Effects of Class III Antiarrhythmic Drugs on Transient Outward and Ultra-rapid Delayed Rectifier Currents in Human Atrial Myocytes

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ABSTRACT
A variety of class III antiarrhythmic agents have been shown to block the delayed rectifier current, but their effects on other K+ currents, particularly in human tissues, are less clear. We studied the concentration-dependent actions of the class III compounds d-sotalol, E-4031 and ambasilide on the transient outward current (Ito) and the ultra-rapid delayed rectifier current (IKur) in human atrial myocytes. d-Sotalol and E-4031 failed to alter Ito or IKur at concentrations up to 500 and 50 μM, respectively. In contrast, ambasilide produced a concentration-dependent inhibition of Ito and IKur with statistically significant effects at 10 μM and maximum effects at 100 μM. The 50% inhibitory concentration of ambasilide averaged 23 ± 2 μM and 34 ± 3 μM for Ito and IKur respectively. Ambasilide did not alter the voltage-dependence of activation or inactivation of Ito, or the voltage-dependence of IKur, and it did not affect Ito recovery from inactivation. On the other hand, ambasilide accelerated Ito inactivation, by introducing a more rapid component that accelerated with increasing drug concentration. Furthermore, block of both Ito and IKur developed over time after the onset of depolarization, with time constants of 5.8 ± 0.8 msec and 2.5 ± 0.4 msec at concentrations of 10 and 50 μM for Ito and 6.1 ± 0.8 msec and 2.1 ± 0.3 msec at 10 and 50 μM for IKur. We conclude that neither d-sotalol nor E-4031 affects Ito or IKur whereas ambasilide produces efficacious open-channel block of both currents, in human atrial myocytes.

Class III antiarrhythmic agents exert their actions by prolonging the cardiac action potential and thereby increasing the refractory period, without altering phase 0 sodium current or conduction velocity. A variety of class III drugs are in current clinical use or in development, including sotalol (Singh and Nademanee, 1987), dofetilide (Rasmussen et al., 1992), E-4031 (Fujiki et al., 1994) and ambasilide (Takanaka et al., 1992). Although data have been presented that suggest that sotalol is a highly selective antagonist of the delayed rectifier K+ current (Carmeliet, 1985), and particularly of the rapid component IKr (Sanguinetti and Jurkiewicz, 1990), other work has suggested that sotalol potently inhibits the transient outward current (Berger et al., 1989). Dofetilide and E-4031 have been characterized as specific blockers of IKr (Rasmussen et al., 1992; Fujiki et al., 1994; Colatsky et al., 1995), largely on the basis of experiments with cells isolated from experimental animals. Ambasilide has been found to inhibit both components of the delayed rectifier: IKr and the slower component IKs (Zhang et al., 1994b). In an experimental canine model of AF, ambasilide was found to be a more potent antiarrhythmic agent than d-sotalol and to prolong refractoriness with much less reverse use-dependence than d-sotalol (Wang et al., 1994b).

Recent work has helped to clarify the ionic currents governing human atrial repolarization. Whereas delayed rectifier K+ currents in human atrium resemble corresponding currents in a variety of animal cells (Wang et al., 1993a;
Wang et al., 1994a), some K+ currents in human atrial cells show important differences from other species. For example, human atrial Ito has quite different kinetic properties from Ito in rabbit atrium (Fermini et al., 1992), and a novel type of delayed rectifier with kinetic and pharmacologic properties resembling those of the cloned human K+ channel Kv1.5 appears to be important in human atrial repolarization (Wang et al., 1993b). The latter current has been designated I(kur) or the ultra-rapid delayed rectifier, because its activation kinetics are two orders of magnitude faster than those of Ito (Wang et al., 1993b).

Relatively little is known about the effects of antiarrhythmic drugs on ionic currents in human heart cells. We have shown that quinidine produces open-channel block of Ito in human atrial cells, whereas flecainide inhibits Ito in a fashion that suggests the highest affinity for the inactivated state (Wang et al., 1995). Quinidine was found to inhibit I(kur) significantly at concentrations in the clinically relevant range, which suggests that I(kur) block may contribute to the drug’s antiarrhythmic properties in the human, whereas flecainide had no detectable effect on I(kur) at concentrations as large as 10 μM (Wang et al., 1995).

