Atrial-selective sodium channel block strategy to suppress atrial fibrillation.

Ranolazine versus propafenone.

Alexander Burashnikov, Luiz Belardinelli, Charles Antzelevitch

Masonic Medical Research Laboratory, Utica, NY (AB, CA) and

*Gilead Sciences, Palo Alto, CA (LB).
RUNNING PAGE TITLE

Running title: Atrial-Selective Sodium Channel Block

Number of:
Pages 26
Table 1
Figures 6
References 38
Words in abstract 223
Words in Introduction 179
Words in Discussion 1727

Address for correspondence:

Dr. Alexander Burashnikov, PhD, FHRS
Masonic Medical Research Laboratory
2150 Bleecker Street
Utica, N.Y. 13501
Phone: (315)735-2217
FAX: (315)735-5648
E-mail: sasha@mmrl.edu

Non-standard abbreviation: ADP = action potential duration; AF = atrial fibrillation; AP = action potential; CL = cycle lengths cycle lengths; CT – conduction time; DTE = diastolic threshold of excitation; ERP = effective refractory period; PRR = post-repolarization refractoriness; RA – right atria; V_{max} = maximum rate of rise of the AP upstroke

Section assignment: Cardiovascular
ABSTRACT

Ranolazine has been shown to produce atrial-selective depression of sodium channel-dependent parameters and to suppress atrial fibrillation (AF) in a variety of experimental models. The present study contrasts the effects of ranolazine and those of a clinically-used anti-AF Class IC agent, propafenone. Electrophysiological and anti-AF effects of propafenone and ranolazine were compared at clinically relevant concentrations (i.e., 0.3-1.5 µM and 1-10 µM, respectively) in canine isolated coronary-perfused atrial and ventricular preparations. Transmembrane action potential and pseudo-ECG were recorded. Both ranolazine and propafenone produced atrial-selective prolongation of action potential duration (APD$_{90}$). Propafenone depressed sodium channel-mediated parameters ($V_{\text{max}}$, conduction time, diastolic threshold of excitation) and induced post-repolarization refractoriness to a greater degree than ranolazine and these effects, unlike those induced by ranolazine, were not or only mildly atrial-selective at normal rates (CL = 500 ms). At fast pacing rates, however, the effects of propafenone on $V_{\text{max}}$ and conduction time become atrial-selective, due to elimination of diastolic interval in atria, but not in ventricles. Propafenone (1.5 µM) and ranolazine (10.0 µM) were effective in preventing the initiation of persistent acetylcholine-mediated AF (6/7 and 9/11 atria), its termination (8/10 and 8/12 atria), and subsequent re-induction (8/8 and 7/8 atria, respectively). Thus, propafenone and ranolazine both suppress AF, but ranolazine, unlike propafenone, does it with minimal effects on ventricular myocardium suggesting a reduced potential for promoting ventricular arrhythmias.
INTRODUCTION:

A limitation of the use of the currently available anti-atrial fibrillation (AF) agents is the risk of induction of ventricular arrhythmias. This has prompted the development of atrial-specific antiarrhythmic agents. We have recently shown that ranolazine, an antianginal agent possessing antiarrhythmic properties (Antzelevitch, et al., 2004), selectively affects sodium-channel dependent parameters in canine atria vs. ventricles and effectively suppresses AF in vitro (Burashnikov, et al., 2007). Similar atrial selectivity of ranolazine as well as its anti-AF efficacy have been demonstrated in the porcine heart in vivo (Kumar, et al., 2009; Carvas, et al., 2010). Consistent with these experimental observations, clinical studies have shown anti-AF efficacy of ranolazine (Scirica, et al., 2007; Murdock, et al., 2008; Murdock, et al., 2009; Murdock, et al., 2010). Propafenone, a potent sodium channel blocker, is used for termination as well as prevention of AF in the clinic (Fuster, et al., 2011; Alboni, et al., 2004). The aim of the present study was to compare the electrophysiological effects of ranolazine and propafenone in isolated canine coronary-perfused atrial and ventricular preparations, and their anti-AF efficacy in an experimental model of AF.
METHODS:

