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Molecular charge associated with antiarrhythmic actions in a series of amino-2-cyclohexyl ester derivatives

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ABSTRACT

A series of amino-2-cyclohexyl ester derivatives were studied for their ion channel blocking and antiarrhythmic actions in the rat and a structure-activity analysis was conducted. The compounds are similar in chemical structure except for ionizable amine groups (pK values 6.1–8.9) and the positional arrangements of aromatic naphthyl moieties. Ventricular arrhythmias were produced in rats by coronary-artery occlusion or electrical stimulation. The electrophysiological effects of these compounds on rat heart sodium channels ($Na_v1.5$) expressed in *Xenopus laevis* oocytes and transient outward potassium currents ($K_v4.3$) from isolated rat ventricular myocytes were examined. The compounds reduced the incidence of ischemia-related arrhythmias and increased current threshold for induction of ventricular fibrillo-flutter (VF_t) dose-dependently. As pK increased compounds showed a diminished effectiveness against ischemia-induced arrhythmias, and were less selective for ischemia- versus electrically-induced arrhythmias. Where tested, compounds produced a concentration-dependent tonic block of $Na_v1.5$ channels. An increased potency for inhibition of $Na_v1.5$ occurred when the external pH (pH_o) was reduced to 6.5. Some compounds inhibited $K_v4.3$ in a pH-independent manner. Overall, the differences in antiarrhythmic and ion channel blocking properties in this series of compounds can be explained by differences in chemical structure. Antiarrhythmic activity for the amino-2-cyclohexyl ester derivatives is likely a function of mixed ion channel blockade in ischemic myocardium. These studies show that drug inhibition of $Na_v1.5$ occurred at lower concentrations than $K_v4.3$ and was more sensitive to changes in the ionizable amine groups rather than on positional arrangements of the naphthyl constituents. These results offer insight into antiarrhythmic mechanisms of drug-ion channel interactions.

1. Introduction

There have been many advances in the non-pharmacological treatment of cardiac arrhythmias with limited success for development of pharmacological interventions for ventricular arrhythmias. A major cause of death globally is ventricular fibrillation (VF) due to myocardial ischemia but there are no effective drugs for use in this arrhythmia. Conventional discovery of new antiarrhythmic drugs has historically focused on the selective blockade of cardiac ion channels to treat arrhythmias (Pugsley, 2002). This rationale was undermined by the increased mortality associated with channel-specific antiarrhythmics in clinical trials (Echt et al., 1991; Waldo et al., 1996). Despite these clinical failures a global need remains for new antiarrhythmics and a renewed focus in their discovery and development.

Our interest remains in the development of compounds that specifically prevent VF due to MI in the early phase of myocardial infarction. A major premise is that the physiological conditions that exist during early onset myocardial ischemia can be utilized to confer selectivity of drug action in ischemic ventricular tissue. This approach presumes that disordered electrical activity within the ischemic tissue is a *sine qua non* for induction of arrhythmias (Cascio, 2001; Cascio et al., 1995; Janse et al., 1980). Since the mechanisms for ischemia and reperfusion arrhythmias are different, our focus is on ischemia-induced arrhythmias. The logic behind such assumptions has been previously discussed (Bain et al., 1997; Beatch et al., 2002).

From the above assumptions, it is hypothesized that cardiac ion channel blockers that are more potent under the conditions found in ischemic cardiac tissue will be selectively antiarrhythmic for ischemia-induced arrhythmias. In particular, ion channel blockade would need to be greater in the low extracellular acid pH (pH_o) and elevated

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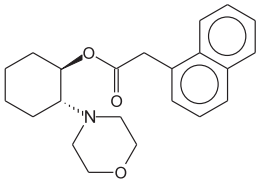
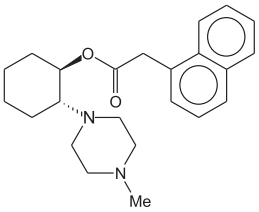
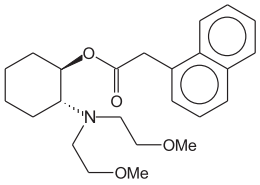


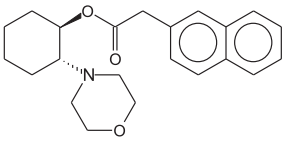
RSD1000 ((±)1,2-trans-1-(naphthylacetoxy)-2-(4-morpholino)cyclohexane monochloride));
pK = 6.1; M.W. 391.9



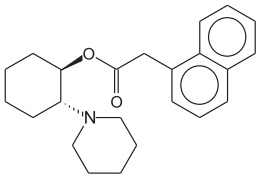
RSD1025 ((±)1,2-trans-1-(naphthylacetoxy)-2-(4-methylpipeazinyl)aminocyclohexane monochloride));
pK = 8.9; M.W. 389.9



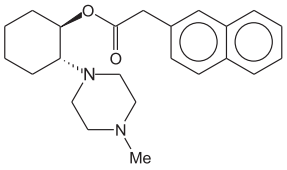
RSD1046 ((±)1,2-trans-1-(naphthylacetoxy)-2-bis-N,N-(2-methoxyethyl)aminocyclohexane monochloride));
pK = 7.0 ; M.W. 437.0



RSD1009 (1R,2R)/(1S,2S)-2-Morpholinyl-1-[(naphthalen-2yl)acetoxy]cyclohexane monohydrochloride;
pK = 6.1; M.W. 391.9



RSD1049 ((±)1,2-trans-1-(1-naphthylacetoxy)-2-piperidinocyclohexane monohydrochoride);
pK = 8.2; M.W. 375.9



RSD1015 (1R,2R)/(1S,2S)-2-(4-Methylpiperazinyl)-1-[(naphthalen-2yl)acetoxy]cyclohexane monohydrochloride;
pK = 8.9; M.W. 389.9

Fig. 1. Chemical structures and physiochemical properties of a series of amino-2-cyclohexyl ester RSD compounds. The pK values were measured according to the protocols described in the Methods section.

potassium conditions associated with the early phase of ischemia (Kleber et al., 1987). More importantly, ion channel blockade would need to include both sodium and potassium channels (Pugsley et al., 2015). Combined Class I and III effects would result in reduced excitability and increased refractoriness. During ischemia development, an ischemia-selective mixed ion channel blocker may render the ischemic tissue electrically quiescent, refractory and incapable of arrhythmogenesis.

We examined a series of structurally-related compounds to RSD1000, a mixed cardiac ion channel blocker with a selective antiarrhythmic effect against ischemia-induced arrhythmias (Yong et al., 1999). Chemical variations in the present series focused on the different ionizable amine groups (molecular pK) (Manallack, 2007) and the positional arrangement of the aromatic moiety. Most conventional sodium channel blocking antiarrhythmics are predominantly charged at physiological pH_o (7.4) because of their high pK values (e.g., quinidine, pK = 8.6; disopyramide, pK = 10.4) and are limited in their usefulness in models of ischemia-induced arrhythmias (Barrett et al., 1995). A limited molecular size has been implicated as a critical determinant for sodium channel binding (Courtney, 1988, 1990) while structure activity relationship (SAR) data for blockade of potassium channels by sodium channel blocking drugs remains limited in the literature. These studies were conducted to evaluate a series of structurally-related compounds and identify chemical attributes that may be associated with antiarrhythmic effectiveness against ischemia- and electrically-induced arrhythmias.

2. Materials and methods

Antiarrhythmic studies were performed in rats (male, Sprague Dawley; 200–300 g) with protocols approved by the Animal Care Committee of the University of British Columbia. The use of rat models for such purposes have been extensively addressed and justified in vivo (Curtis et al., 1987) and in vitro (Curtis, 1998). The study design and animal ethics conform with ARRIVE (Kilkenny et al., 2010) and guidance on experimental design and analysis (Curtis et al., 2018).

2.1. Coronary artery occlusion and ischemia-induced arrhythmias

The rat model of coronary artery occlusion has been extensively used (Curtis and Walker, 1988; Barrett et al., 1995, 2000; Yong et al., 1999). Briefly, rats anesthetized with pentobarbital (60 mg/kg, i.p. initially and supplemented, if necessary) were subjected to cannulation of the trachea, a carotid artery and jugular vein. Body temperature was maintained at 36–38 °C with a heating lamp. A polypropylene thread in a polyethylene guide was loosely placed around the left coronary artery. Rats were allowed 30 min to recover from the adverse effects of surgery prior to blind randomization into treated and control groups. Arterial blood samples were taken before and 15 min after coronary artery occlusion for determination of serum potassium concentrations. Infusion of RSD compounds (μmol/kg/min) was begun 5 min before permanent occlusion of the coronary artery and maintained for a further 15 min.

