electrophysiological effects of dronedarone (sr33589), a noniodinated benzofuran derivative, in the rabbit heart: comparison with amiodarone

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background—to overcome the side effects of amiodarone (am), its noniodinated analogue, dronedarone (sr), was synthesized. in this study, its electrophysiological effects were compared with those of am in rabbit hearts.

methods and results—five animal groups (n = 7 each) for 3 weeks received daily oral treatment of 1 of these regimens: (1) control, vehicle only; (2) am 50 mg/kg (am50); (3) am 100 mg/kg (am100); (4) sr 50 mg/kg (sr50); and (5) sr 100 mg/kg (sr100). eCGs were recorded before drug and at 3 weeks of drug before euthanasia. action potentials were recorded from isolated papillary muscle and sinoatrial node by microelectrode techniques. the short-term effects were studied in controls (n = 5) at various concentrations of sr (0 to 10 µmol/L) in tissue bath. action potential duration at 50% (APD50) and 90% (APD90) repolarization and upstroke dV/dt (Vmax) at various cycle lengths were compared by ANOVA with repeated measures. compared with control, am and sr increased RR, QT, and QTc intervals (P < 0.0001 for all). ventricular APD50 and APD90 were lengthened by 20% to 49% as a function of dose (P < 0.005 to < 0.0001) and cycle length (P < 0.001). SR100 effects were greater than those of AM100 (P < 0.002). Vmax was decreased by both AM100 (P < 0.0001) and SR100 (P < 0.01). sinoatrial node automaticity was slowed in treated groups compared with that of the control group (P < 0.0001 for all).

conclusions—the electrophysiological effects of dronedarone are similar to those of AM but more potent, despite deletion of iodine from its molecular structure, a finding of importance for the development of future class III antiarrhythmic compounds. (Circulation. 1999;100:2276-2281.)

key words: potentials • amiodarone • dronedarone • electrophysiology • drugs

amiodarone (AM) has now emerged as an unusually effective antiarrhythmic agent for controlling ventricular and supraventricular tachyarrhythmias.1 the fact that it might act by prolonging the myocardial action potential duration (APD) after long-term administration was initially suggested in 1970.2 subsequently, it was found that beyond this class III action by inhibiting potassium channels,3,4 the drug exhibited all other known classes of antiarrhythmic mechanisms described by Singh et al.2,5,6 these include antiadrenergic activity and inhibition of fast sodium and slow calcium channels.6 which of these effects might be responsible for the unique clinical antiarrhythmic and low proarrhythmic potentials of the drug remains unclear. although the role of AM now is well entrenched in clinical practice, its side-effect profile remains of concern.7

AM is an iodinated compound. Its major toxicity profile after drug ingestion as a function of time might be due to iodine.1 the development of ocular and serious pulmonary toxicity7 or thyroid dysfunction8,9 has been attributed to the iodinated nature of the molecule.8 however, iodine as an integral component of the AM molecule might have other consequences.10 Singh and vaughan williams2 found that the ventricular APD prolongation in rabbits treated long-term with AM was abolished by administration of thyroxine. there is evidence that the effect of AM might be due in part to cardioselective inhibition of thyroid hormone action in cardiac muscle.11–13 the question arose as to whether the unique long-term electrophysiological effects of AM might stem from its molecular interaction with thyroid hormone receptors independently of iodine in the compound. the development of the noniodinated benzofuran derivative SR33589 (SR), or dronedarone (Sanofi-cherche), structurally related to AM (Figure 1), provided the opportunity to examine this possibility.

The short-term effects of SR are similar to those of AM. In anesthetized animals,14 SR inhibited ischemia-induced arrhythmias, reduced heart rate, and exerted sympatholytic effects characteristic of AM.15 the present study compares the cellular electrophysiological and ECG actions of SR and AM after 3 weeks of oral administration. the short-term
Effects after superfusion with SR in papillary muscles of untreated animals were also examined.

