Rate-Dependent Changes in Intraventricular Conduction Produced by Procainamide in Anesthetized Dogs

A Quantitative Analysis Based on the Relation Between Phase 0 Inward Current and Conduction Velocity

Stanley Nattel and Wuhua Jing

Antiarrhythmic drug effects on maximal upstroke velocity (V_max) are frequency dependent, which implies that the effects of these drugs on conduction should also be rate dependent. Previous in vivo studies have been limited by assumptions about unchanging propagation pathway, and by the empirical use of a first-order recovery model. To explore time-dependent antiarrhythmic drug–induced conduction slowing in vivo, we used 56-electrode epicardial mapping in chloralose–anesthetized dogs with formalin-induced atrioventricular block. Interval-dependent changes in conduction time were assessed under control conditions and then after three loading and maintenance infusions of procainamide. Under control conditions, epicardial activation time (86±26 msec at a basic cycle length of 300 msec) was unchanged (87±24 msec) by pauses up to 6.6±2.2 seconds. Procainamide caused conduction slowing that dissipated as a function of recovery interval, with 94±6% recovery over a maximum pause of 6.7±1.5 seconds, but did not alter activation pattern. Drug-induced changes in conduction were evaluated by use of a mathematical model assuming phase 0 inward current proportional to conduction velocity squared. Conduction changes were better fitted by this "quadratic model" (least sum of squared deviations 3.9×10^-3 by mapping in five dogs, 2.7×10^-2 by use of QRS duration in nine dogs) than by a monoexponential model (sum of squared deviations 5.7×10^-3 by mapping, 3.4×10^-2 with QRS; p<0.01 vs. quadratic model for each). As predicted by theoretical analysis, recovery time constants from the quadratic model were similar to time constants for procainamide-induced changes in V_max in vitro, and significantly longer than values obtained with a monoexponential model. Drug-induced changes in QRS duration were highly correlated with simultaneous changes measured by epicardial mapping (r=0.95, p<0.001), indicating that QRS duration is a valid index of drug effects on ventricular conduction. We concluded that procainamide causes interval-dependent changes in ventricular conduction in vivo that are consistent with a proportional relation between phase 0 inward current and the square of conduction velocity. These observations have important potential implications for the dose-dependent and heart rate–dependent effects of antiarrhythmic drugs. (Circulation Research 1989;65:1485–1498)
shown kinetics of action for sodium channel blockers\(^7\)–\(^9\) and calcium channel blockers\(^10\) similar to previously studied kinetics for blockade of the corresponding inward currents or their indexes in vitro.\(^7\),\(^11\)–\(^14\) Conduction time has been assessed by use of QRS duration,\(^7\),\(^8\) H-V interval,\(^8\) and pairs of epicardial electrodes oriented longitudinally and transversely to fiber orientation.\(^9\) While QRS duration can be measured noninvasively and is clearly related to conduction velocity, its validity as a quantitative index of drug-induced conduction slowing has not been extensively tested. All measurements of conduction require the assumption that the propagation pathway remains constant.

Epicardial activation mapping is a technique that provides precise activation time measurements at many points on the surface of the heart, as well as spacial information about the pattern of ventricular activation.\(^15\) Therefore, it is capable of both quantification of drug-induced changes in conduction and exclusion of major changes in propagation pathways. This approach appears well suited to the description of rate-dependent drug effects on ventricular conduction, but has not, to our knowledge, previously been so applied.

Previous quantitative studies of antiarrhythmic drug kinetics in vivo have used first-order kinetic models empirically for evaluation of drug effects on conduction. Since the recovery from drug effects on sodium and calcium currents are first order,\(^1\)–\(^3\) a first-order recovery model for drug effects on conduction would be expected if there were a linear relation between conduction velocity and inward current. There is no physiological reason that the latter relation should be linear. Approximate solutions of equations describing propagation in a unidimensional cable suggest a linear relation between maximal upstroke velocity (\(V_{ma}\)) or sodium current (\(I_{Na}\)) (normalized to \(C_F\), the foot capacitance of the action potential) and the square of conduction velocity, is a more robust quantitative descriptor of recovery from drug-induced conduction slowing than first-order predictions. Preliminary descriptions of this work have appeared in abstract form.\(^19\),\(^20\)

### Materials and Methods

#### General Methods

Mongrel dogs of either sex weighing 7–15 kg were anesthetized with morphine (2 mg/kg i.m.) and \(\alpha\)-chloralose (100 mg/kg i.v.). Small dogs were selected for facilitation of the production of atrioventricular block by injection of formalin as previously described.\(^7\) 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/min with a tidal volume obtained from a nomogram. Arterial blood gases were measured to ensure adequate oxygenation (\(\text{SaO}_2>90\%\)) and physiological \(\text{pH}\) (7.38–7.45). A right thoracotomy was performed in the third intercostal space, and a pericardial cradle was created.

A bipolar Teflon-coated stainless steel electrode was inserted intramura rally into the right ventricle. Constant current pacing stimuli were delivered by a programmable stimulator and a stimulus isolator using 4-msec square-wave pulses at twice diastolic threshold. A Mingograf paper recorder (Siemens-Elena AB, Solna, Sweden) was used to monitor the six standard surface electrocardiographic leads, arterial pressure, and stimulus artifacts. Electrocardiographic recordings were obtained at 250 mm/sec paper speed, providing a measurement accuracy of \(\pm 2\) msec. 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.

