Effects of Antiarrhythmic Agents on Junctional Resistance of Guinea Pig Ventricular Cell Pairs

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ABSTRACT
Modulation of intercellular coupling through gap junctions can lead to a decrease in conduction velocity and conduction block. Previous studies have suggested that antiarrhythmic agents alter the internal resistance (sum of cytoplasmic and gap junctions resistances) of cardiac fibers. The objective of this study was to directly assess the effect of antiarrhythmic agents on junctional resistance between two isolated cells using the double whole-cell patch-clamp technique. The experimental protocol consisted in holding the membrane potential of each guinea pig ventricular myocyte of a couple cell pair at 0 mV. Then, a junctional voltage gradient was created by changing membrane potential in only one cell. Voltage gradients were varied between −50 to +50 mV in steps of 20 mV. The extracellular medium was set to minimize trans-sarcolemmal currents and the junctional current was recorded in the cell maintained at 0 mV. Drugs tested were quinidine, lidocaine, procainamide, flecainide, propranolol, sotalol, amiodarone and verapamil. Drugs were superfused after a control period of 5 min. during which junctional resistance was observed to be stable. None of the antiarrhythmic agents tested in this study directly affected junctional resistance, although procainamide slightly increased junctional resistance 110 ± 8% after 10 min of exposure. In conclusion, drugs tested in this study, chosen among all classes of antiarrhythmic agents, did not affect junctional resistance of cardiac myocyte cell pairs. However, long-term modulation or indirect effects of antiarrhythmic agents on gap junctions under physiological conditions cannot be excluded.

Electrical coupling through gap junctions is essential for impulse propagation and synchronized contraction of the heart and changes in electrical coupling may lead either to proarrhythmic or antiarrhythmic conditions (Spear et al., 1990; Callana et al., 1992; Boersma et al., 1994; Dhein et al., 1995). Effects of antiarrhythmic drugs on internal resistance (ri) assessed by techniques in which cable parameters of cardiac fibers were measured are not yet clear. Lidocaine and encainide appear to have no effect on ri (Arnsdorf and Bigger, 1975; Schmidt et al., 1981; Arnsdorf et al., 1985; Buchanan et al., 1985; Nattel and Jing, 1989). On the other hand, different studies have shown that procainamide induces either a nonsignificant increase in ri (Arnsdorf and Bigger, 1975) or no apparent effect on ri (Buchanan et al., 1985; Nattel, 1987; Nattel and Jing, 1989) and that quinidine can, in some cases, change ri (Hasegawa et al., 1991) and in others no change was observed (Buchanan et al., 1985; Arnsdorf and Savicki, 1987; Nattel and Jing, 1989). In other studies, an acute application of amiodarone appears to decrease ri of dog epicardial muscles (Quinteiro and Biagetti, 1994). Studies on anisotropic conduction have also shown that quinidine and amiodarone reduce conduction to a similar extent in both longitudinal and transverse directions (Bajaj et al., 1987; Anderson et al., 1989). In contrast, mexiletine has a more pronounced effect on the longitudinal propagation (as expected for a drug inhibiting only the sodium current) (Bajaj et al., 1987). For O-desmethyl encainide, varying results have been obtained depending on the concentration tested (Turgeon et al., 1992).

In this study, we used the double whole-cell voltage-clamp technique for assessment of antiarrhythmic drugs effects on junctional resistance of guinea pig ventricular cell pairs. Results obtained indicate that the antiarrhythmic agents tested (i.e., quinidine, lidocaine, procainamide, flecainide, propranolol, β-sotalol, amiodarone and verapamil) did not affect junctional resistance.

Methods
Isolation of ventricular myocytes. Experiments were performed on pairs of ventricular myocytes obtained from adult guinea pig hearts by use of enzymatic dissociation, which allows a good yield of cell pairs (Daleau and Turgeon, 1997). All solutions used during the cell isolation procedure were oxygenated and maintained at 37°C. Briefly, the hearts were mounted on a Langendorff apparatus

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ABBREVIATIONS: ri, longitudinal resistance of a unit length of fiber; rj, junctional resistance; rm, membrane resistance; lj, junctional current; jm, membrane current; Vt, transjunctional voltage; l, length constant; v, conduction velocity; Vmax, maximal rate of depolarization of the action potential; , inward rectifier potassium current.
containing, respectively, 200 and 500 calcs, Freehold, NJ.). The hearts were reperfused with solutions containing collagenase (final concentration, 300 U/ml; Worthington Biochemicals, Freehold, NJ.). The hearts were reperfused with solutions containing, respectively, 200 and 500 µM CaCl₂. At this point, the ventricles were cut down and minced slightly. After filtration through 200-µm nylon mesh, cells were resuspended in a solution containing 1.8 mM CaCl₂ and maintained at 30°C before use. Experiments were performed on cell pairs with regular striation pattern, in the side-to-side configuration.

