duration (tA). The theoretical analysis suggests that this relation should be linear, with a slope equal to the recovery rate constant (λR) and an intercept equal to the rate constant (λa) for interaction with inactivated channels (for a detailed explanation, see presentation of Equation 8). Recovery time was determined by subtracting tR from the cycle length.

To calculate λa and λR, a modification of the previously described approach is necessary. Because access to the high-affinity state for procainamide occurs predominantly when sodium channels are inactivated, λR is closely related to action potential duration, which varies with changing cycle length. By dividing both sides of Equation 3 by tR, we obtain

\[
\frac{λ^*}{tR} = \lambda_a + \lambda_r \left(\frac{tR}{tA}\right)
\]

Using MAPD as an estimate of tR at each cycle length, we plotted λ*/tR at each BCL as a function of tR/tA at that cycle length. As shown in Figure 2, the resulting relation is linear with a slope of λa and an intercept of λR. The slope averaged 0.29±0.03 sec⁻¹, providing an estimated recovery time constant of 3.4 seconds. This permits graphical solutions for λa and λR, but to avoid statistical distortion resulting from data transformation, nonlinear curve fitting to Equation 3 was used to solve for λa and λR in each experiment using measured values of tR, tA, and λ* at each cycle length. Table 2 shows the individual values for λa and λR obtained in each experiment, along with overall mean values. Theoretically, 1/λa is equal to the recovery time constant, τrec, at least in the absence of use-dependent recovery or activation un-

FIGURE 3. Scatterplots of relation between steady-state block (bss) at each cycle length and γ, a positive function of basic cycle length (for definition, see text). Theoretical considerations suggest that this relation should be linear, with a negative slope. Results are shown from three representative dogs, including the animal (dog 1) whose results are displayed in Figures 1 and 2.

blocking. The overall mean value for λa was 0.26 sec⁻¹, which is equal to a time constant of 3.8 seconds.

Figure 3 is an example of a plot of bss against γ in three dogs, including data for the same dog (dog 1) represented in Figures 1 and 2. Graphical solution results in estimates of a, and r, based on Equation 7 and the subsequent calculation of kS, kR, and kR, based on the four parts of Equation 5. This analysis was performed for each dog studied, resulting in the estimated rate constants presented in Table 2. The mean forward rate constant was more than 20-fold larger for procainamide action during the depolarized phase of the action

### Table 2. Calculated Rate Constants of Procainamide

<table>
<thead>
<tr>
<th>Dog</th>
<th>kS (mmol⁻¹ sec⁻¹)</th>
<th>kR (mmol⁻¹ sec⁻¹)</th>
<th>kS (sec⁻¹)</th>
<th>kR (sec⁻¹)</th>
<th>λa (sec⁻¹)</th>
<th>λR (sec⁻¹)</th>
<th>a</th>
<th>r</th>
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<td>0.42</td>
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</tr>
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<tr>
<td>Mean±SEM</td>
<td>2.6±0.5</td>
<td>0.58±0.07</td>
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<td>0.24±0.03</td>
<td>1.05±0.16</td>
<td>0.26±0.04</td>
<td>0.44±0.03</td>
<td>0.08±0.02</td>
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kS, kR, and kR, binding and unbinding rate constants for the high-affinity and low-affinity procainamide interactions, respectively (for further definition and derivation, see text); a, and r, estimates of the steady-state fractional sodium channel block if cells were continuously depolarized or rested, respectively.
potential ($k_e=2.6\pm0.5 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$) than during electrical diastole ($k_e=0.12\pm0.04 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$). On the other hand, unbinding rate constants were of the same order for the depolarized state ($\xi=0.58\pm0.07 \text{ sec}^{-1}$) and after repolarization ($\xi=0.24\pm0.03 \text{ sec}^{-1}$).