Nine dogs were studied for evaluation of the rate and interval dependence of procainamide's effects on QRS duration. Five separate dogs were studied for evaluation of the effects of procainamide on the pattern and timing of epicardial activation, and for
Activation Mapping

An array of 56 bipolar electrodes with 2-mm interpolar distance, evenly spaced in a mesh sock (Bard Electrophysiology, Billerica, Massachusetts), was used. The sock was placed so as to cover both ventricles, and was fixed in position by sewing the base of the sock to the pericardium. Each signal was filtered with a bandpass of 30–400 Hz, digitized with 12-bit resolution and a 1-kHz sampling rate, and transmitted via duplex fiber-optic cables into a microcomputer (model 286, Compaq Computer, Houston, Texas). 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. Interpolation techniques were used to produce isochronal maps of epicardial activation, but only measured activation times (not interpolated data) were used for quantitative analysis. Each electrogram was analyzed by use of computer-determined peak-amplitude criteria, and was reviewed manually to exclude low-amplitude signals with indiscrete electrograms. The accuracy of measured activation times was ±0.5 msec. The data was downloaded on high-density (1.2-Mbyte) diskettes for subsequent offline analysis. Isochronal maps and activation times for each test activation were recorded by use of an IBM inkjet printer. Hardware and software for the mapping system were obtained from Biomedical Instrumentation, Inc, Markham, Ontario, Canada.

The stimulating electrode was positioned adjacent to a right ventricular epicardial electrode. Conduction time was calculated as 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 in two ways: 1) by observation of the pattern of isochronal activation (qualitative), and subsequently 2) by computation of the relative conduction times to each electrode site for different activations. The relative conduction time was calculated by dividing the conduction time at each electrode site by the conduction time at the site of latest activation. This numerical index of the relative time of activation at each point on the epicardial surface should remain constant for different complexes if the activation pattern is unchanged.

Evaluation of Frequency Dependence

Steady-state drug effects on QRS duration and JT interval were determined after 1 minute of continuous pacing at each of a range of basic cycle lengths. The interval dependence of drug effects on QRS duration and ventricular activation times was evaluated by stimulation of the ventricles at a cycle length (S₁S₂) of 300 msec and study of the response to test (S₃S₄) pause intervals. A basic train of 40 stimuli was used to ensure constant steady-state block before the test interval.

Drug Administration

Loading and maintenance dose infusion regimens (Table 1) were applied for production of a series of stable concentrations for procainamide. Procainamide was chosen for study because its in vitro recovery time constant is in the range of 2 seconds, allowing for almost complete recovery from frequency-dependent sodium channel blockade within the maximum pause range obtainable (generally 4–8 seconds). Electrophysiological studies were begun 20 minutes after the onset of each maintenance infusion. Blood samples for procainamide concentration measurements were drawn before and after electrophysiological studies for confirmation of the stability of drug concentrations during each drug infusion.

Data Analysis

Comparison between quadratic model and first-order recovery of conduction changes. The first-order relation between drug-induced conduction

### Table 1. Procainamide Infusion Regimens and Resulting Plasma Concentrations

<table>
<thead>
<tr>
<th>Loading dose (mg/kg)</th>
<th>Maintenance dose (mg/kg/hr)</th>
<th>Plasma concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose 1 50</td>
<td>12</td>
<td>31.3±7.9</td>
</tr>
<tr>
<td>Dose 2 50</td>
<td>24</td>
<td>58.2±13.0</td>
</tr>
<tr>
<td>Dose 3 50</td>
<td>48</td>
<td>82.8±19.2</td>
</tr>
</tbody>
</table>

Loading doses were administered over 15 minutes. Maintenance dose was begun immediately after completion of loading infusion. All doses are in terms of hydrochloride salt, which was administered as a solution in isotonic saline. Twenty minutes after end of loading dose, a blood sample (prestudy) was obtained for subsequent measurement of plasma procainamide concentration. Electrophysiological studies were then performed, after which a blood sample (poststudy) was obtained for drug assay and next loading dose was begun. Results shown are from five dogs studied by epicardial mapping and nine dogs evaluated for QRS duration.
slowing ($\Delta CT$) and recovery interval ($t$) previously applied in the literature can be written as

$$d(\Delta CT)/dt = k(\Delta CT)$$

(1)

where $k$ is a constant that is quantitatively similar to the rate constant for first-order recovery of drug effects on phase 0 inward current.\(^{7-14}\)

The quadratic model is based on two assumptions: 1) a proportional relation between an index ($IN_{Na}$) of phase 0 inward current and the square of conduction velocity ($CV$), and 2) first-order recovery of drug-induced changes in $IN_{Na}$. These assumptions can be stated by the equations

$$IN_{Na} \propto (CV)^2$$

(2)

$$d\Delta IN_{Na}/dt = k(\Delta IN_{Na})$$

(3)

These equations can be solved simultaneously, as previously shown, in terms of changes in conduction time to yield\(^{18}\)

$$d(\Delta CT)/dt = (k/2) (1+\Delta CT)(2+\Delta CT)/(\Delta CT)$$

(4)

where $\Delta CT$ is the drug-induced change in conduction time (i.e., $[CT_i-CT_i]/CT_c$ where $CT_i$ is the conduction time with drug at a recovery interval $t$ and $CT_c$ is the control conduction time). The first-order model (Equation 1) implies a log-linear relation between conduction time changes and recovery interval. The quadratic model (Equation 4) approaches the first-order equation as $\Delta CT$ tends to 0, and deviates increasingly as $\Delta CT$ becomes larger. Figure 1 shows a series of recovery curves for a range of procainamide concentrations predicted by the quadratic model. On a logarithmic scale, the terminal portion of the curve is linear, and deviations from linearity become evident as drug-induced conduction slowing exceeds 20%. The terminal linear portion of the log plot has a slope (as predicted by the model and shown in the figure) equal to the rate constant for changes in $IN_{Na}$. Other specific predictions of the quadratic model regarding logarithmic plots of $\Delta CT$ against coupling interval include the following: 1) The slope of the terminal linear portion should be independent of drug dose and the magnitude of drug action, 2) the nonlinearity of the overall curve should be more apparent for doses of the drug producing larger effects, and 3) the coupling interval at which nonlinearity appears should be greater for larger doses.

