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Multiple cellular electrophysiological effects of azimilide in canine cardiac preparations

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Abstract

The cellular electrophysiological effect of azimilide (0.1–30 μM) was analyzed in canine ventricular preparations by applying the standard microelectrode and patch-clamp techniques at 37 °C. In papillary muscle, the drug prolonged the action potential duration (APD) in a concentration-dependent manner at a cycle length (CL) of 1000 ms. In Purkinje fibers, at the same CL, the concentration-dependent lengthening of the APD was observed in the presence of up to 3 μM azimilide (at 3.0 μM: 24.1 ± 4.2%, $n = 9$); at higher drug concentration, no further APD prolongation was observed. Azimilide lengthened APD in a reverse frequency-dependent manner in papillary muscle and Purkinje fibers alike. Azimilide (10 μM) caused a rate-dependent depression in the maximal upstroke velocity of the action potential (V_{max}) in papillary muscle. The time and rate constants of the offset and onset kinetics of this V_{max} block were 1754 ± 267 ms ($n = 6$) and 5.1 ± 0.4 beats ($n = 6$), respectively. Azimilide did not prevent the APD shortening effect of 10 μM pinacidil in papillary muscle, suggesting that the drug does not influence the ATP-sensitive K^+ current. Azimilide inhibited the rapid (I_{Kr}) and slow component (I_{Ks}) of the delayed rectifier K^+ current and the L-type Ca^{2+} current (I_{Ca}). The estimated EC_{50} value of the drug was 0.59 μM for I_{Ks}, 0.39 μM for I_{Kr} and 7.5 μM for I_{Ca}. The transient outward (I_{to}) and the inward rectifier (I_{k1}) K^+ currents were not influenced by the drug. It is concluded that the site of action of azimilide is multiple, it inhibits not only K^+ (I_{Kr}, I_{Ks}) currents but, in higher concentrations, it also exerts calcium- and use-dependent sodium channel block.

Keywords: Azimilide; Delayed rectifier K^+ current; Papillary muscle; Purkinje fiber; Action potential

1. Introduction

Since the failure of The CAST (Cardiac Arrhythmia Suppression Trial) trial (The Cardiac Arrhythmia Suppression Trial (CAST) Investigators, 1989), the interest in antiarrhythmic drug development has been shifted from Na^+ channel blocking agents to compounds which lengthen repolarization without interfering with the fast Na^+ channels. Most of these drugs inhibit various K^+ currents, in particular, the delayed rectifier K^+ current (I_K) which has two components in cardiac ventricular muscle of various species, the rapidly (I_{Kr}) and the slowly (I_{Ks}) activating components flowing through HERG (human *ether-a-go-go*-related gene) + MiRP (minK-related peptide) and LQT1 (long QT 1) + minK channels, respectively (Sanguinetti and Jurkiewicz, 1990; Chinn, 1993; Gintant, 1995; Salata et al., 1996; Li et al., 1996). The majority of the available class III antiarrhythmic drugs exert their effects by selective blockade of I_{Kr}, and they prolong the action potential duration more at low than at high stimulation rate. This effect was termed reverse frequency-dependency (Hondeghem and Snyders, 1990), which limits the application of class III antiarrhythmics since at lower heart rate, excessive prolongation of the action potential duration, especially that of Purkinje fibers and midmyocardial ventricular muscle (M-cells-Sicouri and Antzelevich, 1991), may cause

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enhanced inhomogeneity of repolarization and induce early afterdepolarization (EAD) resulting in extrasystoles or life-threatening torsade de pointes ventricular tachycardia.

Most of the class III antiarrhythmic drugs (D-sotalol, dofetilide, E-4031) are methansulphonanilide compounds, which inhibit selectively the I_{Kr} channels. Azimilide (NE-10064) is a chlorophenylfuranyl derivative which blocks both I_{Kr} and I_{Ks} in therapeutic concentrations, and at higher concentration, it may influence other ionic currents and receptors as well (Salata and Brooks, 1997; Brooks et al., 2001). Convincing clinical trials indicate the efficacy of azimilide in preventing atrial fibrillation, atrial flutter or paroxysmal supraventricular tachycardia (ASAP—Azimilide Supraventricular Arrhythmia Program, Karam et al., 1998; Connolly et al., 2001). Currently, a multicenter clinical trial has attracted attention in which the possible prevention by azimilide of sudden cardiac death and the reduction of mortality has been investigated in high-risk patients after myocardial infarction (ALIVE—Azimilide Post-Infarct Survival Evaluation trial; Camm et al., 1998). Recent report from this study showed that azimilide, in contrast to antiarrhythmics applied in the CAST and SWORD trials (Survival With Oral D-sotalol), did not increase all cause mortality in post myocardial infarction patients with low left ventricular ejection fraction or in a subpopulation of patients at high mortality risk as defined by low heart rate variability (Camm et al., 2001).