This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85-23, Revised 1996) and was approved by the ACUC of the Masonic Medical Research Laboratory. Experiments were performed using isolated arterially-perfused canine right atrial (RA) preparations and left ventricular (LV) arterially-perfused wedge preparations (≈ 2x1x1 cm). Detailed methods for isolation and perfusion of these preparations have been described in previous publications (Antzelevitch, et al., 2004; Burashnikov, et al., 2004). Briefly, the preparations were dissected from hearts removed from anesthetized (sodium pentobarbital) adult mongrel dogs (20-25 kg). Unfolded RA with a rim of the right ventricle was cannulated and perfused through the ostium of the right coronary artery and the LV wedge was perfused through a branch of the left coronary artery. Unperfused tissue was removed with a razor blade or scissors. The cut ventricular and atrial branches were ligated using silk thread. After these procedures (performed in cold cardioplegic solution, 4-8°C), the preparations were transferred to a temperature-controlled bath and arterially perfused with Tyrode’s solution by use of a roller pump. The composition of the Tyrode’s solution was (in mM): NaCl 129, KCl 4, NaH2PO4 0.9, NaHCO3 20, CaCl2 1.8, MgSO4 0.5, and D-glucose 5.5, buffered with 95% O2 and 5% CO2 (37±0.5°C, pH=7.35)

Transmembrane action potential (AP) recordings (sampling rate 41 kHz) were obtained using standard or floating glass microelectrodes (2.7 M KCl, 10-25 MΩ DC resistance). A pseudo-electrocardiogram (ECG) was recorded using two electrodes consisting of Ag/AgCl half cells placed in the Tyrode’s solution bathing the preparation, 1.0 to 1.2 cm from opposite ends of the atrial or ventricular coronary-perfused preparations (Fig. 1 and 2). Conduction time was approximated by measuring the duration of the “P wave” complex in atria and the “QRS”
complex in ventricles on the ECG at a level representing 10% and 90% of “P wave” or “QRS” amplitude. **Diastolic threshold of excitation (DTE)** was determined by increasing stimulus intensity in 0.01 mA steps. Maximum amplitude of stimulation used for the study was ten times of the DTE determined in the beginning of each experiment. **Effective refractory period (ERP)** was measured by delivering premature stimuli after every 10th regular beat at a pacing cycle length (CL) of 500 and 300 ms (with 10 ms resolution; stimulation with a 2xDTE amplitude, determined at each CL). **Post-repolarization refractoriness (PRR)** was considered to be present when ERP exceeded APD$_{90}$ in the ventricle and APD$_{75}$ in atria. Under control conditions, ventricular ERP coincides with APD$_{90}$, whereas atrial ERP generally coincides with APD$_{75}$.

**Maximum rate of rise of the AP upstroke (V$_{max}$):** Stable AP recordings and V$_{max}$ measurements are difficult to obtain in vigorously contracting perfused preparations. In coronary-perfused atria and ventricles, the effects of propafenone and ranolazine on V$_{max}$ were determined by comparing the largest V$_{max}$ recorded at any given condition. Due to a substantial inter-preparation variability, V$_{max}$ values were normalized for each experiment and then averaged.