To assess the implications of the binding and unbinding rate constants for rate-dependent conduction changes, we calculated $b_n$ in each dog as a function of BCL, based on the theoretical framework presented above and the estimated values of $k_s$, $\xi$, $k_n$, and $\xi$ presented in Table 2. The only other variable that was necessary for the calculation was $t$, at each BCL. It should be noted that kinetic parameters were derived in part from estimates of $b_n$. Therefore, the simulation of $b_n$ based on kinetic parameters and $t$, does not represent independent validation of the model but rather shows internal consistency and allows for consideration of the continuous $b_n$–BCL relation based on the model.

To obtain a continuous estimate of $t$, as a function of BCL, we assumed that they were related linearly. As shown in Figure 4, this was found to be the case. In 10 experiments, the correlation coefficients were all $>0.99$, with an average intercept of $-0.183\pm0.006$ and a mean slope of $0.90\pm0.01$. Using this relation for each dog along with the estimated binding and unbinding rate constants, we were able to calculate the magnitude of sodium channel block and conduction time slowing predicted for any cycle length.

Figure 5 shows the resulting curves for four dogs. There is a wide range of rate-dependent behavior. Conduction slowing was most rate dependent in dogs 1 and 9, least rate dependent in dog 5, and intermediate in dog 8. Both $b_n$ and corresponding changes in conduction time, calculated from Equation 1, are shown. Although the underlying behavior of the drug is similar in all dogs, differences in rate constants resulted in a range of rate-dependent behavior among dogs. To quantify this variability in rate dependence, we calculated the percent increase in drug effect on conduction time when changes at the shortest BCL available were compared with those at the longest cycle length. These changes were normalized to the range in cycle lengths available for analysis in each dog by dividing by the difference between the longest and shortest cycle length studied (in seconds). Values ranged from 37% to 452% sec$^{-1}$ with a mean value of 190±42% sec$^{-1}$. This index of rate dependence was related linearly to the calculated value of $a_n-\tau_r$, ($r=0.62$, $p=0.05$).

Constancy of conduction pattern is a necessary assumption of the calculation of fractional sodium channel block from conduction data. Conduction pattern should not be altered by drug relative to control, nor should it be affected by the magnitude of drug action. This was assessed quantitatively by comparing relative activation times at each electrode site at a BCL of 300 msec with values after a pause long enough to allow virtually complete recovery from rate-dependent effects. If the conduction pattern is constant, the data should fall close to the line of identity. A similar approach was taken to ensure that conduction pattern in the presence of maximum drug effect (BCL, 300 msec) was unchanged from control at the same cycle length. Figure 6 presents both analyses in a representative experiment and shows that in the presence of procainamide at a cycle length of 300 msec, relative activation time at each electrode site was unchanged compared with results under control at the same cycle length or in the presence of the drug after a 6-second pause.

Figure 7 shows the activation maps on which the analyses illustrated in Figure 6 were obtained. Control results at a cycle length of 300 msec are shown at the top, with results in the presence of procainamide at the same BCL in the middle, and results in the presence of drug after a 6-second pause at the bottom. Procainamide measurably slowed conduction at a BCL of 300 msec, and its effects were almost completely reversed by a 6-second pause. Nevertheless, the overall activation pattern is clearly similar for all three maps.

Results similar to those shown in Figures 6 and 7 were obtained in all 10 dogs. For the analysis of relative conduction times comparing drug with control at a BCL of 300 msec, the regression line was always close to the line of identity (mean slope, 1.00±0.01; mean intercept, $-0.02\pm0.01$; mean $r$, 0.99±0.01). When results at maximum drug effect (BCL, 300 msec) were compared with results in the presence of drug but with minimal effect...
Activation time slowed. Results (CTrPA) electrode site to were $0.99 \pm 0.02$ that the relation between CTr in the presence of the drug at BCL 300 msec (CTrPA) and CTr under control conditions (bottom panel) or CTr after a pause allowing for full recovery of rate-dependent slowing (CTrPA [after pause], top panel) fell along the line of identity. Similar results were obtained in all experiments.

