Electrophysiological and Antiarrhythmic Effects of the Atrial Selective 5-HT\textsubscript{4} Receptor Antagonist RS-100302 in Experimental Atrial Flutter and Fibrillation

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Background—Stimulation of 5-HT\textsubscript{4} receptors increases atrial chronotropic and inotropic responses. Whether other electrophysiological effects are produced is unknown. In humans and swine, 5-HT\textsubscript{4} receptors are present only in atrium. Therefore, the effects of a novel 5-HT\textsubscript{4} receptor antagonist, RS-100302, and the partial agonist cisapride on atrial flutter and fibrillation induced in swine were studied to delineate the role of the 5-HT\textsubscript{4} receptor in modulating atrial electrophysiological properties and the antiarrhythmic potential of RS-100302.

Methods and Results—In 17 anesthetized, open-chest, juvenile pigs, atrial flutter or fibrillation was induced by rapid right atrial pacing with or without a right atrial free wall crush injury, respectively. Atrial effective refractory period (ERP), conduction velocity, wavelength, and dispersion of refractoriness were determined during programmed stimulation via a 56-electrode mapping plaque sutured to the right atrial free wall. Ventricular electrophysiological parameters were also measured. All electrophysiological parameters were measured at baseline and after infusion of RS-100302 and cisapride. In the atrium, RS-100302 prolonged mean ERP (115±8 versus 146±7 ms, \(P<0.01\)) and wavelength (8.3±0.9 versus 9.9±0.8 cm, \(P<0.01\)), reduced dispersion of ERP (15±5 versus 8±1 ms, \(P<0.01\)), and minimally slowed conduction velocity (72±4 versus 67±5 cm/s, \(P<0.01\)). These effects were all partially reversed by cisapride. RS-100302 produced no ventricular electrophysiological effects. RS-100302 terminated atrial flutter in 6 of 8 animals and atrial fibrillation in 8 of 9 animals and prevented reinduction of sustained tachycardia in all animals.

Conclusions—The electrophysiological profile of RS-100302 suggests that it may have atrial antiarrhythmic potential without producing ventricular proarrhythmic effects. \(\textit{Circulation}. 1999;100:2010-2017.\)

Key Words: fibrillation ■ atrial flutter ■ serotonin ■ electrophysiology

Atrial fibrillation (AF) is a common, frequently refractory arrhythmia associated with significant morbidity and mortality.\(^1\) AF is a reentrant arrhythmia and is more likely to occur in the presence of an abnormally shortened atrial effective refractory period (ERP) and increased dispersion of ERP (ERP\textsubscript{disp}).\(^2,3,5\) In addition, abnormally depressed conduction velocity (CV) and anatomic obstacles may play a role in the reentrant mechanism of AF.\(^7\) Experimental studies have suggested that prolongation of atrial wavelength and a reduction in ERP\textsubscript{disp} may be critical determinants of the efficacy of antiarrhythmic drugs in terminating and suppressing reentrant atrial arrhythmias.\(^6-9\) Both of these salutary electrophysiological effects are produced by class III antiarrhythmic drugs, such as sotalol, but not by class I antiarrhythmic drugs, such as quinidine.\(^6,7\) Despite their favorable electrophysiological profile, however, the class III drugs are not more effective than the class I drugs in suppressing AF in humans, with only 50% to 65% of patients in sinus rhythm after 6 months of therapy.\(^10-13\) In addition, the organ toxicity and potential life-threatening ventricular proarrhythmia associated with antiarrhythmic drugs further limit their use for treating AF.\(^14\)