Although some valuable information regarding the electrophysiological effects of azimilide is available, knowledge of the exact cellular electrophysiological mechanism of the action of this compound is still incomplete. Therefore, the recent encouraging clinical results prompted us to further analyse the in vitro electrophysiological effect of azimilide at various concentrations in different canine heart preparations in order to better understand and characterize the mode of action of this promising new antiarrhythmic agent.

2. Methods

2.1. Conventional microelectrode measurements

Adult mongrel dogs of either sex weighing 8–16 kg were used. The animals were kept, treated and the experiments were carried out under conditions delineated in the Guidelines of the Committee on Animal Research (CAR), University of Szeged, Hungary, which complies with the European Community Guidelines for the use of experimental animals.

Following anaesthesia induced by sodium pentobarbital (30 mg/kg i.v.), each heart was rapidly removed through a right lateral thoracotomy and immediately rinsed in oxygenated modified Locke's solution containing (mM/l): NaCl 128.3, $NaHCO_3$ 21.4, KCl 5.0, D-glucose 10.01, $CaCl_2$ 1.8, $MgCl_2$ 0.42. The solution pH ranged from 7.35 to 7.45 when gassed with 95% O_2–5%CO_2 at 37 °C. Purkinje strands obtained from either ventricle and right ventricular papillary muscle tips were mounted individually in a tissue chamber (volume ~ 40 ml). Each preparation was stimulated (HSE stimulator type 215/II) initially at constant cycle length (CL) of 1000 ms (frequency 1 Hz) using rectangular constant current pulses of 2 ms in duration. Transmembrane potentials were recorded using conventional 5–20 MΩ, 3 M KCl-filled microelectrodes connected to the input of a high impedance electrometer (Biologic Amplifier VF 102, Claixe, France). The first derivative of transmembrane voltage with respect to time (V_{max}) was electronically obtained (Biologic Differentiator DV 140, Claix, France).

The maximum diastolic potential (resting potential [RP] in papillary muscle and maximal diastolic potential [MP] in Purkinje fibre), action potential amplitude (APA), conduction time (CT) and action potential duration at 50% and 90% of repolarization (APD_{50} and APD_{90}) were automatically measured using a software developed in our laboratory (Hugo Sachs Elektronik, March-Hugstetten, Germany; Action Potential Evaluation System) running on a 386 microprocessor-based, IBM compatible computer, containing ADA 3300 analog-to-digital data acquisition board (Real Time Devices, PA, USA) with a maximum sampling frequency of 40 kHz.

Extrastimuli were used to study the recovery of V_{max} from the inactivation (offset kinetics of V_{max} block): after the 40th striking of the basic stimulation (S_1), an extrastimulus (S_2) was applied and the S_1–S_2 coupling interval was increased progressively from the end of the refractory period. The diastolic intervals preceding the test action potential were measured from the point corresponding to 90% of repolarization of the preceding basic beat to the upstroke of the test action potential and were increased from −10 to 7000 ms progressively.

The development of frequency-dependent V_{max} block (onset kinetics) was studied in canine ventricular muscle by introducing a stimulus train of 40 beats at a cycle length of 400 ms after a 60-s rest period.

2.2. Patch-clamp measurements

2.2.1. Cell isolation

Ventricular myocytes were enzymatically dissociated from hearts of mongrel dogs as described previously (Varro et al., 2000).