(i.e., immediately after a pause), the average values were $0.99 \pm 0.02$ (for slope), $0.00 \pm 0.01$ (for intercept), and $0.99 \pm 0.01$ (for $r$).

The final assumption requiring validation was the beat-to-beat constancy of action potential duration, since a single value of $t_e$ for each BCL is assumed in the analysis (Equations 3 and 8). Figure 8 shows monophasic action potentials of the first three beats after a 6-second pause in the presence of procainamide, as well as of the 20th beat, at which steady-state conduction changes at this cycle length (300 msec) had occurred. The duration of beat 0 was longer than that at steady state by an average of $19 \pm 2\%$ at a cycle length of 300 msec, which justifies our exclusion of the first postpause beat from analysis. On the other hand, beat 1 had a duration that was very close to values attained at the time of steady-state conduction changes. Similar results were obtained in other experiments, with the ratio between the action potential duration of beat 1 and that of an action potential at which steady-state conduction changes were achieved averaging $1.01 \pm 0.01$.

In patients, it would be impossible to obtain pauses sufficiently long to allow complete unbinding of most antiarrhythmic drugs. The theory presented above suggests that the onset of block when pacing rate is abruptly changed should have the same rate constant ($\lambda^*$) as noted for the initiation of pacing at the same cycle length after a complete unblocking pause. Furthermore, the steady-state level of block ($b_\infty$) also should be identical. We evaluated these ideas in three dogs in whom the onset of block was studied after both a complete unblocking pause and an abrupt change in pacing cycle length. Figure 9 shows a representative experiment. Although the initial level of block ($b_\infty$) is greater with an abrupt rate change, the final level of block is similar and the onset of block is first order as shown by the monoexponential curve fits. Table 3 lists the mean values for $b_{\infty}$, $b_0$, and $\lambda^*$ as determined after a prolonged pause and an abrupt rate change. Although $b_\infty$ was less after the pause, $b_0$ and $\lambda^*$ did not differ significantly between the two protocols.

**Discussion**

We have shown that use-dependent conduction slowing by procainamide in vivo can be quantitatively analyzed on the basis of mathematical models of drug interaction with the sodium channel. This analysis results in the estimation of binding and unbinding rate constants, which in turn allow for the prediction of rate-dependent conduction slowing.

**Comparison With Previous Studies of Procainamide**

A variety of investigators have studied the kinetics of procainamide's effects on $V_{\text{max}}$ in vivo,12,25–29 Recovery time constants in these studies range from 1 to 4.4 seconds, with an average of approximately 3 seconds. According to the mathematical model applied in the present study, $1/\lambda_1$ should equal the recovery time constant for procainamide.16,19 Using the mean value for $\lambda_1$ in all dogs, the estimated recovery time constant is 3.8 seconds and is in the same range as values obtained from $V_{\text{max}}$ changes in vitro.

