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The antiarrhythmic actions of bisaramil and penticainide result from mixed cardiac ion channel blockade

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ABSTRACT

Decades of focus on selective ion channel blockade has been dismissed as an effective approach to antiarrhythmic drug development. In that context many older antiarrhythmic drugs lacking ion channel selectivity may serve as tools to explore mixed ion channel blockade producing antiarrhythmic activity. This study investigated the non-clinical electrophysiological and antiarrhythmic actions of bisaramil and penticainide using *in vitro* and *in vivo* methods. In isolated cardiac myocytes both drugs directly block sodium currents with IC_{50} values of 13μM (bisaramil) and 60μM (penticainide). Both drugs reduced heart rate but prolonged the P–R, QRS and Q–T intervals of the ECG (due to sodium and potassium channel blockade) in intact rats. They reduced cardiac conduction velocity in isolated rat hearts, increased the threshold currents for capture and fibrillation (indices of sodium channel blockade) and reduced the maximum following frequency as well as prolonged the effective refractory period (indices of potassium channel blockade) of electrically stimulated rat hearts. Both drugs reduced ventricular arrhythmias and eliminated mortality due to VF in ischemic rat hearts. The index of cardiac electrophysiological balance (iCEB) did not change significantly over the dose range evaluated; however, different drug effects resulted when changes in BP and HR were considered. While bisaramil is a more potent sodium channel blocker compared to penticainide, both produce a spectrum of activity against ventricular arrhythmias due to mixed cardiac ion channel blockade. Antiarrhythmic drugs exhibiting mixed ion channel blockade may serve as tools for development of safer mixed ion channel blocking antiarrhythmic drugs.

1. Introduction

More than 500,000 deaths occur annually in the United States from sudden cardiac death (SCD) and 60–70% result primarily from ventricular arrhythmias due to coronary heart disease (CHD) [1–3]. CHD remains the leading cause of death attributed to cardiovascular disease [2,4] and remains the principal focus of health care providers [5,6]. While pharmacological treatment of ventricular arrhythmias does not improve survival to hospital discharge; the use of antiarrhythmic drugs such as lidocaine and amiodarone improve patient survival to hospital admission [7,8].

Several classes (I-IV) of antiarrhythmic drugs exist and each act by specifically removing a putative cause of the arrhythmia [9–11]. Class I sodium channel blocking antiarrhythmic drugs (*i.e.*, lidocaine) suppress undesired ventricular automaticity, reduce abnormal cardiac conduction and prevent the development of arrhythmogenic re-entrant circuits [12]. Although some class I antiarrhythmics prevent arrhythmia development, others may precipitate fatal ventricular arrhythmias [13–15].

Drug induced blockade of cardiac sodium channels is highly dependent on the structural nature of the molecule [9]. While the majority of drugs in this class originate as local anaesthetics and are analogs of lidocaine, many other structurally distinct drugs such as flecainide were also developed with potent, selective sodium channel blocking activity [16]. Similarly, other drugs such as the class III antiarrhythmic drug, amiodarone, were developed which have mixed, rather than selective, ion channel blocking actions. This means that although amiodarone blocks repolarizing ventricular potassium channels, it also blocks sodium channels when given at high doses [17] Amiodarone is a highly effective antiarrhythmic drug [18] and listed as an essential medicine by the World Health Organization.

Bisaramil (YUTAC®) is structurally related to the alkaloid

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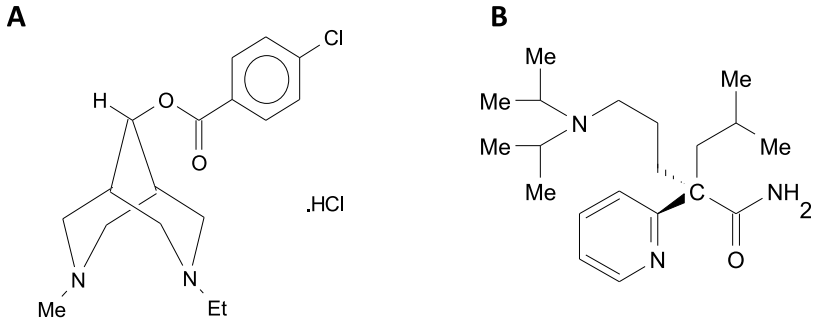

Fig. 1. The chemical structures of bisaramil [(1R,5S)-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]nonan-9-yl] 4-chlorobenzoate (A) and penticainide 2-[2-[di(propan-2-yl)amino]ethyl]-4-methyl-2-pyridin-2-ylpentanamide (B).

antiarrhythmic drug sparteine and shows efficacy against both chemical and ischemic ventricular arrhythmias [19–24]. Bisaramil reduces both intraventricular conduction [25] and ventricular depolarization (V_{max}) [23,26] and also produces use-dependent block [26,27]. Pugsley & Goldin [28] showed that bisaramil has potent, preferential cardiac sodium channel isoform blocking properties.

Penticainide is a class I antiarrhythmic agent and a 2-pyridyl-pentanamide derivative that is chemically-related to disopyramide [29,30]. Penticainide blocks sodium currents [31], reduces V_{max} of the cardiac action potential [32] and exhibits antiarrhythmic drug activity [33,34].

Of the antiarrhythmic drugs available for characterization, this study investigated the electrophysiological and antiarrhythmic actions of the potent but highly structurally dissimilar sodium channel blocking drugs bisaramil and penticainide. An evaluation of the effects of both drugs on the index of cardiac electrophysiologic balance (iCEB), a novel biomarker of cardiac arrhythmias, was conducted. The implications of mechanistic differences in assessment of ion channel blocking profiles is discussed in terms of the potential for the use of these drugs as tools in the study of ion channel function and in the study of the mechanisms responsible for the development of cardiac arrhythmias.

