Class III Antiarrhythmic Drug Action in Experimental Atrial Fibrillation

Differences in Reverse Use Dependence and Effectiveness Between d-Sotalol and the New Antiarrhythmic Drug Ambasilide

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Background Drug therapy to maintain sinus rhythm in patients with atrial fibrillation (AF) is limited by adverse effects and inadequate efficacy. There has been an increased interest in the use of class III drugs to treat AF, and several new agents have been developed, but there is little information available about mechanisms of class III drug action in AF. The present study was designed to compare the effects of two class III agents, d-sotalol and ambasilide, in dog models of experimental AF.

Methods and Results A previously developed dog model of sustained vagotonic AF was used to assess the ability of equal loading doses of d-sotalol and ambasilide (2 mg/kg, followed by maintenance infusions), to terminate AF and prevent its induction. At this dose, ambasilide terminated AF in 12 of 12 dogs and prevented AF induction in 10 of 12 dogs; d-sotalol terminated AF in 1 of 8 dogs (P=.001 versus ambasilide) and prevented AF induction in none of 8 dogs (P=.002). An additional dose of d-sotalol (cumulative load, 8 mg/kg) terminated AF in 7 of 8 dogs and prevented induction in 5 of 8 dogs. In an additional 6 dogs with sterile pericarditis and inducible AF, ambasilide prevented AF induction in all 6. An equal dose of d-sotalol (2 mg/kg) failed to suppress AF induction in any dog, but 8 mg/kg of d-sotalol suppressed AF induction in all.

A variety of antiarrhythmic drugs have been used to terminate atrial fibrillation (AF) and to maintain sinus rhythm after spontaneous or electrical cardioversion.1 Antiarrhythmic drug therapy to produce and maintain sinus rhythm is fraught with a variety of problems, including incomplete efficacy,2 proarrhythmic properties,3-5 and possibly an increased mortality rate.6,7 Since some of the more dangerous proarrhythmic potential of antiarrhythmic drugs appears to be related to sodium channel blocking properties,8-10 there has been increased interest in class III drugs, which act by increasing action potential duration (APD) without blocking sodium channels.

Sotalol, an agent with combined class II and class III properties,11,12 has been used both to terminate arrhythmia13 and prevent its occurrence14,15 among patients with AF. We have found that d,l-sotalol is capable of terminating AF and preventing its reinduction in an experimental dog model of AF but that reverse use-dependent actions on atrial refractoriness appear to limit efficacy of the drug.16 Since the latter experiments were conducted in dogs whose β-adrenergic receptors were blocked with nadolol, presumably only the class III effects of sotalol were manifest. Rensma et al17 showed that similar doses of d-sotalol prevent the induction of nonsustained AF in conscious dogs. To our knowledge, these are the only published studies of class III drug action in experimental atrial fibrillation.

Ambasilide is a relatively new class III agent18 that acts by blocking both the rapid and slow components of delayed rectifier current (I_K) in guinea pig ventricular myocytes.19 It thus differs from many other class III drugs such as sotalol, dofetilide, and E-4031, which act selectively on the rapid component (I_Kr).20 The molecular structures of ambasilide and d-sotalol are shown in Fig 1. The purpose of the present experiments was to evaluate the efficacy of ambasilide in a canine model of AF previously developed in our laboratory21,22 and to compare the electrophysiological and antiarrhythmic effects of ambasilide with those of a comparison class III agent, d-sotalol. We also examined the effects of am-
basilide and d-sotalol in an atrial fibrillation model that does not involve vagal nerve stimulation in order to exclude specific drug-vagal interaction effects that may occur in the vagotonic AF model.