2.2.2. Experimental procedure, drugs and solutions

The membrane currents were recorded in the whole-cell configuration of the patch-clamp technique at 37 °C. HEPES buffered Tyrode's solution served as the normal bath solution (composition in mM: NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES 5.0 at pH of 7.4). The cell capacity was measured by integration of the capacitive transient divided by the amplitude of the voltage step (10 mV). When measuring K^+ currents, nisoldipine (1 μM) (obtained as a gift from the

Table 1
Concentration-dependent effect of azimilide on different parameters of the action potential in canine right ventricular papillary muscle and in canine Purkinje fibre at stimulation rate of 1 Hz

Papillary muscle	Control	0.3 μM	Control	3.0 μM	Control	10.0 μM
	$n=8$		$n=9$		$n=7$	
APD_{90} (ms)	247.9 ± 6.5	257.3 ± 6.8*	246.7 ± 5.8	273.0 ± 8.1*	248.3 ± 9.1	293.1 ± 14.2*
APD_{50} (ms)	216.4 ± 7.0	226.6 ± 6.6*	216.1 ± 5.8	240.9 ± 7.8*	218.0 ± 9.0	258.6 ± 14.2*
APA (mV)	109.5 ± 1.3	109.6 ± 1.2	109.4 ± 1.1	107.1 ± 1.1*	110.4 ± 1.7	106.4 ± 2.1
V_{max} (V/s)	218.4 ± 18.6	220.6 ± 19.4	214.1 ± 15.1	210.2 ± 15.3	191.3 ± 7.0	166.6 ± 9.2*
CT (ms)	5.0 ± 0.2	5.1 ± 0.2	5.1 ± 0.2	5.2 ± 0.2	5.2 ± 0.3	5.9 ± 0.3*
RP (mV)	− 84.2 ± 0.7	− 84.5 ± 0.6	− 84.1 ± 0.6	− 85.1 ± 0.4	− 83.6 ± 0.6	− 84.5 ± 0.8
Purkinje fibre						
APD_{90} (ms)	326.6 ± 19.2	356.6 ± 19.7*	329.8 ± 17.4	405.3 ± 16.0*	330.9 ± 19.7	402.1 ± 16.8*
APD_{50} (ms)	225.6 ± 16.6	237.9 ± 17.3	229.9 ± 15.2	202.1 ± 25.0	231.1 ± 17.1	102.5 ± 27.1*
APA (mV)	118.4 ± 0.8	117.6 ± 0.9	118.8 ± 0.7	114.3 ± 1.5*	118.5 ± 0.8	109.6 ± 1.3*
V_{max} (V/s)	737.3 ± 29.9	737.8 ± 38.1	743.0 ± 25.5	696.3 ± 39.3	751.0 ± 27.4	531.6 ± 36.2*
CT (ms)	2.9 ± 0.3	2.8 ± 0.4	2.9 ± 0.3	3.5 ± 0.3*	3.1 ± 0.3	3.6 ± 0.5
MP (mV)	− 84.6 ± 0.6	− 85.7 ± 0.7	− 85.2 ± 0.5	− 86.6 ± 0.5	− 85.4 ± 0.5	− 86.1 ± 0.6

Mean ± S.E.M.
n = number of experiments.
APD_{50-90} = action potential duration at 50% and 90% of repolarization.
APA = action potential amplitude.
V_{max} = maximal rate of depolarization.
CT = conduction time.
RP = resting potential.
MP = maximal diastolic potential.
* $p<0.05$.

Bayer, Leverkusen, Germany) was placed in the external solution to eliminate inward Ca^{2+} current (I_{Ca}), the internal (pipette) solution contained (in mM): K-aspartate 100, KCl 45, ATP 3, $MgCl_2$ 1, EGTA 10 and HEPES 5 (pH 7.2 by KOH). The rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier K^+ current were separated by using the selective I_{Kr} channel blocker E-4031 (1 μM) or the I_{Ks} channel blocker chromanol 293B (30 μM). When Ca^{2+} current was measured, the pipette solution contained (in mM): CsCl 110, CsOH 40, EGTA 10, HEPES 10, TEACl 20, ATP 5 (pH was adjusted to 7.2 by CsOH).

Azimilide (NE-10064, Procter & Gamble Pharmaceuticals, USA) was diluted from a 1 mM and dofetilide (Drug Research Institute, Budapest, Hungary) from a 10.0 mM stock solution containing 50% DMSO (dimethyl sulfoxide), and concentrations of 0.1, 0.3, 0.5, 1.0, 3.0, 10.0 and 30 μM of the drug were used for conventional microelectrode and patch-clamp measurements.