Standard bath solution. The cells were superfused at 2 ml/min. with the bath solution at 30°C in a 0.5-ml chamber mounted on the stage of an inverted microscope. The bath solution used contained (in mM): NaCl 132, KCl 4.8, MgCl₂ 1.2, CaCl₂ 1.8, HEPES 10 and glucose 5, pH 7.4. Channel blockers of nonjunctional membrane conductance were added (0.5 mM BaCl₂ to minimize the inward rectifier K⁺ current and 0.1 mM Cd²⁺ to eliminate the Ca²⁺ inward current).

Pipette solution. Pipette resistance varied between 2 and 4 MΩ once filled with the following solution (in mM): KCl 120, NaCl 10, MgCl₂ 3, EGTA 5, K₂-ATP 5, HEPES 10, CaCl₂ 20 and TEA-Cl 5 to minimize the delayed rectifier K⁺ current, pH adjusted to 7.2 with KOH.

Double whole-cell patch-clamp technique. When the double whole-cell is established on a pair of cardiac cells, both electronic systems are linked by the intercellular resistance. In a double voltage-clamp, each cell of the pair is independently maintained at a chosen potential (holding potential). The only pathway for current to flow from one cell to the other is through gap junctions. Measured junctional resistance (rj) is isolated from ground potential by seals and peripheral membrane resistances (rₘ₁, rₘ₂) (see fig. 1). To measure the amplitude of current flow through gap junctions (Ij), cells are clamped to the same holding potential. Then, a voltage gradient (Vj) is maintained between the two cells by applying a voltage step to only one cell (V₁, see fig. 2). Current measured in each cell (I₁, I₂) corresponds to:

\[ I₁ = \frac{V₁}{rₘ₁ + \frac{V₁}{r_j}} \]

\[ I₂ = -I₁ \]

where Iₘ₁ is the membrane current of the stimulated cell; junctional resistance is derived from Vj/Ij.

Current recordings. Series resistances arising from the pipette tip and access resistance (due to membrane inside the pipette tip when the whole-cell perforation was performed) were compensated, respectively. Current was measured in the whole-cell configuration of the patch-clamp technique using two separate voltage-clamp amplifiers (Axopatch 200A; Axon Instruments, Foster City, CA). Voltage-clamp command pulses were performed using pClamp software and generated by a 12-bit digital-to-analog converter (Digidata 1200 interface; Axon Instruments). Currents were filtered at 1 kHz and digitized at 2 kHz using pClamp software.

Protocols. Rod-shaped cells with clear cross striations, resting potential of at least −78 mV and a stable junctional resistance as assessed during a base-line period of 5 min were used. Cells that did not maintain their clear cross striations during the experiments were eliminated. The experimental procedure consisted in holding the membrane potential of each cell at 0 mV. One cell of the pair was pulsed for 750 msec to various voltages (every 20 mV between −50 and +50 mV) while the membrane potential of the second cell was kept constant. The current was recorded in the second cell.
(which represents the current flowing through the gap junction = \( I_2 \)). Different cell pairs were used for each experiment.

**Statistical analysis.** Drug effects are presented as mean ± S.D. for non-normalized data and as mean ± S.E.M. for normalized data (percent of base line) and analyzed by Student’s paired t test as normality assumption was not rejected. Level of statistical significance was set at \( P < .05 \).

**Drugs.** All antiarrhythmic agents tested in this study were purchased from Sigma Chemical (St. Louis, MO) except for d-sotalol (Bristol-Myers Squibb, Wallingford, CT) and used at high concentrations (≈10 times the maximal effective therapeutic concentrations: 10⁻⁴ M quinidine, 10⁻⁴ M procainamide, 2 × 10⁻⁴ M lidocaine, 5 × 10⁻⁵ M flecainide, 2 × 10⁻⁴ M propranolol, 10⁻³ M d-sotalol, 10⁻² M amiodarone, 10⁻⁵ M verapamil). Compounds insoluble in water (i.e., lidocaine base, amiodarone and verapamil) were dissolved in ethanol at concentrations 10³ higher than the final concentration tested.