Methods

Adult mongrel dogs of either sex were anesthetized with morphine (2 mg/kg IM) and a-chloralose (100 mg/kg IV) and ventilated with room air supplemented with oxygen. Respiratory parameters were adjusted to maintain physiological arterial blood gases (SaO2 more than 90%; pH 7.38 to 7.44). Catheters were inserted into the left femoral artery and both femoral veins and kept patent with heparinized saline solution (0.9%). A median sternotomy was performed, and a pericardial cradle was created. Body temperature was maintained at 37 to 39°C with a homeothermic heating blanket. Two bipolar Teflon-coated stainless steel electrodes were inserted into the right atrial appendage for recording and stimulation. A programmable stimulator (Digital Cardiovascular Instruments) was used to deliver 4-millisecond pulses at twice threshold current. A demand pacemaker (GBM 5880, Medtronic Inc) was used to pace the right ventricle when the ventricular rate was less than 90 min⁻¹. A P23 1D transducer (Statham Medical Instruments), electrophysiological amplifiers (Bloom Ltd), and a paper recorder (Astromed MT-95000) were used to record six standard surface ECG leads, an atrial electrogram, and stimulus artifacts. Nadolol was administered as an initial dose of 0.5 mg/kg IV, followed by 0.25 mg/kg every 2 hours, as in previous studies.\(^{16,21}\)

Atrial Fibrillation Model

Vagotonic AF was created as previously described\(^ {16,21}\) and studied in 20 dogs. The cervical vagal trunks were isolated and decentralized, and bipolar hook electrodes (stainless steel insulated with Teflon except for the terminal, 1 to 2 cm) were inserted through a 21-gauge needle into the middle of each nerve, with the electrode running parallel to vagal fibers for several centimeters.\(^ {22}\) Bilateral vagal nerve stimulation was delivered by a DS-9F stimulator (Grass Instruments Inc) with a pulse width of 0.1 millisecond and an applied voltage of 5 V. The stimulation frequency was adjusted in each dog to two thirds of the threshold for asystole under control conditions. In the presence of vagal stimulation, a short burst (1 to 3 seconds) of atrial pacing (10-Hz frequency, four times threshold current) induced AF. AF persisted in the presence of vagal stimulation for more than 30 minutes and terminated within seconds after stopping vagal stimulation. AF was defined as a rapid (more than 300 min⁻¹ under control conditions) irregular atrial rhythm with varying atrial electrogram morphology. To control for time-dependent changes in vagal actions, the vagal frequency-response relation was assessed before each AF induction, and the vagal stimulation frequency was adjusted to produce consistent sinus node slowing. A similar procedure was used to establish vagal stimulation parameters during maintenance drug infursion.

In an additional series of 6 dogs, a recently described model of AF associated with pericarditis\(^ {22}\) was studied. A right thoracotomy was performed under sterile conditions and pentobarbital anesthesia (30 mg/kg IV). The pericardium was incised, and the epicardial surfaces of the atria were dusted liberally with sterile talcum powder. The atria were then covered with sterile gauze pads, and the pericardial incision was sutured shut. The pneumothorax was evacuated, and the chest was closed in layers. Dogs were permitted to recover for 48 hours. During this time, they received intravenous antibiotics (benzathine penicillin G, 150 000 U and procaine penicillin G, 150 000 U SC daily) and levorphanol (0.056 mg/kg SC BID) as necessary for pain control. Two days after the initial procedure, the dogs were anesthetized with morphine and a-chloralose, and a right thoracotomy was performed. Atrial fibrillation was induced by a short burst of rapid atrial pacing and defined as described above for the vagotonic model.

Activation Mapping

Previously described mapping techniques were used to assess atrial conduction velocity at various frequencies and activation during AF.\(^ {16,21}\) In brief, five plastic sheets containing a total of 112 bipolar electrodes were sewn to the atria to cover the epicardial surfaces of both atria. Each signal was filtered (30 to 400 Hz), digitized at 1 kHz (12-bit resolution), and transmitted into a microcomputer for subsequent analysis. Maps were later constructed with the use of computer-derived peak amplitude criteria and visual review of all recordings.\(^ {23}\) The mean atrial cycle length during AF was determined as described previously,\(^ {16}\) on the basis of the number of atrial activation cycles over a 1-second interval at 16 widely dispersed atrial sites.

Assessment of Vagal Frequency-Response Relations

Possible antivagal drug effects were assessed on the basis of vagal frequency-response relations for sinus bradyarrhythmic actions before and after drug administration. Vagal stimulation voltage was kept constant, and the vagus nerves were stimulated for 30 seconds at frequencies ranging from 2 to 20 Hz, with a 30-second rest period between stimulations at each frequency. Heart rate was determined over the last 20 seconds of each stimulation period. Change in heart rate was plotted against vagal stimulation frequency, and stimulation frequency was adjusted during each maintenance infusion to produce the same effect on sinus rate as observed under control conditions.