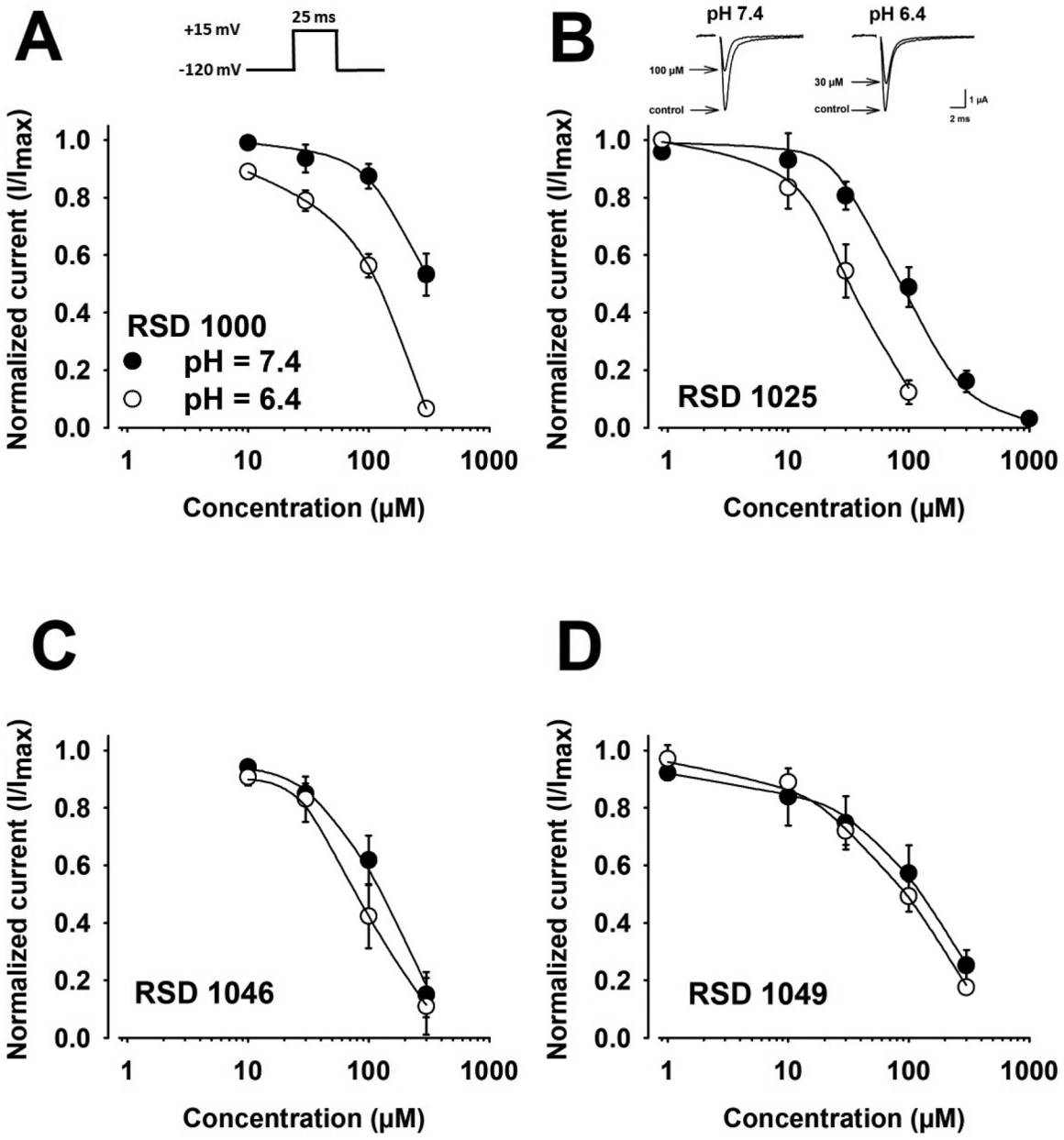


Fig. 2. The inhibitory actions of RSD1000, RSD1025, RSD1046, and RSD1049 on rat $Na_v1.5$ channels in *Xenopus* oocytes. Cells were held at −120 mV and depolarized to − 10 mV using a 20 ms pulse duration. The peak inward current was measured in the absence or presence of the drug at either pH 7.5 (•) or with an extracellular acidosis (pH 6.5, ○) external bath. The current amplitudes were normalized to control and plotted as a function of RSD concentration. Data points (mean ± S.E.M.) were fit according to the logistic Eq. (1) and the estimated IC_{50} values are listed in Table 4.

The incidence and duration of arrhythmias were recorded in the 15 min post-occlusion period. Thereafter, hearts were excised and perfused via the Langendorff technique with buffer containing cardio-green dye (0.2 mg/L Fast Green FCF) to reveal the under-perfused tissue (occluded zone; OZ). The OZ size was calculated as the percentage of the total ventricular weight.

The arrhythmia type and incidence for each animal was recorded and the total arrhythmic history was summarized as an arrhythmia score (AS; Curtis and Walker, 1988). This normally (Gaussian) distributed variable takes into account the occurrence, severity, and duration of arrhythmias. Criteria for arrhythmias followed the guidelines of the Lambeth Conventions (Walker et al., 1988; Curtis et al., 2013).

2.2. Ventricular arrhythmias induced by electrical stimulation

The electrical-induction of ventricular arrhythmias in anesthetized rats was performed according to the method of Walker and Beatch (1988). Rats were prepared as above, but without thoracic surgery. Stimulating electrodes were implanted by a transthoracic route in the apex of the left ventricle. Square wave stimulation (at 7.5 Hz) was used to determine threshold current (It-μA) and pulse width (Tt-ms) for pacing as well as the threshold current for induction of ventricular fibrillo-flutter (VFt-μA) at 50 Hz.

Stimulation variables were measured every 5 min until consistent values were established. Animals were then randomly allocated to vehicle or compound treatment. Infusion of compounds (μmol/kg/min)