Ito and I(kur) appear to play important roles in human atrial repolarization (Shibata et al., 1989; Escande et al., 1987; Wang et al., 1993b) and may therefore be important targets for antiarrhythmic drug action. The effects of class III drugs on I(kur) have not, to our knowledge, been studied. There is limited information about class III drug effects on Ito, and the results that have been obtained, largely relating to sotalol, are somewhat contradictory (Carmeli et al., 1985; Berger et al., 1989). We therefore designed the present study to evaluate the effects of three class III antiarrhythmic drugs, d-sotalol, E-4031 and ambisilide, on Ito and I(kur) in human atrial myocytes.

Methods

Isolation of human atrial cells. Specimens of human right atrial appendage were obtained from the hearts of 36 patients (58 ± 3 years old) undergoing aortocoronary bypass surgery. All patients were free of supraventricular tachyarrhythmias, and the atria were grossly normal at the time of surgery. The procedure for obtaining the tissue was approved by the Ethics Committee of the Montreal Heart Institute. Samples were quickly immersed in nominally Ca2+-free Tyrode’s solution (100% O2, 37°C) of the following composition (mM): NaCl, 126; KCl, 5.4; MgCl2, 1.0; NaH2PO4, 0.33; HEPES, 10; glucose, 5.5; pH adjusted to 7.4 with NaOH. In order to minimize possible contamination from Ito, I(K1), I(Ach), and choline-activated K+ current (Fermini and Nattel, 1994), the following chemicals were present during current recording: TEA (Sigma Chemicals, 10 mM, to inhibit Ito), BaCl2 (Sigma Chemicals, 1 mM, to inhibit I(K1)) and atropine (Sigma Chemicals, 1 μM, to inhibit I(Ach), and choline-activated K+ current). In preliminary experiments and previously published studies (Wang et al., 1993a; Wang et al., 1993b), we found these interventions to be without effect on Ito and I(kur). Sodium current was inhibited with the use of a holding potential of −50 mV and/or equimolar choline replacement of Na+ in the superfusate. CdCl2 (200 μM) was added to the superfusate to inhibit Ca2+ current. 4AP was obtained from Sigma Chemicals, prepared as a 1 M stock solution with pH adjusted to 7.4 with the use of 1 N HCl and added at selected concentrations as specified below. Experiments were conducted at room temperature in order to resolve the rapid activation and deactivation kinetics of I(kur); previous studies have shown that the amplitude of I(kur) at room temperature is similar to that at 37°C (Wang et al., 1993b).

The whole-cell patch-clamp technique was employed to record ionic currents in the voltage-clamp mode. Borosilicate glass electrodes (OD: 1.0 mm) were used, with tip resistances of 2.5 to 5 MΩ when filled with (millimolar concentrations): KC1, 150; MgCl2, 1.0; HEPES, 10; EGTA, 5; Mg-ATP, 5; Na2-creatine phosphate, 5; pH adjusted to 7.4 with KOH, and were connected to a patch-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, CA). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pClamp software (Axon Instruments). Recordings were low-pass filtered at 1 kHz. Currents were digitized at a maximum frequency of 100 kHz (model TM 125, Scientific Solutions, Solon, OH) and stored on the hard disk of a personal computer.

Junction potentials were zeroed before formation of the membrane-pipette seal. Mean seal resistance averaged 11.6 ± 3.9 GΩ (n = 30). Several minutes after seal formation, the membrane was ruptured by gentle suction to establish the whole-cell configuration for voltage clamping. Rm was electrically compensated to minimize the duration of the capacitive surge on the current record and the voltage drop across the clamped cell membrane. Rm along the clamp circuit was estimated by dividing the time constant obtained by fitting the decay of the capacitive transient (τc) by the calculated cell membrane capacitance (the time-integral of the capacitive surge measured in response to 5-mV hyperpolarizing steps from a holding potential of −60 mV divided by the voltage drop).

Before Rm compensation, the decay of the capacitive surge had a time constant of 552 ± 36 μsec (cell capacitance of 87.7 ± 4.6 pF, n = 30). After compensation, the time constant was reduced to 166 ± 3 μsec. The initial Rm was calculated to be 6.3 ± 0.3 MΩ, and Rm was reduced to 2.2 ± 0.1 MΩ after compensation. Currents recorded during this study did not exceed 2 nA. The voltage drop across Rm therefore never exceeded 5 mV. Cells with significant leak currents, manifested as a conductance > 0.6 nS upon 10-mV hyperpolarization and depolarization from −60 mV, were rejected. If leak current changed over the course of an experiment, as indicated by a significant change (> 10 pA) in the holding current at −50 mV or by an increase in the membrane conductance at −60 mV, the experiment was terminated.