2. Materials

2.1. Animals and general animal surgical preparation

Male Sprague-Dawley rats (200–300 g; U.B.C. Animal Care Centre) were used for all studies in Vancouver, Canada while male Wistar rats (200–300 g) were used for all studies in Canberra, Australia. All studies were conducted according Animal Care and Use Committee guidelines at both research sites. All study design and animal ethics conformed to the ARRIVE guideline [35] and a more recent guidance on experimental design and analysis [36].

Rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and the trachea was cannulated for artificial ventilation [37]. The body temperature was maintained at 36 ± 1C using a heating lamp. The right jugular vein was cannulated for drug administration, the left carotid artery for blood pressure recording and the ECG was recorded by the method of Penz et al. [37].

2.2. Isolated ventricular rat cardiac myocytes

Cardiac myocyte isolation was performed according to Pugsley & Saint [27]. Heparinized, male rats were cervically dislocated. Hearts were removed and washed in an oxygenated, cold, calcium-free Tyrode's solution prior to aortic perfusion with a warmed (37 ± 1C) calcium-free Tyrode's solution. The pH 7.4 Tyrode's solution contained (mM): NaCl 134; TES (N-tris-(hydroxymethyl)methyl-2-amino ethanesulphonic acid) 10; KCl 4; NaH_2PO_4 1.2; $MgCl_2$ 1.2; glucose 11. After thorough washing, the heart was perfused with Tyrode's solution containing protease (0.10 mg/mL, Sigma Type XIV), collagenase (1.0 mg/ml, Worthington CLS II), 25 μM calcium and fetal calf serum (1.0 μg/ml). After 40 min the ventricles were removed, cut into pieces in the calcium-Tyrode's solution and the cells dispersed. Cell suspensions were centrifuged, washed (200μM calcium-Tyrode's solution), resuspended (1 mM calcium-containing Tyrode's solution) and plated onto glass coverslips for use in studies.

2.2.1. Solutions and compounds

Electrophysiology studies were conducted at 22–24 °C in a pH 7.4 bath solution containing (mM): NaCl 70; TES 10; KCl 5.4; $CaCl_2$ 2; cholineCl 60; $CoCl_2$ 5; CsCl 5; $MgCl_2$ 1.0; glucose 10. The pipette solution for sodium currents contained (mM): CsF 140; TES 10; $MgCl_2$ 1; K-EGTA 10; $CaCl_2$ 2; ATP-disodium 10 and was pH adjusted to 7.4 with 1.0 M KOH. Sodium currents were evoked by a voltage-step from a prepulse potential of −150 mV to a potential of 0 mV. The voltage step was delivered at 6 s intervals

Bisaramil ([(1*R*,5S)-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1] nonan-9-yl] 4-chlorobenzoate) (a gift from The Chemical Works of Gedeon Richter, Budapest, Hungary) (Fig. 1A) and penticainide (2-[2-[di(propan-2-yl)amino]ethyl]-4-methyl-2-pyridin-2-ylpentanamide) (a gift from Sanofi Recherche, Montpellier, France) (Fig. 1B) were solubilized in 0.9% saline prior to i.v. injection or dissolution in the *in vitro* assay external bath solution. A 1.0 mL plexiglass bath was used for voltage clamp studies and drugs superfused cells at a flow rate of 1–2 ml/min.

2.2.2. Electrophysiological recording methods

Isolated myocytes were perfused with buffer but only quiescent, rod-shaped cells were selected for use in studies. Borosilicate glass electrodes (resistances 1–5 M) were used. Currents were recorded 10 min after whole-cell patch clamp was achieved [27] using an Axopatch 200 A amplifier (Molecular Devices, Union City, CA). At the time of data analysis, final capacitance and leak compensation was conducted by subtraction of a 20 mV hyperpolarizing current pulse. Currents were filtered (5 kHz), sampled (10 kHz) and records saved on a computer system hard drive.

2.3. Langendorff isolated rat hearts

Rat hearts were attached to a Langendorff perfusion cannula [38] at an aortic pressure of 100 mmHg. Carbogenated (5% CO_2 in O_2) Krebs-Henseleit buffer (pH 7.4), at a temperature of 35 ± 1.5C, was used to perfuse the heart. Left ventricular pressure was measured using a saline-filled balloon and the maximum rate of pressure development (+dP/dt_{max}) recorded using a model 7P20C Grass differentiator. Epicardial silver-ball electrodes recorded the ECG. Bisaramil (1–16 μM) or penticainide (2–64 μM) was perfused for 5 min at each concentration into each heart (n = 5) and contractility and ECG measures recorded.