**Experimental Protocols.** The equilibration period for the preparations was 30-60 min. The concentrations of ranolazine (1.0, 5.0, 10.0 µM) and propafenone (0.3 and 1.5 µM) were increased in a step-wise manner, with at least 20 min for ranolazine and 40 min for propafenone at each concentration prior to start collecting the data. This difference reflects the various exposure durations that are required to achieve a steady-state in electrophysiological actions of the agents (Antzelevitch, et al., 2004; Delgado, et al., 1985). The concentrations of the drugs used are within the therapeutic relevant ranges of ranolazine and propafenone achieved when the drugs are prescribed at their recommended doses (Antzelevitch, et al., 2011; Grant, 1996). To compare the antiarrhythmic potential of propafenone and ranolazine, we used an acetylcholine...
(0.5-1.0 µM) - dependent AF model in coronary-perfused right atria, where persistent AF is inducible in 100% of preparations (by a single premature beat or rapid pacing) (Burashnikov and Antzelevitch, 2003; Burashnikov, et al., 2007). We determined the effect of propafenone and ranolazine to prevent (series 1) the induction of AF as well as, in different preparations, the effect of these drugs to terminate (series 2) persistent AF. In the first series, ACh was added to the perfusate ≥40 min after the start of 1.5 µM propafenone and ≥20 min after start of 10 µM ranolazine, followed by attempts to induce arrhythmias using programmed electrical stimulation (PES). In the second series, the effect of propafenone or ranolazine to terminate persistent AF was tested by adding these drugs to the coronary perfusate solution following 5-8 min of persistent AF. After AF termination, re-induction of the arrhythmia was attempted using PES.

**Drugs:** Ranolazine (Gilead Sciences, Palo Alto, CA), propafenone, and acetylcholine (both SIGMA, MO) were all dissolved in distilled water and each was prepared fresh as a stock of 1-10 mM before each experiment.

**Statistics:** Statistical analysis was performed using paired or unpaired t tests and one way repeated measures or multiple comparison analysis of variance (ANOVA) followed by Bonferroni’s test, as appropriate. All data are expressed as mean ± SD.
RESULTS:

Propafenone vs. ranolazine: APD\textsubscript{90}, ERP, PRR, DTE, V\textsubscript{Max} and CT

Both propafenone and ranolazine produced atrial, but not ventricular prolongation of APD\textsubscript{90} (Fig. 1). Propafenone rate-dependently lengthened ERP much more than APD\textsubscript{75-90} in both atria and ventricles, thereby inducing a significant PRR in both chambers (Fig. 2). Ranolazine induced rate-dependent atrial-selective prolongation of ERP and the development of PRR only in atria (Fig. 2).

DTE was significantly increased by propafenone in both atria and ventricles at 500 and 300 CLs (Fig. 3). In contrast, ranolazine increased DTE selectively in atria, with a much greater increase at 300 vs. 500 ms CL (Fig. 3). Propafenone potently depressed V\textsubscript{max} and increased conduction time similarly in atria and ventricles at a CL of 500 ms, but altered these parameters to a greater extent in atria vs. ventricles at a CL of 300 ms (Fig. 4 and 5). Ranolazine produced atrial-selective reduction in V\textsubscript{max} and increase in conduction time, which was more pronounced at faster pacing rates (Fig. 4 and 5). Ranolazine produced a weaker depression of V\textsubscript{max} and conduction velocity, compared to propafenone. Figure 4A reveals the mechanisms contributing to the atrial-selectivity of propafenone and ranolazine to reduce V\textsubscript{max} at rapid pacing rates. Because of the slow repolarization phase of the atrial action potential and the effect of both propafenone and ranolazine to further slow phase 3, acceleration of rate leads to elimination of the diastolic interval in atria but not in the ventricles. Because much of the recovery from sodium channel block occurs during the diastolic interval, greater accumulation of block occurs in atria vs. ventricles at rapid rates of activation.