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Because of the limited efficacy and potential adverse effects of antiarrhythmic drugs that modulate cardiac ion channels, new approaches to antiarrhythmic drug therapy must be developed. One possible approach is the modulation of membrane receptors that play a role in controlling normal cellular electrophysiology. One such receptor, which when stimulated causes increased heart rate in humans, is the 5-hydroxytryptamine, or 5-HT, receptor.\(^15-18\) Extensive research into the cardiac effects of 5-HT has revealed the existence of the 5-HT\textsubscript{1} receptor subtype in human atrium.\(^19,20\) Stimulation of the 5-HT\textsubscript{4} receptor produces positive chronotropic effects and increases cAMP levels, cAMP-dependent protein kinase activity and inotropic force, and onset of muscle relaxation.\(^19,20\) Furthermore, stimulation of 5-HT\textsubscript{4} receptors induces arrhythmic contractions in atrial myocardial tissues.\(^19,20\)
um that are inhibited by selective 5-HT$_4$ antagonists.$^{21-24}$ Similar arrhythmic contractions have been demonstrated with stimulation of atrial $\beta_1$ and $\beta_2$-receptors.$^{25}$ However, it is unknown whether 5-HT$_4$ receptor stimulation, or conversely, its blockade, produces any other electrophysiological effect in the atrium.

Of particular importance is the observation that the 5-HT$_4$ receptor subtype is present in human atrium, but not in the ventricle.$^{26}$ The lack of 5-HT$_4$ receptors in human ventricle suggests that their pharmacological modulation may not cause ventricular proarrhythmia, provided that there is no direct electrophysiological effect of such a pharmacological agent. In contrast, ketanserin, a 5-HT$_2$ receptor subtype blocker, prolongs ventricular action potential duration and may cause ventricular proarrhythmia (ie, torsade de pointes), because this receptor is found in both atrium and ventricle.$^{27}$ Therefore, the electrophysiological and antiarrhythmic effects of the 5-HT$_4$ antagonist RS-100302$^{28}$ and the partial agonist cisapride were studied in pigs, which, like humans, have 5-HT$_4$ receptor activity confined to the atrium$^{29}$ during atrial flutter (AFL) (crush injury) or AF induced by rapid atrial pacing.$^{6-8,30,31}$

**Methods**

Seventeen juvenile pigs weighing 15 to 20 kg were studied after sedation with intramuscular ketamine and salazine and general anesthesia with intravenous pentobarbital 30 to 35 mg/kg bolus plus 0.05 mg·kg$^{-1}$·min$^{-1}$ maintenance infusion. The pigs were intubated and ventilated mechanically with room air. A femoral vein and artery were cannulated via direct cutdown for intravenous drug administration and arterial pressure monitoring, respectively. Ringer’s lactate was infused continuously at $\approx30$ mL/h. The surface ECG was monitored continuously during the study. Body temperature was maintained by a warm-water–circulating pump. A median sternotomy was performed, and the heart was exposed by opening of the pericardium.

**Induction of Arrhythmia**

Bipolar hook electrodes (interelectrode spacing 2 mm) were attached to the right atrial appendage (Figure 1). Ten attempts were made to induce AF (Figure 2) by rapid atrial pacing for 60 seconds at a cycle length of $\approx200$ ms to atrial refractoriness, and if sustained $\geq$10 minutes, AF was studied.$^6$ If sustained AF was not inducible, a crush injury was performed on the atrial free wall (Figure 1) as previously described.$^7$ Attempts were then made to induce AFL (Figure 3) with rapid atrial pacing in a manner identical to that for AF, and if sustained $\geq$10 minutes, AFL was studied. Sustained AF or AFL was then terminated by pacing or DC cardioversion, and baseline electrophysiological measurements were performed (see below).

**Electrophysiological Measurements and Mapping**

A 56-electrode mapping plaque (Figure 1), measuring 3.5×2.5 cm with 3- to 5-mm interelectrode and 2-mm intraelectrode spacing, was sewn in place over the right atrial free wall.$^6,7$ Pacing was performed from the mapping plaque for electrophysiological measurements. A 64-channel mapping system (Bard Electrophysiology, Inc) was used for activation mapping during AFL or AF. Activation maps were also obtained during atrial pacing from 4 electrodes, 1 at each corner of the mapping plaque, at cycle lengths of 200 and 150 ms to determine atrial CV. Right atrial ERP was determined at all 56 electrodes on the mapping plaque by decremental stimuli (S$_1$S$_2$) scanning diastole at 10-ms intervals at a drive cycle length (S$_1$S$_1$) of 200 ms. Right and left ventricular ERPs were similarly determined at drive cycle lengths of 400 and 300 ms via bipolar hook electrodes on the ventricular epicardium. Sinus cycle length (RR interval), QTc (QTC=QT/7214 RR interval), and ventricular CV (estimated by
measurement of QRS duration) were determined during sinus rhythm. After completion of baseline electrophysiological measurements, AFL or AF was reinduced and allowed to sustain for 10 minutes. Experimental study drugs were then administered.

