RSD1000: A Novel Antiarrhythmic Agent with Increased Potency under Acidic and High-Potassium Conditions


Department of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, British Columbia, Canada

Accepted for publication November 16, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

This study reports the use of a novel agent, RSD1000 [trans-(+)-trans-[2-(4-morpholinyl)cyclohexyl]naphthalene-1-acetate monohydrochloride], to test the hypothesis that a drug with pKₐ close to the pH found in ischemic tissue may have selective antiarrhythmic actions against ischemia-induced arrhythmias. The antiarrhythmic ED₅₀ for RSD1000 against ischemic arrhythmias was 2.5 ± 0.1 μmol/kg/min in rats. This value was significantly lower than doses that suppressed electrically induced arrhythmias. In isolated rat hearts, RSD1000 was approximately 40 times more potent in producing ECG changes (i.e., P–R and QRS prolongation) in acid (pHₐ = 6.4) and high [K⁺]₀ (10.8 mM) buffer than in normal buffer (pHₐ = 7.4; [K⁺]₀ = 3.4 mM). In patch-clamped, whole-cell rat cardiac myocytes, inhibition of sodium (I_Na) currents by RSD1000 was pH- and use-dependent. The IC₅₀ for I_Na blockade was lower (P < .05) in acid (0.8 ± 0.1 μM) than in pH 7.3 (2.9 ± 0.3 μM), respectively, whereas the IC₅₀ for blockade of transient outward potassium current (I_TO) at pH = 6.4 and 7.3 was 3.3 ± 0.4 and 2.8 ± 0.1 μM, respectively. Mixed ion channel block in ischemic myocardi um with minimal effects on normal cardiac tissue, as governed by the low pKₐ of RSD1000, may account for its antiarrhythmic activity against ischemia-induced arrhythmias.

Current antiarrhythmic therapy using drugs is often unsatisfactory, particularly for the severe arrhythmias due to myocardial ischemia-infarction (Roden, 1994; see reviews by Cairns, 1997). There are many reasons for this. One relates to the fact that many of the current drugs provide antiarrhythmic protection by virtue of acting upon normal (non-pathological) cardiac tissue (Duff et al., 1988; Abraham et al., 1989) to prevent its participation in arrhythmias. Conventional ion channel blocking antiarrhythmics have poor antiarrhythmic efficacy against ischemia/infarction arrhythmias in both clinical (Echt et al., 1991; Waldo et al., 1995) and experimental (Igwemezie et al., 1992; Barrett et al., 1995) settings. This appears to result from the fact that most of such drugs, apart from Class Ib agents, do not select between pathologically disturbed myocardial tissue and normal myocardium. Secondly, because such drugs only act at doses that affect normal cardiac tissue, they are particularly liable to be proarrhythmic (see review by Roden, 1994) because of over expression of their basic action. Furthermore, by virtue of other cardiac and extra cardiac actions they produce toxicities such as cardiac failure and hypertension, plus other unpleasant side effects (Ravid et al., 1989; Schlepper, 1989). Even Class Ib antiarrhythmics, such as lidocaine, lack sufficient ischemia selectivity and thus their therapeutic use is limited by central nervous system toxicity and hypotension (Feldman et al., 1989; Barrett et al., 1995).

Arrhythmias due to myocardial ischemia/infarction depend upon the electrophysiological abnormalities occurring in the pathologically disturbed (ischemic) tissue (Lazzara and Scherlag, 1984; Janse et al., 1986; Kléber, 1986). Thus in the ischemic region, slowing of conduction and decreases in refractoriness (Lazzara and Scherlag, 1984; Janse et al., 1986; Kléber, 1991) create situations whereby reentry circuits between normal and damaged tissues can occur (Lazzara and Scherlag, 1984; Janse et al., 1986; Kléber, 1991). Such reentry circuits can be terminated by either changing electrophysiological behavior in either normal tissue or in the damaged tissue. Changes in electrophysiology in normal tissue are liable to cause arrhythmias and/or depress cardiac functions. Such considerations lead to the suggestion that effective drugs should act selectively on ischemic tissues to first prevent the reduction of refractoriness due to ischemia and then second abolish all electrical activity in the damaged tissues (Walker and Chia, 1989). It may be possible to accomplish this with a drug that selectively acts upon damaged tissues to block both sodium and potassium channels.