The predictions of the quadratic model were tested in two ways: 1) Observed recovery data was plotted using the format shown in Figure 1, and the accuracy of the above predictions with respect to the shape of the recovery curve and the slope of the terminal line was evaluated; and 2) curve-fitting techniques were developed to fit recovery data to the quadratic model and to a simple exponential relation. The instantaneous slope of the curve predicted by the quadratic model (Equation 4) is determined by the rate constant $k$ and the magnitude of drug-induced conduction slowing ($\Delta CT$) at that time. The entire recovery curve, therefore, can be characterized by $k$ and a single value of $\Delta CT$ at a given coupling interval ($t_i$). Any exponential curve can similarly be characterized by the equation

$$\Delta CT_i = (\Delta CT_x \times e^{kt}) \times (e^{kt})$$

(5)

where $\Delta CT_i$ is the drug-induced conduction slowing ($\Delta CT$) at a specific time $t_i$, and $\Delta CT_x$ is $\Delta CT$ at any time $t$. Thus, both the quadratic model and exponential curves are determined by the same three variables: 1) the rate constant ($k$) and 2) the magnitude of drug effect ($\Delta CT_x$) at 3) a single time point ($t_i$).

We wrote software in BASIC to generate curves using either the quadratic or exponential model for any values of $k$, $\Delta CT_x$, and $t_i$. The ability of any quadratic model or exponential curve to fit a set of experimental data was determined by the sum of squared deviations (SS) of experimental points from the putative curve. The program stepped through a range of values of $k$ and $\Delta CT_x$, using step sizes of 0.01/sec (for $k$) and 0.001 (for $\Delta CT_x$). Since $t_i$ can be any point in the recovery process, it was set at the longest coupling interval studied in each experiment. The curve with the least sum of squared deviations (LSS) represented the best-fit quadratic model or exponential curve for that set of data.\(^{26}\) A nested design was used to test all $\Delta CT_x$ values for...
Inspection of Equations 4 and 1 suggests that an exponential fit should provide a reasonable approximation to data that perfectly obeyed the quadratic model. Figure 2 (left) shows the best-fit curves using the quadratic model (solid line) and exponential model (dashed line) to fit a set of 21 theoretical data points (not shown) generated by the quadratic model for a Δ CTx of 0.03 and k of 0.054. While the curves are not identical, the differences are clearly subtle. Therefore, we would not expect to observe major differences between curves generated by the quadratic and exponential models. For quantitative comparison of the two approaches, we relied on a nonparametric statistical comparison between the LSS of the best-fit quadratic model curves and exponential curves determined in the same way using the number of variables and the same set of data in each experiment. A significantly lesser LSS should indicate superiority of a given model in description of the observed results.

Drug Assays

Plasma procainamide concentration was measured by reverse-phase high-performance liquid chromatography. Procainamide was extracted from plasma by use of 0.1 ml of 1N sodium hydroxide added to 0.5 ml of plasma containing internal standard (ethylmethyl glycinexylidide, Astra Pharmaceuticals, Mississauga, Ontario, Canada) and 2.5 ml of dichloromethane. After vortexing and centrifugation, the solvent layer was dried under 100% N2 and resuspended in mobile phase (45% 0.03 M KH2PO4, 55% acetonitrile, and 1.3 g/l octanesulfonic acid). One milliliter of this solution was injected by use of a Rheodyne loop injector onto a column of Spherisorb 5μ-ODS beads (Chromatography Sciences, Montreal, Canada), and procainamide was detected by ultraviolet absorbance at a wavelength of 210 nm. The ratio of the peak height of procainamide to that of internal standard was used for calculation of plasma procainamide concentration by means of a three-point standard curve generated with control plasma on the same day.

Statistical Methods

Group data are presented as mean±SD. Comparisons between group means were made by two-way analysis of variance (ANOVA) with Scheffe’s test.26 The frequency dependence of steady-state drug effects was evaluated by two-way ANOVA with an F test for interaction.26 Student’s paired t test was used when only two groups of results were compared.26 Wilcoxon’s signed rank test27 was used to compare LSS values for the best-fit quadratic model and exponential curves to each set of recovery data. A two-tailed probability of less than 5% indicated statistical significance.

Results

Frequency-Dependent Effects of Procainamide on QRS Duration and Repolarization

Our loading and maintenance dose regimen produced stable procainamide concentrations (Table 1). No measurable concentrations of N-acetylprocainamide were detected. Procainamide increased steady-state QRS duration in a frequency- and concentration-dependent fashion (Table 2). Drug-induced changes in QRS duration were a monotonic function of basic cycle length, and increased an average of threefold as ventricular activation frequency was increased from 30 to 200/min. While procainamide tended to increase the JT interval slightly, drug-induced changes in JT interval were not statistically significant (Table 2).