2.4. Hemodynamic and ECG measures in anesthetized rats

The dose-response effects of bisaramil and penticainide were

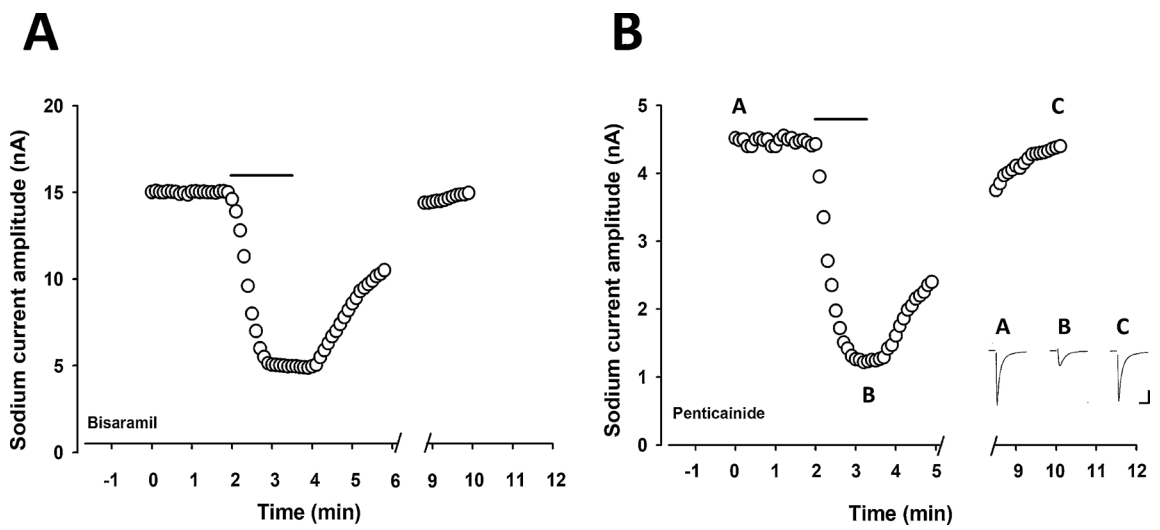

Fig. 2. Bisaramil (panel A) and penticainide (panel B) block of the sodium current in rat cardiac myocytes. Sodium currents were evoked at 6 s intervals and either bisaramil (30μM) or penticainide (120μM) was added to the bath solution as indicated. The panel B inset shows the current amplitude in the absence (A) and presence (B) of penticainide. The re-control current (C) is indistinguishable from the control current.

examined in anaesthetised, ventilated rats (n = 5 per group). Doses of bisaramil (0.01–8.0 μmol/kg, i.v.) or penticainide (0.01–32 μmol/kg, i.v.) were given and each dose was administered over a 2 min period with an inter-dose interval of 10 min. Heart rate was determined from the R−R interval of the ECG recorded using a Grass polygraph (Model 7D, Quincy, Mass., U.S.A.). The ECG intervals (P–R, QRS, and Q–T) were measured directly from the ECG traces.

2.5. Electrical stimulation studies

Electrical stimulation of the left-ventricle of anesthetized rats was conducted as described by Pugsley et al. [10]. Square-wave stimulation was used to determine threshold current (i_t-μA) and pulse-width (t_t-ms) for induction of extrasystoles and ventricular fibrillation threshold (VF_t-μA) levels. The maximum following frequency (MFF-Hz) and effective refractory period (ERP-ms) were also measured using this same procedure [39]. Doses of both bisaramil and pentacainide (1–4.0 μmol/kg, i.v.) were administered over a 5 min period.

2.6. Coronary artery occlusion studies

Rats were anesthetized, ventilated and the left carotid artery was cannulated to determine serum potassium concentrations (Ionetics Potassium Analyzer, Fountain Valley, CA) and blood pressure. A left thoracotomy was produced, the heart exposed and a polyethylene occluder was placed around the left main coronary artery [10]. The chest was closed and animals were monitored during a 30 min post-surgical recovery period.

Animals were given a random and blind infusion of either saline, bisaramil (2.5 μmol/kg) or penticainide (5.0 μmol/kg). Blood pressure and the ECG were recorded during drug infusion and a blood sample (0.25 mL) removed prior to coronary artery occlusion.

Blood pressure, the ECG, arrhythmias, heart rate and animal mortality were monitored for 30 min post-occlusion. Arrhythmias were defined as ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) and used to derive an arryhythmia score (AS) defined according to Curtis & Walker [40]. At the end of the observation period another blood sample was taken. Hearts were removed and perfused with cardiogreen dye (1.0 mg/ml) to reveal the coronary occluded zone (OZ). Studies were performed according to the Lambeth Conventions [41].

2.7. Index of cardiac electrophysiologic balance (iCEB)

iCEB [42,43] is a non-invasive biomarker that has been shown to determine the ratio of potassium to sodium channel blockade observed using standard variables derived from the ECG (*i.e.*, iCEB = QT/QRS). Since both the QRS and QT intervals are dependent upon the rate of contraction and changes in hemodynamics, iCEB was also normalized to both heart rate (*i.e.*, iCEB/HR) and blood pressure (*i.e.*, iCEB/BP), respectively.

2.8. Statistical analysis

Data is shown as the mean ± standard error of the mean (SEM) (for *n* experiments). The NCSS statistical, graphics, and sample size software was used to conduct statistical analyses at an -level of $p < 0.05$. An analysis of variance (ANOVA) followed by the post hoc Duncan's test were used in all experiments except in arrhythmia studies where VPB number was $\log_{10}$ transformed. Mainland's contingency tables were used to determine significance between drug arrhythmic groups and control saline [44].

3. Results

3.1. Isolated ventricular rat cardiac myocytes

While both bisaramil and penticainide produced a concentration-dependent reduction in sodium current in isolated rat myocytes, bisaramil was a more potent current blocker. The half-maximal sodium current block (IC_{50}) for bisaramil (n = 3) was 13μM with a Hill coefficient (n_H) of 1.2. The IC_{50} for penticainide (n = 3) was 60 μM with a Hill coefficient of 1.05. The development of block was similar for both drugs and occurred immediately upon the start of drug perfusion with drug wash-out from block being much slower for both compounds (Fig. 2 A, B).

3.2. Langendorff isolated rat hearts

At concentrations that reduced sodium current in isolated myocytes, bisaramil (16μM) and penticainide (64μM) significantly reduced heart rate by 35% and 32%, respectively (Table 1). Both bisaramil and penticainide also significantly reduced peak systolic pressure by ~50% compared to pre-drug values at the highest concentrations tested (Table 1). In addition to a reduction in ventricular contractility, both

Table 1
The effect of bisaramil and penticainide on Langendorff isolated rat hearts.