ABBREVIATIONS: AS, arrhythmia score; C₅₀, concentration producing 25% change from predrug level; D₅₀, dose producing 50% change from predrug level; I_Na, inward sodium current; I_TO, transient outward current; IT, current threshold; ERPF, effective refractory period; OZ, occluded zone; PVC, premature ventricular contraction; RSD1000, trans(–)-trans-[2-(4-morpholinyl)cyclohexyl]naphthalene-1-acetate monohydrochloride; τ, time constant of I_TO current decay; VT, ventricular tachycardia; VF, ventricular fibrillation; VFf, ventricular fibrillo-flutter threshold.
The question is how to develop drugs that act selectively on damaged (ischemic) tissue. If one had a drug that blocked ion channels only in its charged form from an external site it would be possible to take advantage of the acid conditions found in the extracellular fluid of ischemic tissue (Abraham et al., 1989; Dennis et al., 1991). Acid conditions could be used to ensure that more of the active species of a drug were available in ischemic as opposed to normal tissue. For example, if such a drug had a $pK_a$ close to the pH of about 6.4 found during ischemia (Owens et al., 1996), then the effective concentration of charged form would be higher in ischemic tissue and selective blockade thereby produced.

RSD1000 [(±)-trans-[2-(4-morpholino)cyclohexyl]naphthalene-1-acetate monohydrochloride] is a novel antiarrhythmic agent that blocks sodium and potassium channels and has a $pK_a$ of 6.1 (Fig. 1). We have studied RSD1000 in a variety of rat models to assess both its antiarrhythmic and ion channel-blocking actions. Initial provisional reports have been made of some of the actions of RSD1000 (Yong et al., 1996).

**Materials and Methods**

**In Vivo Studies**

All experiments (approved by The Animal Care Committee of the University of British Columbia) were conducted on male Sprague-Dawley rats weighing 200 to 300 g. Rats were anesthetized with pentobarbitone (60 mg/kg, i.p.). In intact rats, an endotracheal tube (14 Jelco IV catheter) was inserted, the left carotid artery was cannulated for blood pressure recording, and right jugular vein cannulated for drug administration (Harvard Syringe pump, model 55–2222). ECGs were recorded from needle electrodes placed in an approximate lead V2 configuration. Signals were recorded on a Grass polygraph (model 79D) at a standard chart speed of 100 mm/s. Using a Harvard Miniature ventilator pump (model 50–1700) artificial ventilation was set at 10 ml/kg, 60 times a minute. Body temperature was maintained at 36 to 38°C.

**Ischemia-Induced Arrhythmias.** Ischemic arrhythmias were induced by occlusion of the left coronary artery as previously described (Barrett et al., 1995). A specially constructed occluder, consisting of a polypropylene thread (5–0, Ethicon 8720H) inserted into a polyethylene guide (PE-10), was loosely placed around the left coronary artery at the level just below its first bifurcation. Rats were allowed 30 min to recover from surgery before random and blind drug treatment. Arterial blood samples were taken before and after coronary artery occlusion for determination of serum potassium concentrations using a potassium ion selective electrode (Ionetics Potassium Analyzer, Ionetics, CA, USA). After 5 min of drug infusion, the occluder was permanently tightened and drug infusion maintained. All arrhythmias were recorded for 15 min postocclusion. The arrhythmic history of each animal was expressed as an arrhythmia score (AS) (Curtis and Walker, 1988) following the guidelines outlined in the Lambeth Conventions (Walker et al., 1988). At the end of the experiment, hearts were excised and perfused with piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES) buffer containing cardiogreen dye (0.2 mg/liter Fast Green FCP) to differentiate between underperfused (occluded zone) from perfused (green) tissue. The former region was excised and weighed to give the size of the occluded zone as a percentage of the total ventricular mass (%OZ).

**Electrophysiological Studies of Single Rat Ventricular Myocytes.** The methods used to prepare dissociated rat ventricular myocytes generally followed those previously described (Mitra and Morad, 1985); the specific procedures employed have also been previously detailed (McLarnon and Xu, 1995). A constant-flow Langendorff perfusion system was used during the isolation of cells with oxygenated Tyrode’s solution containing: 153 mM NaCl, 3.4 mM KCl, 1.18 mM MgSO4, 1.2 mM NaH 2PO4, 10 mM $N$-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and 11 mM glucose, and pH was adjusted to 7.4 with 10.8 mM KCl and aerated with oxygen. Acid ($pH_e = 6.4$) and raised [$K^+$], buffer was adjusted by titrating with HCl and adding 10.8 mM KCl in place of 3.4 mM KCl.

Rats were overdosed with pentobarbitone (70 mg/kg, i.p. plus heparin, 1000 U). Excised hearts were washed with ice-cold buffer before being perfused at 100 mm Hg and 37°C. A noncompliant balloon, inserted in the left ventricle at an end diastolic pressure of 5 mm Hg, was used to measure left ventricular pressure. Epicardial ECGs were recorded using two silver ball electrodes attached to separate 1-cm circular disks of filter paper. Hearts were stabilized for 20 min in normal buffer before being randomized to normal or “ischemic” buffer containing vehicle or RSD1000. The concentration range of RSD1000 tested was 0.01 to 300 $\mu$M. Each concentration was perfused for 3 min before heart rate, ECG variables, and ventricular pressure were measured.