Sada et al25 used a computer simulation to estimate binding and unbinding rate constants for the various states of the sodium channel according to Hondegem-Katzung modulated receptor theory,3 using data obtained from $V_{\text{max}}$ measurements in guinea pig papillary muscles. The procainamide uptake rate constants they obtained were $0.54 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$ for the inactivated state and $0.054 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$ for the rested state of the sodium channel. Weirich and Antoni29 obtained a value of $0.72 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$ for the inactivated state and did not list a value for the rested state because no resting state block was observed. Estimates that we obtained from in vivo conduction data are somewhat higher than those of the above in vitro studies but of the same order: 2.6 and 0.12 mmol$^{-1} \cdot$sec$^{-1}$, respectively. Although discrepancies between the calculated kinetic constants are to some extent due to the different ways in which they were obtained, some of the difference may be due to interspecies variability in interactions with sodium channels. For example, we have found that the effects of quinidine and flecainide on $V_{\text{max}}$ of atrial action potentials vary among species, with a larger effect occurring on dog tissue compared with guinea pig tissue.30 Although the absolute values of the rate constants differ between our study and that of Sada, the ratio of forward rate constants for the inactivated compared with resting states are of the same order, with a ratio of 10:1 in Sada et al’s study25 and 22:1 in ours, indicating relatively low affinity for the rested state. Because the recovery from procainamide-induced block at the resting potential is determined predominantly by the $\text{ID}\rightarrow\text{I}\rightarrow\text{R}$ (drug unbinding from the inactivated channel followed by return to the resting state) transition according to the Hon-
FIGURE 7. Activation maps from which the data shown in Figure 6 were obtained. Each color represents a 10-msec isochrone, with the scale shown on the left. Maps were obtained under control conditions at a cycle length of 300 msec (top panel), in the presence of procainamide at the same cycle length (middle panel), and in the presence of procainamide after a 6-second pause (bottom panel). Although conduction is slower in the presence of procainamide at the basic cycle length, the overall conduction pattern is similar under all three conditions.
deghem-Katzung formulation, the unbinding rate constant during the diastolic period should be governed by the unbinding constant of inactivated channels. The value estimated by Sada et al for this constant is 0.2 sec⁻¹, which is similar to our corresponding estimate of 0.24±0.03 sec⁻¹.

A variety of groups have shown in a qualitative way that procainamide’s conduction-slowing properties in vivo are rate dependent. In two previous studies in humans, procainamide increased QRS duration by averages of 19% and 16% at maximal cycle lengths of 600 and 700 msec, respectively. Drug-induced QRS prolongation was increased to 38% by ventricular pacing at a cycle length of 300 msec in one study and to 28% at a cycle length of 250 msec in the other study. These results are in the same range as the increases in conduction time (17% and 34% at cycle lengths of 1,000 and 300 msec, respectively) produced by procainamide in our dogs. Although procainamide’s rate-dependent effect on conduction is clear, its magnitude over a given range of cycle lengths can vary widely (Figure 5). This variability occurs despite qualitatively similar behavior in terms of the onset of block and may be due to differences in the discrepancy between estimated equilibrium sodium channel blockade during the plateau and diastole (aₙ−rₙ), as reflected in Equation 7 and the statistical analysis described above. The possibility of different magnitudes of rate dependence of drug action may have implications regarding the determinants and manifestations of drug-induced conduction slowing in individual patients.

The only previous quantitative analysis of procainamide’s effects in vivo of which we are aware is our study of the recovery kinetics of procainamide-induced conduction slowing. The latter research suggested that drug-induced conduction changes were related to the square of underlying phase 0 inward current and that functional sodium channel blockade can be estimated as 1−(CTₕ/CTₑ). The present investigation differs from our previous study in that the previous research examined only the recovery process, whereas the current study examines the onset of block, the dependence of conduction slowing on steady-state cycle length, and possible underlying kinetic interactions with the sodium channel.

**Table 3. Kinetic Variables Measured Using the Onset of Pacing After a Full Unbinding Pause Compared With an Abrupt Decrease in Cycle Length**

<table>
<thead>
<tr>
<th>BCL (msec)</th>
<th>Pause</th>
<th>Abrupt change</th>
<th>Pause</th>
<th>Abrupt change</th>
<th>Pause</th>
<th>Abrupt change</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0.43±0.01</td>
<td>0.44±0.01</td>
<td>0.02±0.02</td>
<td>0.18±0.01</td>
<td>0.32±0.03</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>400</td>
<td>0.36±0.01</td>
<td>0.35±0.01</td>
<td>0.04±0.01</td>
<td>0.17±0.03</td>
<td>0.40±0.02</td>
<td>0.37±0.03</td>
</tr>
<tr>
<td>500</td>
<td>0.30±0.01</td>
<td>0.30±0.01</td>
<td>0.04±0.03</td>
<td>0.15±0.03</td>
<td>0.47±0.02</td>
<td>0.50±0.04</td>
</tr>
</tbody>
</table>

b₀, Steady-state block; bₐ, block of beat 0 (i.e., first postpause beat or last beat of slower pacing for abrupt change); λ⁺, rate constant for onset of block (in beats⁻¹); BCL, basic cycle length.