Methods

Long-Term Studies

New Zealand White rabbits of either sex weighing 1.9 to 2.2 kg were used. Long-term studies were conducted in 5 separate groups, 7 animals per group. Each group was treated orally daily for 3 weeks with 1 of the following regimens: (1) vehicle only, consisting of 7 mL of 75% polyethylene glycol (MW 400) (control group); (2) SR at 50 mg/kg (SR50 group); (3) SR at 100 mg/kg (SR100 group); (4) AM at 50 mg/kg (AM50 group); and (5) AM at 100 mg/kg (AM100 group). The drugs were administered by gavage in a solution prepared fresh every day. Each dose of SR or AM was dissolved in 5.25 mL of 75% polyethylene glycol (MW 400) diluted to 7.0 mL with distilled water before administration. ECGs recorded from conscious restrained rabbits were stored in digitized form. The QTc was obtained by Bazett’s formula. After completion of treatment, the rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV), and hearts were rapidly removed and dissected in cold oxygenated Tyrode’s solution. Tissue blocks (2x3 mm) from the middle part of the sinoatrial (SA) node region and the papillary muscles (0.4 to 0.6 mm in diameter and 3 to 4 mm long) from right ventricle were mounted in a tissue bath (10 mL volume) and superfused with Tyrode’s solution (15 mL/min) at 37°C. Its composition (in mmol/L) was as follows: NaCl 130, KCl 4.0, CaCl2 1.8, MgSO4 0.5, NaH2PO4 1.8, NaHCO3 18.0, and dextrose 5.5. It was bubbled with 95% O2 and 5% CO2, with pH maintained at 7.40±0.02. SA node preparations were administered to be allowed to beat spontaneously, whereas papillary muscles were electrically stimulated through bipolar electrodes at 1 Hz. Standard microelectrode techniques (glass capillaries filled with 3 mol/L KCl, tip resistance 10 to 20 MΩ) were used for recording of membrane action potentials. The electrode was connected by Ag-AgCl wire to a high-input impedance amplifier (Warner E-201). Signals were amplified and displayed on an oscilloscope (Tektronics 2201). The maximum slope of action potential upstroke (Vmax) was obtained by electronic differentiation. The resting membrane potential, action potential amplitude, Vmax, and APD at 50% and 90% repolarization (APD50 and APD90, respectively) were measured from the papillary muscles. Maximal diastolic potential, spontaneous cycle length, and Vmax were measured from the SA node. Frequency-dependent effects of SR and AM in the papillary muscles were evaluated at cycle lengths of 1200, 900, 600, and 300 ms. Action potential recordings were obtained after 5 minutes of steady stimulation at each cycle length. Data were digitized and stored on a computer with pClamp software (Axon Instruments).

Data Analysis

The data are presented as mean±SD. The intergroup comparisons of the cycle length–dependent effects on APD50, APD90, and Vmax in papillary muscles were made by ANOVA with repeated measures, with cycle length as the within factor and the treatment as the grouping factor. By use of this analysis, the effects of treatment, the effects of cycle length, and the interaction between treatment and cycle length were evaluated simultaneously. All other parameters, including the ECG and the SA nodal parameters, were evaluated by 1-way ANOVA. If ANOVA indicated significant differences among the groups, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons. BMDP biomedical statistical software was used (SPSS Inc).

Results

Long-Term Studies

Whole-Animal Data

All animals remained active during treatment and gained weight, an average of 0.59±0.18 kg. There were no significant differences in ECG parameters or RR, PQ, QT, or QRS intervals among the groups before treatment. There was a significant prolongation of RR, QT, and QTc intervals in all drug-treated groups compared with control (Table 1). However, the dose-related and drug-specific changes in the RR, QT, or QTc intervals among the treated groups did not attain statistical significance.

Effects on Ventricular Action Potential Characteristics

The mean values of the parameters measured from the papillary muscles are summarized in Table 2. Representative traces of action potentials at various cycle lengths for control, SR100, and AM100 groups are presented in Figure 2. The mean data on APD50 and APD90 for all groups are plotted against cycle length in Figure 3. Both APD50 and APD90 were prolonged significantly, by 31% to 56% and 28% to 47%, respectively, in the drug-treated groups compared with control (P<0.0001). The patterns of cycle length versus APD curves shown in Figure 3 were significantly different (ie, significant interaction between treatment and cycle length) between treatment groups and control (P<0.001). The effects of drug treatment were significantly cycle-length–dependent in all treated groups. The slopes of the APD50 and APD90 plots against the cycle length of treated groups were not significantly different. The APD50 and APD90 of the SR100 group were significantly more prolonged than those in the AM100
group \((P<0.002)\). At the lower dose, there was a significantly greater prolongation only in the APD\(_{50}\) of the SR-50 group compared with that of the AM-50 group \((P<0.03)\). The prolongations in APD\(_{50}\) and APD\(_{90}\) were significantly dose-dependent for both drugs \((P<0.005 \text{ to } <0.0001)\). The effective refractory period \((ERP)\) measured at 900-ms cycle length was highly correlated with the APD\(_{90}\) across the treatment groups \((R=0.988; P<0.0001)\), with ERP at 84% of APD\(_{90}\). Therefore, ERP data were not analyzed separately.