Fig. 1. Chemical structure of RSD1000; FW: 389.92, (as monohydrochloride salt), 353.54, (as free base); $pK_a$: 6.1.

**In Vitro Studies**

**Isolated Rat Hearts.** The actions of RSD1000 in isolated rat hearts were studied using a modified Langendorff perfusion apparatus (Curtis et al., 1986). “Normal” PIPES buffer was of the following composition: 153 mM NaCl, 3.4 mM KCl, 1.18 mM MgSO4, 11.1 mM d-glucose, 2.52 mM CaCl2; $2H_2O$, and 14.34 mM PIPES. The buffer was titrated to pH 7.4 with NaOH and aerated with oxygen. Acid ($pH_e = 6.4$) and raised [$K^+$], buffer was adjusted by titrating with HCl and adding 10.8 mM KCl in place of 3.4 mM KCl.

Rats were overdosed with pentobarbitone (70 mg/kg, i.p. plus heparin, 1000 U). Excised hearts were washed with ice-cold buffer before being perfused at 100 mm Hg and 37°C. A noncompliant balloon, inserted in the left ventricle at an end diastolic pressure of 5 mm Hg, was used to measure left ventricular pressure. Epicardial ECGs were recorded using two silver ball electrodes attached to separate 1-cm circular disks of filter paper. Hearts were stabilized for 20 min in normal buffer before being randomized to normal or “ischemic” buffer containing vehicle or RSD1000. The concentration range of RSD1000 tested was 0.01 to 300 $\mu$M. Each concentration was perfused for 3 min before heart rate, ECG variables, and ventricular pressure were measured.

**Isolated Rat Ventricular Myocytes.** The methods used to prepare dissociated rat ventricular myocytes generally followed those previously described (Mitra and Morad, 1985); the specific procedures employed have also been previously detailed (McLarnon and Xu, 1995). A constant-flow Langendorff perfusion system was used during the isolation of cells with oxygenated Tyrode’s solution containing: 133.5 mM NaCl, 4 mM KCl, 1.2 mM MgCl2, 1.2 mM NaH 2PO4, 10 mM $N$-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and 11 mM glucose, and pH was adjusted to 7.3 with 1 mM NaOH. Following 15 to 20 min of enzyme treatment (0.07% Type II collagenase, Worthington Biochemical), Tyrode’s solution (with 25 $\mu$M Ca$^{2+}$) was reapplied with gentle agitation of the digested tissue. Cell suspensions were centrifuged and resuspended in fresh Tyrode’s solution. The cells were stored at room temperature between intervals of washing with successively increased Ca$^{2+}$ concentrations (final concentration = 1.8 mM). The morphology of the cells was rod-shaped and quiescent and their percentage yield was in the range of 70%.

**Electrophysiological Studies of Single Rat Ventricular Myocytes.** The procedures used in this laboratory for the recording

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Fig. 1. Chemical structure of RSD1000; FW: 389.92, (as monohydrochloride salt), 353.54, (as free base); $pK_a$: 6.1.
of macroscopic currents from isolated rat ventricular myocytes have been described previously (McLarnon and Xu, 1995; 1997). In the present study, whole-cell transient outward K+ (I_o), inward Na+ (I_Na) and inward calcium (I_{Ca}) currents were recorded. The micropipettes were made from Corning 7052 glass (A-M Systems, Everett, WA) with resistance values between 2 to 4 MΩ. An axopatch amplifier (model 200A, Axon Instruments, Foster City, CA) was used to record the currents with the low-pass filter set at 1 or 2 KHz. Capacitive current and series resistance were compensated using analog circuitry of the amplifier. Holding potentials were from −70 to −100 mV and voltage clamp protocols were operated by computer using pClamp 6 (Axon Instruments). ITO was activated with a depolarizing mV and voltage clamp protocols were operated by computer using circuitry of the amplifier. Holding potentials were from positive current and series resistance were compensated using analog record the currents with the low-pass filter set at 1 or 2 Khz. Capacitor (model 200A, Axon Instruments, Foster City, CA) was used to pettes were made from Corning 7052 glass (A-M Systems, Everett, WA). The patch pipette solution to suppress I_{Ca} and I_{Na}, respectively. The patch pipette solution contained: 120 mM KCl, 0.15 mM CaCl2, 6 mM MgCl2, 5 mM EGTA, 5 mM Na2-ATP, and 10 mM HEPES; pH was adjusted to 7.3 with KOH. Blood pressure (mm Hg) was recorded 5 min after beginning infusion of RSD1000 (in mmole/kg/min) in ischemic arrhythmia study. Values are mean ± S.E.M., n = 7. In sham occlusion study, variables were measured 5 and 20 min after infusion of 8.0 μmol/kg/min RSD1000.