**Drug Studies**

After completion of baseline electrophysiological measurements and induction of sustained AFL or AF, the 5-HT₁ receptor antagonist RS-100302 (Roche Bioscience, Inc) was administered in a dose of 30 μg/kg over 10 minutes. This dose of RS-100302 was previously determined to maximally inhibit 5-HT–induced tachycardia in pigs (unpublished data, Roche Bioscience, Inc). After completion of drug infusion, each animal was monitored for 20 minutes to observe arrhythmia response. If AFL or AF did not terminate spontaneously within 20 minutes after completion of drug infusion, sinus rhythm was restored by burst pacing or DC cardioversion, respectively.
Electrophysiological measurements were then repeated as during control, and a venous blood sample was drawn for plasma levels of RS-100302. Attempts were then made to reinduce AFL or AF as performed at control. Reinduced arrhythmias were defined as sustained if lasting >10 minutes, nonsustained if lasting >30 seconds but <10 minutes, or none if lasting <30 seconds. Sustained arrhythmias were terminated after 10 minutes by burst pacing or DC cardioversion. Cisapride was then administered at a dose of 0.1 mg/kg over 10 minutes, after which electrophysiological measurements were repeated. Attempts were again made to reinduce AFL or AF, and the same definitions as those used after RS-100302 infusion regarding sustained, nonsustained, or no arrhythmias were applied. The study was then terminated, and animals were euthanized by an overdose of pentobarbital (150 mg/kg).

Data Analysis
All electrophysiological parameters, including RR, QTc and QRS intervals; minimum, maximum, and average atrial ERP at all 56 electrodes; atrial CV; wavelength; ERP<sub>disp</sub> and interelectrode dispersion (IE disp); and AFL and AF cycle length (AFLCL and AFCL) are presented as mean±SD for all animals for control and drug conditions. ERP<sub>disp</sub> was defined as the SD of the ERP determined at all 56 electrodes in each animal. IE disp was defined as the number of electrode pairs on the mapping plaque with an ERP difference of ≥20 ms between adjacent electrodes. During pacing from each corner of the mapping plaque at 200- and 150-ms drive cycle length, an effective CV (cm/s) across the mapping plaque was calculated by dividing the length of the mapping plaque (3.5 cm) by the activation time (in seconds) across the mapping plaque. The average CV for each animal was then calculated from the effective CV measured in both directions along the upper and lower halves of the mapping plaque during pacing at each of these 4 electrodes. Wavelength was calculated by multiplying mean ERP by average CV in each animal. Mean AFLCL and AFCL were calculated for each animal by counting the number of local atrial activations during 8 seconds of rhythm at 1 electrode on the mapping plaque with discrete, nonfragmented electrograms. AFLCL and AFCL were determined at control and just before termination of sustained or nonsustained arrhythmia after both drug infusions.

Statistical Analysis
The statistical significances of differences in ERP, CV, wavelength, ERP<sub>disp</sub>, IE<sub>disp</sub>, AFLCL, AFCL, sinus cycle length (RR), QRS, and QTc between baseline and after RS-100302 and cisapride administration were calculated by 1-way repeated-measures ANOVA with Bonferroni’s correction for pairwise multiple comparisons when the normality test failed or Student-Newman-Keuls correction when the

