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Comparison of Bidisomide, Flecainide and Dofetilide on Action Potential Duration in Isolated Canine Atria: Effect of Isoproterenol

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ABSTRACT

Prolongation of action potential duration (APD) and the effective refractory period (ERP) is one mechanism to prevent reentrant atrial and ventricular arrhythmias. Because arrhythmias are usually associated with an elevated sympathetic tone and increase of circulating catecholamines, the potential influence of catecholamines on antiarrhythmic effects of an agent are critical to predicting the potential clinical response. In this study the effects of three different antiarrhythmic agents, bidisomide, flecainide and dofetilide, each of which prolongs atrial ERP, were compared before and after treatment with isoproterenol, a β-adrenergic agonist. Standard intracellular microelectrode recording techniques were used to record action potentials from isolated canine atrial tissue. Bidisomide and flecainide elicited a 20 to 27 msec increase in APD and ERP that was independent of stimulation frequency (1–5 Hz). Dofetilide prolonged APD and ERP at 1 Hz (40 and 37 msec, respectively) but was completely ineffective at 5 Hz. After equilibration with the antiarrhythmic agent, tissues were additionally exposed to isoproterenol. Only bidisomide prolonged APD and ERP in the presence of isoproterenol. In the converse series of experiments, after treatment with isoproterenol, which caused a 20 to 30% reduction in APD and ERP, only bidisomide completely reversed the effect of 1 μM isoproterenol. Bidisomide was inactive in a functional β-adrenergic antagonism assay, thus ruling out β-adrenergic blockade as a potential mechanism. These results indicate that bidisomide, unlike flecainide and dofetilide, was able to prolong APD and ERP in isolated canine atrium even at high stimulation frequencies and in the presence of isoproterenol. These data suggest that bidisomide would be effective in the presence of elevated sympathetic tone and of the agents studied, only bidisomide possessed a unique and desirable antiarrhythmic profile.

Inasmuch as the Cardiac Arrhythmia Suppression Trial (Echt *et al.*, 1991) indicated that antiarrhythmic agents that elicit a potent block of the sodium channel in patients with prior myocardial infarction may increase mortality, more attention has been given to specific prolongation of the cardiac refractory period as an antiarrhythmic approach (*e.g.*, Colatsky and Argentieri, 1994; Roden, 1993; Singh, 1993a). However, a clinical study showed prolongation of ERP by amiodarone and sotalol, both potassium channel blockers (*e.g.*, Roden, 1993; Singh, 1993a), to be antagonized by isoproterenol infusion, indicating a potential lack of effect by these agents on ERP associated with catecholamine release and elevation of sympathetic tone that occurs during arrhythmias (Newman *et al.*, 1993).

Because potassium channels have a role in cardiac repolarization, new specific agents targeting potassium channels are under development as antiarrhythmic agents (Colatsky and Argentieri, 1994; Roden, 1993; Sanguinetti, 1992), many of which block the fast component of the delayed rectifier current termed I_{Kr} (Colatsky and Argentieri, 1994; Roden, 1993; Sanguinetti, 1992; Sanguinetti and Jurkiewicz, 1990).

Dofetilide is a new class III antiarrhythmic under clinical development for ventricular and supraventricular arrhythmias, including atrial fibrillation and flutter (Rasmussen *et al.*, 1992). Dofetilide has been shown to increase ERP in isolated canine ventricle and atrium (Rasmussen *et al.*, 1992; Spinelli *et al.*, 1992). Experiments from isolated tissue have indicated that dofetilide specifically blocks I_{Kr} (Carmeliet, 1992; Jurkiewicz and Sanguinetti, 1993; Kiehn *et al.*, 1994).

Flecainide, a class Ic antiarrhythmic agent, is indicated for paroxysmal atrial fibrillation and flutter and paroxysmal supraventricular tachycardia. Flecainide has been shown to increase canine atrial, but not ventricular, refractoriness with the largest increases at fast stimulation rates (O'Hara *et al.*, 1992). Experiments from single cells indicate that flecainide blocks potassium channels (*e.g.*, Follmer and Colatsky, 1990; Slawsky and Castle, 1994) in addition to sodium channels (*e.g.*, Anno and Hondeghem, 1990).

Bidisomide is a new antiarrhythmic agent that has been tested clinically for paroxysmal supraventricular tachycardia

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ABBREVIATIONS: APD, action potential duration; ERP, effective refractory period; APD_{90}, APD at 90% repolarization.

and atrial flutter/fibrillation. In addition to decreasing action potential upstroke velocity (Martin *et al.*, 1989) and slowing conduction (Garthwaite *et al.*, 1992), bidisomide has been shown to increase canine atrial, but not ventricular, ERP in isolated tissue and intact animal experiments (Garthwaite *et al.*, 1992; Martin and Chinn, 1994).

In the present study, the effects of bidisomide, flecainide and dofetilide on isolated canine atrial action potentials were examined. After treatment with each individual antiarrhythmic agent, tissues were equilibrated with the agent plus the β-agonist, isoproterenol, to mimic catecholamine release. In an additional series of experiments, tissues were first equilibrated with isoproterenol followed by addition with one of the three agents. Bidisomide uniquely prolonged APD_{90} and ERP in the presence and absence of isoproterenol at all stimulation frequencies.