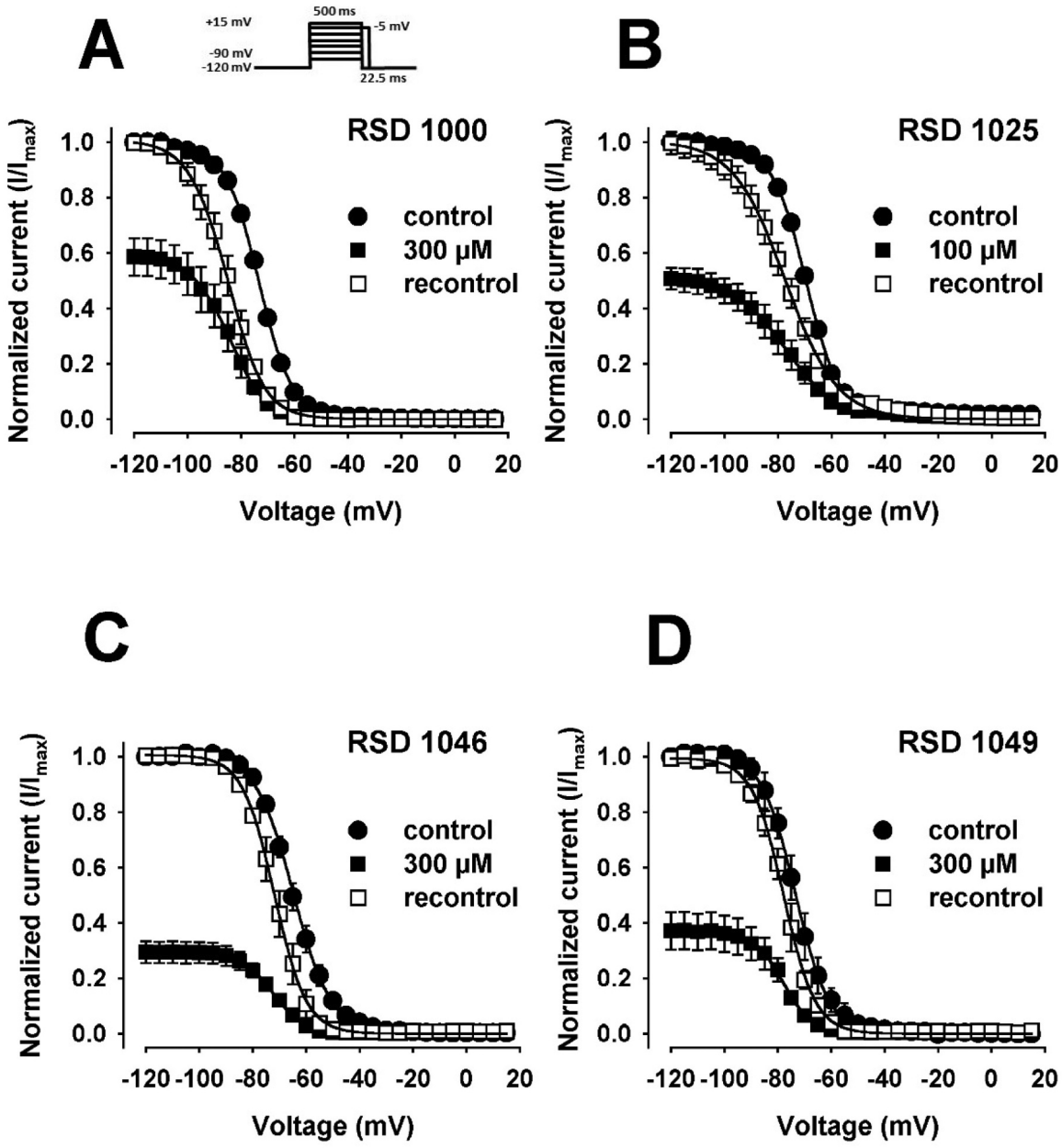


Fig. 3. The effects of RSD compounds on the voltage-dependence of inactivation of rat $Na_v1.5$ channels in *Xenopus* oocytes. Sodium currents were conditioned with a 500 ms pre-pulse from −120 mV to + 15 mV (in 5 mV increments) and this was followed by a test pulse to −5 mV for 22.5 ms, as indicated. Peak current amplitude was normalized to the maximum current amplitude and plotted as a function of the conditioning pre-pulse potential in the absence (•) and presence (■) of IC_{50}. Recontrol curves() are shown. Data were fitted to a two-state Boltzmann equation.

was continuous with successive doubling every 5 min. At the end of each 3rd minute of each infusion, electrical stimulation measurements (in duplicate) were taken over 2 min.

2.3. Expression of cardiac sodium ($Na_v1.5$) channels in Xenopus oocytes

The examination of drug effects on sodium currents was conducted in *Xenopus* oocytes since these cells do not express a large number of additional ion channels and receptors, allowing the sodium channel effects of these novel drugs to be studied without contamination from endogenous channels (Goldin, 1991). Experiments were performed according to guidelines established by the Institutional Animal Care and Use Committee of the University of California, Irvine and used elsewhere to investigate RSD921 (Pugsley and Goldin, 1999). Briefly, $Na_v1.5$ was expressed in *Xenopus* oocytes and studied in room temperature (20–22 °C) in ND96 bath solution of the following composition (in mM): 96 NaCl; 2 KCl; 1.8 $CaCl_2$, and 5 N-[2hyroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES), at pH_o of either 7.5 or 6.5. A low volume (0.7 ml) Plexiglas recording bath well allowed for efficient exchange (20–30 s) between control and compound-containing solutions from gravity-flow reservoirs. Recording microelectrodes contained 3 M KCl in 0.5% low melting point agarose and had resistances between 0.5 and 1 MΩ. Sodium currents were recorded using a virtual ground circuit and data were filtered at 3 kHz on-line and then digitized at a sampling frequency of 12.5 Hz. Currents were recorded and analyzed using pClamp 6.0.3 software (Molecular Devices, San Jose, CA). Capacitance transients and leak currents were corrected by P/4 subtraction with the depolarizations for subtraction applied after each protocol. Non-linear curve fitting was performed using SigmaPlot v. 5.0 (Systat Software, San Jose, CA). Concentration-responses were

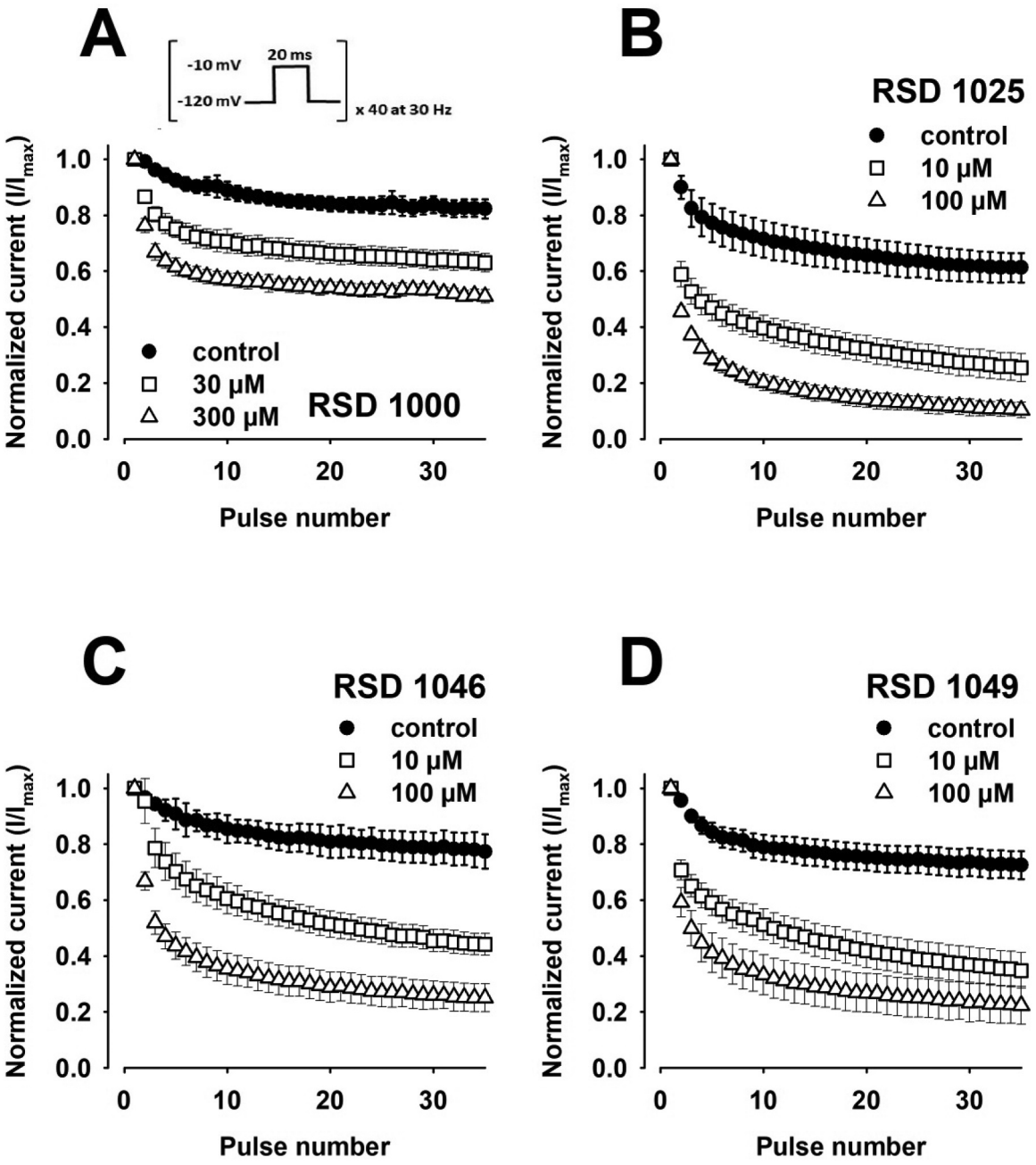


Fig. 4. The effects of RSD compounds on the frequency-dependent inhibition of rat $Na_v1.5$ channels in *Xenopus* oocytes. Cells were held at −120 mV and depolarized (40 runs) to −10 mV with a 25 ms pulse duration. Pulses were delivered at 30 Hz in the absence (•) and presence (10 and 30 μM denoted as □ depending upon compound and 100 μM denoted as Δ) of RSD compounds. Current amplitude during each pulse was normalized to the peak maximal current (1st pulse) and plotted as a function of pulse number.

determined for peak inward currents from cells depolarized from −120 mV to −10 mV. The −120 mV was used to allow for complete recovery from slow inactivation (Pugsley and Goldin, 1998). Currents were allowed to recover from slow inactivation for 10 min before beginning any electrophysiological protocols. The concentration-response curves were fitted to Eq. (1):

$$I_{Na} = [1 + ([A]/IC_{50})^{n}]^{-1} \quad (1)$$

where I_{Na} = fractional block, [A] = concentration of RSD compound, n = Hill coefficient, and IC_{50} = drug concentration blocking 50% of channels.