### Statistical Analysis

Results are presented as the mean ± S.E.M. (vertical lines). When comparing pre- with postdrug values, Student’s t test was used (P < .05). Statistical analysis between vehicle and treated groups was performed by repeated measures ANOVA followed by Tukey’s t test (P < .05) or Dunnett’s test (P < .05 or P < .001). In cases where mortality was the endpoint, statistical analysis was performed using the χ-square test with P < .05. The effects of pH were evaluated by comparing drug changes in acid and normal pH using Student’s paired t test.

### Results

**Effects of RSD1000 on Hemodynamic, ECG Variables and Ischemia-Induced Arrhythmias**

Hemodynamic and ECG variables were measured 5 min after beginning treatment, before coronary artery occlusion. RSD1000 dose dependently decreased blood pressure, heart rate, and prolonged both P–R and Q–T intervals (Tables 1 and 2). Statistically significant effects occurred at 1 μmol/kg/min for lowering blood pressure and 4 μmol/kg/min for increasing P–R and Q–T intervals. Although mean blood pressure was decreased with increasing dose, the mean pulse width was unchanged by RSD1000. In the sham occlusion experiment, RSD1000 at 8 μmol/kg/min demonstrated that the hypotensive action of RSD1000 was not progressive during the duration of the experiment.

The antiarrhythmic action of RSD1000 against ischemia-induced arrhythmias in intact rats is summarized for all arrhythmias in Table 3 and is summarized as a reduction in mean AS in Fig. 2. The incidence of premature ventricular contractions (PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) occurring after coronary artery ligation were decreased in RSD1000-treated animals, relative to control, in a dose-dependent manner. Antiarrhythmic data points were best fit using the equation,

\[ y = x^a + b \]

where x specifies concentration of RSD1000, y denotes AS, a is ED50, and b is the Hill coefficient. The response, y, was
In sham occlusion study, variables were measured 5 and 20 min after infusion of 8.0 μmol/kg/min RSD1000.

Fig. 3. Antiarrhythmic actions of RSD1000 in normal and simulated ischemia buffers. The actions of RSD1000 in normal buffer and a simulated ischemia buffer were investigated in isolated rat hearts. Changes induced by RSD1000 were expressed as percentage increases in ERP in a dose-related manner. Data points were fitted to lines using nonlinear equations and their D50% values were estimated from seven determinations. D50% values for VF, iT, and ERP were estimated to be 15 ± 3.2, 11 ± 1.4, and 7.8 ± 0.9 μmol/kg/min, respectively.

Table 2
Heart Rate and ECG effects of RSD1000
Heart rate and ECG variables were recorded 5 min after beginning infusion of RSD100 (in μmole/kg/min) in ischemic arrhythmia study. Values are mean ± S.E.M.; n = 7.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Heart Rate</th>
<th>P-R</th>
<th>QRS</th>
<th>QT</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/kg/min</td>
<td>bpm</td>
<td>ms</td>
<td>ms</td>
<td>ms</td>
</tr>
<tr>
<td>Vehicle</td>
<td>403 ± 9</td>
<td>59 ± 1</td>
<td>29 ± 1</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>RSD1000</td>
<td>1</td>
<td>376 ± 11</td>
<td>61 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>362 ± 16</td>
<td>63 ± 2</td>
<td>29 ± 1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>349 ± 18a</td>
<td>64 ± 3a</td>
<td>32 ± 1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>305 ± 12a</td>
<td>70 ± 3a</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Sham occlusion</td>
<td>5 min postinfusion</td>
<td>297 ± 14a</td>
<td>69 ± 4a</td>
<td>31 ± 2</td>
</tr>
<tr>
<td></td>
<td>20 min postinfusion</td>
<td>281 ± 8a</td>
<td>71 ± 4a</td>
<td>31 ± 3</td>
</tr>
</tbody>
</table>

a P < .05 versus predrug for each dose (t test).

Table 3
Arrhythmia incidences in presence of RSD1000
Antiarrhythmic activity of RSD1000 against ischemia-induced arrhythmias is shown as number of animals (x) in group (n) having PVC, VT, VF, and/or fatal or irreversible ventricular fibrillation (irrv VF). Number of PVCs was expressed as a log10 transform. Preocclusion serum potassium ([K+]p) and occluded zone size (%OZ) are also presented.