**Results**

Initial experiments were performed to characterize the properties of intercellular resistance in isolated pairs of guinea pig ventricular myocytes. Figure 2 presents a typical experiment in which one cell of a pair is voltage-clamped during 750 msec to various potentials from −50 to +50 mV by steps of 20 mV from a holding potential of 0 mV while the other cell was held at 0 mV. The current recorded in the cell maintained at 0 mV corresponds to the junctional current. In this example, the junctional resistance measured from the slope of the junctional current/transjunctional voltage \((I_j-V_j)\) relationship is 14 MΩ, which corresponds to a conductance of 71 nS. The \(I_j-V_j\) relationship presented in fig. 2B was linear within the range of transjunctional voltages tested. Figure 3 illustrates control experiments showing reversible uncoupling of a cell pair after extracellular application of the prototype uncoupling agent heptanol (Johnston et al., 1980). At a concentration of 2 mM, heptanol decreased dramatically \( I_j \) within 1 min of application \((n = 3)\). This effect was readily reversible on washout. These data are presented to serve as a positive control indicating what would happen if any of the antiarrhythmic agents tested increased junctional resistance.

Figure 4 presents recordings of junctional currents elicited by transjunctional voltage pulses from +50 to −50 mV in 20-mV steps obtained during a control period of 5 min during which \( I_j \) was stable and after a 10-min application of several class I antiarrhythmic compounds. \( I_j \) was unchanged after a 10-min application of 10⁻⁴ M quinidine, 2 × 10⁻⁴ M lidocaine, 10⁻³ M procainamide or 5 × 10⁻⁵ M flecainide. Table 1 summarizes results obtained from all experiments in which effects of these antiarrhythmic drugs were assessed on \( I_j \). Statistical analysis shows that none of the drugs tested significantly modified \( I_j \) of ventricular cell pairs, although procainamide had a tendency to increase \( I_j \) (110 ± 8% of control values; mean ± S.E.M.; \( P = .28 \)). In four of six experiments, the application of procainamide was continued over 15 min, but changes in \( I_j \) were still nonsignificant (107 ± 7% of control values; mean ± S.E.M.; \( P = .69 \)).

Another series of experiments performed to assess effects of class II, III or IV antiarrhythmic compounds on \( I_j \) is presented in figure 5. Recordings obtained after 10 min of superfusion of 2 × 10⁻⁴ M propranolol, 10⁻³ M d-sotalol, 10⁻⁴ M amiodarone and 10⁻⁵ M verapamil show that \( I_j \) was unchanged during the application of these drugs. Results obtained from all experiments performed to assess effects of these compounds on \( I_j \) are presented in table 2. None of these antiarrhythmic drugs changed the junctional resistance of ventricular cell pairs.

**Discussion**

The purpose of the present work was to assess the effect of several antiarrhythmic agents (taken from classes IA, IB, IC, II, III and IV of antiarrhythmic drugs) on junctional resistance of isolated guinea pig ventricular cell pairs using the double patch-clamp technique. Results obtained in this study show that none of the antiarrhythmic drugs tested modified junctional resistance in resting myocytes.

Previous studies have suggested that class I antiarrhythmic drugs may change internal resistance of cardiac fiber by altering electrical coupling through gap junctions. Hasegawa et al. (1991) have shown in stimulated guinea pig papillary
muscles that quinidine alters the relationship between the maximum rate of rise of the action potential and the square of conduction velocity \(V_{\text{max}} \cdot \theta^2\) relationship, suggesting a decrease in \(r_i\) (although the authors concluded that there was an increase in \(r_i\)). Using the technique of intracellular current application and transmembrane voltage recording, Arnsdorf and Bigger (1976) showed an increase in internal resistivity of Purkinje fibers with procainamide, although this effect was not significant. Moreover, it has been shown in studies on anisotropic conduction properties in cardiac preparations that quinidine and O-desmethyl encainide depress both longitudinal and transverse conduction velocities to the same extent (Bajaj et al., 1987; Turgeon et al., 1992). A decrease in sodium current leads to a greater decrease in propagation velocity in the longitudinal vs. transverse direction in cardiac muscle (Spach et al., 1985, 1987), thus an orientation-independent increase in conduction times is not expected for drugs acting solely on sodium channels. In the present study, effects of class I antiarrhythmic drugs (i.e., quinidine, lidocaine, procainamide and flecainide) were assessed at high concentrations (relative to their inhibition of the sodium current) on junctional resistance of coupled guinea pig ventricular cell pairs using the double whole-cell voltage-clamp technique. None of these antiarrhythmic agents significantly modified the junctional resistance over the transjunctional voltage range tested (fig. 4) after a superfusion of 10 min. However, procainamide tended to increase \(r_i\) (110 ± 8% and 107 ± 7% of control values after 10 and 15 min of superfusion, respectively; mean ± S.E.M.; \(n = 6\) and 4, respectively), but this effect was not significant (\(P = .28\) and .69, respectively, for 10- and 15-min superfusion). The difference between our results and previously reported data that suggested an effect of class I antiarrhythmic agents on \(r_i\) may be explained by differences in stimulation conditions. In our experiments, measurements of \(r_i\) were realized in quiescent pairs of cardiac myocytes; in other studies, preparations were contracted by trains of stimulations, which are, for example, known to modulate the intracellular activity of calcium (Lee and Clusin, 1987). However, it was important to verify whether class I antiarrhythmic agents could directly alter junctional resistance independently of mechanisms depending on repetitive contractions.