Data analysis. The amplitude of Ito was measured as the difference between the peak of the transient outward current and the sustained current at the end of the pulse, as previously described (Wang et al., 1993b; Wang et al., 1995). To record I(kur) in the absence of contamination by Ito, we used a 1-sec prepulse to +40 mV to inactivate Ito 10 msec before a depolarizing test pulse, a procedure that we have previously developed and validated (Wang et al.,...
1993b). $I_{K_{ur}}$ was measured in two ways: 1) as described previously (Wang et al., 1993b; Wang et al., 1995), based on the maximum current upon depolarization in the presence of a depolarizing prepulse to inactivate $I_{to}$ and 2) in terms of the tail current upon repolarization from a depolarizing test potential to $-20\text{ mV}$.

Comparisons among groups were performed by ANOVA with Scheffé's contrasts. Single comparisons between base-line and drug data were performed with Student's $t$ test, and a two-tailed probability of 5% was taken to indicate statistical significance. Group data are presented as mean $\pm$ S.E.M. Nonlinear curve fitting was performed using Clampfit in pClamp (Axon Instruments) or Sigmaplot software (Jandel Clump Scientific, San Rafael, CA).

## Results

Effects of d-sotalol and E-4031 on $I_{to}$ and $I_{K_{ur}}$. Concentrations of d-sotalol up to 500 $\mu$M, which fully inhibit $I_{K_{a}}$ (Sanguinetti and Jurkiewicz, 1990) and are substantially higher than the maximum therapeutic concentration in the human (Wang et al., 1986), failed to affect $I_{to}$ recorded on 300-msec depolarizing pulses delivered at 0.1 Hz from $-50\text{ mV}$. Similarly, $I_{K_{ur}}$ elicited by 160-msec pulses from $-50\text{ mV}$ (after a 1-sec prepulse to $+40\text{ mV}$ to inactivate $I_{to}$) was not altered by the drug. Overall, concentrations of 5, 10, 50 and 500 $\mu$M d-sotalol (in five cells at each concentration), produced $-1.2 \pm 0.3, 0.9 \pm 0.4, 1.5 \pm 0.7$ and $-1.4 \pm 0.9$% changes in $I_{to}$ and $0.8 \pm 0.3, -2.1 \pm 0.7, -1.3 \pm 0.4$ and $1.5 \pm 0.9$% changes in $I_{K_{ur}}$, respectively, at $+40\text{ mV}$. When a train of 15 conditioning 90-msec pulses to $+50\text{ mV}$ at a frequency of 1, 2 and 3.3 Hz was introduced before the test pulse to evaluate possible use-dependent actions, no effect of 100 $\mu$M d-sotalol on $I_{to}$ or $I_{K_{ur}}$ was noted. $I_{to}$ averaged $850 \pm 65, 845 \pm 59$ and $831 \pm 62$ pA at $+50\text{ mV}$ at 1, 2 and 3.3 Hz, respectively, before and $849 \pm 62, 846 \pm 61$ and $829 \pm 58$ pA after 100 $\mu$M d-sotalol ($P = \text{NS}$ for d-sotalol vs. control). Similarly, $I_{K_{ur}}$ averaged $499 \pm 52, 488 \pm 49$ and $475 \pm 46$ pA at $+50\text{ mV}$ at 1, 2 and 3.3 Hz, respectively, before and $490 \pm 49, 490 \pm 52$ and $480 \pm 50$ pA after 100 $\mu$M d-sotalol ($P = \text{NS}$ for d-sotalol vs. control).

E-4031 similarly failed to alter $I_{to}$ or $I_{K_{ur}}$—at concentrations of 1, 5 and 10 $\mu$M E-4031 (in five cells at each concentration), E-4031 produced $0.9 \pm 0.2, -1.1 \pm 0.4$ and $0.8 \pm 0.7$% changes in $I_{to}$ and $-1.3 \pm 0.8, 1.2 \pm 0.7$ and $0.9 \pm 0.2$% changes in $I_{K_{ur}}$ respectively at $+40\text{ mV}$. When a train of 15 conditioning 90-msec pulses to $+50\text{ mV}$ at a frequency of 1, 2, and 3.3 Hz was introduced prior to the test pulse to evaluate possible use-dependent actions, no effect of 50 $\mu$M E-4031 on $I_{to}$ or $I_{K_{ur}}$ was noted. $I_{to}$ averaged $798 \pm 60, 788 \pm 69$, and $770 \pm 63$ pA at $+40\text{ mV}$ at 1, 2 and 3.3 Hz respectively before and $789 \pm 58, 781 \pm 56$, and $768 \pm 60$ pA after 50 $\mu$M E-4031 ($P = \text{NS}$ for E-4031 versus control for each). Similarly, $I_{K_{ur}}$ averaged $459 \pm 49, 450 \pm 45$ and $442 \pm 38$ pA at $+40\text{ mV}$ at 1, 2 and 3.3 Hz respectively before and $453 \pm 47, 448 \pm 46$ and $449 \pm 41$ pA after 50 $\mu$M E-4031 ($P = \text{NS}$ for E-4031 versus control for each).