<table>
<thead>
<tr>
<th>Dose</th>
<th>[K+]p (preoccl)</th>
<th>%OZ</th>
<th>log10 PVC</th>
<th>VT</th>
<th>VF</th>
<th>irrv VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/kg/min</td>
<td>x/n</td>
<td>x/n</td>
<td>x/n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.8 ± 0.2</td>
<td>39 ± 1.2</td>
<td>1.9 ± 0.1</td>
<td>19/21</td>
<td>20/21</td>
<td>20/21</td>
</tr>
<tr>
<td>RSD1000</td>
<td>1</td>
<td>3.5 ± 0.4</td>
<td>41 ± 1.4</td>
<td>1.5 ± 0.2</td>
<td>5/7</td>
<td>7/7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.9 ± 0.3</td>
<td>42 ± 1.5</td>
<td>1.6 ± 0.1</td>
<td>4/7</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.8 ± 0.3</td>
<td>36 ± 1.1</td>
<td>1.8 ± 0.1</td>
<td>2/7b</td>
<td>1/7b</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.9 ± 0.2</td>
<td>38 ± 1.4</td>
<td>0.5 ± 0.2a</td>
<td>0/7b</td>
<td>0/7b</td>
</tr>
</tbody>
</table>

a Indicates statistical significance from vehicle (P < .05) using ANOVA with Tukey’s t test; b indicates statistical significance from vehicle (P < .05) using χ2 square test.

Fig. 2. Antiarrhythmic dose-response curve for RSD1000 against ischemia-induced arrhythmias. Data is expressed as group mean arrhythmia score of seven experiments. RSD1000 provided dose-related protection against arrhythmias induced by acute myocardial ischemia with an ED50 of 2.5 ± 0.1 µmol/kg/min.

Fig. 3. Actions of RSD1000 on electrical stimulation in vivo. Each point indicates mean of seven experiments. RSD1000 increased threshold currents for induction of VFt (µA) (■), iT (µA) (□), and ERP (ms) (●) in a dose-dependent manner. D50% values for VFt, iT, and ERP were estimated to be 15 ± 3.2, 11 ± 1.4, and 7.8 ± 0.9 µmol/kg/min, respectively.

Effects on Electrically Induced Arrhythmias
To reveal the possible ion channel blocking actions of RSD1000 in vivo and its effects on arrhythmias in normal myocardium, RSD1000 was tested against arrhythmias produced by electrical stimulation of the left ventricle in intact rats. Figure 3 shows that RSD1000 at infusion levels greater than those in Fig. 2 increased threshold currents for both electrical induction of VFt and extrasystoles (iT) and also increased ERP in a dose-related manner. Data points were fitted to lines using nonlinear equations and their D50% values were estimated from seven determinations. D50% values for VFt, iT, and ERP were estimated to be 15 ± 3.2, 11 ± 1.4, and 7.8 ± 0.9 µmol/kg/min, respectively, demonstrating that at higher doses RSD1000 has actions in nonischemic rat myocardium, possibly related to block of Na+ and K+ currents.

Effects of RSD1000 in Normal and Simulated Ischemia Buffers
The actions of RSD1000 in normal buffer and a simulated ischemic buffer were investigated in isolated rat hearts. Changes induced by RSD1000 were expressed as percentage factors by 6.25 to scale the response to 100% efficacy. The ED50 for antiarrhythmic activity was estimated to be 2.5 ± 0.1 µmol/kg/min (h = −2.8) with complete protection against ventricular tachycardia and fibrillation occurring at 8 µmol/kg/min (Table 3).
of changes from pretreatment and plotted in Fig. 4 in terms of P–R interval and QRS duration. Maximal responses for heart rate, P–R and QRS could not be obtained, because atrioventricular block occurred at the highest concentrations of RSD1000; atrioventricular block occurred at an average RSD1000 concentration of 1 μM (n = 6) in ischemic and 300 μM (n = 6) in normal buffers. RSD1000 produced a concentration-dependent decrease in heart rate and an increase in P–R interval and QRS duration. Drug effects were more pronounced on heart rate (results not shown) and P–R interval. In addition, a slight decrease in ventricular pressure was observed under both buffer conditions (results not shown). Data points were fitted to lines using nonlinear equations and their C25% values were estimated from seven determinations. We compared the relative potency of RSD1000 in each condition and estimated the concentrations required to produce a 25% change from predrug values (C25%). RSD1000 was approximately 40 times more potent in the ischemic buffer. For example, the C25% values in ischemic and normal buffers for P–R interval increases were 0.8 ± 0.3 μM and 34 ± 7 μM, respectively (P < .05).

