Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare but particularly disabling disease that manifests as spontaneous transition to VT associated with increased sympathetic activity. In 2 variants of this disease (CPVT1 and CPVT2), the abnormal electric activity of the heart is associated with genetic defects in the coding of either the ryanodine receptor (type 1) or a related sarcoplasmic reticulum (SR) protein, calsequestrin (type 2). For those cases (20%–30%) unresponsive to β-blocker treatment there were few options available to mitigate the risk of VT/VF (ventricular tachycardia/ventricular fibrillation). However, an article published by the Knollman group in 2009 indicated that these individuals may respond to a direct action on the excitability of cardiac muscle via inhibition of INa. The article provided evidence that in the case of CPVT, its antiarrhythmic action was not via inhibition of INa, but instead via inhibition of the activity of the cardiac ryanodine receptor (RyR2) and subsequent reduction in the proarrhythmic release of Ca2+ during diastole. This interpretation was supported by further work by the Knollman and his collaborators, in particular, the demonstration of direct effects of flecainide and the related more potent local anesthetic R-propafenone on SR Ca2+ release in permeabilized cardiac muscle preparations. However, the interpretation of this result is controversial. Two independent groups have failed to reproduce the effects of flecainide on RyR2 activity in either normal ventricular myocardium or a mouse model of CPVT. The first group reported effects of flecainide that are entirely consistent with a direct action on the excitability of cardiac muscle via I\textsubscript{Na} whereas the second produced evidence that the antiarrhythmic effect is by depressed Na influx through I\textsubscript{Na} and the subsequent effects on cytoplasmic Ca2+ via the Na/Ca exchanger. To date this issue remains unresolved. A detailed mechanistic view of the action of flecainide is essential to design new strategies to counter CPVT. RyR2 inhibition may be a suitable target for drug design, but the form of inhibition is unclear because a novel drug known to depress RyR2 (JTV 519) has been reported to be ineffective at suppressing the CPVT phenotype in some cases. This makes a detailed analysis of the interaction of flecainide with RyR2, a priority for advancement in this area.

In this issue, Bannister et al present a detailed study of the action of flecainide on the isolated human cardiac ryanodine receptor. The authors show that flecainide can bind with the cytoplasmic face of isolated human cardiac ryanodine receptor 2 to reduce open probability (P\textsubscript{o}) when cations (K+) are conducted from cytoplasmic to luminal side of the channel. This block has a half maximal effect =20 μmol/L and is characterized by the appearance of a long-lasting subconductance state of =20% of maximal that supports the sustained conductance of cations in the cytoplasmic to luminal direction. The net effect is to cause an 16% reduction in current flow (cytoplasm to lumen). This contrasts to the lack of effect of flecainide at concentrations ≤50 μmol/L on the current flow through human cardiac ryanodine receptor 2 in the physiological direction (lumen to cytoplasm). The authors demonstrate this current flow asymmetry with equal K+ concentrations either side of the bilayer and varying the direction of current flow by changing the clamp voltage across the bilayer or arranging a lumen-to-cytoplasm concentration gradient of K+ ions and varying the voltage. Furthermore, this effect is also reproduced by R-propafenone and with Ca2+ as the current carrying ion. The presence of a CPVT1 mutation (N4104K) did not alter the sensitivity to flecainide. As regards site of action, the study shows that flecainide competes for effect with a drug (tetrapentyl ammonium) that has a known binding site on the cytoplasmic face of the pore forming unit. A cytoplasmic action was confirmed by directly demonstrating that flecainide had no effect when applied to the luminal face of isolated human cardiac ryanodine receptor 2 in the physiological direction (lumen to cytoplasm). The authors also examined the possibility that flecainide may affect Ca2+ flux through RyR2 channels by modulating the counter ion flow either through the SR K+ channel (trimeric intracellular cation [TRIC]) or through countercurrent flow via RyR2. In the case of the TRIC channel, flecainide was inactive; the reported effect of the drug on countercurrent flow of K+ through RyR2 indicates that under physiological conditions, flecainide would only depress this current by 15% at the highest flecainide concentration (50 μmol/L). This would suggest that at concentrations at which antiarrhythmic effects are seen (5–25 μmol/L), the effect of flecainide on the SR countercurrent magnitude would be minimal.
Finally, the authors examined the effects of flecainide on the RyR2 in cardiac muscle SR by recording Ca\(^{2+}\) sparks and waves in isolated, permeabilized rat ventricular myocytes. Neither the frequency nor the amplitude of these events was affected by flecainide (≤25 µmol/L). They concluded that flecainide cannot alter SR Ca\(^{2+}\) release via a direct action on RyR2 or via modulation of the magnitude of the counter current, a finding that is certain to stimulate the debate on the site of action that is relevant to CPVT.