Studies have also suggested that antiarrhythmic agents taken from classes III and IV may alter internal resistance of cardiac fibers. For example, Quinteiro et al. (1990) and Quinteiro and Biagetti (1994) demonstrated that an acute application of amiodarone decreases \(r_i\) of dog epicardial muscles. Verapamil was also shown to alter the \(\theta^2\)-\(V_{\text{max}}\) relationship to such an extent that lower \(V_{\text{max}}\) was associated with relatively greater \(\theta^2\) compared with control (Kabell, 1988), which is consistent with an increase in cell-to-cell coupling. Thus, another series of experiments was designed to assess whether several agents taken from class III and IV of antiarrhythmic drugs could directly alter junctional resistance. As for class I antiarrhythmic compounds tested in this study, amiodarone, d-sotalol and verapamil did not modify the junctional resistance after a 10-min superfusion (fig. 5). On the other hand, differences between these results and previously reported data demonstrating changes in \(r_i\) with class III and IV antiarrhythmic agents may be explained by the model considered (i.e., constant pacing rate of heart tissue vs. quiescent voltage-clamped cell pairs). On the other hand, several previous studies that described effects of antiarrhythmic agents on \(\theta^2\)-\(V_{\text{max}}\) relationship may also be criticized. Changes in \(V_{\text{max}}\) in the presence of amiodarone (Quinteiro and Biagetti, 1994) were not predicted by the quadratic changes in \(\theta\) during transverse propagation, which suggested to the authors a decrease in \(r_i\) with amiodarone. However, if an increase in \(r_m\) (it was recently shown that amiodarone inhibits the inward rectifier potassium current \(I_{\text{K1}}\); Sato et al., 1994) and a decrease in \(r_i\) are expected, we can anticipate a pronounced increase in \(r_i\) with amiodarone, more pronounced than that reported in their study (mean values reported for \(r_i\) control and amiodarone perfusion period are 0.982 and 1.073 mm, respectively). In addition, effects of quinidine on \(r_i\) from Hasegawa et al. (1991) were assessed on papillary muscles of \(\approx 2.5\) mm in length and 0.5 to 1 mm in diameter. The application of cable analysis in that case can be questionable, especially because measurement of \(V_{\text{max}}\) was sometimes done.

![Fig. 4. Recordings of junctional currents elicited by transjunctional voltage pulses from -50 to +50 mV by 20-mV steps of 750-msec duration. Results obtained in four experiments are shown. Recordings to the left correspond to junctional current families elicited during, respectively, each control period of 5 min, and recordings to the right correspond to currents elicited after 10 min of superfusion of different class I antiarrhythmic agents.](Image)
very close to fiber boundaries (the distance between the microelectrodes for action potential recordings was generally >2 mm). When the propagated activity reaches an extremity of a fiber ended by an infinite resistance, changes in the $V_{\max}-\theta^2$ relationship could occur as is the case for transverse conduction ($r_l\text{trans} > r_l\text{long}$), where $V_{\max}$ can increase simultaneously with a decrease in $\theta$ (see Spach et al., 1990, for an interpretation of this phenomenon).

To complete this study, effects of a class II antiarrhythmic agent, propranolol, were tested. In these experiments, propranolol did not affect $r_l$ after a 10-min superfusion, which is consistent with the absence of changes in $r_l$ during tolamolol application in Purkinje fibers (Arnsdorf and Friedlander, 1976). Beta blockers may, however, influence the modulation of cell coupling by beta agonists (Xiao and De Mello, 1991).