To determine voltage-dependence of sodium channel inactivation, a 500 ms conditioning pre-pulse from −120 mV to +15 mV (in 5 mV increments) was followed by a test pulse to −5 mV for 22.5 ms. Peak current amplitude was normalized to the maximum current amplitude and plotted as a function of the conditioning pre-pulse potential in the absence and presence of IC_{50}. Data were fitted to a two-state Boltzmann equation:

$$I = I_{max} * [1 + (\exp^{(V - V_{½})/k})]^{-1} \quad (2)$$

where I_{max} = maximal current evoked, V = potential of the voltage pulse, $V_{½}$ = voltage at which 50% of the current is inactivated (mid-point of the inactivation curve), and k = slope factor.

Frequency-dependent effects were examined from a holding potential of −120 mV with 40 depolarizations to −10 mV each of 25 ms duration. Pulses were delivered at 1 and 30 Hz in the absence and

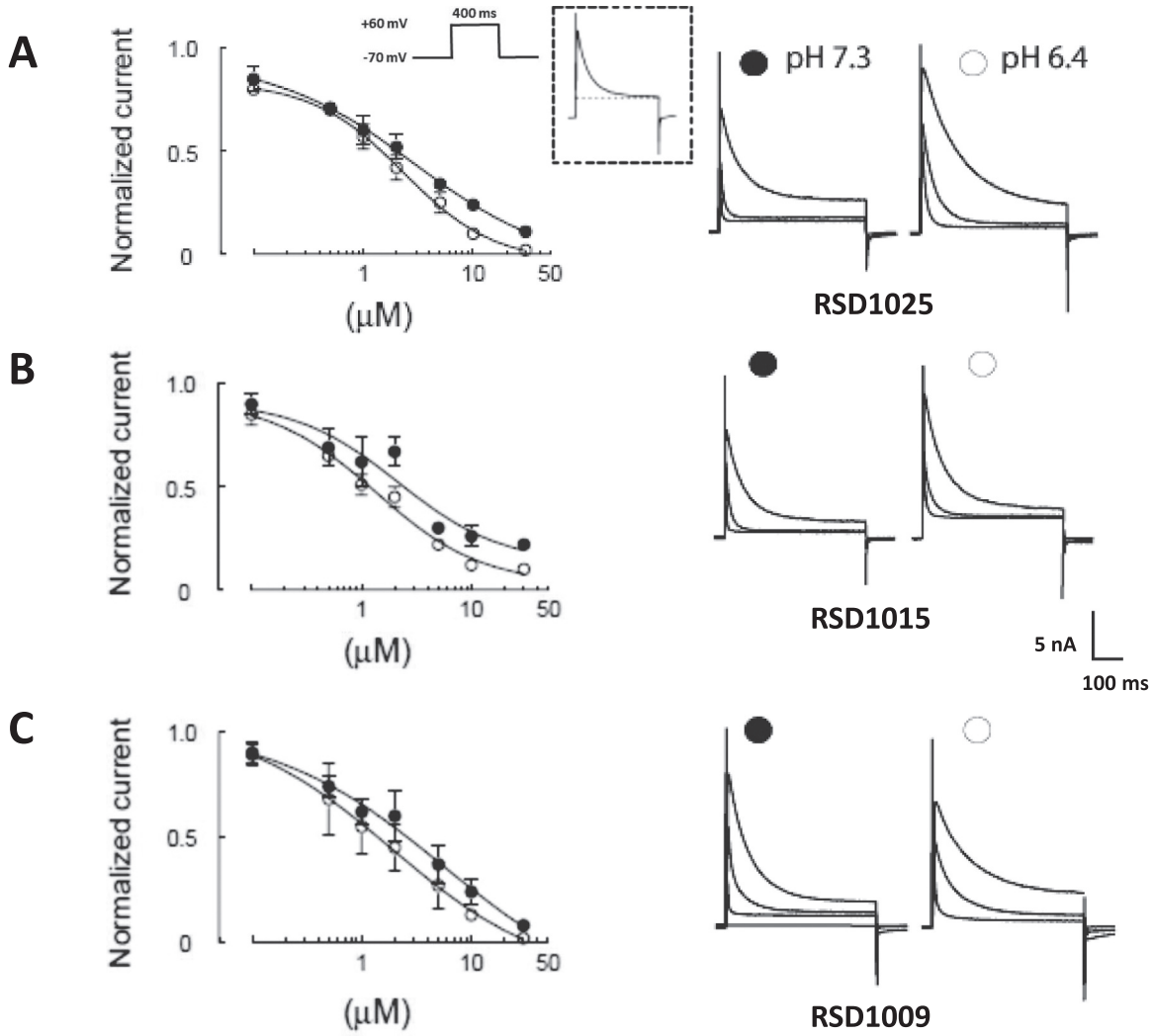


Fig. 5. The effects of RSD compounds on I_{to} currents in rat ventricular myocytes. I_{to} currents were recorded after a depolarizing step to + 60 mV for 400 ms from a holding potential of −70 mV. In the left panels (A-C) the concentration-response relationships illustrate the inhibition of I_{to} as a function of RSD concentration at low (pH 6.3 ○) and normal (pH 7.3 •) bath pH levels. The original current traces for each drug are shown in the right panels from different cells at either pH 7.3 (•) or 6.4 (○). Each current trace is shown in the absence (top trace) and presence of either 2 μM (middle trace) or 30 μM (bottom trace) concentrations of each RSD compound. I_{to} currents were expressed as the integral of the fast inactivating component of I_{to} current ($I_{to,f}$, hatched area of the current trace above the dashed line minus the non-activating component, $I_{to,s}$, see inset current trace) normalized to the integral of control current. Data points are fitted according Eq. (1) and IC_{50} values are listed in Table 7. Traces from RSD1000 are not shown for clarity (see Yong et al., 1999).

presence of RSD compounds. Current amplitude during each pulse was normalized to the peak maximal current (pulse number 1) and plotted as a function of pulse number.

2.4. Isolation of rat cardiac ventricular myocytes

Experiments were approved by the UBC Animal Care Committee. Hearts were excised from pentobarbital-anesthetized (100 mg/kg + 1000 u heparin, i.p.) Sprague-Dawley rats (200–250 g) and perfused via the aorta on a Langendorff perfusion apparatus. An initial washout period of 8 min was performed with Solution I, composed of (in mM): 150 NaCl, 10 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 10 HEPES, and 11 glucose, pH at 7.35. Solution I was bubbled with 100% O_2 and applied at a constant flow rate and temperature of 37°. The heart was then perfused with Solution I containing 1 mg/ml collagenase (317 u/mg, Type II, Worthington Biochemical, Freehold, NJ), 25 μM $CaCl_2$ and 1 mg/ml bovine serum albumin (fatty acid free, Sigma Chemical Co., St. Louis, MO) for 15–20 min before the ventricles were then cut from the atria and placed in 20 ml collagenase-free Solution I with 25 μM $CaCl_2$ and 1 mg/ml BSA (Solution II). The tissue was shaken and triturated gently at 37° to release dissociated myocytes. Cells were harvested by filtering the resulting suspension through a 200 μm nylon mesh, the filtrate was centrifuged at 180*g* in a table-top centrifuge to form a pellet from the cells. The supernatant was drawn off and the pellet was re-suspended in Solution II. Myocytes were stored at room temperature and the calcium concentration was successively increased (~ 10 min intervals) from 25 μM to a final concentration of 1.8 mM in four equal steps. Cells were allowed to settle for 2 h and only those cells that were rod-shaped, quiescent, and exhibited clear cross striations were used for the experiments.

2.5. Transient outward (I_{to}) potassium currents recorded from single rat ventricular myocytes

The protocols used were those detailed previously (McLarnon and Xu, 1995, 1997). In rat ventricular myocytes, two kinetic components have been shown to comprise the I_{to} outward current; one that activates and inactivates rapidly ($I_{to,f}$) and the other which is slower activating and sustained ($I_{to,s}$) (Apkon and Nerbonne, 1991). In this study, the focus was on the rapidly activating and inactivating component ($I_{to,f}$) as

Table 1a
Effects of RSD drugs on hemodynamics and heart rate in anesthetized rats.