Variable	Bisaramil		Penticainide	
	Pre-drug	Post-drug (16 μM)	Pre-drug	Post-drug (64 μM)
Systolic Pressure (mmHg)	151 ± 7.0	69 ± 12*	153 ± 5.0	75 ± 12*
Conduction Velocity (m/s)	2.2 ± 0.1	0.5 ± 0.08*	2.3 ± 0.04	1.2 ± 0.15*
ECG Measures (ms)				
P-R	65 ± 4.0	177 ± 10*	70 ± 4.0	119 ± 9.0*
QRS	31 ± 1.0	47 ± 2.0*	32 ± 0.8	49 ± 2.0*
Q-T	40 ± 1.5	67 ± 3.0*	40 ± 1.0	55 ± 3.0*

Values represent mean ± SEM. * Indicates that post-drug values are significantly different from pre-drug values ($p < 0.05$). Note the post-drug concentation data for both drugs is shown at the highest concentrations perfused (16 μM for bisaramil and 64 μM for penticainide).

bisaramil and penticainide significantly reduced conduction velocity by 77% and 48%, respectively (Table 1). Bisaramil significantly prolonged the P–R interval (170%) compared to penticainide (70%); however, both drugs equally prolonged the QRS width (52% and 58%) relative to control (Table 1). Both drugs also significantly prolonged the Q–T interval by 68% and 36%, respectively, at the highest concentrations tested (Table 1).

3.3. Haemodynamic and ECG measures in anesthetized rats

In all vehicle control (n = 5) animals both blood pressure and heart rate were stable for the dosing duration (Table 2). Bisaramil and pentacainide dose-dependently reduced blood pressure and heart rate in anesthetized rats; however, bisaramil showed a greater potency in overall cardiodepressive actions compared to penticainide. At maximal doses of 8 μmol/kg and 32 μmol/kg, respectively, bisaramil and penticainide significantly reduced blood pressure by 50% and heart rate by 52%, respectively (Table 2).

ECG measures were also dose-dependently changed by bisaramil and penticainide. At the highest doses administered, bisaramil significantly prolonged the P–R interval by 46% whereas penticainide significantly prolonged the P–R interval by 63% (Table 2). Both drugs also significantly prolonged the width of the QRS interval. Bisaramil produced a 72% increase in the QRS interval while penticainide produced an 86% increase at the highest doses given. These changes are consistent with the evidence of sodium channel blockade in both myocytes and isolated hearts (Table 2). However, both drugs also had effects on cardiac repolarization. Bisaramil significantly prolonged the Q–T interval by 38% while penticainide prolonged the Q–T interval by 36%. These effects are consistent with evidence of potassium channel blockade in isolated hearts (Table 2). The vehicle control did not affect any of the ECG measures over the duration of the experiment (Table 2). Fig. 3A describes the iCEB values determined for each drug using the ECG (*i.e.*, iCEB = QT/QRS). The drug iCEB profiles were not easily distinguished from each other (Fig. 3A); however, each drug followed a distinct profile when normalized to changes in both blood pressure (Fig. 3B) and heart rate (Fig. 3C). Importantly, the pressure and/or heart rate normalized iCEB measures were not changed relative to control over cumulative dose ranges examined in subsequent electrical stimulation and ischemic arrhythmia studies.

3.4. Electrical stimulation studies

The patterns of bisaramil and penticainide effects on hemodynamic measures *in vivo* and ECG measures from both *in vitro* and *in vivo* studies indicate drug-induced block of both sodium and potassium channels with differences in drug potency and efficacy. Drug effects on electrical properties of the heart relevant to sodium and potassium channel function were evaluated against electrically-induced arrhythmias in the rat (Table 1). Both drugs significantly (0.01 μmol/kg to 4 μmol/kg) increased thresholds for capture for the induction of ventricular extrasystoles (i_t) and ventricular fibrillation (VF_t), respectively. Both drugs also significantly prolonged the effective refractory period (ERP), an effect consistent with decreases in maximum following frequency (MFF). The vehicle-control did not affect any of the stimulation-based measures over the duration of the experiment. The effects of bisaramil and penticainide on these electrical stimulation measures are shown at the maximal cumulative doses tested (4 μmol/kg) in Table 3.

An analysis of drug effects on both ERP and MFF (measures that are affected by potassium channel blockade) *versus* VF_t (a measure affected by sodium channel blockade) suggested that both drugs produced a dose-related change in the ratio of ERP and MFF relative to VF_t (Fig. 4 A and B). However, the reductions in ERP/VF_t and MFF/VF_t (at the highest dose of drug tested compared to pre-drug values) were greatest for bisaramil at 4-fold and 21-fold, respectively, when compared to values for penticainide which were 2-fold and 11-fold, respectively. In contrast, while both drugs produced dose-related changes in the ratio of ERP and MFF compared to the i_t variable (Fig. 5A and 5B), the reductions in ERP/i_t and MFF/i_t (at the highest dose of drug dose tested compared to pre-drug values) were similar for bisaramil at 1.5-fold and

Table 2
The effect of bisaramil and penticainide on hemodynamic and ECG variables in anesthetized rats.