Measurement of Electrophysiological Variables

Conduction velocity and refractory period were assessed after at least 2 minutes of constant pacing at basic cycle lengths between 200 and 400 milliseconds. The effective refractory period was measured with a train of 15 basic (S1) stimuli followed by a premature (S2) stimulus. The effective refractory period was defined as the longest S1-S2 interval failing to produce a propagated response. Activation maps during steady-state pacing were generated off-line after the experiment, and conduction time was determined between a site adjacent to the stimulating electrode and another site in the direction of rapid propagation. Interelectrode distance (in most experiments, 4.3 cm) was divided by conduction time to calculate conduction velocity. The same sites were used for conduction velocity measurements during control and drug infusion periods after ensuring a constant pattern of impulse propagation.

Experimental Design

Vagotonic Model

After conduction velocity and effective refractory period had been measured at all cycle lengths in the absence of vagal stimulation, AF was induced, and an 8-second window of
Electrogram data was obtained. Vagal stimulation was continued for 30 minutes to verify the stability of AF and then stopped to allow a return to sinus rhythm. Measurements of conduction velocity and effective refractory period were then obtained during vagal stimulation at all cycle lengths.

After the acquisition of baseline data, sustained AF was initiated during vagal stimulation as described above. Five minutes after the onset of AF, either ambasilide or d-sotalol was administered. Ambasilide was kindly provided by Knoll Pharmaceuticals, and d-sotalol was obtained from Bristol-Myers-Squibb Pharmaceuticals. The initial loading dose was 2 mg/kg, administered over 15 minutes, for each drug. This was followed 15 minutes later by a maintenance infusion of 4 mg·kg·hr⁻¹ for ambasilide, and 1 mg·kg·hr⁻¹ for d-sotalol. If AF was terminated, an 8-second window of activation data was acquired, with the trigger for data acquisition set to obtain at least 2 seconds of data before termination. If AF was not terminated within 30 minutes, vagal stimulation was stopped to restore sinus rhythm. AF reinduction then was attempted. The measurements of effective refractory period, conduction velocity (with and without vagal stimulation), and response to vagal stimulation were repeated in the presence of the drug. Before obtaining these measurements, vagal stimulation frequency was readjusted to produce the same sinus bradycardic effect as under control conditions in the absence of drug.

In contrast to ambasilide, which was found to be highly effective in terminating AF at a loading dose of 2 mg/kg, d-sotalol was relatively ineffective. Therefore, after all measurements had been obtained during the first maintenance dose of d-sotalol, AF was reinduced and an additional loading dose of 6 mg/kg was given, followed by a maintenance dose of 3 mg·kg·hr⁻¹. The same procedure and measurements described for the first dose of sotalol were then repeated. A total of 12 dogs received ambasilide, and 8 dogs received both doses of d-sotalol.

**Sterile Pericarditis Model**

A total of 6 dogs with sterile pericarditis were studied. Baseline electrophysiological data were obtained as described above for the vagotonic model. AF induction was then attempted with burst atrial pacing (10 to 20 Hz, four times threshold) a total of 10 times, and the duration of AF induced on each occasion was noted. Ambasilide was then administered, as described above, and electrophysiological variables and AF inducibility were reassessed during the maintenance infusion as under control conditions. Ambasilide infusion was then stopped, and AF induction was reattempted. When the inducibility of AF returned to baseline values, or after 2 hours (whichever was shorter), low-dose d-sotalol was given and measurements repeated during the maintenance dose. The higher dose of d-sotalol was then given, and measurements were once more repeated.

In two dogs, sustained atrial fibrillation (≥30 minutes) was induced under control conditions. Ambasilide was given after AF had been sustained for over 30 minutes. In one of these dogs, sustained AF could once more be induced after ambasilide washout, and the response of AF in this dog to the lower dose of d-sotalol was observed.

