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IS THE DEBATE ON THE FLECAINIDE ACTION ON THE RYR2 IN CPVT CLOSED?

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The class IC antiarrhythmic agent flecainide, developed in the 70s as more stable local anesthetic by incorporating of fluorine, is nowadays recommended as one of the first-line rhythm control therapeutic option in patients without significant structural heart disease or severe hemodynamic instability. Flecainide appears to be effective for managing arrhythmias via blocking Na^+ channel but also acts on voltage-gated K^+ and Ca^{2+} channels[1] and thus can also be classified as class IVb antiarrhythmic[2]. More recently, flecainide has drawn renewed interest on its precise mechanisms of action stimulated from a landmark study[3] demonstrating that flecainide may also block the intracellular Ca^{2+} release channel – the ryanodine receptor (RyR2) and suppress ventricular arrhythmias in both murine models and patients suffering from catecholaminergic polymorphic ventricular tachycardia (CPVT). This life-threatening inherited arrhythmia is caused by mutations in the RyR2 gene, but also in far lesser extent from RyR2 modulators such as calsequestrin-2 (CasQ2), calmodulin (CALM), and triadin (TRDN). Mutations in Trans-2,3-Enoyl-CoA Reductase-Like protein (TECRL) and other ion channels (Kir2.1-inward-rectifier potassium channel, KCNJ2 and Na^+ channel, SCN5A) have also been linked to some CPVT[4]. While the beneficial effect of flecainide in patients with CPVT[5], as well as other RyR2 dysfunction-linked inherited arrhythmias[6–9] is largely accepted, the participation of a direct effect on the RyR2 has been challenged. Objections mainly claim that flecainide only blocks RyR2 at not physiological positive potential, and blunt sarcoplasmic reticulum (SR) cation influx (cytosolic to SR luminal flux) but not SR efflux. Others claim that the main flecainide mechanism of action preventing CPVT arrhythmias is due to the blockade of Na^+ channels, which increases the

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DISCLOURE

None

threshold for triggered activity[10] and/or results in preventing elevated intracellular Ca^{2+} levels via the Na^+/Ca^{2+} exchanger to decrease inappropriate post-depolarization[11, 12]. To add complexity, it has been shown that flecainide might prevent Na^+ current modulation by RyR2 in CPVT atria[13]. As many scientific debates, the conflicting reports look like a ping-pong game where the opponents used different paddles (techniques), balls (dose and application time of flecainide) and play on a different table (normal vs CPVT model), jeopardizing the reconciliation. Whatever the root cause of the discrepancies, and even if a more definitive proof could come with [^{3}H]-ryanodine binding assay to see how flecainide affect RyR2 channel activity, the contrasting studies agree on the fact that flecainide is an open RyR2 channel blocker, altering Ca^{2+} sparks, the elementary RyR2-mediated SR Ca^{2+} release.

In this issue, Kryshtal et al.[14] revisit the contribution of RyR2 blockade action of flecainide in antiarrhythmic activity using analogs that retain Na^+ channel blocking action but have no or significantly less effect on RyR2. Two different flecainide analogs are synthetized, by single or double methylation in the nitrogen of the pyridine ring. The effect on RyR2 was tested in single sheep RyR2 incorporated into lipid bilayers, and in permeabilized cardiomyocytes from calsequestrin knock out mice, a model of type 2 CPVT. Both approaches eliminate the effect on Na^+ channels: the first one because they are not present, and the second because the saponin permeabilization produces big pores in the sarcolemma, clamping [Na^+] at both sides of the membrane, which furthermore loses its negative potential. Saponin affects only cholesterol membranes, so the SR membrane is unaffected and remains functional. Thus the Na^+ channels are inactivated and there is no Na^+ gradient. Flecainide derivatives showed significantly less effect on single RyR2, and virtually no effect on Ca^{2+} waves in permeabilized cardiomyocytes. The effect of methylated flecainide on Na^+ channel was analyzed on HEK cells as was equal to flecainide. Thus, these derivatives are useful to study the antiaryhtmic effect of flecainide which has both actions, and compare it to its derivative, which only retains the effect on Na^+ channels.

In intact cardiomyocytes, Kryshtal et al. show that Na^+ channel inhibition with TTX or with both flecainide derivatives significantly reduced proarrhythmogenic Ca^{2+} waves, but to a lesser extent that flecainide, independently of the presence or absence of TTX, showing an additive action of both Na^+ channel and RyR2 blockade in Ca^{2+} waves. *In vivo* analyses however, only flecainide showed antiarrhythmic efficacy, demonstrating that the effect on RyR2 is needed for its therapeutic action.

Now the intimate anti-arrhythmic mechanism of flecainide action on RyR2 in CPVT patients is still not resolved. Several modes of action might be postulated. One option might be that flecainide affects RyR2 dynamic modulation, including altered interactions with accessory/regulatory proteins[15] and/or phosphorylation. Indeed, CPVT is known to be potentiated by catecholamine release during exercise and β-adrenergic stimulation and flecainide is more potent and efficient on more active RyR2[16]. However, flecainide action has been observed on purified RyR2 and Kryshtal et al[14] did not observe alteration of flecainide action on phosphorylated RyR2. Instead, the authors speculated that RyR2 Ca^{2+} transport might generate an inside-positive SR membrane potential allowing flecainide block. So far, compelling evidences showed that during Ca^{2+} release, the voltage across the SR membrane

is maintained near a resting value close to 0 mV because the transfer of positive charge through the SR membrane carried by Ca^{2+} is supposedly compensated by the available counter-ion conductances[17]. This has been recently clarified using a novel FRET assay in skeletal muscle fibers[18]. Instead and following this direction, one might simply propose that flecainide, by blocking counter SR K^+ influx during RyR2 activation, would very quickly move the SR potential to the Ca^{2+} equilibrium potential limiting thus net Ca^{2+} release[17]. This more plausible, simple and less conflicting explanation has been already noticed by the supporters for the absence of the therapeutic role of RyR2 modulation by flecainide[11, 12]

All these debates might seem futile to some considering flecainide's striking clinical efficacy in patients with inherited cardiac arrhythmias linked to defective RyR2 function. However understanding the molecular defect targets might open the door for the development of a new, more efficacious classes of drugs for a variety of inherited and acquired arrhythmia syndromes that appear to involve defective Ca^{2+} signaling as a central component of their pathophysiology.

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