Variable	Bisaramil		Penticainide		Vehicle	
	Pre-drug	Post-drug (8 μmol/kg)	Pre-drug	Post-drug (32 μmol/kg)	Pre-drug	Post-drug (0 μmol/kg)
Blood Pressure (mmHg)	116 ± 5	58 ± 8*	121 ± 7	60 ± 9*	118 ± 5	128 ± 9
Heart Rate (beats/min)	490 ± 12	235 ± 22*	475 ± 11	230 ± 17*	472 ± 15	470 ± 22
ECG Measures (ms)						
P-R	58 ± 2	85 ± 3*	56 ± 2	91 ± 6*	60 ± 3	56 ± 1
QRS	29 ± 0.5	50 ± 2*	29 ± 2	54 ± 2*	30 ± 1	30 ± 1
Q-T	45 ± 3	62 ± 3*	44 ± 2	60 ± 5*	43 ± 2	40 ± 4

Values represent mean ± SEM. *Indicates that post-drug values are significantly different from pre-drug values ($p < 0.05$). Note although full dose-response curves were determined for both drugs, to enable efficient summary, the post-drug dose data for both drugs is shown only at the highest doses tested (8 μmol/kg for bisaramil and 32 μmol/kg for penticainide).

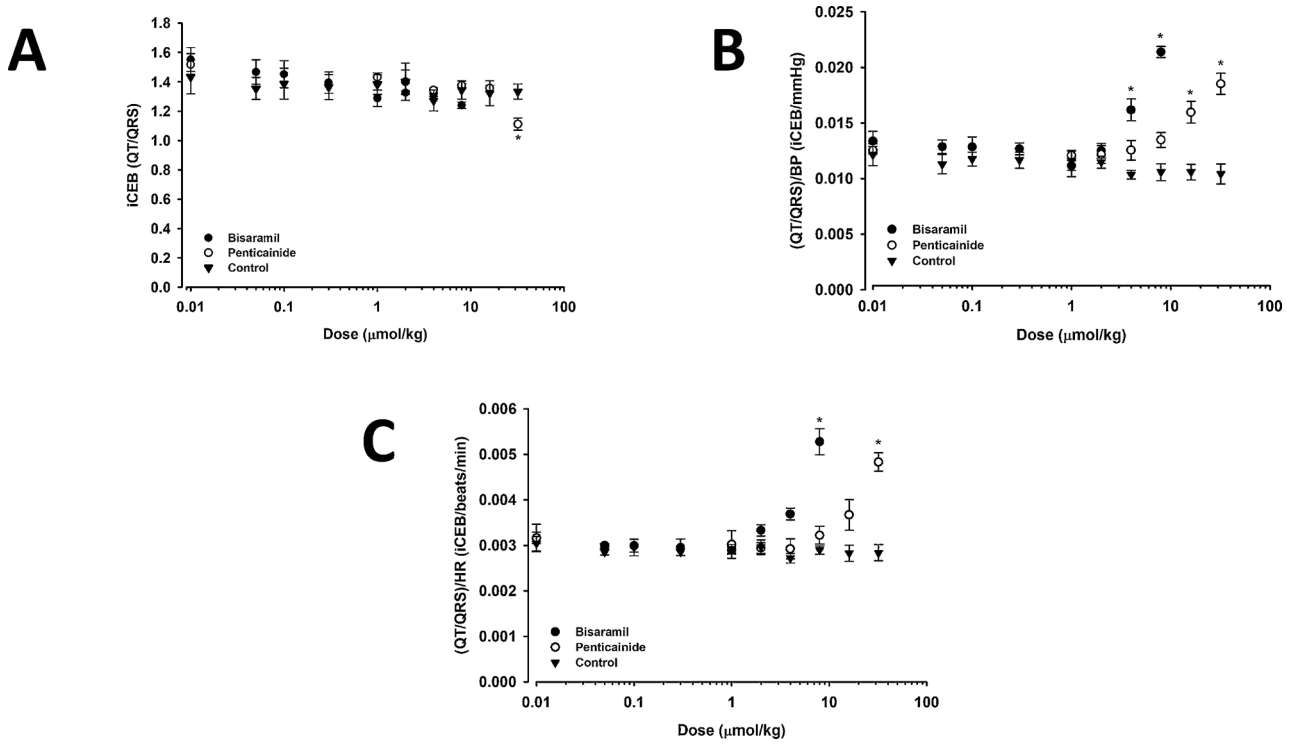

Fig. 3. The dose-dependent effects of bisaramil (●), penticainide (○) and saline control (▼) on the index of cardiac electrophysiological balance, iCEB (A) in pentobarbital anesthetized rats (n = 5). iCEB (= QT/QRS) reflects the balance and imbalance of ventricular depolarization (QRS duration) and repolarization (QT interval). iCEB profiles for each drug were separated from each other but followed normalization to changes in blood pressure (B) and heart rate (C) in anesthetised rats. Values were determined after completion of each dose. Saline control had no effect upon iCEB. *Indicates $P < 0.05$ for difference from saline control.

9-fold, respectively, when compared to values for penticainide at 1.5-fold and 7-fold, respectively.

3.5. Coronary artery occlusion studies

Occlusion of the left anterior descending (LAD) coronary artery produced uniform occlusion zone sizes of 32 ± 5%–35 ± 6% of the left ventricular mass in saline and drug treated animals. Changes in serum potassium both pre- and post-occlusion were not different between treatment groups and ranged between 3.4 ± 0.6 mM - 3.7 ± 0.8 mM before ischemia developed and between 3.6 ± 0.8 mM - 3.9 ± 1.0 mM at the end of the study period. Neither bisaramil or penticainide reduced the incidence of VPB's (Log VPB) (Table 4). However, while bisaramil significantly reduced the occurrence of VT and VF, the effects of pentacainide were limited only to a significant reduction in the incidence of VF (Table 4). Since bisaramil reduced the incidence of both VT and VF, this resulted in a significantly reduced AS and abolition of VF-induced arrhythmia related mortality. While penticainide did not reduce VT incidence or AS, it did eliminate mortality due to ischemia. Neither bisaramil or penticainide altered the maximum elevation or time to maximum elevation of the ST segment of the ECG and neither drug reduced the increase in R wave amplitude associated with ischemia in occluded hearts (data not shown).