Propafenone vs. ranolazine: Anti-AF action
ACh (0.5 µM) alone significantly abbreviated atrial APD_{90} (from 198±17 to 45±9 ms, p<0.001, n=10 for each, PM, CL = 500 ms) and ERP (from 149±12 to 51±7 ms, p<0.001, n=10 for each, PM, CL = 500 ms), permitting the induction of persistent AF in 100% of atria (10/10 atria). Addition of ACh (0.5 µM) to atrial preparations pretreated with propafenone (1.5 µM) and ranolazine (10 µM) abbreviated APD and ERP to an extent that was less than that observed in the absence of propafenone and ranolazine (Table). Under these conditions, both agents were very effective in preventing the induction of persistent ACh-mediated AF, with propafenone being slightly more effective compared to ranolazine (Table). In a different set of atrial preparations, we tested the effect of the two drugs to terminate persistent ACh-mediated AF. The addition of propafenone (1.5 µM) or ranolazine (10.0 µM) to the perfusion solution on the 5-8th min of persistent AF terminated the arrhythmia in 8/10 and 8/12 atria, respectively (Fig. 6, Table). Average time for AF termination was 7±5 min for propafenone and 14±7 min for ranolazine. Both propafenone and ranolazine effectively prevented the re-induction of persistent AF (Table). Brief episodes of non-sustained AF or atrial flutter (< 1 min duration) could still be induced in 3/8 and 5/8 atria in the presence of propafenone and ranolazine, respectively. These anti-AF actions of propafenone and ranolazine were associated with rate-dependent depression of excitability, making it impossible for the atria to beat at rapid rates such as those during AF (Fig. 6).
DISCUSSION

The main result of the current experimental study is that while both ranolazine and propafenone effectively terminate ACh-mediated AF and prevent the induction of the arrhythmia, ranolazine, in contrast to propafenone, does it without producing significant electrophysiological effects in the ventricles.

Atrial selective sodium channel block and AF suppression

The risk of induction of ventricular proarrhythmia and/or organ toxicity is a major limitation of currently clinically available anti-AF agents (Fuster, et al., 2011). The availability of atrial-specific or atrial-selective agents could obviate this problem. Block of $I_{Kur}$ has long been considered to be a promising atrial-selective approach for the management of AF. However, recent studies indicate that “pure” $I_{Kur}$ block is unlikely to be effective in suppressing AF (Burashnikov and Antzelevitch, 2008c; Burashnikov and Antzelevitch, 2008b; Burashnikov and Antzelevitch, 2010; Ravens and Wettwer, 2011; Pandit, et al., 2011).

We recently provided evidence in support of the hypothesis that atrial selective sodium channel block may effectively suppress AF without inducing ventricular arrhythmias (Burashnikov, et al., 2007). This concept stemmed from the finding that certain biophysical properties (e.g., steady-state inactivation) of the sodium channels and of action potential morphology in atria differ from those in the ventricles (Burashnikov, et al., 2007). Both ranolazine and amiodarone were shown to “take advantage” of these distinctions, producing significant depression of sodium channel-dependent parameters in canine atrial, but not in ventricular preparations, thus leading to effective suppression of AF at concentrations causing minimal to no effect on ventricular electrophysiology (Burashnikov, et al., 2007; Burashnikov, et al., 2008; Sicouri, et al., 2009; Antzelevitch, et al., 2011). The atrial selectivity of ranolazine as
well as its anti-AF efficacy have been demonstrated in both in vitro and in vivo animal studies (Burashnikov, et al., 2007; Kumar, et al., 2009; Szel, et al., 2011; Carvas, et al., 2010) Atrial-selective depression of sodium channel-mediated parameters in the canine heart has also been reported with exposure to chronic amiodarone and acute AZD1305 (Burashnikov, et al., 2008; Burashnikov, et al., 2010). In a large clinical study in patients with non-ST segment elevation acute coronary syndrome (MERLIN-TIMI 36), treatment with ranolazine was associated with reduced incidence of supraventricular arrhythmias and a 30% reduction in new onset AF (Scirica, et al., 2007). A number of relatively small clinical studies have shown a potent anti-AF efficacy of ranolazine for termination of paroxysmal AF (Murdock, et al., 2008; Murdock, et al., 2009; Murdock, et al., 2010). In an exploratory (not placebo-randomized control) clinical study, ranolazine was found to be more effective than amiodarone in preventing post-operative AF (AF incidence was 17.5 vs. 26.5%, respectively) (Miles, et al., 2011).