Dose (μmol/kg/min)		Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Heart Rate (bpm)
RSD1000	vehicle	137 ± 5	121 ± 6	384 ± 8
	1	110 ± 2[a]	84 ± 2 [a]	376 ± 16
	2	99 ± 6 [a]	78 ± 4 [a]	367 ± 19
	4	89 ± 4 [ab]	65 ± 2 [ab]	348 ± 19
	8	81 ± 4 [ac]	62 ± 3 [ac]	295 ± 14
RSD1046	vehicle	137 ± 5	119 ± 6	383 ± 11
	0.5	123 ± 3	97 ± 2	358 ± 15 [a]
	1	142 ± 6	103 ± 8	314 ± 10 [a]
	2	124 ± 5	87 ± 4 [a]	276 ± 14 [ab]
	4	116 ± 8	95 ± 5	263 ± 28 [ab]
	8	123 ± 7	87 ± 7 [a]	259 ± 30 [ab]
RSD1049	vehicle	134 ± 5	116 ± 6	372 ± 12
	0.1	134 ± 5	102 ± 2	368 ± 14 [a]
	0.5	105 ± 5	84 ± 3	299 ± 15 [ab]
	1	117 ± 12 [a]	83 ± 7 [a]	267 ± 16 [ab]
	2	111 ± 7 [ab]	73 ± 7 [ab]	242 ± 20 [ac]
	4	91 ± 5 [ac]	49 ± 8 [ac]	207 ± 6 [ac]
	8	–	–	–
RSD1025	vehicle	137 ± 5	121 ± 6	351 ± 6
	0.5	128 ± 6	103 ± 3	331 ± 9 [a]
	1	119 ± 5 [a]	80 ± 2 [a]	332 ± 23 [ab]
	2	109 ± 5 [ab]	78 ± 3 [ab]	227 ± 13 [ac]
	4	95 ± 2 [ac]	58 ± 5 [ac]	207 ± 7 [ac]
RSD1009	vehicle	127 ± 4	103 ± 4	380 ± 8
	1	120 ± 8	98 ± 9	377 ± 13
	2.5	128 ± 8	104 ± 7	374 ± 10
	8	117 ± 6	96 ± 6	324 ± 6 [ab]
	16	118 ± 5	93 ± 2	292 ± 17 [ab]
RSD1015	vehicle	119 ± 5	93 ± 4	342 ± 9
	0.5	124 ± 5	104 ± 5	289 ± 12 [ab]
	1	106 ± 4 [b]	84 ± 5	248 ± 16 [ab]
	2	94 ± 4 [ab]	71 ± 6 [ab]	253 ± 11 [ab]
	4	79 ± 8 [ac]	57 ± 9 [ac]	237 ± 9 [ab]
	8	58 ± 3 [ac]	36 ± 2 [ac]	195 ± 16 [ab]

[a] $P < 0.05$ vs. pre-infusion for each dose (two-sample *t*-test). [b] $P < 0.05$ and [c] $P < 0.01$ vs. vehicle as change from pre-infusion (single-factor ANOVA followed by Dunnett's test).

Table 1b
Effects of RSD drugs on heart rate and the ECG in anesthetized rats.

Dose (μmol/kg/min)		PR Interval (ms)	QRS Interval (ms)	QT Interval (ms)
RSD1000	Vehicle	64 ± 1	28 ± 1	39 ± 1
	1	67 ± 3	27 ± 2	35 ± 2
	2	65 ± 3	31 ± 2	40 ± 2
	4	63 ± 2	32 ± 1	41 ± 1
	8	66 ± 3	29 ± 2	38 ± 2
RSD1046	Vehicle	39 ± 1	30 ± 1	39 ± 1
	0.5	42 ± 1	33 ± 1	42 ± 1
	1	42 ± 1	31 ± 1	43 ± 1
	2	41 ± 2	32 ± 2	47 ± 6
	4	39 ± 1	30 ± 1	49 ± 4 [a]
	8	43 ± 1	35 ± 1[a]	60 ± 6[ab]
RSD1049	Vehicle	40 ± 1	30 ± 1	40 ± 1
	0.1	43 ± 1	33 ± 1	42 ± 1
	0.5	41 ± 1	31 ± 1	42 ± 1
	1	40 ± 1	29 ± 1	43 ± 1 [a]
	2	39 ± 1	34 ± 4	51 ± 6 [a]
	4	40 ± 1	38 ± 2 [ab]	67 ± 9 [ab]
RSD1025	Vehicle	42 ± 2	33 ± 2	43 ± 2
	0.5	43 ± 1	34 ± 1	43 ± 1
	1	40 ± 1	33 ± 1	43 ± 1
	2	43 ± 1	34 ± 1	47 ± 1 [a]
	4	40 ± 1	35 ± 2 [ab]	51 ± 7 [ab]
RSD1009	Vehicle	65 ± 2	30 ± 2	42 ± 2
	1	68 ± 3	29 ± 1	43 ± 2
	2.5	70 ± 2	30 ± 3	42 ± 3
	8	71 ± 4	32 ± 4	41 ± 2
	16	74 ± 3 [a]	30 ± 1	44 ± 3
RSD1015	Vehicle	63 ± 2	31 ± 1	42 ± 2
	0.5	67 ± 3	32 ± 1	43 ± 1
	1	68 ± 1	32 ± 2	42 ± 2
	2	75 ± 1	30 ± 2	41 ± 3
	4	80 ± 4	36 ± 1 [a]	47 ± 1 [a]
	8	86 ± 9 [a]	37 ± 1 [a]	53 ± 5 [a]

The dose response effects of RSD compounds on heart rate (HR; beats/min) and ECG variable are shown. Values are mean ± S.E.M. (n = 5) for heart rate (HR) and ECG variables before and 5 min after commencing infusions of compound. Infusions were cumulative with a higher dose given after 5 min. Note that [a] $P < 0.05$ vs. pre-drug for each dose (*t*-test). [b] $P < 0.05$ vs. vehicle as change from pre-drug (ANOVA and Dunnett's test).

representing predominantly I_{to} (see Fig. 5, inset). Under our experimental conditions, this Ca^{2+}-independent component of I_{to} was studied since external cadmium was added to the perfusing solution and ethylene glycol-bis(β-aminoethyl ether) N,N,N',N-tetraacetic acid (EGTA, 5 mM) was included in the pipette solution thereby blocking $I_{Ca,L}$. I_{to} was activated with a 400 ms depolarizing step to + 60 mV from a holding potential of −70 mV using an Axopatch 200 A amplifier (Axon Instruments, Foster City, CA) with the low-pass filter set at 1 or 2 kHz. Capacitative current and series resistance were compensated using the analog circuitry of the amplifier. The micropipettes were made from Corning 7052 glass (A-M Systems, Everett, WA) and had resistance values between 2 and 4 MΩ when filled with recording solution. Current recordings were performed at room temperature (21–24 °C).

The solution used to perfuse the cells during an experiment to record I_{to} contained (in mM): 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 0.2 $CdCl_2$, 5 glucose, and 10 HEPES, adjusted to pH 7.3 with 5 N NaOH. Cadmium and tetrodotoxin (5 μM) were included in the bathing solution to block inward calcium and sodium currents, respectively. The pipette solution contained (in mM): 120 KCl, 0.15 $CaCl_2$, 6 $MgCl_2$, 5 EGTA, 5 adenosine-5'-triphosphate disodium salt (Na_2-ATP), and 10 HEPES, adjusted to pH 7.3 with 1 N KOH.

2.6. Statistical analysis

Comparisons between each dose level and control groups for each compound were performed using a two-way ANOVA followed by Dunnett's test with significance levels of $P < 0.05$ or $P < 0.01$, where indicated. Electrophysiological data for $Na_v1.5$ are shown as the mean ± S.E.M. for n experiments and analyses were performed using SigmaStat® (Systat Software, San Jose, CA) statistical software with $P < 0.05$ being considered statistically significant. Group data for I_{to} are expressed as mean ± S.E.M. Statistical significance was determined using Student's *t*-test or two-way ANOVA. A nonlinear least-square curve fitting program Sigma Plot 5.0 (Systat Software, San Jose, CA) was used to perform curve fitting procedures.

2.7. RSD compounds

Cardiome Pharma Corp. (Vancouver, B.C., Canada) synthesized all of the RSD compounds used in this study. Their chemical names, structures, and physiochemical properties are summarized in Fig. 1. The monohydrochloride salt of each compound was dissolved in distilled water or a 22%:78% mixture of ethanol: distilled water. All compounds were esters based upon RSD1000 (PubChem CID: 9863383; pK of 6.1). For RSD1009 the only difference with RSD1000 was the configuration of the napthylene ring. Chemical modifications were made on the nitrogen atom attached to the cyclohexyl ring to change the pK of the compound which resulted in pK values varying from 6.1 (RSD1000 and RSD1009) to 8.9 (RSD1015 and RSD1025). The molecular difference between RSD1015 and RSD1025 is similar in nature to that between RSD1000 and RSD1009.

Table 2
Effects of RSD drugs on arrhythmia incidence in anesthetized rats subject to coronary artery occlusion.