Given the range of $pK_a$ for class I antiarrhythmic drugs used in this study (from 7.9 to 9.3), we can calculate (using the Henderson-Hasselbalch equation) that $98\%$ of procainamide or flecainide but $70\%$ of quinidine and lidocaine are protonated at pH 7.4. Therefore, at physiological pH, these compounds are largely confined to the aqueous phase and thus expected to cross membranes less readily. However, it is well known that class I antiarrhythmic drugs block the sodium channel from the cytosol or the lipid membrane but not directly from the extracellular fluid, with a $t_{on}$ on the order of seconds (Campbell, 1983). This can be explained by considering their respective lipid solubility (lipid/water partition coefficient; log P). In fact, it appears that $>99\%$ of the neutral form of quinidine, lidocaine and flecainide and $90\%$ of procainamide are in the lipid phase (Campbell, 1983). Thus, protonation and diffusion (in the cytosol or within the membrane lipids) characteristics of these compounds suggest that the likelihood that they gain access to the gap junctions in the time frame of our experiments is sufficiently high. Respective $pK_a$ and log P values of other compounds tested in this study suggest that >97% of propranolol or d-sotalol is protonated and that their neutral forms are highly lipophilic (>99% in the lipid phase) and lipophobic (~40% in the lipid phase), respectively (Woods and Robinson, 1981). Amiodarone ($pK_a = 6.6$ and log P > 7; Craig, 1990) is very lipophilic and weakly protonated (16%) under our physiological conditions, which suggests that this drug will be rapidly distributed in plasma membrane. Finally, verapamil ($pK_a = 8.92$ and log P = 3.79; Craig, 1990) is 97% protonated but highly liposoluble, which also suggests for this drug a rapid distribution in lipids. In conclusion, regardless of the mechanisms by which the antiarrhythmic compounds tested in this study could reach or modulate gap junctions (i.e., through intracellular pathways or through a direct effect on membrane fluidity, as was shown for heptanol; Bastiaanse et al., 1993), we

### Table 1: Effects of class I antiarrhythmic agents on junctional resistance of isolated guinea pig ventricular cell pairs

<table>
<thead>
<tr>
<th>Cell pair for each drug (pair no.)</th>
<th>Junctional resistance (MΩ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>r_j [a]</td>
<td>24 ± 14</td>
</tr>
<tr>
<td>Percent of baseline [b]</td>
<td>102 ± 3</td>
</tr>
</tbody>
</table>

[a] Mean ± S.D.  
[b] Mean ± S.E.M.

Fig. 5. Recordings of junctional currents elicited by transjunctional voltage pulses from −50 to +50 mV by 20-mV steps of 750-msec duration. Results obtained in four experiments are shown. Recordings to the left correspond to junctional current families elicited during, respectively, each control period of 5 min, and recordings to the right correspond to currents elicited after 10 min of superfusion of different class II, III and IV antiarrhythmic agents.
assume they can reach intracellular milieu and/or gap junctions during the time course of our experiments (especially at the high concentrations tested). Moreover, the time exposure to each drug in our experiments (i.e., 10 min) is longer than the time needed to show an effect of antiarrhythmic drugs in any of the previous works in which an action on junctional resistance was suggested.

In summary, this report presents data demonstrating the lack of effects of antiarrhythmic agents tested, chosen among class IA (quinidine, lidocaine), IB (propranolamide), IC (flecainide), II (propranolol), III (d-sotalol, amiodarone) and IV (verapamil), on junctional resistance of guinea pig ventricular cell pairs. However, indirect effects of antiarrhythmic agents on gap junctions in myocytes that contract regularly at physiological pacing frequencies are not excluded.

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References


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TABLE 2

Effects of class II, III and IV antiarrhythmic agents on junctional resistance of isolated guinea pig ventricular cell pairs

<table>
<thead>
<tr>
<th>Cell pair for each drug (pair no.)</th>
<th>Control</th>
<th>Propranolol (2.10 -3 M)</th>
<th>Control</th>
<th>d-Sotalol (10 -3 M)</th>
<th>Control</th>
<th>Amiodarone (10 -3 M)</th>
<th>Control</th>
<th>Verapamil (10 -3 M)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>19</td>
<td>19</td>
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<tr>
<td>2</td>
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<td>26</td>
<td>22</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
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<td>16</td>
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<td>25</td>
<td>30</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Percent of baselinea</td>
<td>20 ± 5</td>
<td>19 ± 6</td>
<td>18 ± 5</td>
<td>18 ± 6</td>
<td>18 ± 9</td>
<td>18 ± 10</td>
<td>20 ± 6</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>Percent of baselineb</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
<td>94 ± 5</td>
</tr>
</tbody>
</table>

* a Mean ± S.D.
* b Mean ± S.E.M.