**Figure 8.** Recordings after the onset of pacing at a cycle length of 300 msec in a representative dog. Monophasic action potential duration (MAPD) of the first postpause beat, 238 msec, was quite prolonged. By the second postpause beat, MAPD had achieved a value (188 msec) that was very similar to that (190 msec) measured at the time steady-state conduction changes had occurred (beat 20). VEG, ventricular electrogram; MAP, monophasic action potential recording; SA, stimulus artifact; BN, beat number (first postpause beat defined as beat 0). Paper speed, 200 mm/sec.
Relation to Previous Studies of Rate-Dependent Drug Effects on Conduction In Vivo

A variety of investigators have shown that the recovery kinetics from conduction slowing by sodium channel blockers in anesthetized dogs are similar to those of changes in $V_{\text{max}}$ in vitro.6-10 We have recently shown that the onset of conduction slowing produced in humans by ventricular pacing in the presence of amiodarone, quinidine, propafenone, and flecainide55 follows kinetics similar to the corresponding changes in $V_{\text{max}}$ studied in vitro.36-40 The similarity between the kinetics of conduction slowing in vivo and those of $V_{\text{max}}$ in vitro is expected at the drug concentrations studied, despite the nonlinear relation between conduction velocity and $V_{\text{max}}$15,16 but it would not be expected at higher drug concentrations.14

The present evaluation differs from previous in vivo research in this area in two major respects. The first is that instead of using conduction slowing per se as an index of drug effect, we estimated fractional sodium channel blockade based on a relation for which there is both theoretical and experimental evidence.14-16 The second difference is that we have applied a well-developed mathematical approach7-19 that allows for the detailed analysis of the onset of block and the estimation of underlying rate constants for interactions with the sodium channel. Our experimental data show that the model accurately predicts a variety of rate-dependent properties of antiarrhythmic drug-induced conduction changes in vivo that have not been previously evaluated. These include a decrease in the rate constant for the onset of block at faster rates, the linear relation between onset rate constants and recovery time (in the present report, normalized to action potential duration), and the linear relation with a negative slope between steady-state fractional sodium channel block at any cycle length and the derived parameter $\gamma$. Furthermore, the resulting estimates for binding and unbinding rate constants with the sodium channel are in the same range as calculations from more direct in vitro studies.25

Study Limitations

A number of important limitations of the mathematical and experimental approach must be kept in mind. Most important, this report should not be interpreted as suggesting that in vivo experiments are an appropriate way to analyze molecular interactions with the sodium channel. The latter can be accurately assessed only by voltage-clamp methods and/or unitary channel analysis. On the other hand, the only currently available method of evaluating drug effects on sodium current at physiological temperatures relies on the use of $V_{\text{max}}$, an indirect and probably nonlinear index of phase 0 sodium current.41,42 Changes in temperature appear to produce qualitative as well as quantitative changes in the state-dependent properties of drug-induced blockade.43 In addition, our approach depends on a proportional relation between $I_{\text{Na}}$ or $V_{\text{max}}$ and the square of conduction velocity, a relation for which there is experimental evidence,19,23,24 but under limited conditions, and a relation that may break down in the presence of diseased tissue, altered cellular coupling, and so on. Our measure of action potential duration, obtained from a single endocardial MAP catheter, does not precisely reflect ventricular action potential duration in all areas of the heart, particularly the His-Purkinje system. Nevertheless, the overall agreement between in vivo estimates of rate constants and corresponding in vitro data supports the relevance of the latter for understanding rate-dependent drug effects on conduction.