Materials and Methods

Canine Atrial Action Potential Recordings

Canine hearts were removed by left thoracotomy from pentobarbital (30 mg/kg, i. v., Butler Company, Columbus, OH) anesthetized beagles (8–12 kg; Marshall Farms, North Rose, NY or White Eagle, Doylestown, PA) and placed in ice-cold Tyrode's solution containing in mM: NaCl, 127; KCl, 4; NaH_2PO_4, 0.43; $NaHCO_3$, 23.8; $MgCl_2$, 1; $CaCl_2$, 1.8 and dextrose, 5.5. Atrial trabeculae muscles were removed from either right or left atrium and pinned in the tissue bath. The muscles were superfused (1.5 ml/min) with oxygenated Tyrode's solution at 37°C, and stimulated by a bipolar platinum electrode with a 3-msec duration pulse at twice diastolic threshold (A300 Pulsemaster stimulator with a A365 stimulus isolator, World Precision Instruments, Sarasota, FL). Intracellular action potentials were recorded with glass microelectrodes filled with 3 M KCl (10–15 MΩ) via a M-707A amplifier (World Precision Instruments). After a 90 to 120-min equilibration period in which tissues were stimulated at 1 Hz, control records of ERP measured by paired pulse technique observed on an oscilloscope (TDS 420, Tektronix, Rolling Meadows, IL), and APD_{90} (APD at 90% repolarization) computed on-line by a computer (sampling rate of 25 μsec; built at Searle) were measured at 1, 2, 3.3 and 5 Hz in a sequential manner. ERP measurements were made at twice diastolic threshold for each stimulation frequency recorded to the nearest msec (recording electrode located a few mm from stimulating electrode). All values obtained were at steady state (approximately 1–2 min for each stimulation frequency change). Tissues stimulated at 1 Hz were equilibrated with antiarrhythmic agent for 30 min after control records and measurements were repeated at all stimulation frequencies. The agents compared were 30 μM bidisomide (α-[2-[acetyl(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-1-piperidinebutanamide, synthesized at Searle, dissolved in a small volume of 1 N HCl brought to volume with H_2O to achieve a concentration of 10 mM stock), 5 μM flecainide (obtained as acetate salt from Riker 3M Labs, St Paul, MN, dissolved in H_2O to achieve a concentration of 10 mM stock) and 50 nM dofetilide (synthesized at Searle, dissolved in small volume of dimethyl sulfoxide brought to volume with H_2O to achieve a concentration of 1 mM stock). These concentrations were chosen to elicit approximately equal prolongations of APD_{90} and ERP. The interaction with isoproterenol (isoproterenol hydrochloride, Sigma Chemical Company, St Louis, MO, 10 mM stock in H_2O) was examined by equilibration of tissues with individual antiarrhythmic agent for 30 min, followed by addition of 1 μM isoproterenol for an additional 30 min. The converse experiment was performed separately in which tissues were first equilibrated with 1 μM isoproterenol followed by treatment with isoproterenol plus individual antiarrhythmic agent. Volume of isoproterenol or antiarrhythmic agents added was small (<0.5% of total volume) and did not change ionic composition of the Tyrode's solution. Due to contraction of the tissue, most measurements were made from different cells because a single impalement could not be maintained with stable resting membrane potential throughout the entire time-course of the experiment. Time control experiments were performed in which measurements were made every 30 min to determine stability of measurements over time.

Guinea Pig Atria: Functional β-Antagonism Assay

Male guinea pigs (600–700 g, Harlan Sprague Dawley, Inc., Dublin, VA) were killed by pentobarbital overdose (1.5 ml/kg, i.p., Anpro Pharmaceutical, Arcadia, CA) and the right atrium was quickly removed and placed in a 40-ml tissue bath filled with oxygenated (95% O_2/5% CO_2) Krebs's solution of the following composition (in mM): NaCl, 120; $NaHCO_3$, 20; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5 and D-glucose, 11.1. Approximately 500 mg of resting tension was placed on the tissue. The atria were allowed 1 hr equilibration with buffer changes every 15 min. Contractions of the spontaneously beating atria were measured with a force displacement transducer (model FT03, Grass Instrument Co., Quincy, MA) connected to a polygraph (model 7D, Grass Instrument Co.). Spontaneous beating rate was measured with increasing concentrations of bidisomide (3–100 μM). Atrial beats were counted for the 5 sec before the addition of the next higher concentration of compound. In a separate experiment, concentration-response curves to isoproterenol were obtained for atria in which cumulative increasing concentrations of isoproterenol (0.3 nM–1 μM) were added (each higher concentration was given when the previous concentration had reached a maximal effect). Because chronotropic effects of bidisomide alone were minimal, after completion of a control isoproterenol concentration-response curve, bidisomide (100 μM) was incubated with the tissue 15 min before repeating the isoproterenol concentration-response curve. Control experiments were also performed in which a second isoproterenol response curve was repeated in the absence of bidisomide.

Statistical Analysis

Action potential experiments. Statistical analyses were performed with the PROC MIXED test in SAS (SAS Institute, Cary, NC), used to fit mixed linear models describing data from repeated-measures experiments with fixed and random factors. A primary analysis of bidisomide, flecainide, dofetilide or isoproterenol was made for single or combination treatment. To examine whether the amount of change from base line (control) was significantly different from zero for any drug (single or treatment combination) each stimulation frequency mean was compared to zero. To determine whether there were significant differences among stimulation frequencies in the amount of change from base line for any drug or treatment combination, a simultaneous comparison among the four stimulation frequencies was made. If overall significance was found, then individual pair-wise comparisons between conditions were made. To examine whether there were significant differences between the single or combination treatments in the amount of change from base line for any stimulation frequency, pair-wise comparisons between treatment conditions were made for each stimulation frequency. The pair-wise comparisons were made using the denominator degrees of freedom from the Satterthwaite approximation. The degrees of freedom used for the above comparisons were adjusted using Huyhn-Feldt epsilon values obtained from PROC GLM (with the REPEATED statement). The Huyhn-Feldt ϵ was also used in the calculations for the Satterthwaite approximation (Milliken and Johnson, 1984). The 0.05 level of significance was used for all tests and means comparisons (which were all two-sided).