2.3. Statistical analysis

All data are expressed as mean ± S.E.M. Statistical analysis was performed using Student's *t*-test for paired data. The results were considered significant when p was <0.05. One- and two-exponential equations were used to calculate the recovery kinetics of V_{max} block in control and azimilide group, respectively. The actual value of the V_{max} (V_{maxt}) corresponding to each diastolic intervals was compared to that plateau value (V_{maxpl}) which was observable at the longest diastolic interval (7000 ms). The time constant (τ)

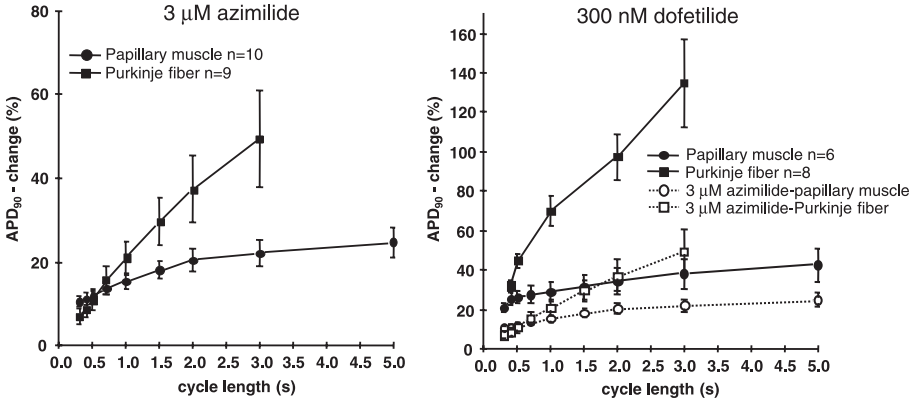

Fig. 1. The reverse frequency-dependent repolarization lengthening effect of azimilide (3.0 μM) and dofetilide (300 nM) in papillary muscle and Purkinje fibre.

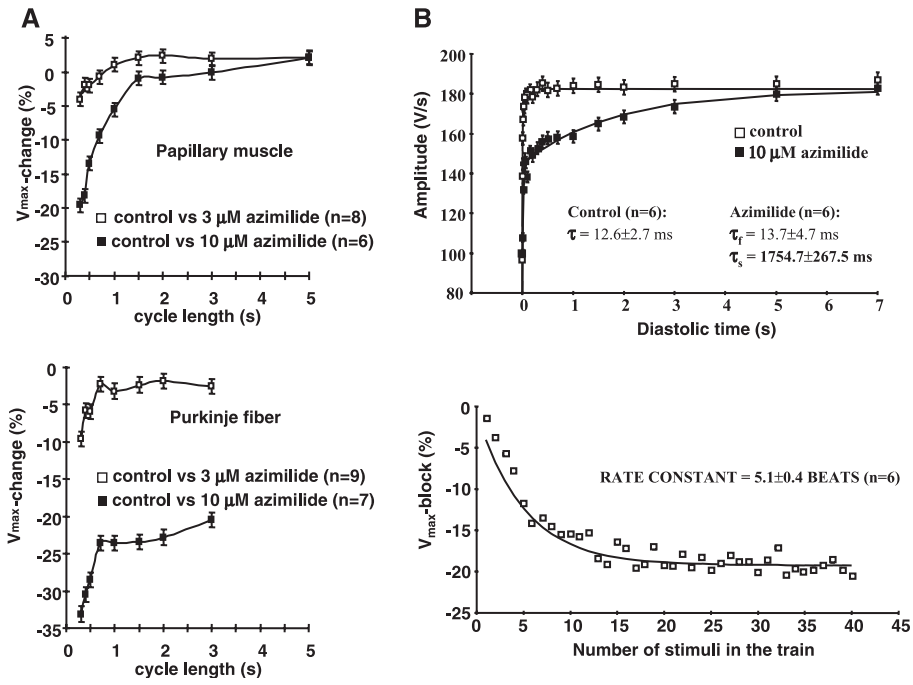

Fig. 2. The frequency-dependent decrease of the maximal rate of depolarization (V_{max}) in the presence of different concentrations of azimilide in right ventricular papillary muscle and Purkinje fiber (panel A). The kinetics of V_{max} recovery from inactivation in control conditions and in the presence of 10 μM azimilide in papillary muscle (top). The onset kinetics of the V_{max} block (bottom) after 3 μM azimilide in papillary muscle (panel B).

was estimated by fitting of exponential equations. One-exponential equation ($V_{maxt} = V_{maxpl} + V_{maxA} * e^{(-t/\tau)}$) for the control group and two-exponential equation ($V_{maxt} = V_{maxpl} + V_{maxAf} * e^{(-t/tf)} + V_{maxAs} * e^{(-t/ts)}$) for the azimilide group were used where t means the diastolic interval, τ the time constant (τ_f for the fast and τ_s for the slow component), V_{maxA} the increasing component (V_{maxAf} for the fast and V_{maxAs} for the slow).