Dose (μmol/kg/min)		VT Incidence	VF Incidence	AS
RSD1000	Vehicle	7/7	7/7	5.9 ± 0.2
	1	5/7	7/7	5.9 ± 0.1
	2	4/7	6/7	4.0 ± 0.9
	4	2/7[a]	1/7[a]	1.5 ± 0.6[a]
	8	1/7[a]	0/7[a]	0[a]
RSD1046	Vehicle	12/12	11/12	6.1 ± 0.3
	0.5	6/7	4/7	4.9 ± 0.6
	1	5/5	4/5	5.7 ± 0.5
	2	2/5[a]	2/5[a]	3.9 ± 0.4[a]
	4	0/5[a]	0/5[a]	0[a]
	8	1/5[a]	0/5[a]	1 ± 1[a]
RSD1049	Vehicle	11/11	11/11	6.0 ± 0.2
	0.1	6/6	6/6	5.3 ± 0.6
	0.5	5/6	3/6[a]	4.5 ± 0.5[a]
	1	2/5[a]	1/5[a]	3.0 ± 0.8[a]
	2	1/5[a]	0/5[a]	1.4 ± 0.9[a]
	4	–	–	–
RSD1025	Vehicle	11/11	9/11	5.8 ± 0.3
	0.5	5/5	1/5 *	3.4 ± 1.1
	1	4/5	2/5	2.8 ± 0.9
	2	4/7	3/7	2.6 ± 1.5[a]
	4	1/7[a]	0/7[a]	1.1 ± 0.4[a]
RSD1009	Vehicle	14/15	13/15	5.7 ± 0.4
	1	7/7	7/7	6.8 ± 0.3
	2.5	5/5	3/5	3.8 ± 0.7
	8	6/9	5/9	2.4 ± 0.6[a]
	16	3/9[a]	1/9[a]	1.5 ± 0.5[a]
RSD1015	Vehicle	12/16	15/16	6.3 ± 0.4
	0.5	7/9	6/9	4.6 ± 0.3
	1	7/9	4/9[a]	2.5 ± 0.7
	2	5/7	4/7	2.3 ± 0.6[a]
	4	2/7[a]	1/7[a]	1.5 ± 0.9[a]

The dose response effects of RSD compounds on arrhythmia incidence is shown. Values for arrhythmia score (AS), defined in the methods, are mean ± S.E.M. after occlusion. Arrhythmias were recorded as VT and VF.

[a] $P < 0.05$ for difference from vehicle. – denotes that at this dose occlusion caused cardiac output failure immediately after ligation of the coronary artery.

2.8. Determination of pK values for compounds

The pK values (i.e., the logarithmic value of the acid dissociation constant, K_a) for the RSD compounds were determined by a standard equivalence point method using (NaOH) titration, in which a pH-titration was made in water and ethanol/water mixtures as described above. The solution was illuminated with a tightly focused beam of light to detect any precipitate of free base. The equivalence point of the test solution was determined graphically from continuous titration curves.

3. Results

3.1. Ischemia-induced ventricular arrhythmias

The antiarrhythmic effects of RSD compounds were tested in intact rats subjected to coronary-artery ligation. Five min post-infusion but prior to coronary occlusion, all compounds produced dose-dependent reductions in blood pressure and heart rate (Table 1a) with RSD1025, RSD1046 and RSD1049 being more potent compared to RSD1000. Similarly, most compounds, at the highest doses tested, except RSD1000, produced effects on the PR, QRS and QT intervals of the ECG (Table 1b). Serum potassium concentrations prior to treatment were not significantly different among groups ($[K^+] = 3.6–4.0$ mM) and remained unchanged in surviving animals 15 min post-occlusion.

The dose-response effects of the compounds on ischemia-induced arrhythmias are shown in Table 2. All of the compounds tested reduced the incidence of ischemic arrhythmias in a dose-dependent manner whether measured as an AS or group incidence of VT or VF. Those compounds with low pK values tended to have greater reductions in these measures compared to those compounds with high pK values. Notably, only RSD1046 and RSD1000 provided near maximal protection against ischemic arrhythmias at 4–8 μmol/kg/min (Table 2). Similar antiarrhythmic protection at this dose range could not be attained with RSD1049 or RSD1025 due to detrimental effects on blood pressure, heart rate (Tables 1a, 1b) and development of atrioventricular

Table 3
Effects of RSD drugs on threshold for induction of ventricular fibrillo-flutter (VF_t) in anesthetized rats.

Compound	Dose (μmol/kg/min)	% VF_t (1000μA)
RSD1000	1	2 ± 0.2
	2	5 ± 0.3[a]
	4	12 ± 2[a]
	8	26 ± 4[a]
	16	41 ± 7[a]
RSD1046	0.5	2 ± 0.4
	1	16 ± 3[a]
	2	25 ± 2[a]
	4	42 ± 5[a]
	8	58 ± 3[a]
RSD1049		
	0.1	5 ± 2
	0.5	30 ± 4[a]
	1	33 ± 6[a]
	2	63 ± 8[a]
	4	82 ± 7[a]
RSD1025		
	0.5	5 ± 2
	1	24 ± 3[a]
	2	44 ± 6[a]
	4	100[a]
RSD1009		
	1	2 ± 0.5
	2.5	5 ± 0.3
	4	22 ± 5[a]
	8	27 ± 5[a]
	16	38 ± 10[a]
RSD1015		
	0.5	0
	1	1 ± 0.2
	2	20 ± 2[a]
	4	38 ± 4[a]
	8	87 ± 6[a]
	16	96 ± 5[a]

Current was applied using square pulses at 50 Hz to produce ventricular fibrillo-flutter (VFt). The minimum current threshold to capture VFt was determined in the presence of RSD compound. This was expressed as a percentage of the maximum current (% VF_t) that was applied (1000 μA).

[a] $P < 0.05$ for difference from vehicle.

Table 4
Inhibition of the rat cardiac sodium channel ($Na_v1.5$) expressed in *Xenopus laevis* oocytes by RSD drugs.

RSD Drug	IC_{50} (μM)		IC_{50} (μM)	
	pH 7.3	n_H	pH 6.5	n_H
1000	335 ± 38	1.7 ± 0.2	94 ± 8[a]	1.6 ± 0.3
1046	141 ± 17	1.6 ± 0.2	83 ± 14	1.0 ± 0.2
1049	125 ± 34	1.3 ± 0.4	95 ± 20	1.2 ± 0.2
1025	86 ± 14	1.6 ± 0.1	33 ± 6[a]	1.6 ± 0.3

The concentration-response curves for the effects of each drug on the rat heart ($Na_v1.5$) sodium channel were determined as described in the Methods in the absence and presence of each drug at the pH_o indicated. The fractional block of sodium current at each concentration was plotted against the log concentration of drugs (Fig. 2) and fit with the Hill equation as described in the Methods. Results are mean ± S.E.M. for at least 4 individual cells.

[a] $P < 0.05$ for difference between IC_{50} values.

Table 5
Effect of RSD drugs on the voltage-dependence of $Na_v1.5$ current inactivation.

RSD Drug	$V_{1/2}$ (mV)	*k* (mV)
RSD1000		
Control	− 73 ± 0.2	6.2 ± 0.2
300 μM	− 85 ± 1.7*	6.7 ± 0.5
RSD1046		
Control	− 66 ± 2.2	6.4 ± 0.5
300 μM	− 72 ± 3.2*	5.6 ± 0.3
RSD1049		
Control	− 72 ± 4.0	5.8 ± 0.5
300 μM	− 77 ± 3.0*	5.4 ± 1.1
RSD1025		
Control	− 70 ± 1.5	6.3 ± 0.3
100 μM	− 78 ± 3.8*	6.7 ± 1.2

Data were fit to a two-state Boltzmann function as outlined in Methods. $V_{1/2}$ is the voltage at which half-maximal current inactivation occurs and k is the slope factor.

* Indicates a statistically significant difference from control at $p < 0.05$.

Table 6
Effects of RSD drugs on the frequency-dependence of $Na_v1.5$ current block.

Drug	1 Hz	30 Hz
RSD1000		
control	2%	17%
30 μM	3%	37%
100 μM	1%	45%
RSD1046		
control	5%	19%
30 μM	8%	43%
100 μM	22%	76%
RSD1049		
control	4%	24%
30 μM	5%	58%
100 μM	8%	76%
RSD1025		
control	5%	26%
30 μM	8%	63%
100 μM	22%	84%

Table 7
The IC_{50} values for inhibition of $K_v4.3$ currents in rat ventricular myocytes by RSD drugs.