Guinea pig atria: functional β-antagonism assay. The effect of bidisomide on spontaneous beating rate of guinea pig right atrium was analyzed by calculating change from baseline using PROC MIXED in SAS to conduct a repeated measures analysis of variance. To examine the effect of bidisomide on isoproterenol concentration-

response curves, EC_{50} were calculated and compared. For each tissue and condition (isoproterenol alone and isoproterenol plus bidisomide), 0% was defined as the lowest response (MIN) and 100% was defined as the highest response (MAX). Each value was then converted to a percent maximum = 100*(value-MIN)/(MAX-MIN). A four-parameter logistic equation was used to fit a sigmoidal-shaped model to each of the concentration-response curves for each tissue. Two parameters were fixed, the lower plateau at 0% and the upper plateau at 100%. The other two parameters were estimated using PROC NLIN in SAS with the Marquardt option. The same analysis was used for the isoproterenol control experiments.

Results

Effects of antiarrhythmic drugs on canine atrial action potentials. Control experiments were performed in which APD_{90} and ERP were recorded from isolated canine atria every 30 min at each stimulation frequency. No significant changes in APD_{90} or ERP were observed at any time or stimulation frequency during the 90-min measurement period used to approximate the duration of subsequent experiments (fig. 1). No differences in APD_{90} or ERP values were observed between right or left atrial trabeculae. Tissues with different action potential configurations, *i.e.*, pacemaker-like were not used in this study.

The effects of bidisomide (30 μM), flecainide (5 μM) or dofetilide (50 nM) on canine atrial action potentials are shown in table 1. Bidisomide and flecainide both prolonged APD_{90} and ERP in a stimulation frequency-independent manner. There was no difference in the prolongation of APD_{90} or ERP by 30 μM bidisomide and 5 μM flecainide. Dofetilide (50 nM) was effective at prolonging APD_{90} and ERP only at low stimulation frequencies and the effect was inversely proportional to frequency, with the largest increase observed at the lowest stimulation frequency.

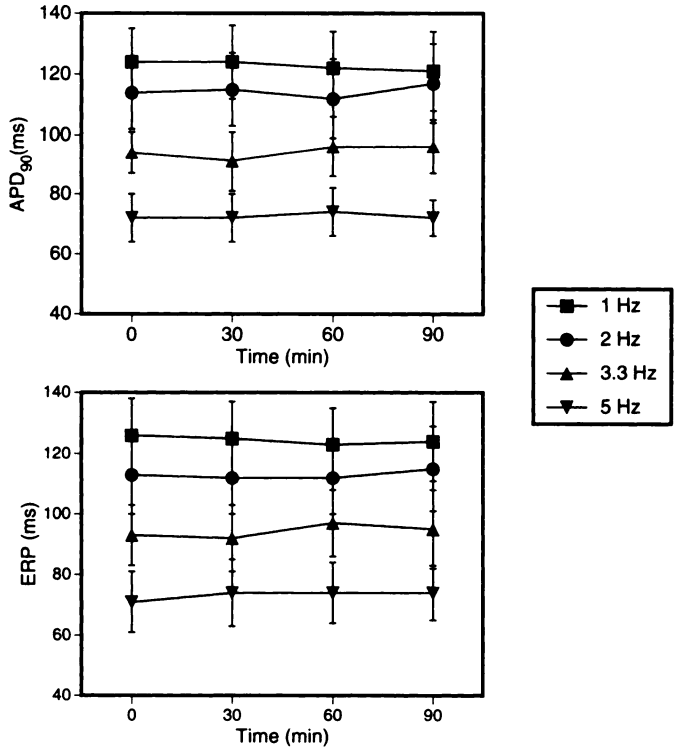

Fig. 1. Control measurements of action potential duration at 90% repolarization (APD_{90}) and effective refractory period (ERP) recorded from isolated canine atria. Values represent mean ± S.E.M. (n = 5) recorded at four stimulation frequencies over time. Time = 0 recorded after 90 to 120-min equilibration period and correlates to control records in subsequent experiments. There were no significant changes from base line at any time for any stimulation frequency.

TABLE 1

Effect of antiarrhythmic agents on action potentials recorded from isolated canine atria

Stimulation Frequency	1 Hz	2 Hz	3.3 Hz	5 Hz
ΔAPD_{90} (ms)				
Bidisomide	24 (3)*	20 (2)*	18 (2)*	18 (1)*
Flecainide	25 (4)*	21 (4)*	24 (4)*	20 (3)*
Dofetilide	40 (4)*[ac]	24 (6)*[b]	9 (4)*[c]	1 (2)[c]
ΔERP (ms)				
Bidisomide	26 (3)*	22 (3)*	21 (3)*	21 (2)*
Flecainide	27 (6)*	25 (4)*	27 (2)*	25 (2)*
Dofetilide	37 (5)*[a]	22 (6)*[b]	7 (6)[c]	0 (2)[c]

APD_{90} represents action potential duration at 90% repolarization. ERP represents effective refractory period. Values represent change from control in msec, mean (S.E.M.), absolute values are shown in Figures 2 and 3. n = 7 for 30 μM bidisomide, n = 5 for 5 μM flecainide and 50 nM dofetilide.

* Significant change from control, two-tailed test with $P < .05$.

[a,b] Significantly different from other stimulation frequencies for individual drug, $P < .05$.

[c] Significantly different from other drugs at that stimulation frequency, $P < .05$.