To more fully explore the effects of procainamide on repolarization, we evaluated changes in monophasic action potential duration (APD) in five additional dogs. Using this more direct measure, we found that procainamide increased APD significantly, by 4–20% (Table 3). Drug-induced APD changes depended on both dose and pacing cycle length, with larger changes occurring at longer pacing cycle lengths. The dose dependency of changes in APD was quite different from that of changes in QRS...
Rate-Dependent Changes in Epicardial Activation

Under control conditions, overall epicardial activation time (measured from the first to the last site activated) and pattern were quite constant over a wide range of rates. Activation time averaged 86 ± 26 msec at a basic cycle length of 300 msec, and 87 ± 24 msec at the longest pause interval possible (6.6 ± 2.2 seconds). Figure 3 shows the similarity of epicardial activation under control conditions at a basic cycle length of 300 msec (top left) and after a 6-second pause (top right) in a representative experiment.

The infusion of procainamide produced substantial, rate-dependent increases in activation time. These were maximal at a basic cycle length of 300 msec (mean 32%, range 13–51%), and were attenuated by increases in diastolic recovery time. Drug-induced changes in activation time observed at a cycle length of 300 msec were reduced by 94 ± 6% at a mean maximum pause interval of 6.7 ± 1.5 seconds. Figure 3 (bottom left) shows the slowing in epicardial activation produced by procainamide at a cycle length of 300 msec in the same experiment described above. After a pause of 4.5 seconds (bottom right), epicardial activation times return very nearly to control.

While procainamide altered the rate of epicardial activation, the overall pattern of activation remained unchanged. The conduction slowing shown in Figure 3 is uniform, with epicardial electrodes continuing to show activation in the same relative sequence. Quantitative analysis of conduction pattern was applied as described in "Materials and Methods." The results of such quantitative analysis of activation pattern in a representative experiment are illustrated in Figure 4. The relative time during a complex at which each site is activated is determined by dividing the conduction time to that site by the overall conduction time for the complex (as determined at the site of latest activation). Results for a beat during steady-state drug effect (at a cycle length of 300 msec) are then compared with results for a beat showing maximum recovery. If the activation patterns of the two beats are equivalent, each site should be activated after the same relative sequence.

<table>
<thead>
<tr>
<th>n</th>
<th>300</th>
<th>500</th>
<th>700</th>
<th>1,000</th>
<th>2,000</th>
<th>F_{ad}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRS duration (msec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose 1</td>
<td>6</td>
<td>67±16</td>
<td>87±21</td>
<td>69±17</td>
<td>83±21</td>
<td>67±16</td>
<td>79±20</td>
</tr>
<tr>
<td>Dose 2</td>
<td>5</td>
<td>64±17</td>
<td>92±24</td>
<td>65±19</td>
<td>88±24</td>
<td>64±17</td>
<td>83±23</td>
</tr>
<tr>
<td>Dose 3</td>
<td>5</td>
<td>66±15</td>
<td>113±26</td>
<td>68±16</td>
<td>99±22</td>
<td>66±14</td>
<td>92±24</td>
</tr>
</tbody>
</table>

JT interval (msec)

| Dose 1 | 6         | 159±25    | 143±27    | 159±31    | 168±40    | 176±37 | 184±46 | 194±43 | 210±59 | 209±61 | 237±89 | 2.0 NS |
| Dose 2 | 5         | 143±26    | 143±16    | 160±23    | 178±29    | 185±35 | 194±32 | 204±41 | 222±43 | 224±56 | 237±57 | 1.0 NS |
| Dose 3 | 5         | 145±26    | 148±35    | 164±32    | 185±36    | 191±34 | 209±48 | 212±39 | 229±56 | 228±53 | 272±99 | 2.5 NS |

All values are mean±SD.

JT, duration from end of QRS complex to end of T wave; n, number of experiments; Ctl, control; F_{ad}, F value from interaction analysis; p, statistical significance of interaction between cycle length and drug effects analyzed by two-way analysis of variance (ANOVA) with an F test for interaction; NS, nonsignificant.

Procainamide significantly increased QRS duration from control values (p<0.01) at all cycle lengths for all doses. JT interval was not significantly altered by any procainamide infusion at any cycle length.

Table 3. Changes in Monophasic Action Potential Duration Produced by Procainamide in Five Dogs

<table>
<thead>
<tr>
<th>Basic cycle length (msec)</th>
<th>300</th>
<th>500</th>
<th>700</th>
<th>1,000</th>
<th>2,000</th>
<th>F_{ad}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monophasic APD (msec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>188±10</td>
<td>224±19</td>
<td>251±19</td>
<td>266±19</td>
<td>286±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose 1</td>
<td>196±5</td>
<td>246±9</td>
<td>276±14</td>
<td>303±22*</td>
<td>316±18*</td>
<td>6.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dose 2</td>
<td>201±5*</td>
<td>256±10†</td>
<td>291±11*</td>
<td>313±18†</td>
<td>321±17¢</td>
<td>11.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dose 3</td>
<td>203±3*</td>
<td>259±8*</td>
<td>296±14*</td>
<td>318±17*</td>
<td>323±17*</td>
<td>14.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are mean±SD; results are from five separate dogs studied to evaluate effects of procainamide on APD. F_{ad}, F value from interaction analysis; p, statistical significance of interaction between cycle length and drug effects analyzed by two-way analysis of variance (ANOVA) with an F test for interaction; APD, action potential duration to full repolarization.