**Data Analysis**

Plasma concentrations of d-sotalol were measured by previously described high-performance liquid chromatography methods. To measure plasma concentrations of ambasilide, 0.5 mL of plasma was added to a test tube containing 0.5 μg of the internal standard (procainamide), 0.1 mL of 1N sodium hydroxide, and 2.5 mL of ethyl acetate. The upper solvent layer was removed and evaporated to dryness with nitrogen. The samples were reconstituted in 60 μL of mobile phase (45% phosphate buffer, 55% acetonitrile, octanesulfonic acid, and glacial acetic acid). Samples were then injected into a Spherisorb 5 μ ODS 2 column (Chromatography Sciences Co) and UV absorption at 268-nm wavelength detected with a spectrophotometer (model 450). The retention time was 3 minutes for the internal standard and 5 minutes for ambasilide. A three-point control calibration curve in blank dog plasma was obtained for each set of experimental samples, and the coefficient of variation averaged 0.94% and 1.84% at 8 and 2 μg/mL ambasilide, respectively.

Group values are presented as mean±SEM values. Comparisons between groups of data were performed by two-way ANOVA with Scheffe’s test, and comparisons between two means only were made by Student’s t test. Comparisons between drugs in terms of frequency of AF termination and prevention of AF induction were performed with a x² test. A two-tailed P<.05 was taken to indicate statistical significance. Wavelength (λ) was calculated with the formula of Wiener and Rosenblueth, according to the relation λ=effective refractory period×conduction velocity.

**Results**

**Dogs With Vagotonic AF**

Ambasilide (2 mg/kg loading dose) terminated AF (Table) in all 12 dogs studied (100% efficacy) and was much more effective than an equal dose of d-sotalol, which terminated AF in 1 of 8 dogs (12.5%, P=.001 versus ambasilide). The addition of a second dose of d-sotalol (8 mg/kg cumulative dose) resulted in AF termination in 7 of 8 dogs (87.5%). The mean plasma concentration of d-sotalol at the time of AF termination was substantially higher for dogs receiving both doses than the concentration of ambasilide required to terminate AF. Plasma concentrations of ambasilide and d-sotalol were kept relatively stable by the maintenance drug infusions (Fig 2). During the maintenance infusion, sustained (>30 seconds) AF could be induced in the presence of low-dose d-sotalol in all 8 dogs but in only 2 of 12 dogs (17%) receiving ambasilide (P=.002).

**Efficacy of Ambasilide and d-Sotalol in Terminating Vagotonic AF and Preventing Its Reinduction**

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. Terminated</th>
<th>Time, min</th>
<th>Conc, mg/L</th>
<th>Prevention of AF Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambasilide</td>
<td>5/5*</td>
<td>12.0±1.6</td>
<td>5.5±1.5</td>
<td>10/12†</td>
</tr>
<tr>
<td>d-Sotalol (2 mg/kg)</td>
<td>1/8</td>
<td>21</td>
<td>6.5</td>
<td>0/8</td>
</tr>
<tr>
<td>d-Sotalol (6 mg/kg)</td>
<td>7/8</td>
<td>7.7±1.7</td>
<td>22.9±1.8</td>
<td>5/8</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; Conc, concentrations.

Results shown are for 5 dogs in which vagal stimulation was readjusted to compensate for antivagal effects of ambasilide before drug infusion. The efficacy of ambasilide in these 5 dogs was significantly greater than that of an equal (2 mg/kg) dose of d-sotalol (P=.02). Overall, ambasilide was given to 12 dogs and terminated AF in all.

†P=.002 for efficacy of ambasilide in preventing AF induction during maintenance dose vs lower dose of d-sotalol. Vagal frequency was adjusted during maintenance dose infusion in all dogs to maintain the same level of sinus bradycardic effect in the presence of ambasilide as observed in its absence.

Waters UV spectrophotometer (model 450). The retention time was 3 minutes for the internal standard and 5 minutes for ambasilide. A three-point control calibration curve in blank dog plasma was obtained for each set of experimental samples, and the coefficient of variation averaged 0.94% and 1.84% at 8 and 2 μg/mL ambasilide, respectively.

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**Effects on Conduction, Refractoriness, and Wavelength**

To assess potential mechanisms determining drug efficacy, effects on atrial effective refractory period (AERP), conduction velocity (CV), and wavelength were established as a function of atrial basic cycle length (BCL). In the absence of vagal stimulation (Fig 3), both
ambasilide and d-sotalol significantly prolonged AERP. The degree of prolongation of AERP by d-sotalol was determined by BCL, with substantially smaller effects noted as BCL decreased. In contrast, the actions of ambasilide on AERP were not significantly altered as BCL was reduced. Similar findings were noted for drug effects on atrial refractoriness in the presence of vagal stimulation (Fig 4); however, while effects of ambasilide were better preserved than those of sotalol at short cycle lengths, statistically significant rate-dependent effects of ambasilide were noted. As observed in previous studies of other drugs, these drug effects on AERP were substantially greater in the presence of vagal stimulation than in its absence.