Effect of β-adrenergic stimulation on antiarrhythmic drugs. The effects of each agent and subsequent combination with isoproterenol on APD_{90} and ERP are shown in figures 2 and 3. The addition of isoproterenol (1 μM) did not affect the prolongation of APD_{90} or ERP by bidisomide (30 μM) at 3.3 to 5 Hz. There was some reduction of APD_{90} and ERP at 1 to 2 Hz, but the values were still significantly greater than control. The prolongation of APD_{90} and ERP by flecainide (5 μM) was completely reversed by addition of isoproterenol and APD_{90} at 1 Hz was even significantly less than control. The prolongation of APD_{90} and ERP at 1 to 2 Hz by dofetilide (50 nM) was also completely reversed by isoproterenol. There was no change from control for dofetilide or dofetilide plus isoproterenol at stimulation frequencies of 3.3 to 5 Hz.

APD_{90} and ERP data from the converse series of experiments in which tissues were preequilibrated with isoproterenol (1 μM) followed by combination with antiarrhythmic agent are shown in figures 4 and 5. For all tissues tested, 1 μM isoproterenol caused a 20 to 30% reduction in APD_{90} and ERP. Addition of bidisomide (30 μM) completely reversed the shortening of APD_{90} and ERP at all stimulation frequencies (no significant differences from control values). The prolongation of APD_{90} and ERP obtained at 5 Hz was statistically greater than that obtained at the lower stimulation frequencies. Flecainide (5 μM) did not reverse the effect of 1 μM isoproterenol on APD_{90} or ERP. However, there was a small, but statistically significant, prolongation of APD_{90} (1, 2 Hz) and ERP (1, 2, 5 Hz) over isoproterenol alone, although all values were still significantly less than control. Dofetilide (50 nM) caused some prolongation of APD_{90} and ERP at 1 to 2 Hz after equilibration with isoproterenol, but the values were still significantly less than control.

Functional β-antagonism assay in guinea pig atrium. The stability and reproducibility of the isoproterenol concentration-response curves on spontaneous beating rate was examined by performing two sequential concentration-response curves for isoproterenol in each tissue. There were

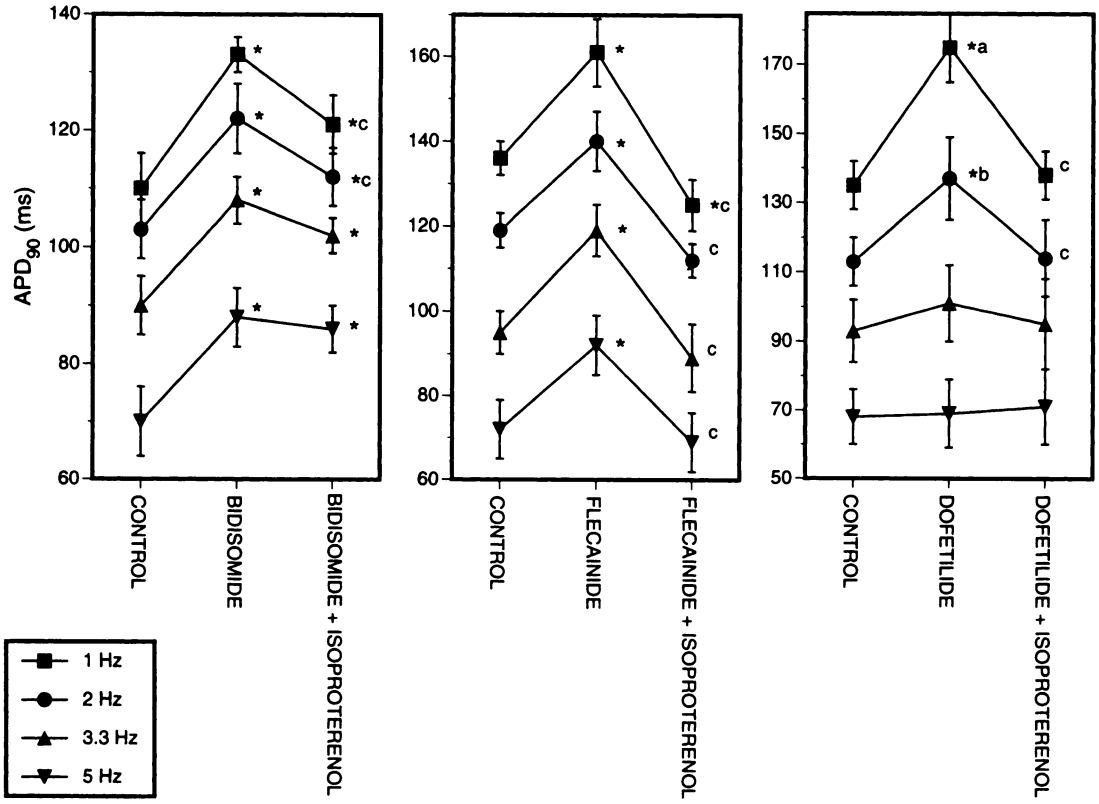

Fig. 2. Effects of bidisomide, flecainide or dofetilide on action potential duration at 90% repolarization (APD_{90}) recorded from isolated canine atria before and after challenge with 1 μM isoproterenol. Values represent mean ± S.E.M. recorded at four stimulation frequencies (1, 2, 3.3 and 5 Hz). Different tissues were used for each experiment, for 30 μM bidisomide, n = 7; for 5 μM flecainide, n = 5 and for 50 nM dofetilide, n = 5. Equilibration time for each agent and isoproterenol was 30 min. *Significant change from control, $P < .05$. [a,b]Significantly different from other stimulation frequencies for individual agent. [c]Significantly different from agent alone at that stimulation frequency.

no significant differences in the EC_{50} obtained in the first and second isoproterenol concentration-response curves (n = 4, first EC_{50} = 2.7 ± 0.7 and second EC_{50} = 1.7 ± 0.2 nM).

The effects of bidisomide on spontaneous beating rate are presented in table 2. The change from base line was significantly different from zero for bidisomide (100 μM), but this was not significantly different from the rundown observed in the matched vehicle control.