When APD data were compared at the shortest cycle length (300 ms), the APD\(_{50}\) and APD\(_{90}\) of the AM-50 group were not significantly prolonged compared with control, whereas the APD of SR-50, SR-100, and AM-100 were significantly prolonged over control (Figure 3). The relative prolongation of APD over mean control values in the treated groups are presented in Figure 4. The percent prolongation of APD over control at 300 ms in the SR-100 group was significantly greater than that in the AM-100 group \((\text{APD}_{50}: 33.6\% \text{ versus } 18.1\% \text{ (both } P<0.0001)); \text{APD}_{90}: 27.0\% \text{ versus } 15.2\%; P<0.05)\). Thus, APD prolongation caused by SR was more prominent at shorter cycle lengths than that due to AM.

The \(V_{\text{max}}\) values of the papillary muscle preparations were significantly lower with the shortening of the cycle length in all groups \((P<0.0001)\). However, the relative differences among all groups, including the control group, were not significantly cycle-length–dependent (Figure 5). Significant reduction of \(V_{\text{max}}\) compared with that in the control was observed in SR-100 \((P<0.0001)\) as well as AM-100 groups \((P<0.01)\). The dose-dependent reduction of \(V_{\text{max}}\) was significant in the case of SR \((P<0.01)\), but not AM \((P=\text{NS})\).

### Effects of SR and AM Treatments on the SA Nodal Preparations

The mean data are summarized in Table 3. Representative traces of relevant action potential recordings are presented in Figure 6. There were no differences among the groups with respect to maximum diastolic potential, action potential amplitude, or \(V_{\text{max}}\) of the SA nodal preparations. However, the spontaneous cycle length was significantly prolonged in the treated groups compared with those in the control group \((P<0.0001 \text{ for all})\). Spontaneous cycle length was significantly more prolonged with SR than with the corresponding dose of AM \((P<0.0005)\) at both the lower \((50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) and higher \((100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) doses.

### Short-Term Studies

The results are summarized in Figure 7. In contrast to long-term studies, both APD\(_{50}\) (Figure 7A) and APD\(_{90}\) (Figure 7B) were shortened in a dose-dependent manner over the range of 1 to 10 \(\mu\text{mol/L}\) SR concentration and 300- to 1200-ms stimulation cycle lengths. However, consistent with the long-term study, \(V_{\text{max}}\) measured at a stimulation cycle length of 900 ms decreased in a dose-dependent manner over the entire range of concentrations (Figure 7C).

### Table 1. Effect of Long-Term Oral Administration of AM or SR on Rabbit Surface ECG Parameters in the Conscious State

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RR, ms</th>
<th>PQ, ms</th>
<th>QRS, ms</th>
<th>QT, ms</th>
<th>QTc, ms</th>
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<tr>
<td>Vehicle</td>
<td>235±20</td>
<td>57±7</td>
<td>43±4</td>
<td>140±9</td>
<td>289±15</td>
</tr>
<tr>
<td>AM, mg · kg(^{-1}) · d(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50</td>
<td>283±18*</td>
<td>59±5</td>
<td>46±4</td>
<td>176±9*</td>
<td>330±17*</td>
</tr>
<tr>
<td>100</td>
<td>285±18*</td>
<td>58±7</td>
<td>47±4</td>
<td>181±12*</td>
<td>338±11*</td>
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<td>SR, mg · kg(^{-1}) · d(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>286±17*</td>
<td>60±6</td>
<td>47±4</td>
<td>177±10*</td>
<td>331±14*</td>
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<tr>
<td>100</td>
<td>288±20*</td>
<td>59±5</td>
<td>47±4</td>
<td>183±9*</td>
<td>340±11*</td>
</tr>
</tbody>
</table>

Values are mean±SD, \(n=7\) for each group. Drugs were administered for 3 weeks.

\*\(P<0.0001\) vs vehicle group.

### Table 2. Effects of Long-Term Oral AM or SR on Transmembrane Action Potentials of Rabbit Papillary Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RP, mV</th>
<th>APA, mV</th>
<th>(V_{\text{max}}, \text{V/s})</th>
<th>APD(_{50}), mV</th>
<th>APD(_{90}), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>−85±5</td>
<td>101±5</td>
<td>210±20</td>
<td>103±13</td>
<td>130±15</td>
</tr>
<tr>
<td>AM, mg · kg(^{-1}) · d(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−85±4</td>
<td>101±4</td>
<td>200±21</td>
<td>124±24†</td>
<td>155±27†</td>
</tr>
<tr>
<td>100</td>
<td>−85±6</td>
<td>101±5</td>
<td>191±21†</td>
<td>138±27†</td>
<td>172±29†</td>
</tr>
<tr>
<td>SR, mg · kg(^{-1}) · d(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−85±5</td>
<td>101±6</td>
<td>199±22*</td>
<td>133±23†</td>
<td>160±24†</td>
</tr>
<tr>
<td>100</td>
<td>−85±5</td>
<td>101±5</td>
<td>183±20†</td>
<td>154±25†</td>
<td>183±28†</td>
</tr>
</tbody>
</table>