Neither ambasilide nor d-sotalol significantly altered atrial conduction (Fig 5). Consequently, changes in wavelength (Figs 6 and 7) parallel those in AERP, with the actions of d-sotalol showing significant reverse use dependence and those of ambasilide lacking a statistically significant dependence on BCL in the absence of vagal stimulation. In the presence of vagal stimulation,
ambasilide increased wavelength more at slower rates, but the reverse use dependence was less marked than in the case of d-sotalol.

Both ambasilide and d-sotalol increased ventricular refractoriness slightly but significantly (Fig 8), with larger changes occurring at longer cycle lengths. Doses of sotalol that were effective in AF increased ventricular refractory period (VERP) at a cycle length of 400 milliseconds to a greater extent than ambasilide. For both drugs, changes in ventricular refractoriness were smaller than those in atrial refractoriness, making detailed analysis of the former difficult. While changes in VERP caused by d-sotalol showed significant reverse use dependence ($P<.05$ at each dose), the effects of ambasilide on VERP were not significantly rate dependent.

**Antivagal Effects**

Neither the lower nor the higher dose of d-sotalol altered the sinus node response to vagal stimulation (Fig 9, top). On the other hand, ambasilide significantly attenuated the bradycardic response to vagal stimulation (Fig 9, bottom), indicating antivagal actions. Antivagal actions were unlikely to have been involved in the effects of ambasilide studied during maintenance infusions (eg, effects on AF inducibility, conduction, wavelength, and refractoriness, as shown in Figs 3 through 8.

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**Fig 6.** Wavelength for reentry (WL) in the absence of vagal stimulation as a function of basic cycle length (BCL) under control conditions (CONT.) and in the presence of ambasilide (AMB.) or d-sotalol (2 mg/kg loading dose, 2 mg, or 6 mg/kg loading dose, 6 mg). Data are mean±SEM from all experiments. Top, Raw data; bottom, percent change relative to control at each cycle length. *P<.05, **P<.01, ***P<.001 vs control at same cycle length; †††P<.001 for frequency-dependence of effect.

**Fig 7.** Wavelength for reentry (WL) in the presence of vagal stimulation as a function of basic cycle length (BCL) under control conditions (CONT.) and in the presence of ambasilide (AMB.) or d-sotalol (2 mg/kg loading dose, 2 mg, or 6 mg/kg loading dose, 6 mg). Data are mean±SEM from all experiments. Top, Raw data; bottom, percent change relative to control at each cycle length. *P<.05, **P<.01, ***P<.001 vs control at same cycle length; †††P<.001 for frequency-dependence of effect.

**Fig 8.** Ventricular effective refractory period (VERP) before and after ambasilide and d-sotalol. Data are mean±SEM from all experiments. *P<.05, **P<.01, ***P<.001 vs control at same cycle length; ††P<.05 for frequency-dependence of effects. See Fig 4 for other abbreviations.

**Fig 9.** Changes in sinus rate (A-heart rate) caused by vagal stimulation at various frequencies in the absence and presence of d-sotalol (top) and ambasilide (AMB, bottom). ***P<.001 vs control response at same frequency. CONT. indicates control conditions.
The antivagal actions of ambasilide contributed to AF termination by its direct electrophysiological actions at the 2 mg/kg dose. HR indicates heart rate.

**Effects on Activation During AF**

Both d-sotalol and ambasilide slowed atrial activation during AF (Fig 11). The increase in the mean atrial cycle length produced by 2 mg/kg d-sotalol was significantly less than that caused by the same dose of ambasilide (P<.001). Ambasilide increased the cycle length during AF to a greater extent than did either dose of d-sotalol. Effective doses of ambasilide and d-sotalol terminated AF by reducing the number of reentry circuits. As in previous studies, AF under control conditions was characterized by numerous (five to seven) zones of reentry at any time. In the presence of ambasilide and the higher dose of d-sotalol, the number of circuits was greatly reduced, consistent with the change in the wavelength for reentry. The mechanisms of AF termination were similar to those we have previously reported, and there were no apparent differences in the mechanisms of AF termination between ambasilide and d-sotalol. Since these mechanisms have already been described extensively in previous works, illustrative activation maps will not be reproduced in this presentation.