The effect of bidisomide on the isoproterenol concentration-response curve was examined. There were no significant differences in the EC_{50} for isoproterenol obtained before (n = 4, EC_{50} = 4.1 ± 1.4 nM) or after (2.5 ± 1.1 nM) exposure to 100 μM bidisomide. The maximal response to isoproterenol was the same in the presence or absence of bidisomide (increase of 153 ± 16 beats/min and 159 ± 8 beats/min, respectively).

Discussion

In this study, three different antiarrhythmic agents that increase atrial refractoriness were compared, bidisomide, flecainide and dofetilide.

Bidisomide is new antiarrhythmic agent that has been tested clinically for paroxysmal supraventricular tachycardia and atrial flutter/fibrillation. Bidisomide has been shown to reduce action potential upstroke velocity in a frequency-independent manner in isolated tissue (Martin *et al.*, 1989; Martin and Chinn, 1991), as well as slow conduction in intact animal experiments (Garthwaite *et al.*, 1992). Bidisomide increased canine atrial, but not ventricular, ERP in isolated tissue and intact animal experiments (Garthwaite *et al.*, 1992; Martin and Chinn, 1994). Bidisomide had no effect on APD and ERP of isolated guinea pig atrium and shortened APD and ERP in isolated guinea pig ventricle and canine Purkinje fiber (Martin *et al.*, 1989; Martin and Chinn, 1994).

Flecainide, although also indicated for paroxysmal atrial fibrillation and flutter and paroxysmal supraventricular tachycardia, has long been characterized by its class Ic antiarrhythmic activity in the ventricle. Flecainide slows action potential upstroke velocity in a use-dependent manner (Borchard and Boisten, 1982; Campbell and Vaughan Williams, 1983) and has been shown to be a potent blocker of activated sodium channels (Anno and Hondeghem, 1990; Kojima *et al.*, 1989; Rouet and Ducouret, 1994). In anethestized dog experiments, flecainide has been shown to increase atrial refractoriness with the largest increases at fast stimulation rates, but had no effect on ventricular ERP (O'Hara *et al.*, 1992). Flecainide has been shown to prolong APD and ERP in isolated canine ventricle and atrium but to shorten APD and ERP in canine Purkinje fiber (Ikeda *et al.*, 1985; Wang *et al.*, 1990). Experiments from single cells indicate that flecainide also blocks the delayed rectifier (probably I_{Kr}) (Follmer and Colatsky, 1990; Follmer *et al.*, 1992; Slawsky and Castle, 1994; Yang *et al.*, 1994) and the transient outward current (Slawsky and Castle, 1994; Wang *et al.*, 1995) in addition to sodium channels.

Dofetilide is a new class III antiarrhythmic under clinical

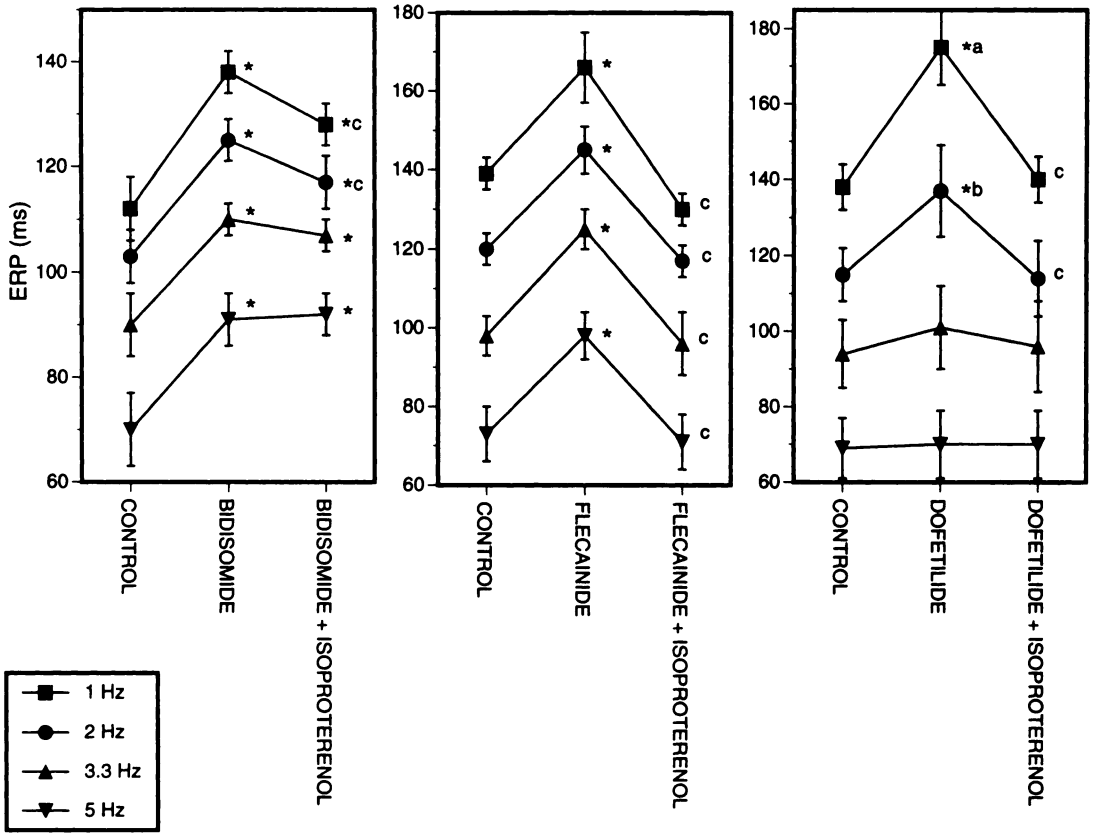

Fig. 3. Effects of bidisomide, flecainide or dofetilide on effective refractory period (ERP) recorded from isolated canine atria before and after challenge with 1 μM isoproterenol. Values represent mean ± S.E.M. recorded at four different stimulation frequencies (1, 2, 3.3 and 5 Hz). These data obtained from the same experiment as figure 2, for 30 μM bidisomide, $n = 7$, for 5 μM flecainide, $n = 5$ and for 50 nM dofetilide, $n = 5$. Equilibration time for each agent and isoproterenol was 30 min. *Significant change from control, $P < .05$. [a,b]Significantly different from other stimulation frequencies for individual agent. [c]Significantly different from agent alone at that stimulation frequency.

