Role of cardiac Na^+ channel blockers and Mg^{2+} in inhibiting the cardiac calcium release channel

D. Mehra, D.F. van Helden and D.R. Laver, Department of Biomedical Sciences and Pharmacy, University of Newcastle and HMRI, Callaghan, NSW 2308, Australia.

The cardiac ryanodine receptors (RyR2) are the calcium release channel in the sarcoplasmic reticulum (SR). Class I anti-arrhythmic drugs are essentially divided into Ia, Ib and Ic. They block by binding to the Na^+ channel in the activated, inactivated and open states (Liu *et al.*, 2003). Our previous work showed that the Class I agents blocked the RyR2, thus reducing the spontaneous Ca^{2+} release in an inherited arrhythmia called catecholaminergic polymorphic ventricular tachycardia (Hwang *et al.*, 2011). Here we investigate the mechanisms of inhibition on RyR2 shown by these cardiac Na^+ channel blockers.

Sheep were euthanized according to the University of Newcastle Animal Care & Ethics Committee guidelines. RyR2 was isolated from sheep heart as described previously (Laver *et al.*, 1995). RyR2 was incorporated into artificial lipid bilayers to measure channel gating using single channel recording. RyR2 open and closed times were measured in the presence of various Class I agents (5-500μmol/l), 2 mmol/l ATP and varying cytoplasmic Ca^{2+} and Mg^{2+} concentrations. We found that these drugs had four inhibiting actions on RyR2 with distinct kinetics.

1. Mexiletine (Class 1b) at concentrations >200 μmol/l reduced the channel conductance by >20%. Class Ia and Ic agents do not show this effect.
2. Mexiletine (200 μmol/l, Class Ib) and pilsicainide (100 μmol/l, Class Ic) caused voltage-independent, brief closures to the fully closed state.
3. Propafenone, flecainide and encainide (Class Ic) induced brief closures (~1 ms, IC_{50} ˜ 50 μmol/l at +40 mV) from the main open state (O, 450 pS) to a substate (S, 85 to 115 pS depending on the drug) and from the substate to the closed state (C). The closing rates from O to S and S to C are proportional to concentration whereas the corresponding opening rates are concentration independent. This is consistent with a tri molecular reaction in which substate events correspond to periods where a drug molecule is bound to the RyR and complete closures correspond to periods where two molecules are bound. The rates of drug binding and dissociation are voltage dependent, producing stronger inhibition at positive membrane potentials.
4. The presence of cytoplasmic Mg^{2+} revealed another, voltage-dependent inhibition by flecainide which was mediated by long (5 s) closed events (IC_{50} = 25 μmol/l at +40mV). The frequency of closed events was proportional to flecainide concentration and showed a hyperbolic dependence on [Mg^{2+}] (K_a = 2.5 mmol/l). The duration of long closures was independent of flecainide and Mg^{2+} concentrations. This is consistent with a molecular reaction in which the combined binding of a flecainide molecule and a Mg^{2+} ion induce channel closure.

Overall, Class Ic drugs are more potent compared to Class Ia and Ib. This study illustrates the manifold mechanisms of RyR2 block that are specific for each class of drug. Our results show that Mg^{2+}, at physiological concentrations, makes flecainide a more potent inhibitor of RyRs by inducing an additional inhibition mechanism. This could provide mechanistic understanding for therapeutic efficacy in cardiac ischemia since Mg^{2+} increases by factor 2 from normal physiological levels (Murphy *et al.*, 1989)

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.

Laver DR, Roden LD, Ahern GP, Eager KR, Junankar PR & Dulhunty AF. (1995). *Journal of Membrane Biology* **147,** 7-22

Murphy E, Steenbergen C, Levy LA, Raju B, London RE. (1989). *Journal of Biological Chemistry* **264,** 5622-5627.