4. Discussion

'Sudden death' resulting from fatal ventricular arrhythmias remains the most common cause of death in the U.S. [4,45]. The underlying causes of arrhythmias are problematic to ascertain because they arise acutely but are slow to develop as a result of slowly developing atherosclerosis in coronary arteries. Non-clinical studies show that death, resulting from VF, is the likely first symptom of an acute MI episode. However, since clinical trials with Class I and Class III antiarrhytmic drugs were stopped due to unanticipated high incidence of deaths in drug treatment groups [13,14], research efforts ceased for ion-channel related antiarrhythmic drug development. Similarly, these findings have seriously restricted the use of most approved antiarrhythmic drugs because of serious drug safety issues [4,46]. However, the optimism for development of novel, clinically effective antiarrhythmic drugs with a better safety profile persists. Many reviews

Table 3
The effect of bisaramil and penticainide on electrical properties of the heart in anesthetized rats.

Variable	Bisaramil		Penticainide		Vehicle	
	Pre-drug	Post-drug (4 μmol/kg)	Pre-drug	Post-drug (4 μmol/kg)	Pre-drug	Post-drug (0 μmol/kg)
Threshold current for capture (i_t; μA)	59 ± 1	135 ± 10*	58 ± 1	160 ± 10*	58 ± 1	58 ± 9
Ventricular fibriliation threshold (VF_t; μA)	84 ± 5	490 ± 20*	83 ± 7	375 ± 19*	81 ± 7	89 ± 21
Effective refractory period (ERP; ms)	46 ± 3	79 ± 7*	55 ± 2	112 ± 4*	46 ± 4	50 ± 4
Maximum following frequency (MFF; Hz)	18.4 ± 1.0	5.1 ± 1.1*	17 ± 0.2	6.5 ± 0.6*	17 ± 1	17 ± 0.2

Values represent mean ± SEM. *Indicates that post-drug values are significantly different from pre-drug values ($p < 0.05$). Note although full dose-response curves were determined for both drugs, to enable efficient summary, only the post-drug dose data for both drugs is shown at the highest dose tested (4 μmol/kg).

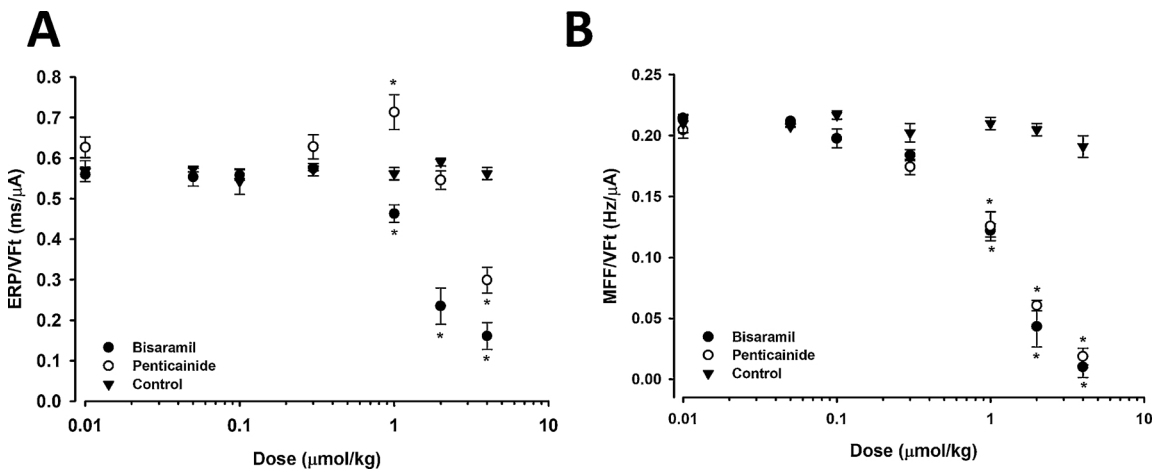

Fig. 4. The dose-dependent effects of bisaramil (●), penticainide (○) and saline control (▼) on the ratio of electrical stimulation measures of refractoriness in cardiac muscle, the effective refractory period (ERP) and maximum following frequency (MFF) to ventricular fibrillation threshold (VF_t) in pentobarbital anesthetized rats (n = 5). Both drugs dose-dependently decreased the ERP/VF_t (A) and MFF/VF_t (B) ratios while saline control had no effect. Values were determined after completion of each dose. *Indicates P < 0.05 for difference from saline control.

suggest that drugs with a wider spectrum of pharmacological activity, *i.e.*, that block multiple ion channels, may have the desired efficacy and safety combination [47]. This approach was used to characterize the safety profile of a novel fluoroquinolone antimicrobial drug [48]. An examination of the non-clinical and clinical cardiovascular studies conducted with JNJ-Q2 provided a safe cardiovascular profile with comparable or reduced potential to prolong the QT interval compared to moxifloxacin. The findings in this study reflect the importance of sodium and calcium channel blocking activity in counterbalancing drug-induced potassium channel blocking activity [48]. Currently this concept is being evaluated through the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative for cardiovascular drug safety. In a cross-pharmaceutical company consortium, drug safety scientists are currently validating a mechanistic-based assessment of the proarrhythmic risk of drugs [49–51]. The blocking effects of a set of test drugs on hERG (or I_{Kr}), calcium, and sodium currents will be determined and findings will be subsequently integrated into a computer model of the human cardiomyocyte to predict proarrhythmic risk [49]. If these validated methods predict the proarrhythmic potential of test drugs these studies will likely be a supplement to current drug safety pharmacology studies [50].