*p<0.05; †p<0.01, ‡p<0.001 compared with control at same basic cycle length.
FIGURE 3. Isochrone activation maps under control conditions (top panels) and in presence of procainamide (bottom panels). Scale of 10-msec isochrone colors is shown at left of each map. Electrode positions are shown by white dots, and positions of coronary arteries are shown diagrammatically. Results during steady-state pacing at a cycle length of 300 msec are shown in left panels, and results after a pause of 6 seconds (control) and 4.5 seconds (procainamide) are shown in right panels. Activation patterns were not significantly altered by the pause under control conditions. In the presence of procainamide, there is substantial conduction slowing at basic cycle length (left), which is almost completely reversed after a 4.5-second pause (right). Conduction slowing by procainamide is uniform, leaving sequence of activation unchanged.
conduction time for either complex, and the resulting points should fall along the line of identity. For all experiments using activation mapping, the regression lines fitting data plotted as in Figure 4 were close to the line of identity in each case, and had a mean slope of 1.00±0.05, an intercept of 0.01±0.01, and a correlation coefficient of 0.99±0.02.

**Quantitative Analysis of Interval-Dependent Changes in QRS Duration and Conduction Time**

As was the case for epicardial activation time, QRS duration under control conditions was constant over a wide range of cycle lengths and coupling intervals. Conduction slowing was observed over a narrow range of coupling intervals (<50 msec) just beyond the refractory period, but over a wide range of coupling intervals from 211±46 msec to 3.9±1.9 seconds, QRS duration was equal to the duration at a cycle length of 300 msec.

In the presence of procainamide, changes in coupling interval produced substantial changes in QRS duration. As the coupling interval increased, procainamide-induced conduction changes were reduced as a function of the S1S2 interval. By the end of the next train of 40 basic (S1S2) beats, the QRS duration had returned to the prepause value, with the standard deviation of the QRS of the last basic (S1) beat before the pause averaging 3.0±1.3% of the steady-state value.

Quantitative analysis of interval-dependent changes in QRS duration or epicardial conduction time was obtained at a basic cycle length of 300 msec in all experiments. Results were available from 11 drug infusions in nine dogs in the study of QRS duration, and from eight drug infusions in five dogs in the study of epicardial activation. Conduction time changes were analyzed in experiments using epicardial mapping in terms of the conduction time to the last site of epicardial activation.

Figure 5 shows the relation between coupling interval and the logarithm of changes in QRS duration in a representative experiment. The bottom panel shows the line of best fit to the terminal linear portion of each set of data. The correlation coefficients for these lines are 0.990 and 0.995 for doses 2 and 3, respectively, and clear deviations from the terminal lines are evident at coupling intervals showing over 25% conduction slowing. The deviation from linearity occurred at coupling intervals of 450 msec and 900 msec for doses 2 and 3 in this experiment, and the nonlinearity of the overall relation is clearer for the larger dose. The top panel shows the same results using the quadratic model to describe the data, indicating better agreement with the overall shape of the recovery curve. In all experiments showing over 20% conduction slowing at the basic cycle length, similar observations were made; that is, the terminal portion of the log plot was linear, deviations from linearity were more apparent and occurred at longer coupling intervals at doses producing larger effects, and the terminal slope was independent of drug dose. The terminal log-linear fit resulted in measured time constants.
Techniques for determining exponential relation (right panel). Techniques for determination of best-fit curve were identical for both curve-fitting approaches (see "Materials and Methods"), and conduction time was measured by epicardial activation mapping. While the differences between fits are subtle, in all cases the model fitted the experimental data better, as indicated by a smaller sum of squared deviations of experimental data from best-fit curve. In this experiment, least sum of squared deviations was 0.0093 for model fit compared with 0.0093 for exponential fit. dCT/CTw drug-induced increases in conduction time normalized to control conduction time; S1S2, coupling interval S1S2.

that averaged 2.10±0.19 seconds for dose 1, 2.04±0.58 seconds for dose 2, and 2.33±0.42 seconds for dose 3 in all experiments studying QRS duration.

While logarithmic plots support a variety of qualitative predictions of the quadratic model, logarithmic transformation distorts the data and, therefore, is not ideal for quantitative analysis. Figure 6 shows the best-fit curves using the quadratic model and an exponential approach to describe nontransformed conduction changes measured by use of epicardial mapping in a representative experiment. The quadratic model fits the data better, as indicated by a smaller least squares ($0.0051$) than for the exponential fit ($0.0093$). The differences between the two fits are subtle, including an underestimate by the exponential fit at long and short coupling intervals, and an overestimate at the midportion of the curves. A better appreciation of this difference can be obtained by consideration of Figure 2. The left panel of this figure shows the predicted best-fit model and exponential curves to a set of theoretical data that perfectly obeys the quadratic model. The right panel of the figure shows the best-fit model and exponential curves to the experimental data shown in Figure 6. The similarity between the curves generated by fitting experimental data and those describing behavior generated by the theoretical model is striking. Therefore, the lack of major discrepancies between model and exponential fits is consistent with theoretical predictions of the quadratic model, and a statistical comparison of the two methods is necessary. In all experiments the quadratic model fit the observed data better than a monoexponential approach, as indicated by a smaller LSS. The mean LSS was significantly smaller with the quadratic model fit than with the exponential fit for both QRS and mapping data ($p<0.01$ for each, Table 4). The mean time constants from the model fits were comparable with those obtained from the

Table 4. Kinetic Characteristics of Recovery From Procainamide-Induced Block Using QRS Duration as an Indicator of Conduction Time and Conduction Times From Epicardial Mapping

