Inhibition of cardiac Ca^{2+} release channels as therapy for arrhythmia

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The ryanodine receptors (RyR2) are the calcium release channels in sarcoplasmic reticulum (SR) which is the main Ca^{2+} store in the heart. Mutations in RyR2 or calsequestrin cause arrhythmias as a result of increased diastolic Ca^{2+} release *via* RyR2. Such an abnormality manifests in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), a form of cardiac arrhythmia which is the focus of our research.

Flecainide, a sodium channel blocker of the Class I anti arrhythmic group was found to prevent arrhythmia in CPVT by inhibiting RyR2 (Watanabe *et al.,* 2009). However, it is not clear how inhibition *per se* has an anti arrhythmic action because another inhibitor, tetracaine, has a pro-arrhythmic action (Watanabe *et al.,* 2009). It was noted that flecainide decreased channel mean open time whereas tetracaine increased channel closed times (Hilliard *et al.,* 2010). Therefore, we test the hypothesis that the therapeutic inhibition of RyR2 relies on reducing channel open times without affecting its closed times. Our approach is to measure the actions of a range of class 1 drugs on RyR2 open times in single channel recording and correlate this with their therapeutic action determined in isolated cardiomyocytes and in a CPVT mouse model.

RyR2 was isolated from sheep and human hearts as described previously (Laver *et al.,* 1995). RyRs were incorporated into artificial lipid bilayers and channel gating was measured by single channel recording. RyR2 open and closed times were measured in the presence of diastolic [Ca^{2+}] (100 nmol/l cytoplasmic and 0.1 mmol/l luminal). SR Ca^{2+} release was measured using confocal microscopy on intact ventricular cardiomyocytes isolated from hearts of calsequestrin knockout mice (CPVT mouse model). Fura2-AM was used to measure the effect of Class I anti arrhythmic drugs on isoproterenol-induced calcium waves.

RyR2 open time was decreased by the Class Ic anti-arrhythmic drugs whereas Classes Ia and 1b had no significant effect at concentrations up to 50 μmol/l (see Table). None of the Class Ic drugs had any significant effect on RyR2 closed durations. The ability of the drugs to reduce RyR2 open times correlated with their ability to prevent the Ca^{2+} waves in cardiomyocytes; an indicator for their anti-arrhythmic efficacy. Both flecainide and propafenone suppressed ventricular tachycardia in calsequestrin knockout mice.

The data suggest that potency of RyR2 open time reduction rather than just a reduction in open probability determines efficacy of class I agents for the prevention of CPVT. This may lead to a paradigm shift in drug development process by directing strategies away from discovering high affinity compounds to searching for low affinity compounds with short residence time in the channel.

Comparison of the potency (IC_{50}) of class 1 drugs on the mean open time of cardiac RyRs and their IC_{50}s for reduction in frequency of Ca^{2+} waves in intact cardiomyocytes. n.d. = not done. n in parentheses.

	compound	IC_{50} (μmol/l) in RyR2 open time	IC_{50} (μmol/l) Ca^{2+} waves
Class 1a	quinidine	>50 (5)	>6 (28)
	procainamide	>50 (5)	>15 (28)
	disopyramide	>50 (7)	>6 (28)
Class 1b	lidocaine	>50 (5)	>50 (28)
	mexilitine	>50 (6)	>6 (28)
Class 1c	flecainide	16.7 ± 4.0 (14)	2.0 ± 0.2 (36)
	R-propafenone	8.7 ± 0.6 (13)	1.1 ± 0.5 (36)
	S-propafenone	17.3 ± 1.6 (14)	5 ± 1 (36)
	encainide	22.5 ± 1.2 (9)	n.d.

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