Table 4
The effect of bisaramil and penticainide on arrhythmias in rats subject to coronary artery occlusion.

Drug (μmol/kg)	Log VPB	VT (%)	VF (%)	Arrhythmia Score (AS)	Mortality (%)
Saline	1.9 ± 0.3	80	60	5.6 ± 2.0	60
Bisaramil (2.5)	2.1 ± 0.2	0*	10*	1.5 ± 0.4*	0*
Penticainide (5.0)	2.5 ± 0.4	100**	0*	4.3 ± 1.2**	0*

The antiarrhythmic properties of bisaramil and penticainde on arrhythmia incidence during coronary artery occlusion (n = 5/group). Arrhythmias were recorded as ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF). Log VPB is the $\log_{10}$ transformation of the number of VPB's. Arrhythmia Score (AS) is defined in references provided in Section 2.6 of the Methods. *Indicates P < 0.05 for difference from saline control. **Indicates a significant difference between drug treatments.

We characterized the antiarrhythmic and cardiac electrophysiological profile of bisaramil and penticainide using a complementary variety of validated non-clinical models. From the patterns of effects observed from the *in vitro* and *in vivo* studies conducted, both

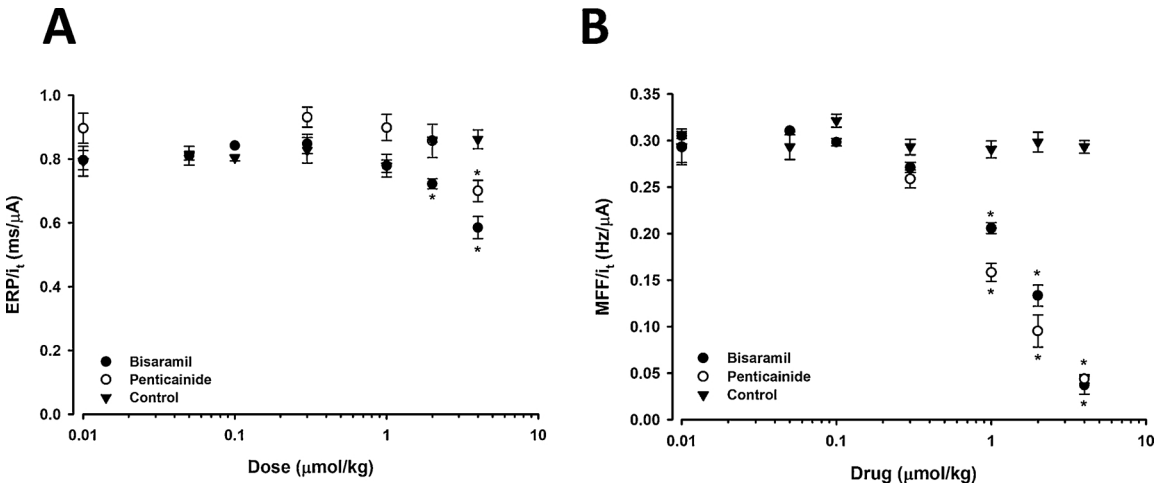

Fig. 5. The dose-dependent effects of bisaramil (●), penticainide (○) and saline control (▼) on the ratio of electrical stimulation measures of refractoriness in cardiac muscle, the effective refractory period (ERP) and maximum following frequency (MFF) to threshold current for induction of extrasystoles (i_t) in pentobarbital anesthetized rats (n = 5). Both drugs dose-dependently decreased the ERP/i_t (A) and MFF/i_t (B) ratios while saline control had no effect. Values were determined after completion of each dose. *Indicates P < 0.05 for difference from saline control.

bisaramil and penticainide directly block sodium and potassium channels in the heart and may also block calcium channels. These mixed ion channel blocking patterns are not uncommon with cardiac drugs and were originally discerned by Vaughan-Williams [9].

In addition to ECG and blood pressure changes *in vivo* and ECG and cardiac contractility changes in isolated Langendorff hearts, both drugs significantly reduced ischemia-induced arrhythmia mediated mortality and selectively reduced high frequency arrhythmias (*i.e.*, VT and VF) compared to low frequency arrhythmias (PVC). Previously, it was suggested that sodium channel block with bisaramil and penticainide primarily accounted for the antiarrhythmic properties of these drugs [22,27,30,32,52–54]. Our data supports these observations [27,28,30] but highlights the importance of the blockade of other channels in the heart. Our studies show that drug changes in repolarization parameters (Q–T, ERP, MFF) occur along with those that produce sodium channel block (PR, QRS, V_{max}, i_t, VF_t). The potassium channel blocking actions that occur are likely beneficial to sodium channel blockade alone at conveying antiarrhythmic efficacy since slowed conduction due to sodium channel blockade is a defined cardiac safety liability [55]. This effect has been described using iCEB, a novel non-invasive biomarker that determines the ratio of sodium to potassium channel blockade using both the QRS and QT intervals derived from the ECG (*i.e.*, iCEB = QT/QRS) [42]. iCEB may provide a novel means with which to determine the potential for development of drug-induced cardiac arrhythmias in the non-clinical [42] and possibly clinical assessment of drugs [43]. iCEB is increased with drugs that predispose to arrhythmias such as torsade de pointes (TdP) and is decreased with drugs that predispose to non-TdP mediated arrhythmias (*i.e.*, VT/VF). For bisaramil and penticainide the iCEB pre-drug ratio values (QT/QRS) were 1.6 and 1.5, respectively. At the highest administered doses (*i.e.*, 8 and 32 μmol/kg) the iCEB ratio values were reduced to 1.2 and 1.1, respectively. From Lu et al. [42] drugs that only slightly change iCEB may be safer than drugs that significantly increase or decrease iCEB; however, a greater evaluation of this novel index is needed. For instance, both drugs examined in this study produced increases in the blood pressure and heart rate adjusted iCEB but not at doses producing effects on electrical stimulation variables or at doses that were shown to be antiarrhythmic. It is possible that at higher doses producing greater changes in iCEB that arrhythmia's may occur; however, no arrhythmias were observed in any of the animals not subjected to coronary artery occlusion or electrical stimulation.