**How Can We Reconcile This Study With the Rest of the Literature?**

**Effect of Flecainide on Isolated RyR2**

As pointed out by Bannister et al, the data on isolated human cardiac ryanodine receptor 2 are consistent with those of earlier studies which used symmetrical monovalent cations (Cs\(^{+}\)) as a charge carrier and held the membrane potential at positive potentials, ensuring cytoplasm-to-lumen current flow.\(^1\) The more recent detailed study by this group\(^7\) shows multiple modes of action of flecainide not detailed by Bannister et al, but the single channel currents are generated by fluxes in the cytoplasm-to-lumen direction. Therefore, the value of the featured article is clear in showing, in relatively unequivocal terms, that flecainide does not directly affect normal human RyR2 function when current flow is in the physiological direction and that any effects on countercurrent mechanisms will be minor.

**Effect of Flecainide on SR Ca\(^{2+}\) Release**

This is much harder to reconcile, as there is disagreement between reports showing no effects\(^4\) and direct effects.\(^2\) In the current study, the spread of the frequency data on Ca\(^{2+}\) spark and Ca\(^{2+}\) waves was relatively large (SD approximately equal to the mean) such that small effects (≈15% changes) could not be resolved. Yet the changes previously reported at equivalent concentrations of flecainide were relatively large, an ≈50% reduction. Although published studies on permeabilized cells were done under comparable conditions, there seems to be a large variation in the baseline activity of Ca\(^{2+}\) sparks and Ca\(^{2+}\) waves between studies. This may reflect differences in the background intracellular Ca\(^{2+}\) concentration, which is difficult to standardize in lightly buffered solutions. Furthermore, permeabilizing cells using the cholesterol precipitating agents β-escin or saponin can result in variable degrees of permeabilization depending on the concentration, duration of exposure to the agent, and the time taken for subsequent measurement. Disruption of the structure and loss of cytosolic proteins by dialysis are unavoidable risk. Loss of small molecule constituents will be relatively fast, but there is also evidence for significant loss of small proteins during a 10- to 15-minute time period.\(^8\)

It remains to be resolved whether flecainide can affect SR Ca\(^{2+}\) release via an indirect action. A perfectly plausible option is that flecainide affects SR Ca\(^{2+}\) release not just via sub-sarcolemmal Na\(^{+}\), and therefore Ca\(^{2+}\) via Na\(^{+}\)/Ca\(^{2+}\) exchanger, \(^5\) but also via binding to one of the numerous cytoplasmic proteins that normally modulate RyR2 directly. The list of small proteins modulators of RyR2 is long and includes sorcin, FKBP12 and FKBP12.6, calmodulin, and S100A1. In this context, it is worthwhile noting the recent report of a reduction in calmodulin binding to cardiac RyR2 because of the R2474S mutation, which was thought to be responsible for altered RyR2 activity and the associated CPVT. Therefore, a potential action of flecainide is to bind calmodulin and increase binding to RyR2, reversing the CPVT phenotype. An indirect effect similar to this would explain the absence of direct effects of flecainide in studies of isolated RyR2 or in permeabilized cell studies where either the Ca\(^{2+}\) next to RyR2 or the calmodulin concentration was not within physiological ranges.

One final issue to keep in mind when investigating means of counteracting the proarrhythmic state observed in CPVT is a characteristic of the VT experienced by humans\(^10\) and mouse models\(^11\) in which the phase of the QRS complex shifts 180° on each beat alternately. This has been interpreted as activation originating alternately at left ventricular and right ventricular sites, most likely at the level of the His-Purkinje network.\(^12\) It would therefore be interesting to study the effect of flecainide on Purkinje cells, particularly in light of the studies that show that the control of spontaneous Ca\(^{2+}\) release is mediated by both RyR2 and inositol trisphosphate receptors within the SR\(^13\) and therefore a host of other potential targets for flecainide.

In summary, the article by Bannister et al usefully challenges previous ideas about the mode of action of flecainide on CPVT and illustrates the difficulty in attributing the action of a drug to a single molecular target.

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**References**


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The Direct Actions of Flecainide on the Human Cardiac Ryanodine Receptor: Keeping Open the Debate on the Mechanism of Action of Local Anesthetics in CPVT

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