development for ventricular and supraventricular arrhythmias, including atrial fibrillation and flutter (Rasmussen *et al.*, 1992). Dofetilide has been shown to increase ERP in intact canine ventricle and atrium (Rasmussen *et al.*, 1992; Spinelli *et al.*, 1992), as well as ERP and APD in isolated canine ventricle and Purkinje fibers. (Gwilt *et al.*, 1991; Knilans *et al.*, 1991). Experiments from isolated guinea pig or rabbit ventricle have indicated that dofetilide specifically blocks the fast component of the delayed rectifier, I_{Kr} (Carmeliet, 1992; Jurkiewicz and Sanguinetti, 1993; Kiehn *et al.*, 1994).

Effects on canine action potentials. In our study, the concentrations of the three agents studied (bidisomide at 30 μM, flecainide at 5 μM and dofetilide at 50 nM) were chosen to approximate a similar prolongation of canine atrial ERP. Bidisomide at 30 μM has been shown to prolong atrial ERP 20 to 25 msec in isolated canine atrium (1–5 Hz) (Martin and Chinn, 1994), although in the intact animal, a plasma concentration range of 42 to 52 μM produced a 47-msec increase in atrial ERP (3.3 Hz) (Garthwaite *et al.*, 1992). Bidisomide has been shown to terminate atrial flutter in a chronically instrumented dog model at a plasma concentration of approximately 24 μM (Spinelli and Hoffman, 1989). Flecainide, in the same atrial flutter model, was effective at terminating the arrhythmia at a plasma concentration of approximately 7 μM (Spinelli and Hoffman, 1989). In isolated canine atrial tissue, 4.5 μM flecainide prolonged APD by 20 to 30 msec (Wang *et al.*, 1993; Wang *et al.*, 1990). The concentration of dofetilide was approximated based on effects in isolated canine ventricular ERP (72 msec prolongation at 1 Hz with 100 nM dofetilide) (Gwilt *et al.*, 1991).

In our experiments, there were no significant differences in the prolongation of ERP among the three antiarrhythmic agents at 1 to 2 Hz stimulation frequencies at the concentrations tested. Bidisomide and flecainide prolonged APD_{90} and ERP (expressed in msec, not percent) to the same degree and in a frequency-independent manner. Importantly, both drugs increased refractoriness at high stimulation frequencies that is important and desirable for effectiveness at the elevated heart rates observed during an arrhythmia.

Flecainide has been reported to prolong atrial ERP in a positively rate-dependent manner in isolated canine atrium (Wang *et al.*, 1993; Wang *et al.*, 1990). Data presented here were examined as absolute values and not as % control so as to be a more rigorous test of "reverse use dependence" (interpreted as APD_{90} or ERP prolongation, not as channel block) (Hondeghem and Snyders, 1990). The prolongation of ERP by flecainide in our experiments, expressed as percentage increase in ERP, ranged from 19% at 1 Hz to 34% at 5 Hz, similar to previous reports (Wang *et al.*, 1993; Wang *et al.*, 1990). Prolongation of APD_{90} and ERP by dofetilide, expressed as increase in msec, exhibited "reverse use dependence" in which the prolongation obtained at 1 Hz was more than 2 Hz which was greater than the lack of prolongation at 3.3 to 5 Hz. Several studies in isolated tissues, primarily ventricular, from different species have indicated that the

