LETTERS TO THE EDITOR

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Triple mode of action of flecainide in catecholaminergic polymorphic ventricular tachycardia

There has been a resurgence of interest in the class I_C antiarrhythmic agent flecainide fuelled by recent work identifying the drug as an effective treatment for catecholaminergic polymorphic ventricular tachycardia (CPVT).[1–3] CPVT is caused by mutations in the sarcoplasmic reticulum (SR) Ca^{2+} release channel (RyR2), or the associated proteins calsequestrin-2 or triadin, which lead to an increased propensity for spontaneous SR Ca^{2+} waves during exercise or stress. These in turn induce a transient inward current due to Ca^{2+} extrusion via the Na/Ca exchanger (NCX), delayed afterdepolarizations (DADs) and triggered action potentials. While it is increasingly accepted that flecainide is an effective treatment for CPVT, a degree of controversy has emerged regarding its mechanism of action.

Watanabe *et al.* provided the first evidence that, in addition to known inhibitory effects on the Na^+ current (I_{Na}), flecainide also acts directly on RyR2 to prevent pro-arrhythmic SR release, thereby providing a dual mode of action.[1] Subsequent work demonstrated that flecainide only inhibits RyR2 gating when the channel is in the open state.[4] The functional consequence of this effect on RyR2 is that the Ca^{2+} flux associated with each diastolic Ca^{2+} spark is reduced, while event frequency increases, such that there is no change in the SR Ca^{2+} leak, SR Ca^{2+} content, or systolic Ca^{2+} release. On the basis of these findings, we proposed a new, primary antiarrhythmic mechanism, whereby the flecainide-induced decrease in the spark mass reduces the probability that Ca^{2+} sparks will undergo salutatory propagation between junctional Ca^{2+} release sites, which is the basis of pro-arrhythmic Ca^{2+} waves.[4]

The importance of flecainide's action on RyR2 was recently questioned by Priori and colleagues, who reported that flecainide did not inhibit Ca^{2+} waves in a mouse model of CPVT.[5] Instead, the antiarrhythmic effects of flecainide were attributed to inhibition of I_{Na} and a decrease in the probably of DADs triggering action potentials. However, as considered previously,[6] diastolic spark frequency was sufficiently high in this study to suggest that the cells were very Ca^{2+} overloaded. Under Ca^{2+}-overload conditions, we also find that flecainide is much less able prevent Ca^{2+} waves and effects mediated via I_{Na} inhibition would then dominate.[7]

In this volume, a study by Sikkel *et al.*[8] also aims at addressing the mechanism by which flecainide influences Ca^{2+} sparks and waves. In contrast to the findings by the Priori group[5] and consistent with our findings,[4] the authors report a *decrease* in Ca^{2+} wave frequency in normal rat ventricular myocytes after exposure to flecainide. However, as other I_{Na} blockers were equally effective in reducing Ca^{2+} waves, the authors conclude that RyR2 inhibition is not relevant, and propose a mechanism whereby inhibition of I_{Na} is linked to indirect effects on Ca^{2+} sparks and waves via NCX. Unfortunately, contrary to the stated conclusions, the experimental conditions used by Sikkel *et al.* preclude any inferences regarding the role of RyR2 modulation by flecainide, for the following reasons:

Sikkel *et al.* use rapid pacing trains that will increase $[Na^+]_i$ and consequently $[Ca]_i$[9] to elicit spontaneous Ca^{2+} sparks and Ca^{2+} waves in normal rat ventricular myocytes. While rapid pacing trains are a classic paradigm for triggering Ca^{2+} waves and DADs, studies in the 1980s have already shown that specific I_{Na} blockers, such as tetrodotoxin and lidocaine, can suppress pacing-train induced DADs in isolated Purkinje fibres, albeit without clarifying the underlying mechanism.[10] The study by Sikkel *et al.* provides an explanation for the 30-year old results: I_{Na} blockers limit Na^+ influx and hence cytosolic Ca^{2+} loading during rapid pacing trains, and thereby reduce Ca^{2+} sparks and Ca^{2+} waves. Since any form of I_{Na} inhibition is expected to reduce Ca^{2+} waves resulting from the rapid pacing protocol, the experiments were not designed to test the additional contribution of RyR2 inhibition by flecainide. This is also evidenced by the finding by Sikkel *et al.* that flecainide *reduced* Ca^{2+} spark frequency (which is the opposite of the direct effect of flecainide on RyR2 channels[4,11]), indicating that any direct effect of flecainide on RyR2 was mitigated by the reported reduction in diastolic Ca^{2+} due to I_{Na} block. It is well established that reduced cytosolic $[Ca^{2+}]$ will reduce the rate of spontaneous RyR2 openings and Ca^{2+} sparks.[9]

Previous studies in intact myocytes from CPVT mice and isolated perfused rabbit hearts have shown that class I_C agents that also inhibit RyR2 (flecainide and R-propafenone) are significantly more effective at blocking Ca^{2+} waves and associated arrhythmias than drugs that only inhibit I_{Na} (e.g. lidocaine and procainamide).[12,13] Why then did Sikkel *et al.* not observe a more potent suppression of Ca^{2+} waves by flecainide compared with other I_{Na} inhibitors that lack RyR2 channel function (i.e. tetrodotoxin, lidocaine)? One important difference is that unlike Sikkel *et al.*, Hwang *et al.*,[7] and Lee *et al.* [12] kept the pacing rate constant and used beta-adrenergic stimulation with isoproterenol to induce Ca^{2+} waves, experimental conditions that reduce the influence of Na^+ channel block on cellular Ca^{2+} loading. Furthermore, Sikkel *et al.* applied 5 μM flecainide only for 5 min in the experiments comparing different Na^+ channel blockers in the voltage clamp studies at −40 mV, the only experiment designed to test the contribution of RyR2 block on Ca^{2+} sparks and waves. However, as considered previously, flecainide enters myocytes slowly,[1,14] and it takes up to 30 min to reach maximal effects on Ca^{2+} waves in intact myocytes.[15] Hence, in all previous studies evaluating the role of RyR2 modulation, intact cells were exposed to 6 μM flecainide between 15 and 30 min before the assessment of Ca^{2+} wave properties.[1,4,7] Sikkel *et al.* argue that 30 min exposure had no more effects on Ca^{2+} wave frequency than 5 min exposure (Supplementary material online, *Figure SIII*). But those experiments were done in the same myocytes, and did not control for time dependent changes in wave frequency. In contrast to the results by Sikkel *et al.*, we do not find any effect of flecainide on Ca^{2+} waves after 5 min flecainide exposure, [15] indicating that experimental conditions of rapid pacing induced Ca^{2+} waves are not comparable with our studies.