The inward sodium current responsible for Phase 0 depolarization (V_{max}) of the action potential is blocked by both bisaramil and penticainide. Drugs interact in a voltage- and time-dependent manner with the sodium channel [55]. Drug associated channels produce shifts in channel kinetics and are manifest as changes in the voltage-dependence of inactivation [55,56]. Bisaramil produces a hyperpolarizing shift the inactivation curve for the sodium current suggestive of a drug association with the inactive state of the channel [27,28]. Our current findings indicate that the onset to block and recovery from block occurred rapidly and thus bisaramil may have an affinity for the active (open) channel [27]. Such effects are frequently observed with drugs such as quinidine, a class Ia antiarrhythmic drug [56,57]. Examination of the VF_t data suggests that bisaramil is frequency-dependent since a marked increase in the fibrillation threshold was observed as was a reduction in the ratio of ERP/VFt and MFF/VF_t (*e.g.*, enhanced dose-related effect on VF_t over measures of repolarization). The effect of penticainide on VF_t was less pronounced at equivalent maximal doses as was the reduction in ERP/VF_t and MFF/ VF_t despite very similar profiles for dose-related changes in ERP/i_t and MFF/i_t and reductions in these ratios for both drugs. Taken together these results suggest that while both drugs may have had a similar ability to prevent the triggering of ventricular arrhythmia's at a given level of refractoriness (*i.e.*, log VPB, ERP/i_t), the apparently greater frequency dependent actions of bisaramil may have permitted for an enhanced ability of the drug to prevent arrhythmia degeneration of the incidence of VT to fatal VF.

Bisaramil prolongs both ERP and APD in guinea pig myocytes [19,20]. Paróczai & Kárpáti [20] showed that bisaramil prolonged ventricular conduction time and ERP and slowed atrioventricular conduction time. This effect is reflected as a prolongation of the P–Q interval of the ECG and suggests drug-induced calcium channel blockade. Sunami et al. [26] found that bisaramil directly blocked the cardiac calcium current. Clinically, bisaramil at a high dose (150 mg) prolonged the P–Q, QRS, and Q–T intervals of the ECG and reduced the rate of development of supraventricular tachycardias [58] confirming an effect on calcium channels.

Penticainide reduced cardiac V_{max} and slowed cardiac conduction in a concentration, voltage and rate-dependent manner [29,31]. While both Carmeleit [30] and Coraboeuf et al. [59] showed that penticainide reduced automaticity and blocked multiple cardiac currents, Sassine et al. [60] showed that penticainide inhibited the slow inward calcium current. Clinically, penticainide produces a negative inotropic effect [61], prevents reentrant tachycardia [62] and markedly affects depolarization and repolarization in patients with ventricular arrhythmias [63].

Thus, blockade of cardiac sodium current occurs primarily for bisaramil and penticainide; however, these drugs also block potassium (and likely calcium) currents which provides for a more balanced blockade of both inward and outward currents supporting antiarrhythmic, not proarrhythmic, activity. This balanced ion channel blockade, reflected by the minimal changes in the iCEB biomarker, limits the potential for the development of severe adverse events associated with sodium channel blockade alone which has been associated with increased mortality in patients [55]. The data obtained from the use of the iCEB biomarker could be the basis for the conduct of additional validation studies that assess the relationship between drug-induced sodium channel block, slowed conduction and pro-arrhythmic liability associated with these drug effects. Methodical comparisons between pure ion channel blocking drugs and drugs with mixed ion channel blocking properties using validated assays are also needed to complement the ongoing CiPA initiative. Proarrhythmic liability resulting from sodium channel blockade and mechanisms responsible for the developed arrhythmia will require the equivalent level of investigation that was used for drug-induced QT prolongation. Now that drug safety scientists have eradicated drug-related hERG signals from their drug discovery pipelines, drug effects on cardiac conduction may take on a more prominent role in the non-clinical evaluation of cardiac liabilities.

5. Conclusion

This study compared the antiarrhythmic and electrophysiological actions of bisaramil and penticainide, using *in vitro* and *in vivo* methods. Both drugs altered ECG measures indicative of sodium channel blockade (QRS interval) but also potassium (QT interval) and likely calcium channel blockade (P–R interval). In isolated hearts, both bisaramil and penticainide reduced conduction velocity but prolonged the Q–T interval of the EKG. In electrical stimulation studies both drugs increased indices of sodium and potassium channel blockade and were antiarrhythmic. In ischemic ventricular arrhythmias at doses exhibiting sodium and potassium channel blockade, both drugs significantly reduced the incidence and mortality due to ischemic arrhythmias. iCEB values did not change significantly over the dose range evaluated; however, drug separation occurred when changes in BP and HR were considered. In totality, these findings indicate that both bisaramil and penticainide are antiarrhythmic and produce a spectrum of activity in the heart indicative of mixed ion channel blocking effects.

Conflict of interest

This publication reflects the views of the authors and does not represent views or policies of any affiliated organization or company.

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