Effects of ambasilide on $I_{to}$. The response of $I_{to}$ to ambasilide is illustrated in figure 1. Figure 1A shows representative currents recorded in one cell under control conditions. Ambasilide produced a slight decrease in $I_{to}$ at a concentration of 10 $\mu$M (fig. 1B). At a higher concentration (50 $\mu$M, fig. 1C), ambasilide decreased $I_{to}$ substantially and caused apparent acceleration in the initial decay of $I_{to}$ after peak values were attained. $I_{to}$ inhibition by ambasilide was almost fully reversed after 20 min of drug washout (fig. 1D).

Mean (± S.E.M.) $I_{to}$ current amplitude in eight cells studied under control conditions, in the presence of 10, 50 and 100 $\mu$M ambasilide, and after 20 min of drug washout are shown in figure 2A. We were unable to study ambasilide concentrations higher than 100 $\mu$M because of limited drug solubility. The drug produced a concentration-related inhibition of $I_{to}$, which was fully reversed by washout. Drug effects were significant at $P < .01$ at all voltages for the two higher concentrations and at $P < .05$ at all voltages at the 10 $\mu$M concentration. Current amplitudes upon washout were not significantly different from those under control conditions at any voltage.

Figure 2B shows the mean percentage change in $I_{to}$ at each TP for each drug concentration. Although significant changes from control were seen at all voltages (asterisks in figure), the effects of the drug were voltage-independent at all concentrations. Figure 2C shows the concentration dependence of drug inhibition of $I_{to}$ on depolarization to $+40\text{ mV}$. The curve in figure 2C is the best-fit equation of the form

$$E = E_{\text{max}}\{1 + (EC_{50}/C)^n\}$$

where $E$ is the effect at any concentration $C$, $E_{\text{max}}$ is the maximal effect, $EC_{50}$ is the concentration for half-maximal effect and $n$ is the Hill coefficient. This equation was applied to data from each of the eight experiments in which $I_{to}$ was recorded before and after ambasilide at each of the four concentrations shown. The mean $EC_{50}$ was 22.6 ± 1.9 $\mu$M, with an average $E_{\text{max}}$ of 62.4 ± 6.5% inhibition and a mean $n$ of 1.6 ± 0.2.

The voltage-dependence of $I_{to}$ activation and inactivation was evaluated in six myocytes each with the voltage protocols (applied at 0.1 Hz) shown in figure 2D. Inactivation was
evaluated with 1-sec CPs from voltages between −120 and +20 mV, followed by a 1-sec test pulse to +60 mV. Activation was analyzed on the basis of tail currents on repolarization to −30 mV after a 5-msec conditioning pulse from V1/2 and k under control conditions were −33.5 ± 3.4 mV and −4.5 ± 0.6 mV, respectively, for inactivation and were +20.4 ± 2.2 mV and +11.5 ± 0.9 mV for activation. In the presence of 50 μM ambasilide, corresponding values were −34.1 ± 3.3 mV and −4.5 ± 0.5 mV for inactivation and +20.8 ± 2.1 mV and +11.6 ± 0.9 mV for activation, respectively. Thus ambasilide did not alter the voltage dependence of Iκr.

**Effects of ambasilide on Iκr.** The effects of ambasilide on Iκr in a representative myocyte are illustrated in figure 3A. Iκr was recorded with the use of the protocol shown in the inset, including a 1-sec prepulse to +40 mV to inactivate Iκr, followed by a 160-msec test pulse delivered at 0.1 Hz to a variety of potentials between −40 and +50 mV (results at +50 mV are shown in the figure). Current under control conditions (fig. 3A) shows the rapid activation with little or no inactivation typical of Iκr. Ambasilide (100 μM) caused a substantial reduction in both step current elicited by depolarization and tail current at −20 mV. The effect of ambasilide was qualitatively similar to, although quantitatively somewhat less than, that of 4AP at a concentration (5 mM) we have previously shown to block Iκr fully (Wang et al., 1993b). The drug-sensitive difference currents shown in figure 3B indicate the similar morphology of the current inhibited by ambasilide and 4AP. Figure 3C shows the mean ± S.E.M. current-voltage relationships of step current sensitive to 100 μM ambasilide (▴) and 5 mM 4AP (△), which were quite similar in form.