**Chronic Pericarditis Model**

Under control conditions, AF was induced during each attempt at induction in all dogs and lasted for an average of 187±90 seconds (range, 6 seconds to 36 minutes). Ambasilide (mean concentration, 5.0±0.5 mg/L) prevented AF induction in all dogs. In the presence of the drug, no AF could be induced in 4 dogs, and in 2 dogs 1 of 10 induction attempts produced AF that lasted for 20 seconds in 1 dog and 16 seconds in the other (mean AF duration during 10 inductions before drug averaged 407±189 and 329±112 seconds in the same dogs, respectively). In the 2 dogs with sustained AF ≥30 minutes, ambasilide terminated AF in both cases, after 7.5 minutes in one dog and 3.5 minutes in the other, at plasma concentrations averaging 8.9±1.5 mg/L.

After drug washout (mean ambasilide concentration, 1.4±0.2 mg/L), AF was once more induced by all 10 induction attempts in all dogs and had a mean duration of 89±85 seconds. After d-sotalol (2 mg/kg loading dose), AF continued to be inducible during all trials and had a mean duration of 39±33 seconds (P=NS versus predrug control). In one dog in which sustained AF could be induced after ambasilide washout, low-dose d-sotalol failed to terminate AF. After a second dose of d-sotalol (cumulative loading dose, 8 mg/kg), AF could no longer be induced in any dog.

Fig 12 shows the changes in AERP and wavelength caused by ambasilide and d-sotalol in dogs with pericarditis and inducible AF. Raw data are shown at the left and percentage changes at the right. Values of conduction velocity remained unchanged after either drug and are not shown. As was the case in the vagotonic dog studies, the effects of d-sotalol were strongly attenuated as cycle length decreased. At the shortest cycle length, AERP and wavelength were not significantly altered by low-dose d-sotalol despite the fact that the drug caused substantial increases at longer cycle lengths. In contrast, the ability of ambasilide to increase AERP and wavelength did not depend on pacing cycle length and was quite appreciable at the shortest cycle lengths tested.

**Discussion**

In the present study, we have compared the actions of two class III drugs, ambasilide and d-sotalol, in experi-
mental models of AF. We found that, for the same dose, the newly developed agent ambasilide was considerably more effective than d-sotalol, although at larger doses d-sotalol was also highly effective. The effectiveness of ambasilide was associated with a lesser degree of reverse use dependence of action on AERP as compared with d-sotalol.

**Comparison With Previous Studies of Antiarrhythmic Drug Actions in AF**

Relatively few studies have examined the actions of antiarrhythmic drugs in experimental AF, and fewer still have specifically addressed class III agents. Renema et al.\(^{27}\) showed that a variety of drugs that increase the atrial wavelength for reentry, including d-sotalol, are effective in preventing the induction of atrial reentrant arrhythmias (including AF) in conscious dogs. The latter study is the only published experimental assessment of a pure class III drug in AF of which we are aware. Kirchhof et al.\(^{27}\) showed that a new class IC agent, 0RG 7797, prevents AF induction by increasing the refractory period and wavelength at the minimum atrial cycle length. We found that flecainide terminates vagotonic AF in the dog by producing tachycardia-dependent increases in AERP\(^{28}\) and that the efficacy of propafenone, procainamide, and d,l-sotalol in the same model are similarly determined by changes in wavelength at rapid atrial rates.\(^{16,26}\)