3. Results

3.1. Action potential measurements

Table 1 contains the concentration-dependent effects of azimilide on different action potential parameters at a stimulation rate of 1 Hz in right ventricular papillary muscle and Purkinje fibre.

In papillary muscle, the drug significantly lengthened the APD_{90} and APD_{50} at each concentration. In the presence of 3 μM azimilide, APA decreased and CT increased. V_{max} was significantly decreased by the highest concentration (10 μM) of azimilide. In Purkinje fibers, APD_{90} was significantly increased by the drug at each concentration but APD_{50} was shortened at higher concentrations. Also, the voltage level during the plateau phase was shifted to the negative direction. APA was considerably decreased by 3 and 10 μM azimilide. V_{max} was decreased significantly by the highest concentration of the drug. CT was significantly lengthened by 3 μM azimilide.

Fig. 1 (left) demonstrates the frequency-dependent repolarization lengthening effect of 3.0 μM azimilide in papillary

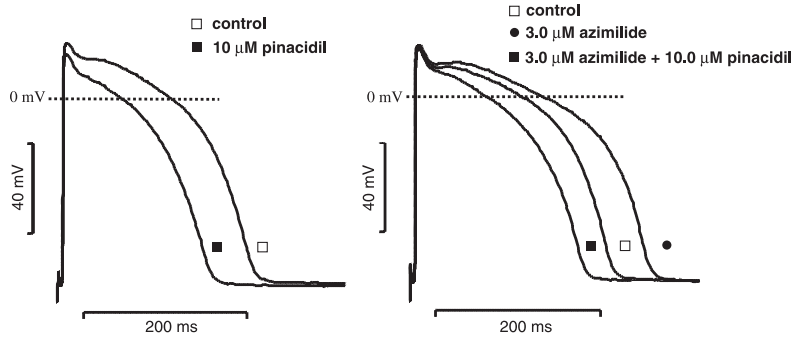

Fig. 3. Lack of effect of azimilide on the ATP-sensitive K^+ current. Azimilide at concentration of 3 μM did not prevent the APD shortening effect of 10 μM pinacidil in right ventricular papillary muscle.

muscle and Purkinje fibre. The effect on APD is more pronounced at lower than at higher stimulation rate and this reverse rate-dependent effect of the drug is more prominent in Purkinje fibers.

In Fig. 1 (right), for comparison, the effect of the selective I_{Kr} blocking "pure" class III antiarrhythmic compound dofetilide on the rate-dependent APD in dog right papillary muscle and Purkinje fibers is also demonstrated. Dofetilide (300 nM) induced marked reverse rate-dependent APD prolongation in both ventricular muscle and Purkinje fibers. It is important to note that dofetilide enhanced inhomogeneity of repolarization between ventricular muscle and Purkinje fibers much more than observed with azimilide.

As Fig. 2A shows, the V_{max} was decreased in a frequency-dependent manner in both papillary muscle and Purkinje fibers after superfusion with increasing concentrations of azimilide.

The offset and onset kinetics of V_{max} block in the presence of 3 and 10 μM azimilide was studied in papillary muscle. In control conditions, V_{max} recovered almost completely from the inactivation at the first 50 ms of the diastole, but after application of azimilide (10 μM), its recovery was substantially slowed. The complete recovery of V_{max} from the inactivation took more than 2 s which resulted in a slow component of the V_{max} recovery curve with a time constant of 1.754 ± 267 ms ($n = 6$) corresponding to the offset kinetics of the drug. The rate constants of the onset kinetics of the V_{max} block after 3 μM azimilide was 5.1 ± 0.4 beats ($n = 6$) (Fig. 2B).

The possible effect of azimilide on the ATP-sensitive K^+ current was studied in papillary muscle by opening of the ATP sensitive K^+ channels by 10 μM of pinacidil which in turn shortens the action potential duration (by 31.9 ± 3.4%, $n = 5$, at a stimulation rate of 1 Hz). Upon washout of pinacidil from the tissue bath, the APD shortening returned to the baseline value. When 3 μM of azimilide was given to the tissue bath, the APD_{90} was lengthened by 12.9 ± 3.0%. In the continuous presence of azimilide, repeated exposure to 10 μM pinacidil resulted in an APD_{90} shortening effect (32.2 ± 4.5%) similar to that measured before azimilide application, suggesting that the ATP-sensitive K^+ channels were not influenced by azimilide (Fig. 3).