Figure 3D shows an analysis of the concentration-dependence of ambasilide effects on Iκr at different test potentials, based on step currents recorded with the protocol illustrated in figure 3A. Ambasilide produced a concentration-dependent inhibition of Iκr that was reversible upon drug washout. The percent change in current produced by the drug is shown as a function of test potential in figure 3E. Although ambasilide produced significant changes relative to control at all concentrations and every voltage (indicated by the asterisks), there was no significant voltage-dependence of drug action. Figure 3F shows the best-fit concentration-response relation for inhibition of Iκr, step current at +50 mV (open diamonds) with the use of a relation of the form $E = E_{\text{max}} \left(1/(1 + (EC_{50}/C^\#))\right)$, as presented above. When fitted to data in each experiment, this relation provided mean values of 75 ± 7% for $E_{\text{max}}$ and 34.2 ± 2.9 μM for $EC_{50}$ in six cells.

One problem in analyzing Iκr step current data is that in measuring relative to the zero current level, it is assumed that no other currents are present. Although a variety of currents, including Iκp, Iκo−k1, IκACh, IκNa and Iκer are inhibited by the contents of the superfusate and/or the voltage
protocol, other conductances, such as that of the time-independent nonselective cation channel (Crumb et al., 1995), can carry current upon depolarization. To obtain a more precise indication of the amplitude of $I_{Kur}$ step current, and of the effect of ambasilide on it, we analyzed the effect of 100 μM ambasilide on 4AP-sensitive current obtained as illustrated in figure 3A to C. Because a depolarizing prepulse was used, $I_{to}$ was suppressed and the only 4AP-sensitive component was $I_{Kur}$. Figure 3B shows 4AP-sensitive current ($a$), obtained by digital subtraction of currents in the presence of 4AP from control, and ambasilide-sensitive current ($b$), obtained by subtraction of currents in the presence of ambasilide from control. For each cell, ambasilide's effect on $I_{Kur}$ can be calculated specifically by relating ambasilide-sensitive current ($b$) to 4AP-sensitive current ($a$) and expressing the ambasilide effect (in terms of percent change in $I_{Kur}$) as $(b/a) \times 100$. When this is done, the results shown by the filled diamonds in figure 3B are obtained. When the corrected concentration-response data were fitted by the $E_{\text{max}}$ equation given above, the $EC_{50}$ averaged 34.7 ± 3.1 μM, and $E_{\text{max}}$ for $I_{Kur}$ inhibition averaged 103% ± 9%.

In order to obtain an independent estimate of the magnitude of the specific ambasilide effect on $I_{Kur}$, we analyzed time-dependent tail currents as shown in figure 4. $I_{Kur}$ tail currents were recorded with 180-msec conditioning depolarizations to potentials between −40 and +50 mV, followed by repolarization to −20 mV for 120 msec. Because $I_K$ is negligible as a result of the short pulse duration, the presence of TEA in the superfusate, and study at room temperature, and because $I_{to}$ is fully inactivated by the end of the conditioning pulse, $I_{Kur}$ is the only component that gives rise to the tail current. Figure 4A shows typical currents recorded with this protocol and indicates a concentration-dependent inhibitory effect of the drug on tail currents. Figure 4B shows the relation between tail current and CP potential in five cells under control conditions, in the presence of various ambasilide concentrations and after drug washout. The drug produced a concentration-dependent inhibition of tail current at all voltages, an effect that was completely reversible upon washout. Drug effects were significant at all voltages and concentrations and were similar at all voltages (fig. 4C). Figure 4D shows the concentration-dependence for inhibition of $I_{Kur}$ tail current elicited by a depolarization to +50 mV, as determined in five cells. The best-fit $E_{\text{max}}$ equation is shown by the solid line, which agrees very closely with the concentration-response relation obtained from corrected step currents (filled diamonds in fig. 3F), whose best-fit concentration-response curve is reproduced as the dashed line in figure 4D. Overall, the tail current analysis provides mean values of 104 ± 10% for $E_{\text{max}}$ and 34 ± 3 μM for $EC_{50}$. 