Effective doses of ambasilide and d-sotalol increased the wavelength in the presence of vagal stimulation at a short cycle length (200 milliseconds) to values in the range of 12 cm (Fig 7). The latter are similar to control values in the absence of vagal stimulation (Fig 6), when sustained AF cannot be induced, and are substantially longer than the critical wavelengths needed for AF induction in conscious dogs.\(^{17}\) Both d-sotalol and ambasilide increased the cycle length during vagotonic AF, enlarged reentry circuits, and reduced their number, leading to termination of reentry. These effects on atrial activation during AF can all be explained by increases in AERP and wavelength. Increases in AERP cause a prolongation in the cycle length of functional, “leading circle” reentry,\(^{28}\) as demonstrated to occur in isolated heart models.\(^{29}\) Similarly, increases in wavelength are expected to increase the size of individual circuits and thereby reduce the number of simultaneous reentry circuits that are possible.\(^{17}\) When the number of simultaneous circuits decreases beyond a critical value, AF is unlikely to sustain itself and termination occurs.\(^{30,31}\)

In the present study, we found that the actions of d-sotalol on AERP show reverse use dependence, similar to our previous observations regarding d,l-sotalol.\(^{16,26}\) Ambasilide is a new class III drug, which has not previously been evaluated for efficacy against AF. Although at long cycle lengths, the effects of ambasilide on AERP were less than or equal to those of high-dose d-sotalol (Figs 3, 4, and 12), the drug showed less reverse use dependence of action and produced larger relative increases than d-sotalol at short cycle lengths. This finding may explain the larger increases in AF cycle length produced by ambasilide (Fig 11), since the AF cycle length is determined by the AERP at the rapid rates of AF.\(^{16,32}\) Larger increases in AERP during AF by ambasilide probably contributed to the greater effectiveness of ambasilide than equal doses of d-sotalol in terminating AF.

**Potential Implications of Our Findings**

We have previously shown that the effectiveness of antiarrhythmic drugs in experimental AF is related to the changes in wavelength for reentry that they produce at rapid activation rates.\(^{16,21,26}\) Reverse use-dependent actions on AERP reduce the ability of d,l-sotalol to increase wavelength as rate increases, and appear to limit the ability of the drug to terminate experimental AF.\(^{16,26}\) Flecainide and propafenone increase AERP with positive use dependence, an action that appears to be important to their efficacy in experimental AF.\(^{16,21}\) However, their sodium channel blocking actions are associated with an undesirable risk of serious proarrhythmic reactions. Positive use-dependent APD prolongation without sodium channel blockade would seem to be an ideal profile of antiarrhythmic drug action.\(^{33,34}\)

Ambasilide is considered a class III antiarrhythmic drug,\(^{19}\) a classification consistent with the significant increases in AERP and VERP and lack of change in atrial conduction velocity that we observed in the present experiments. Our results indicate that class III agents may exhibit different patterns of frequency-dependent action on atrial refractoriness. Unlike d-sotalol, the effects of ambasilide on both AERP and VERP did not show significant reverse use dependence in the absence of vagal nerve stimulation (Figs 3, 8, and 12). We were unable to locate published studies of the effects of ambasilide on atrial action potentials, but Takanaka et al.\(^{27}\) showed that ventricular action prolongation by ambasilide was independent of stimulation frequency.

Amiodarone has recently been found to produce use-independent prolongation of human ventricular monophasic action potentials.\(^{35}\) Like ambasilide,\(^{19}\) amiodarone blocks the slowly activating component of delayed rectifier current, \(I_{Kr}\).\(^{36}\) In contrast, class III agents like sotalol and dofetilide, which specifically block the rapid component of the delayed rectifier \(I_{Kr}\), typically manifest reverse use-dependent actions on APD,\(^{30,40}\) \(I_{Kr}\), but not \(I_{Kr}\), appears to be enhanced.
at increased rates because of incomplete interpulse deactivation$^{38}$ and may therefore contribute to rate-dependent action potential abbreviation. The absence of reverse use dependence of actions by amiodarone on human ventricular repolarization$^{37}$ and the lesser frequency dependence of effects of ambasilide on AERP and VERP compared with sotalol in the present study may be due to an inhibition of $I_{Ks}$-induced action potential abbreviation at rapid rates. While ambasilide blocks $I_{Ks}$, it also appears to inhibit $I_{Kf}$ to a significant extent.$^{19}$ The development of pure $I_{Ks}$ blockers may be a useful approach to the introduction of novel class III agents with a more desirable profile of rate-dependent action on atrial refactoriness.