In *permeabilized* myocytes, the effect of flecainide on Ca^{2+} sparks is rapid, consistent with the removal of the sarcolemma as a barrier to diffusion.[4] Sikkel *et al.* argue that effects of flecainide on RyR2 in permeabilized rat myocytes require cytosolic concentrations that are above those normally found within the plasma (i.e. 25 μM).[8] However, in a relevant disease model of CPVT myocytes, we find a flecainide IC_{50} for reducing Ca^{2+} wave frequency of 7 μM in *permeabilized* myocytes,[11] and of 2 μM in *intact* myocytes after 30 min incubation.[13] Moreover, previous

reports have shown a marked accumulation of flecainide in the ventricular myocardium.[1,16]

Taken together, the experimental conditions (rapid pacing induced $[Na]_i$ loading and short incubation times) chosen by Sikkel *et al*. strongly favour the detection of effects of I_{Na} inhibition on Ca^{2+} waves and hence the suppression of DADs, a property of Na^+ channel blockers that had been observed several decades ago.[10] On the other hand, the experiments by Sikkel *et al*. were not designed to detect the contribution of RyR2 block by class I_C agents to their antiarrhythmic effects on Ca^{2+} wave triggered arrhythmia, a contribution which has been confirmed independently by three groups.[7,12,17] We would caution that the absence of evidence does not constitute evidence for the absence of the therapeutic role of RyR2 modulation by flecainide. Rather, the mechanism of Ca^{2+} wave suppression by flecainide described by Sikkel *et al*. is in addition to Ca^{2+} wave suppression due to flecainide's direct action on RyR2 channels reported by us and others, and the reduced probability of DADs triggered action potentials described by the Priori group. This triple mode of action likely explains flecainide's striking clinical efficacy in CPVT patients.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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Triple mode of action of flecainide in catecholaminergic polymorphic ventricular tachycardia: reply

We thank Steele *et al*. for their interest and comments regarding our recent study focusing on the effects of I_{Na} reduction on spontaneous sarcoplasmic reticulum (SR) Ca^{2+} release.[1] In the context of the authors' previous publications regarding the mechanism of action of flecainide at the cardiac ryanodine receptor (RyR2),[2–4] it appears they have interpreted our study as an attempt to infer that flecainide has no effect on RyR2. We would like to clarify this misinterpretation.

Our work demonstrates that a reduction in Na^+ influx into the cardiomyocyte can, via the enhancement of Ca^{2+} efflux through the Na^+/Ca^{2+} exchanger, reduce $[Ca^{2+}]_i$ in the vicinity of the RyR2 and thus reduce the frequency of spontaneous SR Ca^{2+} release events. We demonstrate that this is a class effect of I_{Na} blockers, and that flecainide causes no reduction in SR Ca^{2+} leak when I_{Na} is eliminated by altering the holding potential via voltage clamp. This mechanism also appears to be relevant in whole-heart models of arrhythmia induced by increased SR Ca^{2+} release as evidenced by recent data from Radwanski *et al*.[5]

Our data should not be taken as a dismissal of the effects of flecainide at RyR2. We merely conclude that, under our experimental conditions, I_{Na} blockade confers a greater reduction in spontaneous SR Ca^{2+} release than effects at the RyR2. Flecainide has been shown by Steele and co-workers[2–4,6] to act at the RyR2 under certain experimental conditions, and we do not doubt the validity of these results.

Steele *et al*. suggest that our use of 5 Hz pacing trains to induce Ca^{2+} waves in normal rat cardiomyocytes may bias our experiments towards finding wave reduction to be mediated by I_{Na} block. We used this technique since we have found it to be reproducible over time within a single myocyte, thus allowing paired data collection in a cross-over design. It also negates requirements for additional pharmacology. As highlighted in our original manuscript, the study of Diaz *et al*.[7] revealed that increased $[Na^+]_i$ is one of the reasons for the elevation of Ca^{2+} wave frequency in stimulated (0.5 Hz) vs. non-stimulated myocytes. This relevance of $[Na^+]_i$ accumulation (and its prevention by I_{Na} blockade) even at low pacing rates is emphasized in our study, since at a stimulation frequency of 0.5 Hz we find a significant reduction in Ca^{2+} spark frequency following application of TTX. We agree that rapid pacing could cause a greater elevation of $[Na^+]_i$ which would be more markedly inhibited by I_{Na} blockade. However, enhanced significance of the mechanism during rapid pacing does not render it irrelevant considering that, in the clinical setting, many ventricular arrhythmias follow periods of tachycardia.[8–10] Use-dependency of I_{Na} block, as exhibited by flecainide,[11] may be especially useful in this setting. Steele *et al*. propose a mechanism that predominates under conditions of elevated sympathetic drive but constant heart rate (1 Hz). *In vivo*, any increase in sympathetic drive leads to