<table>
<thead>
<tr>
<th></th>
<th>k (sec)</th>
<th>$\tau$ (sec)</th>
<th>LSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRS duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal line*</td>
<td>-0.49±0.16</td>
<td>2.24±0.65</td>
<td></td>
</tr>
<tr>
<td>Quadratic model fit†</td>
<td>-0.51±0.17</td>
<td>2.14±0.54</td>
<td>0.027±0.026</td>
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<tr>
<td>Exponential fit</td>
<td>-0.63±0.17†</td>
<td>1.66±0.37‡</td>
<td>0.034±0.032§</td>
</tr>
<tr>
<td>Epicardial mapping</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal line*</td>
<td>-0.66±0.16</td>
<td>1.60±0.37</td>
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</tr>
<tr>
<td>Quadratic model fit†</td>
<td>-0.67±0.14</td>
<td>1.58±0.25</td>
<td>0.0039±0.0023</td>
</tr>
<tr>
<td>Exponential fit</td>
<td>-0.82±0.16‡</td>
<td>1.26±0.23‡</td>
<td>0.0057±0.0039§</td>
</tr>
</tbody>
</table>

Values for $k$ and $\tau$ were compared by analysis of variance (ANOVA), while LSS for quadratic model was compared with LSS for exponential fits by Wilcoxon's signed rank test. Results shown are for 11 infusions in nine experiments studying QRS duration and for eight infusions in five experiments studying epicardial activation.

$k$, kinetic rate constant of procainamide (as determined by curve-fitting techniques or slope of terminal log-linear relation); $\tau$, time constant of recovery from procainamide-induced block (1/k); LSS, least sum of squares of differences of experimental data from best-fit quadratic model or exponential curves (a smaller least sum of squares indicates a better fit to observed results).

*Linear terminal portion of curve relating logarithm of changes in QRS duration or conduction time to coupling interval (see Figure 1). According to quadratic model, slope of this line should equal rate constant for changes in phase 0 sodium current.

†Best-fit curves to quadratic model and to a monoexponential relationship were obtained as described in "Materials and Methods."

§$p<0.001$, $\&p<0.01$ for differences comparing exponential fit results to values obtained either from terminal linear portion of log (A CT)–S1S2 curve or quadratic model fit; results for $k$ and $\tau$ obtained from terminal line and quadratic model curve were not significantly different from each other.
Slopes of the terminal log-linear relations in each experiment, as predicted by the model, and were significantly longer than the time constants obtained from exponential fits (Table 4).

An additional testable prediction of the quadratic model is that a simple exponential approximation should fit the recovery curve well for low doses of drug, but should become increasingly inadequate at larger doses that produce greater steady-state conduction slowing. Figure 7 shows recovery data obtained by epicardial mapping at two dose levels in one dog. The quadratic model fits the data well at both doses. The exponential approach fits the low dose data quite reasonably, but deviates in the predicted fashion from the higher-dose results. The quadratic model fits the data well at both doses. The exponential approach fits the low dose data quite reasonably, but deviates in the predicted fashion from the higher-dose results. The relative ability of the quadratic and exponential models to fit a given set of data points can be assessed by the ratio of the LSS for the best-fit exponential curve to the LSS of the best quadratic model fit. A ratio of 1 indicates fits that are equally good, a ratio less than 1 indicates that the exponential model fits the data better, and a ratio greater than 1 indicates the extent to which the quadratic model fit is superior. Figure 8 shows the relation between this LSS ratio and the percent increase in activation time produced by procainamide at a basic cycle length of 300 msec. This ratio expresses relative ability of either model to fit observed recovery data in each experiment, with values >1 indicating degree of superiority of quadratic model. As predicted by theoretical considerations, the difference between the approaches is larger for infusions producing greater magnitudes of rate-dependent conduction slowing as indicated by percent increase in activation time. (Results shown are from eight infusions in five dogs studied by epicardial mapping.)

**Discussion**

In the present study, we applied epicardial activation mapping to analyze the rate-dependent effects of procainamide in vivo. We have shown that the recovery of drug-induced conduction changes in vivo is qualitatively and quantitatively consistent with a proportional relation between conduction velocity and the square root of phase 0 inward current. To our knowledge, this is the first study to activation mapping, although the latter was quantitatively more accurate. To analyze the concordance between these methods, we compared drug-induced increases in conduction time with corresponding changes in QRS duration in four experiments in which both parameters were measured simultaneously. Changes in these two indexes were closely related (r=0.95) and fell near the line of identity (Figure 9).

**Relation Between Drug Effects on QRS Duration and Epicardial Activation**

The results of recovery data obtained by use of QRS duration as an index of conduction time were qualitatively similar to the results with epicardial mapping, although the latter was quantitatively more accurate. To analyze the concordance between these methods, we compared drug-induced increases in conduction time with corresponding changes in QRS duration in four experiments in which both parameters were measured simultaneously. Changes in these two indexes were closely related (r=0.95) and fell near the line of identity (Figure 9).
apply epicardial activation mapping to the quantitative analysis of rate-dependent drug action in vivo, and the first to test quantitatively potential implications in the intact heart of a squared relation between phase 0 inward current and conduction velocity. Furthermore, we have shown that drug-induced changes in QRS duration behave in a very similar fashion to changes in ventricular conduction time monitored by epicardial mapping, supporting the validity of QRS duration as a noninvasive index of drug effects on ventricular conduction. Since changes in conduction velocity are an important effect of antiarrhythmic agents that can mediate both antiarrhythmic and arrhythmogenic properties, such findings that promote a more detailed understanding of rate-dependent drug effects on conduction are of great potential clinical importance.