A second important consideration is the limited nature of the mathematical analysis. Although sodium channel blockers interact with channels in open, inactivated, and resting states, the model presented considers interactions with only two discrete states. Theoretically, the model could be readily generalized to consider three channel states; however, it is most unlikely that the increased number of characterizing constants (total of six) could be estimated with any degree of accuracy from the limited data (distinct values of $\lambda^*$ at a maximum of six to eight BCLs) obtainable in vivo. Therefore, this analytical approach can be applied only to drugs that, like procainamide4,25 and quinidine,19,44 show preferential association with one predominant state of the sodium channel.

The precise molecular mechanisms of use-dependent sodium channel blockade are under intense and ongoing investigation.45-48 The model applied in the present study is not dependent on specific assumptions about the details of underlying mechanisms. Its only premises are that the channel exists in two discrete states during the cardiac cycle and that the affinity of a blocker for the channel is different for the two states. Whether state-dependent interactions are due to true differences in affinity between the blocker and its receptor as a function of channel state1-3,4 or differences are due to voltage- and state-dependent access functions2 does not alter the applicability of the model. The types of experiments we performed are certainly incapable of testing the relative merits of different molecular models of use-dependent drug action.

The approach used assumes that key modulating variables, such as take-off potential, are constant at different cycle lengths. Although phase 3 block is excluded by observing full MAP repolarization well in advance of the succeeding activation at all cycle lengths, the possibility that resting potential is affected by pacing rate cannot be fully excluded.

The plasma concentrations that we studied are approximately threefold the upper limit of the clinical therapeutic range. However, we have shown previously
in isolated preparations that higher antiarrhythmic drug concentrations are needed to produce the same effect in canine tissues compared with human samples. The conduction changes that we observed are compatible with previous findings regarding the concentration-dependent actions of procainamide in canine ventricular tissues and are similar to those observed in patients treated with therapeutic doses of procainamide.

**Potential Significance**

The results presented in this report advance our ability to understand in a quantitative way use-dependent conduction slowing produced by antiarrhythmic drugs in vivo. We have shown that the rate of onset of block (as estimated from effects on conduction) and the frequency dependence of steady-state conduction slowing follow closely the predictions of basic mathematical models.

The approach that we have taken is, with some modification, applicable to the study of use-dependent antiarrhythmic drug action in humans. Although it is impossible in humans to study pauses long enough for full recovery from rate-dependent sodium channel blockade by most drugs, sufficient data may be obtainable by studying abrupt changes in activation rate. To calculate \( \lambda^* \) at any rate according to Equation 2, all that is necessary is a level of block before rate change (\( b_b \)) that is sufficiently different from the level after rate change (\( b_a \)) to allow the rate constant for the transition from \( b_b \) to \( b_a \) to be determined. Figure 9 and Table 3 indicate that, as predicted by theoretical reasoning, values of \( \lambda^* \) and \( b_a \) after an abrupt change in pacing rate are similar to those after a prolonged pause. If \( \lambda^* \) and \( b_a \) could be calculated accurately at several cycle lengths in humans, all of the kinetic variables derived in the present report can be obtained. We have already demonstrated the existence of such behavior and the possibility of quantifying it in humans for a variety of antiarrhythmic drugs. The type of information obtained using this approach may provide valuable insight into the clinical pharmacodynamics of antiarrhythmic agents and may provide data useful for monitoring drug therapy. Furthermore, the ability to estimate recovery time constants from data obtained at several pacing cycle lengths allows for the assessment of kinetic parameters otherwise unobtainable in humans because of the prolonged pause durations that would be necessary for many antiarrhythmic agents.

Use-dependent actions of antiarrhythmic drugs have been shown to play a major role in their effects on arrhythmias. Marchlinski et al demonstrated that procainamide's ability to slow ventricular tachycardia was related to its use-dependent conduction slowing properties. Other research has shown that the arrhythmogenic and antiarrhythmic actions of drugs in various animal arrhythmia models of clinical relevance are related to use-dependent conduction slowing. The present study provides a link between underlying channel-blocking properties at the cellular level and the resulting effects on conduction that are responsible for clinical actions.

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