Effects of RSD1000 in Single Ventricular Myocytes

Inward Sodium Current (I\textsubscript{Na}) Inward sodium currents were elicited from a resting potential of −70 mV by initiating a hyperpolarizing prepulse to −140 mV (to remove inactivation) and depolarizing to −20 mV (see pulse protocol, Fig. 5). Original traces in Fig. 5A illustrate the control currents (bottom traces) and the effects of 2 μM RSD1000 on I\textsubscript{Na} currents at pH 7.3 and 6.4 (top traces). Concentration responses are shown in Fig. 5B for n = 4 cells with bath solutions at pH 7.3 or 6.4. The lines are best fits using eq. 1 (see above) and the EC\textsubscript{50} values were estimated to be 2.9 ± 0.3 μM (h = −1.1) at pH 7.3 and 0.8 ± 0.1 μM (h = −0.8) at pH 6.4. These results show that the blocking action of RSD1000 on I\textsubscript{Na} was significantly enhanced (P < .05) under external acid conditions.

The current-voltage (I-V) relationship of RSD1000 on I\textsubscript{Na} was investigated by eliciting a series of depolarizing steps from −100 to +30 mV at a frequency of 0.5 Hz (V\textsubscript{H} = −100 mV). Figure 6 shows peak I\textsubscript{Na} amplitudes as a function of depolarizing potentials in the absence (closed symbols) and presence (open symbols) of 5 μM RSD1000 at both pH 7.3 and 6.4. The data are shown with least square best fit of the Boltzman equation in the form:

\[
I_{\text{Na}} = \frac{G_{\text{max}}(V-E_{\text{rev}})}{1+e^{(V-V_{\text{50}})/k}}
\]

(2)
Use-dependent inhibition of $I_{Na}$ was studied using a series of 20 depolarizing steps to +20 mV from a holding potential of 100 mV applied at a frequency of 20 Hz (Fig. 7). We first determined whether any use-dependent effects occurred in the absence of RSD1000. Our results showed that there was no change in $I_{Na}$ amplitude during the sequence of pulses for either pH 7.3 or 6.4 (data not shown). Original sample traces of $I_{Na}$ currents associated with the 1st, 2nd, 5th, 10th, and 20th pulses are shown in Fig. 7A for both pH values in the presence of 5 μM RSD1000. At pH 7.3 there was a small decrease in $I_{Na}$ amplitude with increasing pulses. In this cell, the amplitude of the last current was 70% relative to the initial current evoked in the series. Overall, at pH 7.3 the amplitude of the 20th evoked $I_{Na}$ was 71 ± 8% of the initial current ($n = 4$). In contrast, use-dependent inhibition of $I_{Na}$ by RSD1000 was very prominent when external pH was 6.4. In Fig. 7A, the amplitude of the final current was 36% of the initial amplitude. Overall, at pH 6.4 the amplitude of the last $I_{Na}$ was 37 ± 5% relative to the initial current of the series ($n = 4$). A plot of the normalized current for each of the 20 steps is presented in Fig. 7B for both pH conditions in the absence (open symbols) and presence (closed symbols) of 5 μM RSD1000. These results show that use-dependent block of $I_{Na}$ was significantly enhanced when myocytes were exposed to an external solution with pH 6.4 compared with pH 7.3 ($P < .05$).

**Transient Outward Current (I_{TO}).** In Fig. 8A, sample traces of $I_{TO}$ are shown following depolarizing steps to +60 mV.

where $I_{Na}$ is the maximal sodium current, $G_{max}$ is the maximal channel conductance, $E_{rev}$ is the membrane potential at which the current is zero, $V'$ is the membrane potential at which one half maximal activation occurs, $k$ is the slope factor for the voltage dependence of activation. In control pH 7.3 and 6.4, the threshold for $I_{Na}$ activation was −60 mV with peak $I_{Na}$ amplitudes of approximately 16 to 18 nA between −40 and −30 mV. At pH 7.3, RSD1000 decreased $I_{Na}$ amplitude without changes in threshold and peak potentials. Threshold (−50 mV) and peak (−20 mV) potentials were both shifted to more positive potentials by RSD1000 in pH 6.4, whereas $I_{Na}$ amplitude was decreased ($P < .05$) to half control maximum.

**Fig. 6.** Current-voltage relationship for peak $I_{Na}$ under pH 7.3 (A) and 6.4 (B) in control ([ ]) and in the presence ( [ ]) of 5 μM RSD1000. Peak inward currents were plotted as a function of test potentials elicited from −100 to +30 mV at 10-mV increments at a frequency of 0.5 Hz. Threshold activation of $I_{Na}$ for both pH 7.3 and 6.4 was −60 mV with maximum peak $I_{Na}$ amplitudes between −40 and −30 mV. At pH 7.3, RSD1000 decreased $I_{Na}$ amplitude without changes in threshold and peak potentials. Threshold (−50 mV) and peak (−20 mV) potentials were both shifted to more positive potentials by RSD1000 in pH 6.4, whereas $I_{Na}$ amplitude was decreased ($P < .05$) to half control maximum.