Consideration of the Model

Evidence supporting the validity of the quadratic model was obtained from recovery curves plotted in both a logarithmic and linear fashion. The log plots showed 1) a consistently linear relation for portions of the curve showing under 20% conduction-time prolongation, with gradually increasing slope as conduction slowing increased; 2) a time constant for the terminal linear portion that was independent of dose; and 3) time constants similar to in vitro estimates of recovery times for procainamide effects on $V_{\text{max}}$.

Analysis of data without log transformation showed that the LSS of quadratic model fits was consistently and significantly smaller than the LSS obtained with a simple exponential approach, and that the difference between the fits was not random but was related to the magnitude of drug effect as predicted by the quadratic model. Comparison between the quadratic model and a simple exponential is particularly rigorous, since the quadratic model itself predicts that an exponential curve should be a good approximation of model-predicted behavior. Finally, time constants derived from quadratic model fits were similar to those measured from the terminal log-linear slope, and significantly greater (by ANOVA, Table 4) than those that used a single exponential, as predicted by the quadratic model.

The two assumptions on which the quadratic model is based are 1) an index of phase 0 inward sodium current that is proportional to conduction velocity squared, and 2) first-order recovery from drug effects on that index. Changes in $V_{\text{max}}$ have been found to vary with the square of conduction velocity in vitro, as predicted by approximate solutions of linear cable equations. Furthermore, first-order recovery of procainamide's effects on $V_{\text{max}}$ in vitro are well established. Therefore, there is good experimental and theoretical support for the derivation of the model using $V_{\text{max}}$ as an index of phase 0 current. An advantage of considering $V_{\text{max}}$ is that there is extensive data on the time course of recovery from drug-induced changes in $V_{\text{max}}$ in vitro. A disadvantage is that $V_{\text{max}}$ may be a nonlinear indicator of peak phase 0 sodium current, although $V_{\text{max}}$ nonetheless accurately reflects the net maximal inward current contributing to the depolarization of an action potential. The relation between $I_{\text{Na}}$ and conduction velocity has not been tested experimentally, but theoretical considerations suggest that $I_{\text{Na}}/C_f$ should be proportional to the square of conduction velocity. Since procainamide does not alter membrane capacitance, $I_{\text{Na}}$ should be proportional to conduction velocity squared in the presence of the drug. Procainamide's rate-dependent effects on $I_{\text{Na}}$ have not been studied, but another sodium channel blocker, lidocaine, has been found to depress $I_{\text{Na}}$ in a first-order fashion. Therefore, there is evidence to support the assumptions of the model using $I_{\text{Na}}$ as an index of phase 0 sodium current.

Relation to Previous Electrophysiological Studies of Procainamide

Procainamide caused small but statistically significant increases in APD as measured by monophasic action potentials. As in previous in vitro work with other antiarrhythmic drugs, changes in APD were rate dependent, with smaller drug effects noted at faster stimulation rates. The magnitude of APD changes we observed was compatible with the results of previous in vitro studies of procainamide. All recovery curves were obtained at a pacing cycle length of 300 msec, at which repolarization was complete at all doses well before the shortest S1-S2 interval studied (300 msec). This excludes the possibility that phase 3 block played a role in our recovery data.

The procainamide concentrations that we evaluated were greater than the commonly accepted clinical therapeutic range. The usual "therapeutic" range of plasma procainamide concentrations increases QRS duration in man by less than 10% during sinus rhythm, and substantially larger doses (producing concentrations up to 35 mg/L, in the range we studied) are frequently needed to control recurrent ventricular tachyarrhythmias in patients. Moreover, the conduction time changes that we observed were comparable with the mean QRS prolongations of 42% and 28% produced clinically by therapeutic procainamide concentrations at cycle lengths in the range of 300 msec in humans. Our results are consistent with the magnitude of changes in $V_{\text{max}}$ and conduction velocity in canine tissues exposed to comparable procainamide concentrations in vitro.

Relation to Previous In Vivo Studies of Use-Dependent Antiarrhythmic Drug Effects

Previous studies have shown that conduction slowing in vivo by amitriptyline, lidocaine, and mexiletine can be described as a monoeponential function of recovery interval. The recovery time constants of these agents in vivo were in the same
range as recovery time constants for $V_{\text{max}}$ changes produced by the same drugs in vitro.\textsuperscript{7,11-13} Our results produced by compatible with these findings, in that our recovery data could be fitted by a single exponential. As suggested by Bajaj et al\textsuperscript{9} and predicted by the quadratic model, the monoexponential model tends to underestimate the recovery time constant, particularly at larger magnitudes of drug effect. Nonetheless, the magnitude of underestimation is relatively small if recovery can be followed to near-completion. Under almost all circumstances, the monoexponential fit is as good as the quadratic fit, with important exceptions being at higher drug concentrations and shorter recovery intervals. Therefore, our theoretical considerations, confirmed by experimental observation, explain the underlying mechanisms of previous in vivo observations that have heretofore been simply empirical.\textsuperscript{7-9}

**Limitations of the Model**

Effects of procainamide mediated by mechanisms other than changes in phase 0 current would introduce factors not accounted for by the quadratic model. Sada et al\textsuperscript{24} showed that procainamide's effects on guinea pig papillary muscles at concentrations up to 200 mg/l (about twice our maximum) were limited to changes in $V_{\text{max}}$ and APD. The changes in APD that they saw were in the same range as those we measured using monophasic action potentials, and would not have affected our recovery data. They did not observe any effect of procainamide on resting membrane potential, which is consistent with our observation of virtually complete recovery from procainamide's effects on conduction after long pauses. While changes in passive electrical properties could have affected our results, passive properties controlling conduction are little changed by procainamide.\textsuperscript{34} Finally, Buchanan et al\textsuperscript{17} showed that $V_{\text{max}}$ is proportional to the square of conduction velocity in guinea pig papillary muscles at concentrations up to 66 mg/l. If important extraneous electrophysiological effects were present, such a relation should not have held.