| Table 1: Atrial Electrophysiological Effects of RS-100302 and Cisapride |
|-----------------|-----------------|-----------------|
| Baseline (n=17) | RS-100302 (n=17) | Cisapride (n=17) |
| AERP<sub>200ms</sub> (mean) | 115±8 | 146±7* | 130±5† |
| AERP<sub>200ms</sub> (max) | 139±10 | 161±7* | 148±7† |
| AERP<sub>200ms</sub> (min) | 81±16 | 128±9* | 108±11† |
| ERP<sub>Disp</sub> | 15±5 | 8±1* | 11±3† |
| IE<sub>disp</sub> | 30±10 | 7±3* | 17±5† |
| CV<sub>200ms</sub> | 72±4 | 67±5* | 70±5† |
| CV<sub>150ms</sub> | 62±7 | 55±7* | 58±7† |
| WLA<sub>200ms</sub> | 8.3±0.9 | 9.9±0.8* | 9.1±0.7† |
| AFLCL | 134±15 | 161±22* | 150±17† |
| AFCL | 99±13 | 142±9* | 129±9† |

AERP indicates atrial ERP; CV<sub>200</sub>, CV<sub>150</sub>, conduction velocity at 200 and 150 msec pacing cycle length; and WL, wavelength.

*P<0.01 for RS-100302 and cisapride vs control; †P<0.01 for cisapride vs RS-100302.
normality test passed. For all tests, a value of \( P < 0.05 \) was considered to be statistically significant.

**Results**

**Atrial Electrophysiological Effects of RS-100302**

RS-100302 significantly prolonged mean ERP from 115±8 to 146±7 ms (\( P < 0.01 \)), maximum ERP from 139±10 to 161±7 ms (\( P < 0.01 \)), and minimum ERP from 81±16 to 128±9 ms (\( P < 0.01 \)) (Table 1). Average CV at pacing cycle lengths of 200 and 150 ms decreased slightly, but significantly, after RS-100302 infusion, from 72±4 to 67±5 cm/s (\( P < 0.01 \)) and from 62±7 to 55±7 cm/s (\( P < 0.01 \)), respectively. RS-100302 prolonged atrial wavelength from 8.3±0.9 to 9.9±0.8 cm (\( P < 0.01 \)). RS-100302 reduced ERP disp (Figure 4) from 15±5 to 8±1 ms (\( P < 0.01 \)) and IE disp from 30±10 to 7±3 ms (\( P < 0.01 \)).

**Reversal of Atrial Electrophysiological Effects of RS-100302 by Cisapride**

Cisapride shortened mean ERP from 146±7 to 130±5 ms (\( P < 0.01 \)), maximum ERP from 161±7 to 148±7 ms (\( P < 0.01 \)), and minimum ERP from 128±9 to 108±11 ms (\( P < 0.01 \)) (Table 1). Average CV at pacing cycle lengths of 200 and 150 ms increased slightly but significantly after cisapride infusion, from 67±5 to 70±5 cm/s (\( P < 0.01 \)) and from 55±7 to 59±7 cm/s (\( P < 0.01 \)), respectively. Cisapride shortened wavelength from 9.9±0.8 to 9.1±0.7 cm (\( P < 0.01 \)). Cisapride increased ERP disp (Figure 4) from 8±1 to 11±3 ms (\( P < 0.01 \)) and IE disp from 7±3 to 17±5 ms (\( P < 0.01 \)).

**Effects of RS-100302 and Cisapride on ECG and Ventricular Electrophysiological Parameters**

There were no significant effects of either RS-100302 or cisapride infusion on sinus cycle length, QRS duration, QTc.
TABLE 1. Effects of RS-100302 and Cisapride on AFL and AF

<table>
<thead>
<tr>
<th></th>
<th>RS-100302 (n=17)</th>
<th>Cisapride (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCL (RR)</td>
<td>560±98</td>
<td>596±112</td>
</tr>
<tr>
<td>QRS</td>
<td>602±103</td>
<td>53±9</td>
</tr>
<tr>
<td>QTc</td>
<td>241±29</td>
<td>491±105</td>
</tr>
<tr>
<td>LVERP400 ms</td>
<td>182±21</td>
<td>184±19</td>
</tr>
<tr>
<td>RVERP400 ms</td>
<td>244±29</td>
<td>246±27</td>
</tr>
<tr>
<td>RVERP300 ms</td>
<td>185±22</td>
<td>187±20</td>
</tr>
<tr>
<td>SBP</td>
<td>119±7</td>
<td>118±7</td>
</tr>
<tr>
<td>DBP</td>
<td>73±9</td>
<td>71±8</td>
</tr>
</tbody>
</table>

