Role of cardiac Na^+ channel blockers in inhibiting the cardiac calcium release channel

D. Mehra,[1] D.F. van Helden,[1] H.S. Hwang,[2] B. Knollmann[2] and D.R. Laver,[1] [1]Department of Biomedical Sciences and Pharmacy, University of Newcastle and HMRI, Callaghan, NSW 2308, Australia and [2]Department of Medicine, Division of Clinical Pharmacology, Vanderbilt Vanderbilt University, Nashville, Tennessee 37235, USA.

The cardiac ryanodine receptors (RyR2) are the calcium release channels in the sarcoplasmic reticulum (SR). Mutations in RyR2 result in increased diastolic calcium release *via* these receptors. Abnormality in RyR2 is manifested in catecholaminergic polymorphic ventricular tachycardia (CPVT). Class I anti-arrhythmic drugs are essentially divided into Ia, Ib and Ic due to their intermediate, fast and slow association and dissociation with the Na^+ channel respectively. They block the Na^+ channels by binding to the channel in the activated, inactivated and open states (Liu *et al.*, 2003). Our previous work has showed that that the potency of RyR2 open probability determines efficacy of class I agents for the prevention of CPVT (Hwang *et al.*, 2011). We have proposed that the efficacy of class I anti-arrhythmic drugs is a combination of reduced membrane excitability *via* its known use dependent Na^+ channel block and stabilization of SR Ca^{2+} release by a recently discovered RyR2 block (Hilliard *et al.*, 2010). Here we report RyR2 blocking kinetics of other class I anti-arrhythmic drugs.

Sheep were euthanized according to the University of Newcastle Animal Care & Ethics Committee guidelines. RyR2 was isolated from sheep heart and incorporated into artificial lipid bilayers. Channel gating was measured by single channel recording. RyR2 open and closed times were measured in the presence of diastolic [Ca^{2+}] (1 mmol/l cytoplasmic and 0.1 mmol/l luminal with 2 mmol/l ATP).

Class I drugs inhibit RyR2 by reducing channel open probability by induction of brief ~1 ms substates and by reducing the conductance of the fully open state. The substate conductance induced by all class I drugs tested lay between ~85 to 115pS (*c.f.* 450 pS for the open state). Class Ic caused longer substate durations than did class Ib and class Ic (Table). Class Ic anti-arrhythmic drugs are more potent in decreasing RyR2 open probability than classes Ia and Ib as shown in the Table. Flecainide is a monovalent cation at pH7.4 (pKa 9.2). The ability of flecainide to induce substates decreased by 80% on raising pH to 9.5 indicating that the cation form of flecainide inhibits RyR2.

The drugs in each subclass have fast association and dissociation of block with RyR2 showing no particular distinction as seen in its Na channel block. In addition, the fast flicker block shown by all agents suggests that these drugs have a short residence time in the channel characteristic of low affinity compounds.

Table: Comparison of the substate duration and conductance, over the voltage range of −20 to −60 mV and the potency (IC_{50}) of class I drugs (mean ± SEM of N experiments) n.d. = not done.

	Compound	Substate duration (ms)	Substate conductance (pS)	Substate level (pA)		IC_{50} (μmol/l)
				−20mV	−60mV	
Class 1a	Quinidine	0.8 ± 0.1 (4)	102 ± 3 (4)	−1.1	−6.5	723 ± 0.5 (4)
	Procainamide	0.5 ± 0.1 (4)	86 ± 2 (6)	−1.6	−4.6	737 ± 0.3 (4)
	Disopyramide	0.9 ± 0.1 (4)	108 ± 3 (4)	−1.4	−6.3	552 ± 0.3 (4)
Class 1b	Tocainide	n.d.	114 ± 3 (4)	−1.8	−5.9	663 ± 0.2 (4)
	Mexilitine	1.2 ± 0.2 (4)	93 ± 5 (4)	−1.6	−-1.6	578 ± 1.9 (4)
Class 1c	Flecainide	2.5 ± 0.3 (4)	87 ± 2 (7)	−1.4	−6.4	16.7 ± 4.0 (14)
	R-propafenone	1.8 ± 0.1 (4)	75 ± 3 (4)	−1.5	−7.5	8.7 ± 0.6 (13)
	S-propafenone	0.7 ± 0.1 (4)	n.d.	n.d	n.d	17.3 ± 1.6 (14)
	Encainide	3.6 ± 0.3 (4)	101 ± 2 (6)	−1.2	−5.1	22.5 ± 1.2 (9)

Liu H, Atkins J, Kass RS. (2003) *Journal of General Physiology* **121(3):** 199-214.

Hwang HS, Hasdemir C, Laver D, Mehra D, Turhan K, Faggioni M, Yin H, Knollmann BC. (2011) *Circulation. Arrhythmia and Electrophysiology* **4:** 128-35.

Hilliard FA, Steele DS, Laver D, Yang Z, Le Marchand SJ, Chopra N, Piston DW, Huke S, Knollmann BC. (2010) *Journal of Molecular and Cellular Cardiology* **48:** 293-301.