Directional changes in impulse propagation could have altered our results, either by changing path length or via directionally different actions of antiarrhythmic drugs,\textsuperscript{9,37,42} which result from changes in the uptake of sodium channel-blocking drugs.\textsuperscript{43} Our quantitative analysis of epicardial activation showed it to be unaltered by procainamide. While we did not record activation transmurally, it is unlikely that major changes in endocardial or transmural activation sequence would have occurred while an identical pattern of epicardial activation was maintained. The doses of procainamide that we used were not high enough to produce the conduction block and Wenckebach periodicity associated with directional differences in propagation produced by sodium channel blockers.\textsuperscript{43}

Accurate analysis of the model requires that recovery be followed to near-completion, or that there be no tonic block. In our mapping experiments, we observed $94\pm6\%$ recovery of drug-induced conduction changes over an average maximum pause of 6.7 seconds. Values for procainamide's time constant in vitro range from 1 to 4.4 seconds with a mean of 2.8 seconds.\textsuperscript{12,13,20-25} With these time constants, the predicted extent of recovery over 6.7 seconds in the absence of tonic block ranges from 78\% to 99\%, with a mean value of 92\%. Therefore, our observations are compatible with the requirements for testing the model. Nonetheless, it should be emphasized that the "time constants" derived from our analyses are simply the inverse of rate constants obtained from mathematical analyses of the recovery of conduction changes, and do not necessarily directly reflect the underlying rate constant for drug effects on sodium channels.

**Implications of the Results**

The dose dependence and rate dependence of antiarrhythmic drug effects on conduction are related to the recovery kinetics of conduction changes and to the way in which sodium channel blockade alters conduction velocity. The quadratic model predicts, and experimental evidence supports, a recovery process that becomes increasingly rapid as conduction time changes are increased. For example, Equation 4 suggests that the rate of time-dependent recovery of drug-induced conduction slowing at a dose that doubles the QRS duration will be three times as fast as predicted by a first-order model using the time constant for $V_{\text{max}}$. This implies that severe conduction slowing due to sodium channel-blocking drugs should be particularly sensitive to changes in heart rate. In fact, toxic conduction slowing and spontaneous ventricular arrhythmias induced by amitriptyline are very sensitive to heart rate change, and can be dramatically reversed by slowing of the underlying rate.\textsuperscript{44} Similarly, flecainide-induced ventricular conduction slowing can be greatly enhanced by the sinus tachycardia of exercise,\textsuperscript{44} causing de novo ventricular tachyarrhythmias in predisposed patients.\textsuperscript{44,45} In addition, the conduction slowing of premature beats will be enhanced in the presence of antiarrhythmic drugs by the nonlinear recovery process, with potential implications for mechanisms of antiarrhythmic action.

Our observations support previous studies that used interelectrode conduction time in dogs,\textsuperscript{46} suggesting that QRS duration may be used as a valid index of drug-induced conduction changes in vivo. Since QRS duration can be measured noninvasively and accurately in humans, this allows for direct, quantitative studies of clinical rate-dependent drug action. This approach has been used recently to show that the onset of flecainide-induced conduction slowing in humans directly parallels its kinetic effects on $V_{\text{max}}$ in vitro.\textsuperscript{44} The QRS duration is often used as a clinical indicator of the effects of sodium channel-blocking drugs. A squared relation between...
I_{Na}$ and conduction velocity implies that sodium channel blockade can be estimated from in vivo conduction time changes as $(1-Ct_a/CTd)$, where $CTd$ and $CTa$ are values under drug and control conditions, respectively. Increases in QRS duration under 10% are difficult to detect, but according to the above considerations can correspond to up to 17% sodium channel blockade, indicating that QRS duration per se is a relatively insensitive index of sodium channel–blocking action. A doubling of sodium channel blockade from 17% to 34% would lead to a 24% increase in QRS duration from control, thus bringing QRS changes from undetectable values to a near- toxic level with a relatively moderate change in sodium channel blockade. Further study of antiarrhythmic pharmacodynamics in humans by use of estimated sodium channel blockade as an index of drug action is warranted.

The development of biophysical models of subcellular antiarrhythmic drug action allows for new insights into the drugs’ mechanisms of electrophysiological and antiarrhythmic actions. Quantitative studies of the relevance of these ideas to drug effects in vivo provide an important link between basic theory and potential clinical implications. The present study demonstrates the applicability of this approach in the precise description of the effects of an important antiarrhythmic drug on ventricular conduction in intact animals.

Acknowledgments

The authors thank Carol Matthews, Randi Eliturv-Feder, Christine Villemaire, and Kathleen Kay for providing technical assistance; Lise de Repentigny for typing the manuscript; and Astra Pharmaceuticals (Canada) for providing the internal standard (ethylmethyl glycinexylidide) for procainamide assay.

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KEY WORDS • antiarrhythmic drugs • conduction, cardiac • sodium current • cardiac electrophysiology • procainamide • cardiac arrhythmia, treatment of
Rate-dependent changes in intraventricular conduction produced by procainamide in anesthetized dogs. A quantitative analysis based on the relation between phase 0 inward current and conduction velocity.

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Circ Res. 1989;65:1485-1498
doi: 10.1161/01.RES.65.6.1485

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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