RSD Drug	IC_{50} (μM)					
	pH 7.3	*n*	n_H	pH 6.5	*n*	n_H
1025	2.1 ± 0.2	6	1.1	2.6 ± 0.2	7	0.8
1015	1.4 ± 0.3	5	0.8	2.0 ± 0.1	5	0.6
1009	1.5 ± 0.3	5	0.9	3.0 ± 0.6	5	1.1
1000	3.4 ± 0.3	6	0.8	4.1 ± 0.2	6	1.1

Values are mean ± S.E.M. of number (n) of individual cells at pH_o 7.3 and 6.4 along with the Hill coefficient (n_H) from Eq. (1).

block (AVB) immediately upon ligation of the coronary artery resulting in cardiovascular collapse and mortality (Table 2). No proarrhythmic effects were observed for any of the compounds.

3.2. Electrically-induced ventricular arrhythmias

All compounds were tested for antiarrhythmic actions against electrically induced arrhythmias using the threshold for induction of ventricular fibrillo-flutter (VF_t). While all compounds produced dose-dependent increases in VF_t (Table 3) RSD1049, RSD1025 and RSD1015 provided greater antiarrhythmic activity (i.e., increases in VF^t) than RSD1046, RSD1000 or RSD1009 suggesting that these compounds with higher pK values provide greater efficacy against electrically-induced arrhythmias (Table 3).

In reviewing the spectrum of activity of these compounds in both ischemia and electrically-induced arrhythmias, the major observation was that the low pK compounds provided the best protection against ischemia-induced arrhythmias. However, while the high pK compounds tended to have better activity against electrically-induced arrhythmias, they have a reduced safety margin in ischemic hearts for drug-mediated development of AVB and mortality. In an attempt to explain these findings electrophysiological studies were performed to assess the effects of these compounds on cardiac ionic currents.

3.3. Blocking actions of RSD compounds on $Na_v1.5$ current in Xenopus Oocytes

Since these compounds are novel derivatives of RSD1000, and it has been shown that RSD1000 reduces the incidence of cardiac arrhythmias by blockade of cardiac sodium channels (Yong et al., 1999) we examined the effects of these compounds on the electrophysiological properties of wild-type $Na_v1.5$ cardiac channels expressed in *Xenopus* oocytes using a two-electrode voltage clamp. Oocytes were held at −120 mV and currents were evoked by depolarizations to −10 mV every 6 s and perfused with compounds at physiological (7.3) and acidic (6.5) pH_o. This intermittent pulse protocol was used to show drug interactions with either the rested or open sodium channel state, thus, minimizing the effects of frequency-dependent block and providing a reasonable estimation of the extent to which these compounds produce tonic block of the sodium current. Fig. 2 shows the inhibition of $Na_v1.5$ at pH_o 7.3 and 6.5 as a function of concentration with best fits to the Hill equation (Eq. (1)). The Hill equation fitting parameters are given in Table 4. The potency order was RSD1025 > 1046 = 1049 > 1000. The stoichiometry of inhibition was not significantly different for any of the compounds (n_H in Table 4) suggesting that only one compound molecule is needed to block the channel. Fig. 2 shows that there was a limited increase in the potency for all compounds at pH_o 6.5. Both RSD1000 and RSD1025 showed a ~3.5 and ~2.6-fold change in potency, respectively, while RSD1046 and RSD1049 showed a ~1.3–1.6-fold change in potency.

3.4. The effects of RSD compounds on the voltage-dependence of inactivation of $Na_v1.5$ current

Lidocaine and many other antiarrhythmic drugs preferentially bind to the inactive state of the sodium channel and all generally produce a negative shift in the steady-state voltage-dependence of inactivation (Bean et al., 1983). Thus, in order to determine whether the RSD compounds produce blockade of the inactivated state of the sodium channel we characterized the effect of these drugs on the voltage-dependence of inactivation. Inactivation was examined using a two-pulse protocol with a 500 ms inactivation pre-pulse to ensure that all of the channels were inactivated. The RSD compounds were tested at concentrations sufficient to provide marked current block. The effects of a 300 μM concentration of RSD1000, RSD1046, and RSD1049 and 100 μM concentration of RSD1025 are shown in Fig. 3 as curves of best fits to a two-state Boltzmann function (Eq. (2)). For RSD1000, a −12 mV hyperpolarizing shift in the voltage-dependence of the inactivation curve was observed. Under control conditions, half-maximal current inactivation ($V_{1/2}$) occurred at −73 ± 0.2 mV (n = 4), which was shifted to 85 ± 1.7 mV in the presence of RSD1000. RSD1046, RSD1049, and RSD1025 also produced hyperpolarizing shifts in the voltage-dependence of inactivation that are summarized in Table 5. None of the compounds significantly changed the slope factor for $Na_v1.5$. Therefore the data indicates that all of the compounds tested significantly shift the voltage-dependence of sodium channel inactivation in the hyperpolarizing direction and that these compounds likely associated with the inactivated state of the cardiac sodium channel.

3.5. Frequency-dependent activity of RSD compounds on $Na_v1.5$ current

Differential block of sodium channels in closed, open or inactivated states can result in frequency- or use-dependent block, an important characteristic for antiarrhythmic drug efficacy against high frequency arrhythmias. We examined frequency-dependent block using rapid trains of depolarizing pulses in the absence and presence of each compound (Fig. 4). Steady-state tonic block of $Na_v1.5$ was achieved using a single depolarizing pulse every 6 s in the absence and presence of each compound. Two concentrations of each compound was utilized to obtain minimal current block ($\sim EC_{10}$) and marked current block ($\sim EC_{75}$). Following steady-state drug (tonic) block, trains of depolarizing pulses were delivered to assess the extent of phasic block at different frequencies (1 and 30 Hz). The results are shown in Fig. 4 for each compound examined at a pulse frequency of 30 Hz. The percent reduction of peak current block at the 35th pulse is shown at frequencies of 1 and 30 Hz in Table 6. During the 1 Hz series of depolarizing pulses, all sodium channels were minimally blocked by all compounds at the low and high concentrations examined. It was only at a very high rate of channel stimulation (30 Hz) that a frequency-dependent component of block was revealed for all compounds.

3.6. Effects of RSD compounds on the inhibition of Kv4.3 (I_{to}) in isolated rat ventricular myocytes

The effects of the enantiomeric pairs RSD1000/RSD1009 and RSD1025/RSD1015 on I_{to} currents were examined in isolated rat ventricular myocytes perfused with compounds at physiological (7.3) and acidic (6.5) pH_o. All compounds produced concentration-dependent reductions in the maximal amplitude of the current and also accelerated the rate of I_{to} current decline. Representative I_{to} currents recorded with pH_o 7.3 and 6.4 are shown in Fig. 5 in the absence and presence of 2 and 30 μM concentrations of RSD1025, RSD1015, and RSD1009 (Fig. 5A-C). The IC_{50} values are summarized in Table 7 and show that the compounds tested were equally effective at pH_o 7.3 and 6.4. The Hill coefficient values were all close to unity suggesting that the binding of one RSD molecule is sufficient to block potassium channel permeation.

4. Discussion

This study evaluated a series of structurally-related compounds for antiarrhythmic actions against ischemia- and electrically-induced arrhythmias and how these properties related to their blocking actions on sodium and potassium currents. The RSD compounds were all amino-2-cyclohexyl esters of the prototype, RSD1000. The hypothesis was that compounds with low pK values would exhibit greater antiarrhythmic activity for ischemia- versus electrically-induced arrhythmias compared to higher pK compounds. This physicochemical aspect of drug development was suggested by Beatch et al. (2002) and Bain et al. (1997). For the compounds studied this tended to be the case regardless of the nature of the chemical group used to alter the pK value of the core nitrogen. Other positional changes on the major lipid group did not significantly change ion channel potency or antiarrhythmic effectiveness.