3.2. Effects on transmembrane ionic currents

The possible effects of 1 μM azimilide superfusion on the slow (I_{Ks}) and rapid (I_{Kr}) components of the delayed rectifier outward K^+ current were also investigated. In these measurements, the extracellular solution contained 1 μM nisoldipine to completely block I_{Ca}.

The I_{Ks} and I_{Kr} currents were activated from the holding potential of −40 mV with 1 s (I_{Kr}) and 5 s (I_{Ks}) long depolarizing test pulses of −20 to 50 mV at pulse frequency of 0.05 Hz (I_{Kr}) and 0.1 Hz (I_{Ks}). The amplitude of the tail current measured upon returning to the holding potential

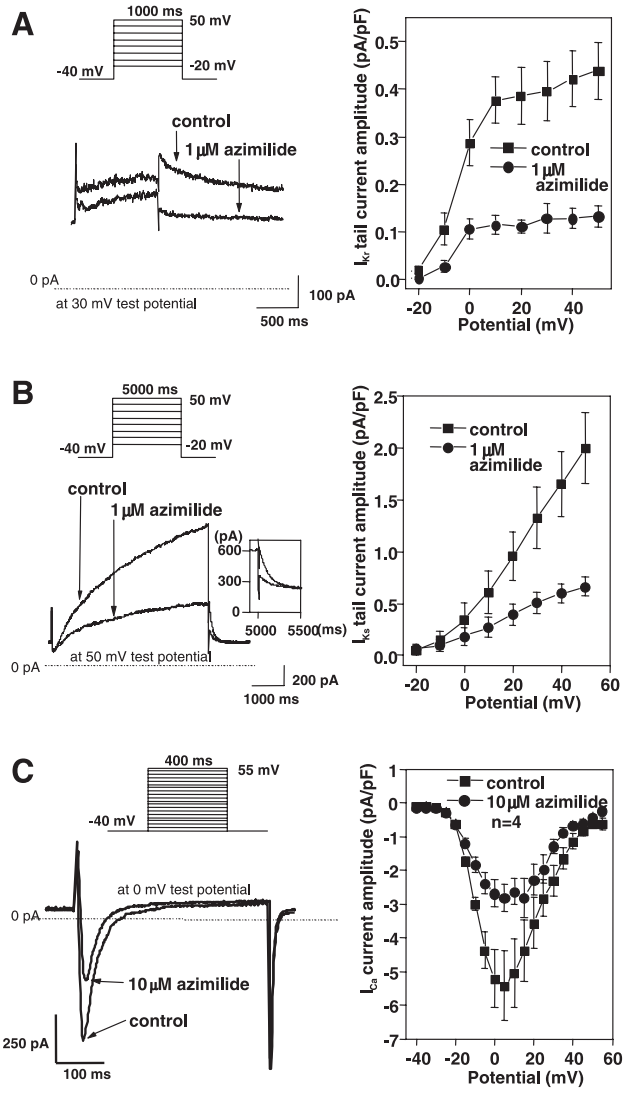

Fig. 4. Effect of azimilide on the rapid component (I_{Kr}) of the delayed rectifier K^+ current (panel A). Representative current traces under control conditions and after application of 1 μM azimilide (left). Current–voltage relationship of I_{Kr} under control conditions and in the presence of 1 μM azimilide (right). The current was activated by 1000 ms long depolarizing voltage pulses from holding potential of −40 mV to various test potentials ranging from −20 to 50 mV in 10-mV increments (top left). Effect of azimilide on the slow component (I_{Ks}) of the delayed rectifier K^+ current (panel B). Representative current traces under control conditions and after application of 1 μM azimilide (left). Current–voltage relationship of I_{Ks} under control conditions and in the presence of 1 μM azimilide (right). The current was activated by 5000 ms long depolarizing voltage pulses from holding potential of −40 mV to various test potentials ranging from −20 to 50 mV in 10-mV increments (top left). Effect of azimilide on L-type Ca^{2+} current (I_{Ca}) (panel A). Representative current traces under control conditions and after application of 10 μM azimilide (left). Current–voltage relationship of I_{Ca} under control conditions and in the presence of 10 μM azimilide (right). The current was activated by 400 ms long depolarizing voltage pulses from holding potential of −40 mV to various test potentials ranging from −40 to 55 mV in 5-mV increments (top left).