RP indicates resting transmembrane potential; APA, action potential amplitude. The \(V_{\text{max}},\) APD\(_{50},\) and APD\(_{90}\) values were averaged over 4 cycle lengths \((1200, 900, 600, \text{ and } 300 \text{ ms})\). Values are mean±SD. \(n=7\) for each group. Drugs were administered for 3 weeks.

\*\(P<0.05\), †\(P<0.001\) vs vehicle group.
Major Findings
The main findings of the study indicate that despite the deletion of iodine from the molecule compared with that in AM, the major electrophysiological properties of SR are very similar to those of AM. During short-term superfusion, SR shortened the APD, as reported for AM, but reduced the ventricular \( V_{\text{max}} \). In contrast, after 3 weeks of oral administration of both drugs, there was significant slowing of the sinus frequency in vivo and in vitro associated with a significant prolongation of ventricular APD. In the sinus node, the rate slowing after long-term treatment was due to the depression of phase 4 depolarization and lengthening of the APD. Both SR and AM produced comparable degrees of depression of \( V_{\text{max}} \) as an index of inhibition of the ventricular myocardial sodium channel activity. Thus, the overall data show that SR is at least as potent as AM in its ability to alter the electrophysiological properties of ventricular muscle and those of the sinus node.

Frequency-Dependent Electrophysiological Effects
It is well known that the APD-lengthening effect of most class III antiarrhythmic drugs is reduced by increases in rate and duration of stimulation of cardiac muscle. Such an effect has been described as reverse rate- and use-dependency, in contrast to the increases in the effects of class I agents on blocking sodium channel function. Hondeghem and Snyder suggested that reverse use-dependency may be responsible for a high incidence of torsade de points associated with most class III antiarrhythmic agents. This is especially so in the case of those agents that exert their predominant repolarization-blocking effects by inhibiting the rapid component of the delayed rectifier K current, \( I_{\text{Kr}} \). In this regard, the long-term effects of AM differ from those of most other class III agents in inducing a negligible incidence of torsade de points, an effect that has been attributed to marked inhibition of the slow component of the delayed rectifier K current, \( I_{\text{Ks}} \). Whether SR might also act by a similar or identical action on the \( I_{\text{Ks}} \) is currently under study. However, our present study showed that SR and AM both prolonged APD\(_{50}\) and APD\(_{90}\) in a cycle length–dependent manner while exhibiting a minimal degree of reverse use-dependency. An unusual observation was that the percent prolongation at the shortest cycle length (300 ms) studied in our experiments was significantly greater with SR than that with AM at the higher drug dose of 100 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\) tested. Thus, under the conditions of our study, SR exhibited even less reverse use-dependency of repolarization than that found with AM, which has been shown to display minimal reverse use-dependency under in vivo conditions.

Discussion

Figure 2. Typical microelectrode recordings from rabbit papillary muscles after long-term treatment with vehicle, AM 100 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\), and SR 33589B. Preparations were stimulated at 4 cycle lengths: A=1200, B=900, C=600, and D=300 ms. Upper traces are transmembrane action potentials; lower traces, first derivative of upstroke (for \( V_{\text{max}} \)) of action potential.

Figure 3. Plots of mean APD\(_{50}\) (A) and APD\(_{90}\) (B) from rabbit papillary muscles at stimulation cycle lengths ranging from 300 to 1200 ms, after 3 weeks of treatment with test drugs. See text for details.

Figure 4. Plots of mean APD\(_{50}\) (A) and APD\(_{90}\) (B) from rabbit papillary muscles of treated groups expressed as percent prolongation over control values at each cycle length. SR-treated groups show marked relative prolongation at shorter cycle lengths, especially for APD\(_{90}\).