The present study is the first to evaluate the effects of pure class III drugs in animal models of sustained AF. It indicates the significance of the actions of these drugs on atrial refactoriness at rapid rates and the potential importance of rate-dependent AERP changes in governing drug efficacy in AF. Reverse use dependence could render a drug ineffective in terminating AF, but the drug might still be able to prevent AF recurrence at the slow rate of sinus rhythm. A recent clinical study$^{44}$ showed that sotalol is relatively ineffective in terminating AF but effective in maintaining sinus rhythm after electrical cardioversion, confirming these expectations. Our data suggest that ambasilide might be more effective than $d$- or $d,l$-sotalol in terminating clinical AF. This possibility would be interesting to assess in prospective clinical trials. Our study is also the first to analyze class III drug action on activation of the fibrillating atrium and shows that the mechanism of AF termination by class III drugs is consistent with observed changes in AERP and wavelength and with current concepts of functional reentry mechanisms.$^{28,29}$

### Potential Limitations

We measured changes in atrial and ventricular refactoriness. While the latter are closely related to action potential duration and neither sotalol nor ambasilide significantly altered conduction velocity to suggest sodium channel blockade, we cannot be sure that changes in refactoriness result directly and solely from corresponding changes in action potential duration. Furthermore, changes in repolarization of ventricular muscle may not parallel those in the His-Purkinje system, which appears to be the primary target for drug-induced arrhythmogenic early afterdepolarizations.$^{41}$ While actions of ambasilide on ventricular repolarization are rate independent,$^{18}$ its effects on Purkinje fiber repolarization show reverse use dependence,$^{18}$ like those of other class III agents such as bretylium,$^{42}$ sotalol,$^{40}$ and $N$-acylpro- cainamide.$^{43}$ It has been suggested that bradyarrhythmia-dependent actions to delay Purkinje fiber repolarization may play an important role in proarrhythmic reactions to drugs that prolong action potential duration.$^{34,41,42}$ Therefore, our observation that actions of ambasilide on AERP and VERP in the absence of vagal stimulation are rate independent do not necessarily indicate a reduced risk of proarrhythmic potential.

Although we adjusted vagal stimulation frequency to compensate for antiarrhythmic actions of ambasilide, we cannot be sure of the quantitative precision of the correction because changes in sinus rate were used to adjust vagal tone, and vagal effects on the atrium may not exactly parallel those on the sinus node. Furthermore, some of the effects of sotalol and ambasilide on vagotonic AF could be due to inhibition of $I_{KAC}$, an action that might be relatively much less important in nonvagal AF. We therefore evaluated the actions of the drugs in a model of AF (sterile pericarditis) in which vagal tone is not enhanced. In the latter model, ambasilide was uniformly effective and low-dose sotalol ineffective in preventing AF induction, results that are qualitatively very similar to those in the vagotonic model. One limitation of our studies in dogs with pericarditis is that sotalol was studied in the presence of low concentrations of ambasilide rather than in a separate series of animals. On the other hand, the presence of ambasilide should have, if anything, enhanced the efficacy of $d$-sotalol, so that any bias should have been to overestimate the effectiveness of $d$-sotalol in terminating AF or preventing its induction.

A final comment relates to the applicability of our results to atrial arrhythmias in general. Since the actions of $d$-sotalol are quite rate dependent, the drug could be more effective in terminating atrial reentry with slower intrinsic rates than AF. This may explain the ability of $d$-sotalol to terminate atrial flutter in an experimental model, as shown by Feld et al.$^{45}$

### Conclusions

We have shown that ambasilide, a new class III drug with both $I_{Kf}$- and $I_{Ks}$-blocking actions,$^{19}$ is more effective against experimental AF than the $I_{Ks}$ blocker $d$-sotalol at an equal dose. In contrast to sotalol, ambasilide has no significant reverse use-dependent properties in the absence of vagal stimulation and shows lesser use dependence in the presence of strong vagal stimulation. These results indicate that the rate dependence of class III drug effects on AERP may differ and that drugs that block $I_{Ks}$ may have a more desirable profile of rate-dependent action. Ambasilide may be a useful drug in the treatment of clinical AF, and $I_{Ks}$ blockade may be a desirable mechanism of antiarrhythmic drug action.

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Class III antiarrhythmic drug action in experimental atrial fibrillation. Differences in reverse use dependence and effectiveness between d-sotalol and the new antiarrhythmic drug ambasilde.

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