Ranolazine can inhibit early \( I_{Na} \), late \( I_{Na} \), \( I_Kr \), and late \( I_{Ca} \) (Antzelevitch, et al., 2004; Burashnikov, et al., 2007). Inhibition of early \( I_{Na} \) by ranolazine is atrial-selective and markedly rate-dependent, which is consistent with the differential effect (atria vs. ventricle) of ranolazine on \( V_{max} \) (Figure 4), P-wave and QRS duration (Figure 5) (Zygmunt, et al., 2011). At therapeutically relevant concentrations (1-10 µM) ranolazine possesses anti-arrhythmic properties in the ventricles, primarily due to its potent effect to inhibit late \( I_{Na} \) (Antzelevitch, et al., 2004; Wu, et al., 2004; Antzelevitch, et al., 2011), whereas in the atria, principally due to its effect to inhibit early \( I_{Na} \) (Burashnikov, et al., 2007). Unlike block of peak \( I_{Na} \), inhibition of late \( I_{Na} \) does not directly affect peak \( I_{Na} \)-mediated parameters such as \( V_{max} \), PRR, and DTE.

Propafenone is a well-studied and clinically used Class IC antiarrhythmic agent, which suppresses AF and prevents its recurrence, due largely to its ability to potently block early \( I_{Na} \)
Within a therapeutic range of concentrations, in addition to $I_{Na}$, propafenone also produces relatively mild inhibition of $I_{Kf}$, $I_{to}$, $I_{Ca}$, and β-adrenoreceptors (Grant, 1996). We demonstrate an effect of propafenone to potently depress $I_{Na}$-mediated parameters in both atrial and ventricular preparations at moderate to slow pacing rates. This is in sharp contrast to the effects of ranolazine to produce a potent depression of $I_{Na}$-mediated parameters only in atria. However, the anti-AF efficacy of ranolazine was only slightly less than that of propafenone (Table).

The electrophysiological effects of both propafenone and ranolazine at clinically-relevant concentrations, pacing rates, and temperature are due largely to inhibition of peak $I_{Na}$ and $I_{Kf}$. The functional potency of these drugs to inhibit peak $I_{Na}$ and $I_{Kf}$ depends critically on the heart chamber studied as well as pacing rate. Physiologically relevant IC$_{50}$ values for comparison of ranolazine and propafenone are not available at present. Previous studies have reported IC$_{50}$ values for ranolazine inhibition of peak $I_{Na}$ of 285±170 and 286±150 µM for atrial and ventricular cells, respectively; however, the data were obtained at a slow pacing rate, 15°C, and -140 mV holding potential (Zygmunt, et al., 2011). Judging from the effects of the drugs on peak $I_{Na}$-mediated parameters ($V_{max}$, PRR, DTE, etc), the potency of propafenone to block peak $I_{Na}$ in the ventricles is much greater than that of ranolazine. Differences in the relative potency of the two agents to suppress peak $I_{Na}$ in the atria is less obvious, particularly at a CL of 300 ms. The potency of propafenone and ranolazine to inhibit $I_{Kf}$ appears roughly comparable in that both agents produce a similar prolongation of APD$_{90}$ in atria and no change in the ventricles.

The ranolazine data reported in the current study are very similar to those previously published by our group (Burashnikov, et al., 2007) (both studies were conducted in canine coronary-perfused right atrial preparations and utilized similar experimental protocols).
Atrioventricular electrophysiological differences on the effect of propafenone are poorly investigated, but the available data are consistent with the results of the present study. Propafenone reduces $V_{\text{max}}$ and induces PRR similarly in atrial and ventricular guinea pig superfused preparations (Delgado, et al., 1985). GE-68, an analog of propafenone, selectively prolongs atrial APD, but depresses $V_{\text{max}}$ to a similar extent in atrial and ventricular guinea pig superfused preparations (Lemmens-Gruber, et al., 1997).