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**Fig. 3.** A) $I_{Kur}$ recorded from one cell with the protocol shown in the inset of panel B, under control conditions and then after exposure to 100 μM ambasilide and 5 mM 4AP. B) Currents sensitive to 5 mM 4AP (a) and to 100 μM ambasilide (b), based on digital subtraction of currents in the presence of drug from those in the absence of drug in panel A. C) Current-voltage relation of 4AP- and ambasilide-sensitive current obtained as illustrated in panels A and B. D) Current-voltage relations for step current under control, 10 (■), 50 (▲) and 100 (Ç) μM ambasilide and washout conditions (mean ± S.E.M., n = 8). E) Percent reduction in $I_{Kur}$ (relative to control at the same voltage). *P < .05; **P < .01 vs. control (n = 8). F) Concentration-response relation for ambasilide inhibition of $I_{Kur}$ at +50 mV. Open symbols represent experimental data (mean ± S.E.M., n = 8 cells exposed to all concentrations); solid lines are best-fit concentration-response equations. Filled symbols show data corrected for nonspecific component by calculating ambasilide’s effects as $(b/a) \times 100\%$, where $b$ is current inhibited by the drug at a given concentration and $a$ is current inhibited by 5 mM 4AP, obtained as shown in figure 3B.
Possible effects of ambasilide on $I_{Kur}$ activation were evaluated by fitting tail current data in each experiment with the Boltzmann distribution equation provided above. Under control conditions, values for $V_{1/2}$ and $k$ averaged $24.0 \pm 0.4$ mV and $7.5 \pm 0.8$ mV, respectively, whereas in the presence of 100 $\mu$M ambasilide, the corresponding values were $23.9 \pm 0.4$ mV and $7.2 \pm 0.7$ mV, respectively. Thus ambasilide did not alter the voltage dependence of $I_{Kur}$ activation.

State-dependence of ambasilide actions. The above results show that ambasilide inhibits $I_{to}$ and $I_{Kur}$ in a concentration-dependent and voltage-independent fashion. To evaluate further the possibility of state-dependent blocking actions, we assessed the time-dependence of block. If ambasilide interacted preferentially with open or inactivated $I_{to}$ channels with recovery from the rested state slower than spontaneous recovery from inactivation, then slowed recovery after a depolarizing pulse would result. Figure 5A presents an analysis of the time-dependent recovery of $I_{to}$ as determined with the two-pulse protocol shown in the inset. Mean values for $I_{to}$ of the test pulse ($P_2$) normalized to current during a basic pulse ($P_1$, at 0.1 Hz) are shown as a function of the $P_1$ to $P_2$ interval in six cells. The best-fit exponential curves to each set of data are shown; they are very similar. Exponential curve fitting to recovery data in each cell provided recovery time constants of $31.8 \pm 2.9$ and $33.2 \pm 3.0$ msec under control and 100 $\mu$M ambasilide conditions, respectively, which indicated no change in $I_{to}$ recovery kinetics.

The results shown in figure 5A do not exclude state-dependent actions of ambasilide; they simply indicate that if such actions exist, then unblocking upon repolarization cannot be substantially slower than spontaneous recovery from inactivation. Visual inspection of $I_{to}$ recordings in figure 3 suggests that $I_{to}$ attains a peak and inactivates more rapidly in the presence of higher concentrations of ambasilide than in the absence of the drug. Figure 5B shows an analysis of the time from the onset of depolarization to peak $I_{to}$ before and after exposure to 100 $\mu$M ambasilide in six cells. Ambasilide significantly decreased the time to peak current at all voltages, a result consistent with an open-channel blocking action (Wang et al., 1995).

To evaluate further the possibility that ambasilide causes open-channel block of $I_{to}$, we evaluated the inactivation of $I_{to}$ in the absence and presence of ambasilide. Figure 6A presents an analysis of the time-dependent recovery of $I_{to}$ as determined with the two-pulse protocol shown in the inset. Mean values for $I_{to}$ of the test pulse ($P_2$) normalized to current during a basic pulse ($P_1$, at 0.1 Hz) are shown as a function of the $P_1$ to $P_2$ interval in six cells. The best-fit exponential curves to each set of data are shown; they are very similar. Exponential curve fitting to recovery data in each cell provided recovery time constants of $31.8 \pm 2.9$ and $33.2 \pm 3.0$ msec under control and 100 $\mu$M ambasilide conditions, respectively, which indicated no change in $I_{to}$ recovery kinetics.
50 and 100 μM ambasilide. Mean rate constants for six cells studied under control conditions and at each drug concentration are shown in figure 6D. In the presence of 10 μM ambasilide, the time constant of inactivation was not altered. At higher concentrations, there is a slower component with a time constant similar to that under control conditions and a faster component whose time constant decreases with increasing drug concentration. These results are consistent with high-affinity open-channel block causing rapid current decay in a concentration-dependent fashion.