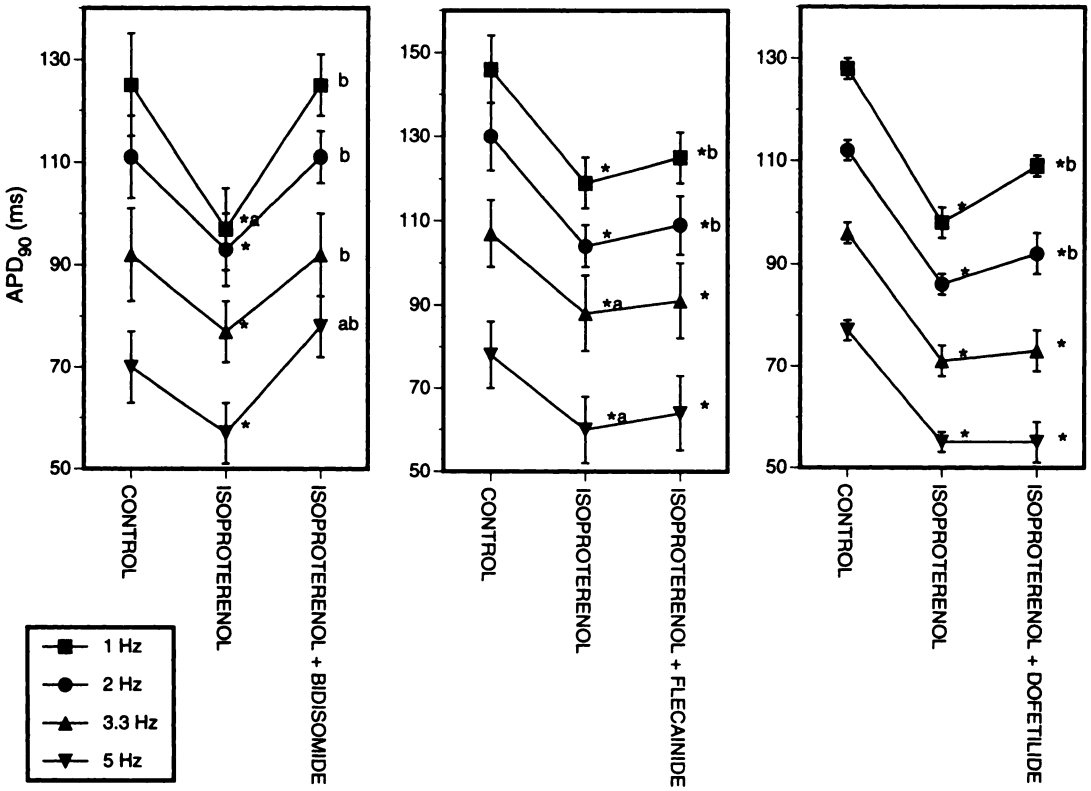

Fig. 4. Effects of 1 μM isoproterenol alone and in the presence of bidisomide, flecainide or dofetilide on action potential duration at 90% repolarization (APD_{90}) recorded from isolated canine atria. Values represent mean ± S.E.M. measured at four different stimulation frequencies (1, 2, 3.3 and 5 Hz). Different tissues were used for each experiment, for 30 μM bidisomide, $n = 7$; for 5 μM flecainide, $n = 5$ and for 50 nM dofetilide, $n = 5$. Each equilibration period was 30 min. *Significant change from control, $P < .05$. [a]Significantly different from other stimulation frequencies for individual agent. [b]Significantly different from isoproterenol alone at that stimulation frequency.

prolongation of APD or ERP by dofetilide decreases with an increase in stimulation frequency (over a range of stimulation frequencies of ≤1–≥3 Hz) (Baskin *et al.*, 1991; Gwilt *et al.*, 1991; Jurkiewicz and Sanguinetti, 1993; Knilans *et al.*, 1991; Spinelli *et al.*, 1993; Tande *et al.*, 1990). In one clinical study in which atrial refractoriness was measured at two different stimulation frequencies, dofetilide (approximately 9 nM plasma concentration) prolonged atrial refractory period more at the higher stimulation frequency (2.22 Hz *vs.* 1.67 Hz) (Sedgwick *et al.*, 1992). These results may suggest important species differences, different effects based on the model used, or that higher stimulation frequencies in humans are needed for correlation with *in vitro* animal data.

Effect of β-adrenergic stimulation. The sympathetic nervous system has been shown in a variety of different studies to be an important factor in atrial and ventricular arrhythmogenesis (Corr *et al.*, 1986; Daly and Sole, 1990; Podrid *et al.*, 1990; Scher and Arsura, 1989). This is exemplified by the popularity of β-adrenergic blocking agents to prevent lethal arrhythmias, especially because of CAST (Coumel *et al.*, 1986; Echt *et al.*, 1991; Kennedy *et al.*, 1994; Singh, 1993b). Circulating catecholamines and subsequent β-adrenergic stimulation have many different effects on the human heart that make it a complicated system to modulate in treating arrhythmias (Clarkson *et al.*, 1995). On a cellular level, β-adrenergic stimulation affects many different ionic currents including calcium, potassium, sodium and chloride currents (*e.g.*, Bennett and Begenisich, 1987; Giles *et al.*, 1989; Gintant and Liu, 1992; Harvey *et al.*, 1990; Kirstein *et al.*, 1991; Matsuda *et al.*, 1993; Schackow and Ten Eick, 1994; Shumaker *et al.*, 1991). Isoproterenol has been shown to affect the APD in a concentration-dependent manner with an increase of APD at low concentrations (presumably an increase in calcium current and possibly chloride current) and a shortening of APD at high concentrations (presumably due to activation of potassium currents) (Priori and Corr, 1990). In our study, a concentration of 1 μM was chosen because it caused 20 to 30% decrease in APD, and it prevented prolongation of refractory period by E-4031, an agent that specifically blocks I_{Kr} (Jurkiewicz and Sanguinetti, 1993; Liang *et al.*, 1985; Priori and Corr, 1990).

In our experiments, canine atrial tissues were equilibrated with antiarrhythmic agent followed by the addition of isoproterenol. Prolongation of APD_{90} or ERP with flecainide or dofetilide was reversed by the addition of isoproterenol, although the effects of bidisomide were not. In the converse set of experiments, canine atrial tissues were equilibrated with isoproterenol, which produced the expected reduction of APD_{90} and ERP. Addition of flecainide or dofetilide in the presence of isoproterenol did not return APD_{90} or ERP values to pretreatment values. There was some prolongation of APD and ERP by flecainide and dofetilide, especially at low stimulation frequencies, over isoproterenol alone, presumably due to I_{Kr} block, although that current is not increased by isoproterenol (Sanguinetti *et al.*, 1991). Bidisomide, however, completely reversed the reduction of APD_{90} and ERP caused by isoproterenol. Bidisomide was inactive in a model of functional β-adrenergic antagonism, similar to that previously