Propafenone is used for both conversion of paroxysmal AF and for long term maintenance of sinus rhythm in AF patient with relatively healthy hearts (Fuster, et al., 2011;Alboni, et al., 2004). One of the clinical uses of propafenone is in the so-called “pill-in-the-pocket” approach (Alboni, et al., 2004). A disadvantage of propafenone is that its use is contraindicated in patients with structural heart disease (conditions encountered in many patients with AF) due to the risk of induction of life-threatening ventricular arrhythmias. Recent clinical studies suggest that a single dose of 2000 mg ranolazine may be effective as a “pill-in-the-pocket” approach, converting 77% of AF patients, including patients with structural cardiac disease(s), with no significant adverse reactions (Murdock, et al., 2009;Murdock, et al., 2010). Considering the safety of ranolazine in patients with structural heart diseases (Koren, et al., 2007;Wilson, et al., 2009), the “pill-in-the-pocket” approach utilizing ranolazine may prove to have a much wider applicability than previously used Class IC antiarrhythmic agents (i.e., propafenone and flecainide).

**Atrial-selective APD prolongation potentiates atrial selectivity of $I_{\text{Na}}$ block**

The primary anti-AF mechanism of propafenone and ranolazine in our study is related to the action of these drugs to block peak sodium channel current, $I_{\text{Na}}$, especially at fast atrial rates.
The prolongation of APD$_{90}$ in atria by both agents likely contributes to the anti-AF effect of these agents both directly and indirectly (i.e., by enhancing the block of early $I_{Na}$, Fig. 4). Atrial-selective prolongation of APD$_{90}$ by ranolazine and propafenone is likely due to their effect to block $I_{Kr}$ (Antzelevitch, et al., 2004; Grant, 1996). Indeed, specific $I_{Kr}$ block with E-4031 produces atrial-predominant prolongation of APD$_{90}$ and ERP in canine preparations at a CL of 500 ms (Burashnikov, et al., 2008). A similar atrial-predominant effect of $I_{Kr}$ blockers to prolong ERP has been demonstrated in vivo in both canine and porcine hearts (Spinelli, et al., 1992; Wiesfeld, et al., 1996). It is also noteworthy that neither propafenone (Gross and Castle, 1998) nor ranolazine (Zygmunt and Antzelevitch, unpublished) block $I_{Kur}$ and that $I_{Kur}$ inhibition abbreviates instead of prolonging APD$_{90}$ in “healthy” atria (Burashnikov, et al., 2004; Wettwer, et al., 2004).

Atrial selective prolongation of APD$_{90}$ induced by propafenone and ranolazine contributes to the abbreviation of the diastolic interval at rapid pacing rates in atria but not ventricles (Fig. 4). Because much of the recovery of the sodium channels from block occurs during the diastolic interval (Whalley, et al., 1995), the atrial selective prolongation of APD$_{90}$ enhances the effect of both drugs to depress $I_{Na}$ and $I_{Na}$-mediated parameters in atria at rapid rates, thus potentiating their effects to suppress AF.