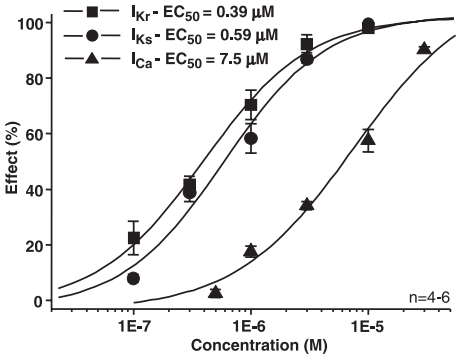

Fig. 5. Concentration–response relationship of the azimilide evoked I_{Kr}, I_{Ks} and I_{Ca} block.

was used to define I_{Ks} and I_{Kr}. To study the effect of azimilide on I_{Ks}, 3–5 μM E-4031 was used to block I_{Kr}. When the effect of azimilide on I_{Kr} was measured, 30 μM chromanol 293B was used to block I_{Ks}. As Fig. 4B shows, 1 μM azimilide significantly decreased the amplitude of the I_{Ks} tail current, and the same applies to the I_{Kr} tail current (Fig. 4A). The magnitude of the I_{Ks} and I_{Kr} depression were 58.4 ± 5.2 % and 70.4 ± 5.3 %, respectively (at 50 mV test potential from 473.9 ± 80.4 pA to 183.7 ± 26.6 pA, $n=6$, $p<0.05$ for I_{Ks} and at 30 mV test potential from 118.3 ± 37.0 pA to 31.3 ± 7.8 pA, $n=5$, $p<0.05$ for I_{Kr}).

L-type inward Ca^{2+} current (I_{Ca}) was evoked by 400 ms long depolarizing test pulses (ranging from −40 to 55 mV) from −40 mV holding potential. The amplitude of I_{Ca} was defined as the difference between the peak inward current at the beginning of the pulse and the current at the end of the pulse. In these measurements, the KCl content of the pipette was replaced by CsCl to suppress K^+ currents. As Fig. 4C shows, 10 μM azimilide, after 3–5 min superfusion, largely decreased I_{Ca}. The magnitude of the I_{Ca} depression at 0 mV was 57.5 ± 4.0 % (from −860.3 ± 102.3 to −377.8 ± 70.8 pA, $n=4$, $p<0.05$), which was only partially reversible even after 10 min washout.

In Fig. 5, the concentration–response relationship of the azimilide-evoked I_{Kr}, I_{Ks} and I_{Ca} block is displayed. The estimated EC_{50} value was 0.59 μM for I_{Ks}, 0.39 μM for I_{Kr} and 7.5 μM for I_{Ca}. The effect of azimilide on the transient outward (I_{to}) and inward rectifier (I_{k1}) K^+ currents was also investigated and azimilide, even at high concentration (10 μM), did not apparently influence I_{to} and I_{k1} currents.

4. Discussion

The main finding of our study is to show that azimilide exerts multiple electrophysiological effects which are as follows: (a) reverse rate-dependent APD prolongation and use-dependent Na^+ channel block, (b) inhibition of both rapid and slow delayed rectifier K^+ currents and the inward Ca^{2+} current without affecting the inward rectifier, transient outward and ATP-sensitive K^+ channels.

We applied azimilide in the concentration range of 0.1–30 μM. These concentrations are either within or exceeding the therapeutic range and were, in part, also applied earlier by other in vitro experiments (Salata and Brooks, 1997).

The efficacy of azimilide to prevent recurrent atrial fibrillation, atrial flutter and paroxysmal supraventricular tachycardia (SVT) was investigated in more than 1000 patients randomly assigned to receive placebo or escalating doses of oral azimilide for 6–9 months (Pritchett et al., 1999a,b; Connolly et al., 1999). In preliminary data on the first 367 patients with atrial fibrillation or atrial flutter randomly assigned to placebo or azimilide, a significant reduction was shown at the higher dose level in median time to arrhythmia development. In other analysis of three trials of atrial fibrillation and atrial flutter examining 906 patients, azimilide significantly prolonged the hazard ratio for arrhythmia recurrence.

The effect of azimilide was also evaluated in patients with paroxysmal SVT. Azimilide significantly prolonged the arrhythmia-free interval (Page et al., 1999, 2002). The overall incidence of torsade de pointes in the supraventricular tachycardia studies has been less than 0.8%.