SCL indicates sinus cycle length; QRS, QRS duration; QTc, QT corrected by Bazett’s formula; LVERP400 and RVERP400 and 300; left and right ventricular refractory periods, respectively, at S1, pacing cycle lengths of 400 and 300 ms; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

TABLE 2. ECG and Ventricular Electrophysiological Effects of RS-100302 and Cisapride

<table>
<thead>
<tr>
<th></th>
<th>RS-100302 (n=17)</th>
<th>Cisapride (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Terminated</td>
<td>Reinduced</td>
</tr>
<tr>
<td>AF</td>
<td>8 (S)</td>
<td>6</td>
</tr>
<tr>
<td>AF</td>
<td>9 (S)</td>
<td>8</td>
</tr>
</tbody>
</table>

S indicates sustained (>10 minutes of arrhythmia); NS, nonsustained (>30 seconds, <10 minutes of arrhythmia); and NO, none (<30 seconds of arrhythmia).

or left and right ventricular ERP at either 400- or 300-ms paced cycle lengths (Table 2). The systolic and diastolic blood pressure decreased slightly, but significantly, after infusion of cisapride but not RS-100302.

Effects of RS-100302 and Cisapride on AFL and AF

RS-100302 significantly increased AFLCL, from 134±6 to 151±22 ms (P<0.01) and AFCL, from 99±13 to 142±9 ms (P<0.01), respectively (Table 1). Cisapride partially reversed AFCL from 161±22 to 150±17 ms (P<0.01) and AFLCL, from 142±9 to 129±9 ms (P<0.01), respectively.

At baseline, 8 pigs had inducible sustained AFL and 9 had AF (Table 3, Figures 2 and 3). During or after infusion of RS-100302, AFL terminated in 6 of 8 pigs, whereas AF terminated in 8 of 9 pigs. After infusion of RS-100302, AFL was no longer inducible in 5 of 8 pigs, whereas nonsustained AFL was reinducible in 3. AF was no longer inducible in 6 of 9 pigs, whereas nonsustained AF was reinducible in 3. After infusion of cisapride, nonsustained AFL was reinducible in 6 of 8 pigs, and there was no AFL in 2 pigs. Nonsustained AF was reinducible in 6 of 9 pigs, and there was no AF in 3 pigs.

Discussion

Electrophysiological and Antiarrhythmic Effects of RS-100302

The 5-HT4 receptor antagonist RS-100302 produced significant electrophysiological and antiarrhythmic effects in pacing-induced AFL and AF in pigs. These electrophysiological and antiarrhythmic effects were comparable to those produced by class III antiarrhythmic drugs such as dofetilide and d-sotalol in similar arrhythmia models in the dog.6,9 For example, RS-100302 prolonged average ERP by 27%, whereas CV was slowed by only 7% at a pacing cycle length of 200 ms, thus producing a 19% increase in wavelength. RS-100302 also reduced ERP at 47% and IE at 77%. These electrophysiological effects resulted in termination of AFL in 75% of animals and AF in 89% of animals and prevented reinduction of sustained AFL or AF in all animals. However, RS-100302 had no effect on sinus or AV node function or any ventricular electrophysiological or hemodynamic effects. Cisapride, which is known to be a partial agonist of the 5-HT4 receptor, incompletely reversed all of the electrophysiological effects of RS-100302, confirming the role of the 5-HT4 receptor in mediating the action of RS-100302.