**Atrial selectivity of $I_{Na}$ blockers: rapid vs. slow dissociation kinetics?**

Both propafenone and ranolazine are predominantly open state sodium channel blockers (Whalley, et al., 1995; Wang, et al., 2008; Zygmunt, et al., 2011). Amiodarone and AZD1305 are also atrial selective sodium channel blockers (Burashnikov, et al., 2008; Burashnikov, et al., 2010). While amiodarone is primarily an inactivated-state sodium channel blockers (Whalley, et
al., 1995), AZD1305 is a potent tonic blocker (i.e., inhibits sodium channel at the resting state) (Burashnikov, et al., 2010). Ranolazine produces little to no tonic block at normal resting membrane potential (Zygmunt, et al., 2011). Thus, the available data suggest that preferential binding to a given state of the channel (i.e., open, inactivated, or resting) does not necessarily determine atrial selectivity of INa blockers. The unbinding kinetics of propafenone and ranolazine appear to play a determining role. Propafenone dissociates slowly (τ ≳ 8 sec (Whalley, et al., 1995)), whereas ranolazine dissociation is relatively rapid (τ = 1.6 sec (Burashnikov, et al., 2007)). Consistent with this hypothesis, amiodarone, which has recently been shown to be an atrial-selective sodium channel blocker (Burashnikov, et al., 2008), unbinds rapidly from the sodium channel (Whalley, et al., 1995). Other factors that contribute to atrial selective inhibition of INa include a more negative voltage-dependence of steady-state inactivation of the sodium channels, a more positive resting membrane potential, and a much slower phase 3 of the action potential in atria vs. ventricles (for detailed discussion see (Burashnikov and Antzelevitch, 2008a; Burashnikov and Antzelevitch, 2009; Burashnikov and Antzelevitch, 2010)).

**Study Limitations.**

Extrapolation of our results obtained from in vitro to in vivo animal models or to the clinic should be done with great caution. The absence of autonomic and hormonal influences, which can significantly modulate cardiac electrophysiology and thereby the pharmacological response to drugs, are among the limitations of in vitro preparations. In addition, our experiments were carried out using “healthy” atria and ventricles, whereas AF normally occurs in electrically and structurally remodeled atria. Atrial remodeling can significantly affect pharmacological responses.
ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Judy Hefferon and Robert Goodrow.

AUTHORSHIP CONTRIBUTION:

Participated in research design: Burashnikov A and Antzelevitch C.

Conducted experiments: Burashnikov A.

Contributed new reagents or analytic tools: Burashnikov A. Belardinelli L, Antzelevitch C.

Performed data analysis: Burashnikov A and Antzelevitch C.

Wrote or contributed to the writing of the manuscript: Burashnikov A, Antzelevitch C, Belardinelli L.
REFERENCES


FOOTNOTES

FUNDING SOURCES: This study was supported by grants from Gilead Sciences, Inc., National Institute of Health [HL-47687-to CA], and the New York State and Florida Free and Accepted Masons.

RELATIONSHIP WITH INDUSTRY: Dr. Antzelevitch received research support and is a consultant to Gilead Sciences. Dr. Belardinelli is an employee of Gilead Sciences, Inc. Palo Alto, CA.
LEGENDS for FIGURES:

**Figure 1.** Effects of propafenone and ranolazine on transmembrane action potentials (APs) from various atrial and ventricular regions.

Shown are representative examples of APs and summary data of the effect of propafenone and ranolazine on APD$_{90}$ in atrial and ventricular preparations stimulated at a cycle length of 500 ms. CT = crista terminalis, PM = pectinate muscle, M cell – subendocardial region, Epi = epicardium. * p < 0.05 vs. respective control. n=6-18.

**Figure 2.** Effects of propafenone and ranolazine on effective refractory period (ERP), action potential duration (APD), and post-repolarization refractoriness (PRR, numbers) in atrial and ventricular preparations. PRR was measured as the difference between ERP and APD$_{75}$ in atria and between ERP and APD$_{90}$ in ventricles (ERP is coincident with APD$_{75}$ in atria and APD$_{90}$ in ventricles). Note, while APD$_{90}$ was prolonged by the agents, APD$_{75}$ was not (see fig. 1).

Ventricular data were obtained from epicardium and atrial data from pectinate muscle.

* p<0.05 vs. control. † p<0.05 vs. APD$_{75}$ values in atria and APD$_{90}$ in ventricles. N=6-18.

**Figure 3.** Ranolazine, but not propafenone, causes an atrial-selective rate-dependent increase in diastolic threshold of excitation (DTE). The increase in DTE caused by propafenone is greater than that of ranolazine.

DTE measurements obtained from crista terminalis and pectinate muscle are combined under “atria” and those from ventricular epicardium and endocardium are combined under “ventricles”.