Azimilide is also being studied as prophylaxis in patients with recent high-risk myocardial infarctions. The Azimilide Post-Infarction Survival Evaluation Trial (Camm et al., 1998, 2001; SoRelle, 2001) is a large, multinational study comparing azimilide and placebo in the reduction of mortality in patients with recent (within 21 days) myocardial infarctions who have low left ventricular ejection fraction (15–35%) and were defined to be at risk for sudden death. The study enrolled 3381 patients, and investigators are encouraged to maximize use of angiotensin-converting enzyme inhibitors and β-blockers (75% of patients were treated with β-blockers). Azimilide did not affect all cause mortality in recent post myocardial patients with low left ventricular ejection fraction or in a subpopulation of patients at high mortality risk as defined by low heart rate variability. Additionally, fewer patients developed atrial fibrillation or atrial flutter on azimilide compared to placebo. These data provide further support for the development of azimilide as a treatment for atrial arrhythmias in patients with structural heart disease.

Previous investigations yielded conflicting results regarding the effect of azimilide on the rate-dependent APD prolongation. Our results are in good agreement with the data published by Gintant (1994), McIntosh et al. (1994), Fermini et al. (1995) and Yao and Tseng (1997) but different from those reported by Qi et al. (1996, 1999) and Restivo et al. (1996) who found that the azimilide-evoked APD lengthening was rate-independent. It is possible that the latter investigators did not study the effect of azimilide at stimulation frequency lower than the expected physiological heart rate.

We first demonstrated that azimilide (3–10 μM) decreased V_{max} in papillary muscle and Purkinje fibers use-dependently. This is consistent with some earlier reports which showed inhibition of the Na^+ channel by the drug (Conder et al., 1994; Bril et al., 1996; Yao and Tseng, 1997). The offset kinetics of the V_{max} block by azimilide was also first determined in the present study, which can be regarded as intermediate kinetics between Class I/A and I/B actions.

In our study, azimilide concentration-dependently blocked the rapid and slow component of the delayed rectifier K^+ current which is consistent with some earlier findings (Fermini et al., 1995; Salata et al., 1996; Yao and Tseng, 1997; Gintant, 1998). In our hands, the effect of azimilide on I_{Ca} was stronger (EC_{50} of 7.5 μM for I_{Ca}) than that measured by Yao et al. (EC_{50} of 17.8 μM) which suggests that this subsidiary effect may considerably contribute to the antiarrhythmic profile of the drug. Our observation that azimilide does not prevent the repolarization shortening effect of pinacidil has not been described previously and strongly suggests that the drug, even at high concentration (10 μM), does not influence the ATP-sensitive K^+ current.

The reverse frequency-dependence of class III antiarrhythmic agents may limit their therapeutic use because of its reduced effectiveness at rapid frequencies and enhanced proarrhythmic potential at slow rates as a consequence of excessive action potential prolongation. This can increase inhomogeneity of repolarization and produce early afterdepolarization (EAD). In the present study, although azimilide produced reverse rate-dependent APD prolongation in both papillary muscle and Purkinje fibers, this effect was less marked than that evoked by 300 nM dofetilide, which is known to block selectively I_{Kr}. It is also an important difference that the inhomogeneity of repolarization between ventricular muscle and Purkinje fiber was much less with azimilide than after application of dofetilide. This is probably due to the inhibition by azimilide of the I_{Na} and I_{Ca}. Similar mechanism was postulated with amiodarone, a drug which lengthens repolarization, but like azimilide, inhibits I_{Ca} and I_{Na} (Kodama et al., 1999; Papp et al., 1996). It has also been shown that reverse rate dependency of action potential prolongation induced by class III agents could be reduced by the simultaneous administration of Ca^{2+} channel antagonists (Bril et al., 1998) and Na^+ channel blockers (Varró and Lathrop, 1990). In addition, these drugs could inhibit drug-induced afterdepolarizations (Nattel and Quantz, 1988; Varro and Lathrop, 1990) at slow stimulation rates so that the incidence of torsade de pointes type polymorphic ventricular arrhythmias would also be decreased. Therefore, the Na^+ channel and L-type Ca^{2+} current blocking effect of azimilide could attenuate the prolongation of repolarization in Purkinje fibers produced by K^+ channel blockade without a marked effect on the conduction velocity at normal heart rate as observed previously with amiodarone (Papp et al., 1996). This complex electrophysiological effect of azimilide seems similar, in many ways, to that of amiodarone and may explain the reduced proarrhythmic potential found with azimilide (Camm et al., 2001).

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