The possibility of open-channel block was further pursued with the analysis shown in figure 7. The pulse protocols shown were used to record $I_{\text{to}}$ (fig. 7A) and $I_{\text{Kur}}$ (fig. 7B) under control conditions and in the presence of 10 and 50 μM ambasilide. Drug-induced block was then plotted as a function of time after the onset of the pulse. For $I_{\text{Kur}}$, the results shown were obtained in a cell lacking $I_{\text{to}}$, which we have shown to have the same $I_{\text{Kur}}$ properties as cells possessing $I_{\text{to}}$ (Wang et al., 1993b), in order to avoid potential complicating effects of the prepulse. Block was found to develop in a time-dependent fashion, with an exponential onset as shown by the curve fits in the figure. The rate of block development increased with increasing concentration; time constants at 10 and 50 μM averaged 5.8 ± 0.8 and 2.5 ± 0.4 msec for $I_{\text{to}}$, and 6.1 ± 0.8 and 2.1 ± 0.3 msec for $I_{\text{Kur}}$, respectively. In the case of $I_{\text{to}}$, there was also a slower time-dependent unblocking phase during sustained depolarization, similar to previous observations with 4AP (Wang et al., 1995).

The time-dependent onset of block is consistent with an open-channel blocking mechanism. The rate constant for block onset should equal $kD + l$, where $k$ is the rate constant for drug binding to open channels, $D$ is drug concentration, and $l$ is the unbinding rate constant. With the use of the experimentally determined mean values indicated above, $k$ and $l$ for ambasilide block of $I_{\text{to}}$ and $I_{\text{Kur}}$ can be estimated, and doing so yields values of 0.0057 μM$^{-1}$ msec$^{-1}$ and 0.166 msec$^{-1}$, respectively, for $I_{\text{to}}$ and $0.0078$ μM$^{-1}$ msec$^{-1}$ and 0.156 msec$^{-1}$ for $I_{\text{Kur}}$. The rate constants for drug binding and unbinding can be used to estimate the dissociation constant for the drug-channel interaction, and doing so yields values of 20 μM for $I_{\text{Kur}}$ and 29 μM for $I_{\text{to}}$. These values are of the same order of magnitude as the directly (and independently) determined EC$_{50}$ values of 34 and 23 μM for $I_{\text{Kur}}$ and $I_{\text{to}}$.

**Discussion**

We have shown that the class III antiarrhythmic agents E-4031 and d-sotalol do not alter $I_{\text{to}}$ or $I_{\text{Kur}}$ in human atrial myocytes. On the other hand, the experimental class III drug ambasilide inhibited both currents. These results point to the
possibility of developing class III antiarrhythmic agents with a different profile of channel blocking actions from previous class III drugs, whose use has been limited by proarrhythmic properties (Hondeghem and Snyders, 1990).

**Comparison with previous studies of ionic mechanisms of the drugs studied.** Several studies have indicated that E-4031 is a highly selective blocker of I_Kr, in animal cells (Sanguinetti and Jurkiewicz, 1990; Colatsky et al., 1990). The present study extends the specificity of E-4031 action by excluding a blocking effect on I_Kur and I_to in human atrium. The specificity of sotalol’s blocking action has been less clear. Carmeliet (1985) showed a high degree of selectivity for I_K but significant effects on other currents at higher concentrations. Sanguinetti and Jurkiewicz (1990) showed a high degree of sotalol selectivity for the I_Kr component, but other studies have suggested relatively strong effects on I_to (Berger et al., 1989). The present study indicates that E-4031 and d-sotalol, even at very high concentrations, have no effect on human atrial I_to or I_Kur.

Zhang et al. (1992) showed that ambasilide affects I_K in guinea pig ventricular myocytes in a fashion that differs from that of E-4031 and indicates significant block of I_Kr. The present observations indicate that ambasilide also causes significant inhibition of human atrial I_to and I_Kur at concentrations comparable to those that affect I_K in guinea pig (Zhang et al., 1992).

**Potential significance of our findings.** Several newer class III agents appear to block the rapid component of I_K with relatively high selectivity (Colatsky et al., 1990). These agents tend to have strong reverse use-dependent effects on repolarization (Wang et al., 1994b; Hondeghem and Snyders, 1990; Jurkiewicz and Sanguinetti, 1993; Colatsky and Argenti, 1994), which are associated with important risks of proarrhythmic reactions because of excessive delays in repolarization at slow HR (Hondeghem and Snyders, 1990; Nattel and Zeng, 1984). Balser et al. (1991) and Zhang et al. (1992) have suggested that class III agents without selectivity for I_Kr may have a more favorable profile of rate-dependent actions. In vivo studies suggest that amidodarone and ambasilide do, indeed, have less reverse use-dependent actions on repolarization than highly selective I_Kr-blocking compounds (Wang et al., 1994b; Sager et al., 1993). The present work points to another potentially interesting action of class III drugs: blockade of currents particularly important in repolarizing human atrial cells. Both I_to and I_Kur have been shown to play important roles in human atrial repolarization (Shibata et al., 1989; Escande et al., 1987; Wang et al., 1993b). Furthermore, I_Kur appears to be absent in human ventricle (Li et al., 1996). Therefore, blockade of I_Kur and/or I_to may be an advantageous property for class III compounds.