**Fig. 7.** Use-dependent inhibition of $I_{Na}$. Currents were elicited by a series of 20 depolarizing steps to +20 mV from a holding potential of +100 mV at stimulation frequency of 20 Hz. A, current traces showing actions of 5 μM RSD1000 at pH 7.3 and 6.4. Traces show responses to 1st, 2nd, 5th, 10th, and 20th steps of the series. B, plot of normalized $I_{Na}$ current as a function of episode number (1–20) at pH 7.3 and 6.4 in the absence (open symbols) and presence (closed symbols) of 5 μM RSD1000.
Concentration-response curves for RSD1000 actions on inward, the time constant of current decay (τ) in six cells, RSD1000 reduced the decay time constant of I_{TO} to 67.3 and 3.3 s with amplitudes between 2 to 2.5 nA were recorded with 60-ms depolarizing steps to +30 mV from a holding potential of −70 mV. RSD1000 (30 μM) showed no evident effect to alter either the amplitudes or the time courses of calcium currents (n = 6 cells).

**Discussion**

The results of this study showed that RSD1000 provided almost complete protection against arrhythmias due to regional myocardial ischemia following coronary artery occlusion in rats. Furthermore, such protection occurred at doses that were lower than those that protected against electrically induced arrhythmias or depressed blood pressure. In vivo studies in normal hearts suggested that at higher doses RSD1000 blocked sodium currents (in a frequency-dependent manner) as well as potassium currents. This view was confirmed in studies with isolated myocytes in which RSD1000 was shown as a potent inhibitor of both I_{Na} and I_{TO} (Figs. 5 and 8). Using a high Ca^{2+}-containing solution (see Materials and Methods), inward Ca^{2+} currents with amplitudes between 2 to 2.5 nA were recorded with 60-ms depolarizing steps to +30 mV from a holding potential of −70 mV. RSD1000 (30 μM) showed no evident effect to alter either the amplitudes or the time courses of calcium currents (n = 6 cells).

**Fig. 8.** Inhibition of I_{TO} by RSD1000. I_{TO} currents were recorded with a depolarizing step to +60 mV from a holding potential of −70 mV. A, original I_{TO} current traces at pH 7.3 and 6.4 in the absence (top trace) and presence of 2 μM (middle trace) and 30 μM RSD1000 (bottom trace). B, concentration-response curves for decaying time course of I_{TO} by RSD1000, where C denotes control currents. Normalized data are for n = 5 cells, which were studied at both pH 7.3 and 6.4 at concentrations of RSD1000 shown.

mV from a holding level of −70 mV with pH 7.3. The effects of RSD1000, applied at concentrations of 2 (middle trace) and 30 μM (bottom trace) on I_{TO} are shown. In this cell, the time constant of current decay (τ) was diminished to 70% (with 2 μM RSD1000) and 15% (with 30 μM RSD1000) of control value. The same experiments were also repeated at pH 6.4 in the absence or presence of RSD1000. The sample traces at pH 6.4 (Fig. 8A) relative to those shown at pH 7.3 illustrate an equipotent suppression of I_{TO} at concentrations of 2 μM (middle trace) and 30 μM (bottom trace) RSD1000. Concentration-response curves for RSD1000 actions on inactivation time course of I_{TO} are shown in Fig. 8B for both pH values (n = 5 cells). Overall, the EC_{50} was 2.8 ± 0.1 μM at pH 7.3 and 3.3 ± 0.4 μM at pH 6.4 and were not significantly different (P > .05). There was also no significant difference between potency of RSD1000 at the two different pH values if the area under the curve was used as an index of effect (data not shown). A previous study on the benzopyran compound, terikalant, also showed no difference in potency if the area under the curve or τ was used as a measure of response (McLarnon and Xu, 1995).

In the experiments described above the holding potential was −70 mV and it was possible that at this level not all I_{TO} was available for activation. To study this point we also carried out additional experiments using a concentration of RSD1000 (10 μM) with V_H = −80 mV (external pH at 7.3). In six cells, RSD1000 reduced the decay time constant of I_{TO} to 66 ± 6% of control (data not shown). This result can be compared with a reduction of 69 ± 3% found at V_H = −70 mV and indicates that there was no significant difference in the effects of RSD1000 to τ at holding potentials of −70 or −80 mV (P > .05).

**Inward Calcium Current (I_{Ca}).** We also investigated the effects of RSD1000 on I_{Ca} currents in rat myocytes. Figure 9 shows the original current traces before and after superfusion of 30 μM RSD1000. This concentration was chosen because at this level RSD1000 strongly inhibited both I_{Na} and I_{TO} (Figs. 5 and 8). Using a high Ca^{2+}-containing solution (see Materials and Methods), inward Ca^{2+} currents with amplitudes between 2 to 2.5 nA were recorded with 60-ms depolarizing steps to +30 mV from a holding potential of −70 mV. RSD1000 (30 μM) showed no evident effect to alter either the amplitudes or the time courses of calcium currents (n = 6 cells).