* p<0.05 vs. control, † p<0.05 vs. respective values in ventricles. n=6-10.
Figure 4. Rate-dependent effects of propafenone and ranolazine to depress the maximal rate of rise of the AP upstroke ($V_{\text{max}}$) in atrial and ventricular preparations.

A: Representative examples of action potentials and respective $V_{\text{max}}$ recorded prior to and upon acceleration of pacing rate from a CL of 500 to 300 ms in atria and ventricles in the absence (control) and presence of propafenone (the AP tracings were taken from the same atrial and ventricular preparations).

B-C: Graphs summarize the changes in $V_{\text{max}}$ of atrial and ventricular APs paced at a CL of 500 and 300 ms. Control $V_{\text{max}}$ values at a CL of 300 ms were normalized to the respective control $V_{\text{max}}$ value obtained at a CL of 500 ms. “Atria” includes combined PM and CT data. “Ventricles” includes combined Epi and M cell data recorded from the ventricular wedge. * $p<0.05$ vs. control. † $p<0.05$ vs. respective values in ventricles. n=6-10.

Figure 5. Rate-dependent effects of propafenone and ranolazine on conduction time in atrial and ventricular preparations. Atrial and ventricular conduction times were estimated by measuring by the duration of “P wave” and “QRS” complexes of ECG recordings from coronary-perfused atrial and ventricular preparations. * $p<0.05$ vs. respective control. † $p<0.05$ vs. respective values in ventricles. n=6-10.

Figure 6. Propafenone suppresses AF and prevents induction/re-induction of the arrhythmia in coronary-perfused right atria.

A: Persistent AF induced in the presence of acetylcholine (ACh, 0.5 µM). B: Propafenone (1.5 µM) terminates the arrhythmia. C: Attempt to re-induce AF fails due to development of use-dependent block of sodium channels (evident from progressive reduction in $V_{\text{max}}$) leading to the development of post-repolarization refractoriness and 2:1 activation failure.
TABLE: Ranolazine (10 µM) vs. propafenone (1.5 µM) to suppress acetylcholine (ACh, 0.5 µM)-mediated persistent AF in the isolated canine coronary-perfused right atria.

<table>
<thead>
<tr>
<th></th>
<th>APD$_{90}$ (ms)</th>
<th>ERP (ms)</th>
<th>Shortest $S_1-S_1$</th>
<th>Induction of persistent AF</th>
<th>Termination of persistent AF</th>
<th>Prevention of AF Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>45±9</td>
<td>51±7</td>
<td>64±8</td>
<td>100% (10/10)</td>
<td>0% (0/10)</td>
<td>-</td>
</tr>
<tr>
<td>ACh + Ranolazine</td>
<td>69±16*</td>
<td>112±23*†</td>
<td>163±46*</td>
<td>18% (2/11)</td>
<td>66% (8/12)</td>
<td>75% (6/8)</td>
</tr>
<tr>
<td>ACh + Propafenone</td>
<td>83±23*</td>
<td>142±37*†</td>
<td>241±69*</td>
<td>14% (6/7)</td>
<td>80% (8/10)</td>
<td>100% (8/8)</td>
</tr>
</tbody>
</table>

APD$_{90}$ and ERP data presented in the table were obtained from the pectinate muscle region of coronary-perfused atria at a CL of 500 ms. Shortest $S_1-S_1$ - the shortest CL permitting 1:1 activation (at a DTE x 2 determined at a CL of 500 ms). * < 0.05 vs. respective values at ACh alone; † P< 0.05 vs. respective APD$_{90}$. n=6-12
Figure 1
Figure 2
Figure 3

Propafenone

CL = 500 ms

- Atria
- Ventrices

CL = 300 ms

Ranolazine

CL = 500 ms

- Atria
- Ventrices

CL = 300 ms
Figure 4
Figure 5
Figure 6