**Mechanisms of channel blocking action.** We found that ambasilide produced time-dependent block of I_to and I_Kur, a result that suggests an open-channel blocking mechanism. These properties are similar to blocking mechanisms we noted previously for quinidine on I_to and I_Kur (Wang et al., 1995). Koidl et al. (1996) recently reported effects of ambasilide on rapidly and slowly inactivating components of I_to in human atrial cells. They observed inhibiting effects of the drug on each component, which they interpreted as representing blocking actions on I_to and I_Kur, respectively. They noted that ambasilide accelerated I_to inactivation, decreasing the time constants of both phases. The acceleration of I_to inactivation that they noted is compatible with an open-channel blocking action, as demonstrated in the present study. Although Koidl et al. (1996) suggested that the effect of ambasilide on the slowly inactivating component of I_to may be due to an effect on I_Kur, they did not study the latter directly. Our findings indicate that the effects of ambasilide they hypothesized on I_Kur do, in fact, occur and that the acceleration of inactivation they noted may be due to open-channel blockade. Unlike Koidl et al. (1996), we did not observe a slowly inactivating component of I_to in human atrial myocytes. The difference is probably due to differences in bath temperature. We studied currents at room temperature in order to observe accurately the rapid activation of I_Kur (Wang et al., 1993b), whereas Koidl et al. (1996) worked at 37°C, at which temperature I_Kur inactivation might accelerate enough to be measurable during a 300-msec depolarizing pulse.

**Potential limitations.** Studies in native myocytes always present difficulties in terms of isolating the currents of interest. I_to is relatively distinct in terms of its rapid inactivation. I_Kur is more difficult to isolate, and we have used two previously described approaches (Wang et al., 1993b): a depolarizing pulse to inactivate I_to and sensitivity to 4AP to identify the highly 4AP-sensitive I_Kur component. In addition, we analyzed effects on I_Kur tail currents and obtained results that were qualitatively consistent with the different methods used. The use of cloned channels in expression systems allows for clearer study of single currents but is limited by uncertainties regarding the relationship between cloned channels and their native counterparts, as well as by potential distortions due to differences between model expression systems and native tissues in the properties of membranes and in important intracellular regulatory processes.

I_to inhibition by ambasilide was incomplete even at maximally effective drug concentrations. This finding may have been due in part to limited solubility of the drug, which prevented us from using higher concentrations. On the other hand, like previous drugs that we have studied (Wang et al., 1995), ambasilide required channel opening in order to produce block. Activation of I_to was faster than the rate of block development, and recovery from block was rapid. Therefore, at the time of peak I_to, ambasilide block was significantly less than when steady state was reached later in the pulse, so changes in peak current underestimate steady-state drug effects on the current at any given concentration.

The EC_{50} values for ambasilide block of I_to and I_Kur were in the range of 20 to 30 μM, and statistically significant effects on both were observed at a concentration of 10 μM. We were unable to find published reports of the drug’s therapeutic concentrations in the human, but in previous studies in a dog model of atrial fibrillation (Wang et al., 1994b), we found that the drug’s therapeutic concentration was approximately 15 μM. Thus effective ambasilide concentrations would be expected to inhibit I_to and I_Kur significantly. Ambasilide also inhibits I_Kr with 50% inhibition of I_Kr, at about 5 μM and about 20% reduction in I_Kr at 10 μM (Zhang et al., 1992). The drug’s effect at therapeutic concentrations is therefore likely to result from actions on a variety of K⁺ currents, including both components of I_K as well as I_Kur and I_to. It remains to be determined whether block of I_Kur and/or I_to gives clinical advantages to IK-blocking class III drugs, such as ambasilide, over class III drugs (such as E-4031 and d-sotalol) that block
$I_R$ without inhibiting $I_{Kur}$ or $I_{to}$. Furthermore, it remains to be established whether class III drugs can be developed that act exclusively on $I_{Kur}$ and/or $I_{to}$, without affecting $I_K$.

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References


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