![Fig. 9.](attachment:image.png) Effects of RSD1000 on I_{Ca}. Original traces elicited by depolarizing to +30 mV from a holding potential of −70 mV before and after exposure to 30 μM RSD1000.
cardium by minimizing the concentration of the cationic species in nonischemic and, possibly, extra-cardiac tissues. This was accomplished by minimizing its degree of ionization at physiological pH relative to the raised extracellular [H+] during acute myocardial ischemia. The N-morpholinogroup of RSD1000 (Fig. 1) is the tertiary nitrogen that contributes to the overall pKₐ value of 6.1. In acid pH (6.4), the majority of RSD1000 is protonated, whereas at pH 7.3 only about 5% is charged. In acutely ischemic myocardial tissue, this should result in a local increase in the concentration of the protonated form of RSD1000. Determination of tissue levels of RSD1000 in ischemic versus nonischemic hearts was not performed in this study. It should be noted, however, that sufficient infusion time was given to achieve a “pseudo” steady-state level of RSD1000 in the heart before coronary artery ligation. In the period following ligation, compound levels in the uninvolved zone should continue to increase (up to a maximum), whereas those in the involved zone should remain unchanged or, alternatively, “trapped” because there was no apparent blood flow for drug removal. The low incidence of collateral flow present in the rat heart (Maxwell et al., 1981; Velebit et al., 1982; Echt et al., 1991; Morganroth et al., 1993) and, indeed, in clinical settings (Pentecost et al., 1993) reported a positive ~5 to 10 mV shift in INa activation when pH was changed from 7.8 to 6.8. Alternatively, the binding site may not be in the electric field, but rather, the electric field acts on the macromolecule (and on other ions in it) to alter the affinity or availability of the site (Woodhull, 1973). The charged form of RSD1000 at pH 6.4 may perhaps bind to a binding site during the activation process, i.e., through the channel pore, resulting in a voltage-dependent decrease in INa (Fig. 9). The voltage-dependent action of RSD1000 arises when the rate-limiting step of the binding process may be the increased energy barrier height of the channel pore, i.e., increased electric field, such that at higher potentials more channels are free and available to conduct. The independence of block at different potentials at pH 7.3 may be due to the neutral form of RSD1000 accessing its binding site via a hydrophobic route. These results may be consistent with those of use-dependent INa, blocking actions of RSD1000 at different pHs (Fig. 7), whereby a greater proportion of the charged form at pH 6.4 is present during the activation process. Thus, the pH-dependent action of RSD1000 is voltage-dependent inasmuch as the availability of the binding site is governed by an electric field from changing the membrane potential. Shifts of the INa voltage dependence by changes in ionic strength, divalent ion concentration, and pH, were recognized, therefore, further studies are required to fully elucidate the interaction of RSD1000 and the INa channel under different pHs.

The mixed blocking actions of RSD1000 on INa and Ito, as well as potentiated effects on INa in acid pH, may be sufficient to explain the selective antiarrhythmic activity of RSD1000 against ischemia-induced arrhythmias. Such a profile would presumably prevent ischemia-induced arrhythmias by virtue of preventing the action potential narrowing due to ischemia and at the same time potentiating the sodium current depressant actions of ischemia. Acting in concert, the actions of RSD1000 would prevent ischemic tissue from participating in re-entry circuits and thereby be antiarrhythmic. The potentiation of action potential modulation in the involved versus uninvolved zone is such that electrical heterogeneity between zones would be unlikely to occur or be reduced. This may explain how RSD1000 prevents ischemia-
induced arrhythmias at doses that have no discernible actions on the nonischemic tissue. The fact that the ED\textsubscript{50} dose for antiarrhythmic protection against ischemia-induced arrhythmias (8 \(\mu\)mol/kg/min) produced some effects on the ECG and on electrically induced arrhythmias does not argue against this, only that RSD1000 has a relative, rather than absolute, selectivity for ischemic tissue.

It would appear, then, that interactions of external H\textsuperscript{+} with protonatable agents possessing \(pK_{a}\)s that approximate external pH of ischemic myocardium may serve as one important determinant in the design of pathology-targeted antiarrhythmic agents. This method of drug design appears optimal when the drug \(pK_{a}\) is below physiological pH so as to limit the cationic drug form in non ischemic myocardium but sufficient for ionization in acid pH. Under these conditions, blockade of both sodium and potassium channels may prove more useful than inhibition of a single type of ion channel.

Acknowledgments

We thank Dr. R.A. Wall and Susan Doan for their chemical analysis of RSD1000. We also thank Eugene Lam for his technical support. We also express our gratitude to Nortran Pharmaceuticals Ltd/Rhythm Search Developments for their generous contribution of RSD1000.

References