Possible Mechanism of the Electrophysiological Effects of RS-100302

Whereas the class III antiarrhythmic drugs produce significant electrophysiological effects by modulating potassium ion channels, such as ICaL,32 RS-100302 produces electrophysiological effects via the novel mechanism of 5-HT4 receptor blockade. The exact mechanism by which 5-HT4 receptor modulation produces electrophysiological effects is unknown, but previous studies suggest 1 possibility. Stimulation of the 5-HT4 receptor increases L-type Ca2+ channel current in human atrial myocytes via a cAMP-dependent protein kinase.33 The resultant increase in intracellular Ca2+ may cause further release of Ca2+ from sarcoplasmic reticulum.34,35 In response to increased intracellular Ca2+, the delayed rectifier potassium current, IK, may be augmented via a calmodulin-dependent pathway.36 Thus, 5-HT4 receptor stimulation could theoretically cause atrial arrhythmias, including delayed afterdepolarizations, as a result of increased intracellular Ca2+ concentration or even AF as a result of shortening of atrial refractory period due to increased delayed rectifier potassium current.33–36 Conversely, 5-HT4 receptor blockade could have antiarrhythmic effects by decreasing intracellular Ca2+ concentration and delayed rectifier potassium current and prolonging atrial refractory period.33–36 Our data are consistent with such a mechanism, in that blockade of the 5-HT4 receptor by RS-100302 was associated with prolongation of atrial ERP and atrial arrhythmia effects.

The observation that RS-100302 produced significant electrophysiological effects suggests that the 5-HT4 receptor must have significant resting tone in vivo. The mechanism by which such resting tone might exist is unknown, but studies comparing atrial myocardium from patients with and without prolonged exposure to β-blockers demonstrated that exposure to β-blockers caused 5-HT4 receptor–mediated hyperresponsiveness to 5-HT.24,37 This β-blocker–induced hyperresponsiveness to 5-HT4 receptor stimulation has been shown to increase both maximum inotropic force and cAMP levels in atrial myocytes.37 These findings suggest that there may be intracellular cross talk between receptor populations within individual cells.24 Although the pigs in this study did not
receive β-blockers, it is possible that sufficient resting 5-HT1 receptor tone was present, as a result of stimulation by local or circulating levels of 5-HT, such that RS-100302 produced significant electrophysiological effects.

Potential Mechanisms of Antiarrhythmic Action of RS-100302
The electrophysiological determinants of termination and prevention of sustained AFL and AF by RS-100302 in the pig appear to be similar to those of the class III antiarrhythmic drugs dofetilide and sotalol in similar arrhythmia models in the dog. Specifically, the antiarrhythmic effects of RS-100302 were associated with prolongation of atrial ERP and wavelength and reduction in dispersion of refactoriness, electrophysiological effects that were partially reversed by cisapride. However, it is unknown whether the antiarrhythmic effects of RS-100302 were solely due to its class III-like electrophysiological effects or whether other effects of the compound played a role. For example, Ca2+ channel blockers have been shown to reduce the frequency of AF recurrence after cardioversion, possibly by preventing the shortening of atrial ERP or electrical remodeling that occurs during AFL or AF as a result of increased intracellular calcium concentration. Thus, the antiarrhythmic effects of RS-100302 might be due in part to a reduction in intracellular Ca2+ loading during the rapid rates associated with AFL or fibrillation and in part to prolongation of atrial repolarization via an indirect effect on ER.

Clinical Implications of the Study
The data from this study support previous speculation that 5-HT1 receptors may play a role in atrial arrhythmogenesis and that 5-HT1 receptor antagonists may have atrial antiarrhythmic effects without ventricular proarrhythmic effects.15,24,39

Study Limitations
Several aspects of the methods may limit conclusions regarding the mechanisms of antiarrhythmic action of RS-100302. First, the mapping plaque did not cover all atrial surfaces. Thus, the electrophysiological characteristics in other areas of the atria were not determined, although the effects of RS-100302 and cisapride are not likely to have been different. Second, although epicardial measurement of CV has limitations owing to the 3D nature of the heart, this is not as problematic in the thin-walled atrium as in the ventricle. Last, thoracotomy and suturing of the mapping plaque to the atrium might have caused serotonin release and increased background 5-HT1 receptor stimulation. This potentially could have shortened baseline atrial ERP and increased ERPdisp, such that infusion of a 5-HT1 receptor blocker could then have produced a significant change compared with these baseline values. Whether a significant degree of resting tone of the 5-HT1 receptor exists at baseline without such surgery, in pigs or humans, is unknown and may deserve further study.

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References


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