Our original supposition was that a change in the pK of a molecule changes the proportion of charged species under different pH conditions which should result in an increased efficacy for low pK compounds against ischemic arrhythmias. This property is well characterized for many local anesthetics since they predominantly have pK values near 7 and exist in both neutral and charged forms at physiological pH. Thus, this chemical property should confer a selective action against ischemic but not electrical arrhythmias since no pH change is associated with the latter. Simple calculations illustrate this idea. According to the Henderson-Hasselbalch equation, the percentage of ionized species of RSD1025 (pK 8.9) at pH_o 7.4 and 6.4 would be ~97% and ~99%, respectively, whereas for RSD1009 (pK 6.1), it would be ~5% and ~34%. Thus, at a concentration of 10 μM, a change of one pH unit (i.e., from 7.4 to 6.4) would change the concentration of charged RSD1025 from 9.7 to 9.9 μM; however, for RSD1009 a change of one pH unit would change the drug concentration from 0.5 to 3.4 μM. Thus, a high pK compound would exist in a predominantly charged form whereas the converse would be true for low pK compounds where the concentration of the charged form would be approximately 7 (34%/5%) times higher in ischemic tissue where pH_o is reduced due to reduced blood flow, accumulation of metabolic waste products and low oxygen levels in the tissue. Such considerations would predict reduced activity for cardiac effects for low pK compounds at normal pH and higher potency at acid pH thereby conferring 'selectivity' for ischemic versus electrical arrhythmias. A critical assumption to the argument is that it is the charged species that is the active antiarrhythmic compound. Evidence for such a view has accumulated beginning with the observation that quaternary lidocaine analogs only block sodium currents if applied inside nerves (Narahashi et al., 1970). The potency for low pK compound activity against electrical arrhythmias was ~6 times less than against ischemic arrhythmias whereas high pK compounds were equipotent against these arrhythmias. A lack of selectivity of the high pK compounds likely resulted in narrow cardiac safety margins limiting their protective ischemic action. Most antiarrhythmic sodium channel blockers are charged at physiological pH (Gintant et al., 1983). Thus, the RSD compounds with high pK values share a similar narrow therapeutic margin as most sodium channel antiarrhythmics (Pugsley, 2002).

Despite the findings from the antiarrhythmic data, some uncertainty existed with regard to the increased potency for sodium channel blockade for low pK compounds during extracellular acidosis (low pH_o). No marked increase in potency was observed for $Na_v1.5$ current blockade by any compound under acidotic conditions (Fig. 2), although high pK compounds tended to be more potent at either pH. The latter is consistent with antiarrhythmic drugs since an ionizable moiety on a drug molecule is a fundamental tenet for the modulated receptor hypothesis (Hille, 1977a; Hondeghem and Katzung, 1977). An increase in potency for sodium channel blockade in an acidic environment is explained by delayed recovery kinetics for the protonated blocker (Wendt et al., 1993; Chernoff and Strichartz, 1990; Mooran et al., 1986; Broughton et al., 1984; Grant et al., 1982). Our results, particularly for the high pK compounds, are consistent with this observation but an acid-induced rate limiting recovery process in vitro does not explain the different antiarrhythmic profiles observed in vivo. These differences likely relate to variances in pharmacokinetics (but no assays were developed to quantitate these compounds) or effects on other cardiac ion channels.

All compounds exhibited frequency- and inactivation-dependent effects and findings suggest preferential binding to the inactive state of the sodium channel. Drugs targeting these physiological changes in cardiac tissue reduce excitability and abolish conduction within ischemically-depressed tissue (Janse, 1992) where there is a depolarized resting membrane potential and an increased proportion of inactivated sodium channels (Bean et al., 1983). Similarly, frequency-dependent kinetics for sodium channel block is another important property for a drug. The interaction between blocking drugs and the sodium channel is modulated by higher binding affinities to the active or inactivated state compared to the resting state of the channel (Hondeghem and Katzung, 1977; Pugsley, 2002). Thus, the degree of blockade is potentiated at elevated heart rates compared with normal heart rates (Hondeghem and Snyders, 1990; Davis et al., 1986). The decrease in sodium current at high rates of stimulation results from an accumulation of drug-associated channels, since sodium channels spend more time in the active and inactivated states as the inter-pulse interval shortens (Pugsley and Goldin, 1999). Drugs such as lidocaine become highly effective under these conditions by reducing excitability and abolishing conduction within ischemically-depressed tissue (Janse,

1992). A similar mechanism likely explains the antiarrhythmic activity associated with these RSD compounds on sodium channels in addition to pK mediated differences in ischemia-selectivity.

With respect to $K_v4.3$, the major repolarizing current in the rat ventricle, blockade was seen with RSD1025, RSD1015, and RSD1009 that was similar to that reported for RSD1000 (Yong et al., 1999). No compounds increased channel blocking potency with extracellular acidosis. Structurally, blockade was independent of the positional arrangement of the naphthyl group as well as the pK of the ionizable nitrogen group. The blockade of $K_v4.3$ with tedisamil, a Class III antiarrhythmic drug, is protective against ischemia-induced arrhythmias but only at doses causing marked QT interval widening ($>$ 135 ms) in the rat (Dukes and Morad, 1989; Beatch et al., 1990; Adaikan et al., 1992). Unlike tedisamil, the RSD compounds blocked the I_{to} current but antiarrhythmic efficacy resulted at doses that only slightly prolonged the QT interval (by $\sim$ 20 ms) in this species.

When the RSD compounds are contrast with conventional sodium/potassium channel blockers the easiest chemical comparators are the local anesthetic and Class I antiarrhythmic. Structurally, the RSD compounds have the same three major chemical domains: an aryl ring, a hydrophilic amine and a linker that includes an ester, amide or ether (Sheldon et al., 1994; Hill et al., 1988). The amine moiety is most likely to bind to the voltage-sensitive region of the channel (Courtney, 1988), whereas the necessity of an aryl ring suggests the importance of interaction with a domain within the channel that accommodates the ring (Lloyd and Andrews, 1986). Indeed, little difference in antiarrhythmic efficacy was seen between the 2-naphthyl, RSD1009, and the naphthyl homologue, RSD1000. Sodium channel pores are small in diameter ($\sim$ 4.1 Å), water-filled (Courtney, 1987; Payandeh et al., 2011) and narrow toward the selectivity filter (Heinemann et al., 1992; Favre et al., 1996) suggesting sodium channel pores "restrict" drug blocker-binding interactions. Recently, Buyan et al. (2018) conducted a molecular dynamics examination of the binding of six compounds (three electrically neutral and three existing in neutral and positively charged forms at pH 7.0) that block sodium channels. While both forms partition into the lipid bilayer, findings suggest the existence of two possible binding sites. While neutral molecules preferentially bind to the S6 site on the sodium channel, charged compounds bind to an uncharacterized site in which the positively charged moiety directly interacts with the selectivity filter of the channel and competes with the binding of sodium ions. Thus, the existence of an additional blocking site may assist in rationalizing the differences observed between compounds with difference pK values.

Unlike sodium channels, the voltage-gated potassium channel is a channel pore with an extracellular-end cavity that is $\sim$ 10 Å across (Doyle et al., 1998). The larger pore size of potassium channels accommodates drugs of different dimensions, thus it is highly promiscuous with respect to the potential of a chemical compound to block the channel.

These studies show noticeable differences in the antiarrhythmic profile between related compounds due to the pK of the central nitrogen of the compound. These differences cannot be wholly explained by selective ion channel blockade for acidotic conditions associated with ischemia. These findings suggest that the additional potassium channel blockade afforded by the molecule complements sodium channel blockade and imparts antiarrhythmic activity. While this is one potential mechanism another possibility not investigated may include ischemia-dependent potentiation of potassium channel blockade by a mechanism not involving altered physiological (i.e., pH) buffer conditions. A comparison with known antiarrhythmic drugs, however, allows for a re-examination of the proportion of charged species that block multiple cardiac ion channels and their role in antiarrhythmic activity. One notable comparison is with quinidine which has a pK of 8.6 and blocks both sodium and potassium currents. In the rat arrhythmia model the antiarrhythmic dose-response curve for quinidine (Barrett et al., 1997) has a greater similarity to that for RSD1025 (pK 8.9) than for RSD1000 (pK 6.1). Thus, for reasons that remain to be fully addressed, low pK compounds in the series of molecules characterized provided better selectivity and efficacy against ischemia-induced arrhythmias. Studies that characterize the toxicology and safety pharmacology of these novel compounds are required to be conducted in order to determine the drug safety profiles prior to phase I clinical testing. Should the safety profiles be sufficient, clinical development will continue and such findings may have relevance to those also searching for novel potential clinical drugs to prevent fatal myocardial ischemia. Note that compounds similar to those described here have ischemia-selective actions in other non-clinical animal species (Beatch et al., 2002) encouraging continued development of drugs selective for fatal ischemia-induced arrhythmias.

CRediT authorship contribution statement

Michael K. Pugsley: Conceptualization, Writing - original draft, Data curation, Visualization, Investigation. **Sandro L. Yong:** Methodology, Writing - original draft, Visualization, Investigation. **Alan L. Goldin:** Methodology, Software, Writing - review & editing, Supervision. **Eric S. Hayes:** Conceptualization, Writing - original draft, Data curation, Investigation. **Michael J.A. Walker:** Supervision, Conceptualization, Writing - review & editing.

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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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