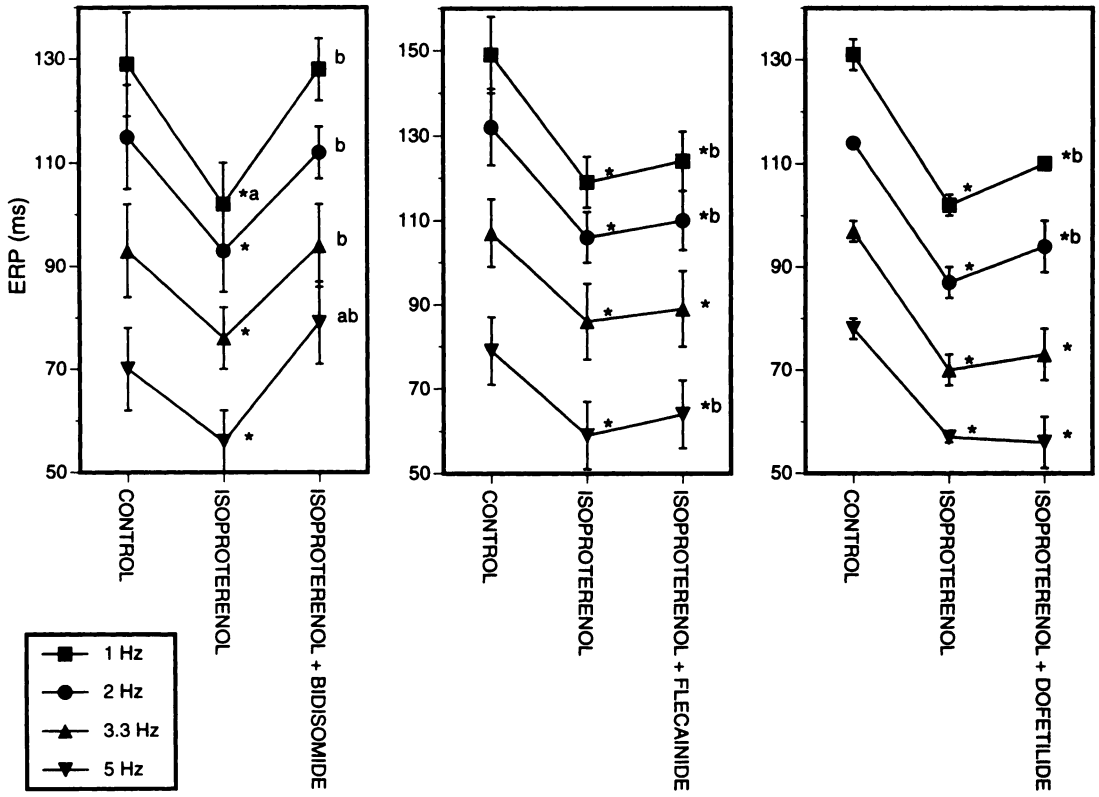

Fig. 5. Effects of 1 μM isoproterenol alone and in the presence of bidisomide, flecainide or dofetilide on effective refractory period (ERP) recorded from isolated caine atria. Values represent mean ± S.E.M. measured at four different stimulation frequencies (1, 2, 3.3 and 5 Hz). These data from the same experiment as figure 4, for 30 μM bidisomide, $n = 7$; for 5 μM flecainide, $n = 5$ and for 50 nM dofetilide, $n = 5$. Each equilibration period was 30 min. *Significant change from control, $P < .05$. [a]Significantly different from other stimulation frequencies for individual agent (for bidisomide experiment, 2 Hz value in isoproterenol alone was not different from 1 Hz or 3.3 and 5 Hz). [b]Significantly different from isoproterenol alone at that stimulation frequency.

TABLE 2

Effect of bidisomide on spontaneous beating rate of guinea pig atria

	Concentration (μM)			
	3	10	30	100
Bidisomide	3 (3)	−6 (3)	−9 (3)	−21 (6)[a]
Vehicle control	−9 (9)	−6 (6)	−12 (10)	−12 (13)

Values represent change from control or base line in beats/min, mean (S.E.M.), $n = 4$. Baseline values were 144 ± 8 and 153 ± 19 beats/min for bidisomide and vehicle experiments, respectively.

[a] Significant change from control, two-tailed test with $P < .05$.

reported for dofetilide, indicating its mechanism was not via simple receptor antagonism (Yang *et al.*, 1991).

A clinical study reported that infusion of isoproterenol prevented prolongation of ventricular monophasic APD and ERP induced by class III agents, suggesting that a pure class III effect [such as block of I_{Kr}, which is not enhanced with isoproterenol (Sanguinetti *et al.*, 1991)] would be undesirable in a clinical setting (Newman *et al.*, 1993). A comparison of *d*-sotalol, which blocks I_{Kr} and has little β-blocking effect, to *dl*-sotalol, which blocks I_{Kr} and has significant β-blocking activity, indicated that only *dl*-sotalol was effective at prolonging APD_{90} with the addition of isoproterenol (Groh *et al.*, 1995). Our results would indicate that flecainide and dofetilide, both of which have been shown to block I_{Kr}, may not be effective in conditions in which sympathetic tone is elevated. It is interesting that pretreatment of isolated canine atria with dofetilide prevented a reduction of APD_{90} or ERP by isoproterenol at 3.3 to 5 Hz (see figs. 2 and 3). This action could be a useful antiarrhythmic mechanism if, at elevated heart rates, dofetilide prevents a reduction of refractoriness upon β-adrenergic stimulation.

Clinical and animal studies indicate that a combination of a class III antiarrhythmic agent with a β-adrenergic blocking agent may be an effective treatment for arrhythmias via prolongation of refractory period (Newman *et al.*, 1993; Sanguinetti *et al.*, 1991), and it has been suggested that perhaps a compound with multiple mechanisms may be the most useful antiarrhythmic agent (Singh, 1993a). Our data indicate that bidisomide could have the advantages of a "β-blocker" combined with a class III antiarrhythmic effect on atrial tissue, even at elevated heart rates. Bidisomide has been shown to be effective at prolonging refractoriness at high stimulation frequencies, both with and without β-adrenergic stimulation in isolated canine atria, which would be necessary during elevated heart rates observed before and during arrhythmias.

In summary, these results suggest that bidisomide would be effective in the presence of elevated sympathetic tone. Thus, of the agents tested, only bidisomide possessed a unique and desirable antiarrhythmic profile.

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