Figure 5. Effect of long-term treatment with AM (A) and SR (B) on \( V_{\text{max}} \) of rabbit papillary muscle.
TABLE 3. Effects of Long-Term Oral Administration of AM or SR on the Transmembrane Potential of Rabbit SA Nodal Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDP, mV</th>
<th>APA, mV</th>
<th>$V_{\text{max}}, \text{V/s}$</th>
<th>SCL, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>$-63\pm 6$</td>
<td>$73\pm 7$</td>
<td>$10\pm 3$</td>
<td>$425\pm 9$</td>
</tr>
<tr>
<td>AM, mg $\cdot$ kg$^{-1} \cdot$ d$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50</td>
<td>$-62\pm 7$</td>
<td>$74\pm 7$</td>
<td>$10\pm 3$</td>
<td>$461\pm 11^*$</td>
</tr>
<tr>
<td>100</td>
<td>$-62\pm 6$</td>
<td>$73\pm 7$</td>
<td>$10\pm 3$</td>
<td>$496\pm 13^\dagger$</td>
</tr>
<tr>
<td>SR, mg $\cdot$ kg$^{-1} \cdot$ d$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>$-62\pm 7$</td>
<td>$73\pm 7$</td>
<td>$10\pm 3$</td>
<td>$487\pm 16^\ddagger$</td>
</tr>
<tr>
<td>100</td>
<td>$-62\pm 9$</td>
<td>$74\pm 6$</td>
<td>$10\pm 3$</td>
<td>$548\pm 11^\ddagger\ddagger$</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential of SA nodal cells; APA, action potential amplitude; and SCL, spontaneous cycle length of the SA nodal preparation. Values are mean $\pm$ SD. n=7 for each group. Drugs were administered for 3 weeks.

$^*$Significantly prolonged vs control group (P<0.0001).
$^\dagger$Significantly prolonged vs the lower-dose group (P<0.001).
$^\ddagger$Significantly prolonged vs the corresponding dose of AM (P<0.005).

Significance of Blocking Myocardial Sodium Channels

In the present studies, the $V_{\text{max}}$ values of papillary muscle transmembrane action potentials were significantly reduced by both AM and SR, indicating inhibition of the fast Na channel. Whether such an additional property might contribute to the overall antiarrhythmic actions of these drugs remains uncertain. In AM, the associated class I antiarrhythmic effect is of moderate potency, but its rate-dependency has not been as compellingly uniform. In AM, the associated class I antiarrhythmic effect is of moderate potency, but its rate-dependency has not been as compellingly uniform. In AM, the associated class I antiarrhythmic effect is of moderate potency, but its rate-dependency has not been as compellingly uniform. In AM, the associated class I antiarrhythmic effect is of moderate potency, but its rate-dependency has not been as compellingly uniform. AM and its noniodinated derivative SR demonstrated here resemble those of hypothyroidism. In this regard, the effect of SR also interacts with $\beta$-adrenergic receptors of the rat heart at intracellular sites.

Potential Mechanisms of Heart Rate Slowing

Although the long-term in vivo effects of AM and SR in terms of increases in RR, QT, and QTc intervals showed trends similar to those of the in vitro data, the differences between the drugs did not attain statistical significance. Also, there were no significant differences between the 2 doses (50 and 100 mg $\cdot$ kg$^{-1} \cdot$ d$^{-1}$) tested, suggesting a saturation effect. However, our data did not address the issue of whether a more prolonged drug exposure might lead to further increases in the RR intervals. In the case of AM and SR, the slowing of the sinus rate might be attributable to the lengthening of the cardiac action potential. Our data in the present study demonstrating that the long-term effects of SR, a nonhalogenated benzofuran derivative, closely resemble those of hypothyroidism. Thus, in the clinical setting, SR therapy
may not have the same proclivity to induce altered thyroid state or the iodine-related complications seen with AM. Conversely, the similarity between the molecular structure of SR and thyroid hormone, as in the case of AM, does not exclude the possibility that the compound might exert its potentially beneficial electropharmacological effect on cardiac muscle by cardioselective blockade of T₃ receptors in cardiac muscle.

Conclusions
The results of this study demonstrate that the major short-term and long-term electrophysiological properties of the noniodinated derivative (SR) of AM on cardiac muscle are very similar to those of the parent compound. After 3 weeks of oral administration, AM and SR reduced sinus frequency in vivo and in vitro with a significant prolongation in the APD in the rabbit ventricular myocardium. This was accompanied by a corresponding increase in the ERP. Both SR and AM produced comparable degrees of depression of the V₉max as an index of inhibition of the myocaridial sodium channels. Thus, the overall data show that SR is at least as effective as AM in its ability to alter the electrophysiological properties of ventricular muscle and those of the sinus node. Its actions are not mediated by the presence of iodine, but the similarity between the molecular structure of SR and thyroid hormone, as in the case of AM, suggests the possibility that its beneficial effect may stem from other mechanisms, which may include cardioselective blockade of T₃ receptors in cardiac muscle.

Acknowledgments
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