Chapter III

Appendix I

Prolongation of minimal action potential duration in sustained fibrillation decreases complexity by transient destabilization

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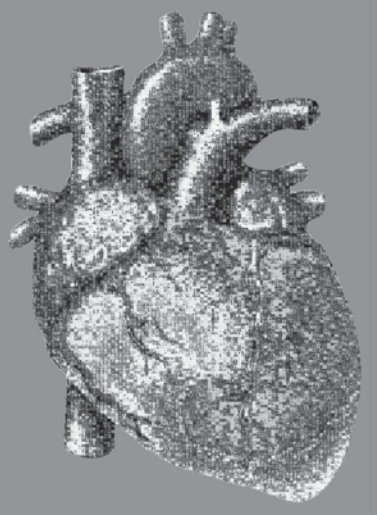

TO THE EDITOR:

We read with great interest the article by Bingen et al.[1] entitled 'Prolongation of minimal action potential duration in sustained fibrillation decreases complexity by transient destabilization'. This paper referred to our report that 2-aminoethoxydiphenyl borate (2APB) induces fibrillation in perfused rat hearts2 (http://youtu.be/pDsm0UKQvt4, http://youtu.be/J0q_YPZyBwk). It also extended our work[3–7] and that of Huo et al.[8] by establishing the arrhythmicity of 2APB in monolayers of neonatal rat ventricular myocytes. Bingen proposed that 2APB provokes electrical instability by inhibiting cardiac connexins which leads to impulse re-entry. They also concluded that prolonging the action potential with Bay K 8644 or barium reduces ectopic complexity to a few re-entrant sources. We would like to highlight four points that are inconsistent with these analyses and suggest an alternate, possibly complementary, explanation for 2APB arrhythmicity.[7]

First, the concentrations of 2APB that initiate sporadic ectopy (10 μM)1,3 do not affect connexin 43 and 45.9 Furthermore, 15–20 μM 2APB which induces tachycardia and fibrillation[1,2] should not appreciably affect connexin 43 while reducing connexin 45 activity about one-half.[9] Transgenesis shows that only large decreases in connexin activity provoke re-entrant arrhythmia.[10] Since arrhythmogenic concentrations of 2APB directly affect connexins to a limited degree, it is not clear how they would so decrease conduction velocity and provoke re-entry.[1] Electromechanical uncouplers and voltage-sensitive dyes themselves decrease impulse conduction.[11,12] This may contribute to the low conduction Bingen reports at doses of 2APB that do not greatly affect connexins.

Secondly, Bingen's voltage-mapping studies expand our data which demonstrated that Bay K 8644 induces organized, high-frequency ectopy in left atria or left ventricular papillary muscles treated with 2APB.[4,5,6] While Bingen concluded that the prolongation of the action potential duration underlies this effect, FPL-64176, isoproterenol, and ouabain also provoke high-frequency ectopy to similar extents.[4] FPL-64176 prolongs the action potential much more than Bay K 8644,[13] isoproterenol increases this variable to a similar degree as Bay K,14 whereas ouabain reduces action potential duration.[15] Thus it would be of interest to test if action potential duration correlates with re-entrant complexity in myocyte cultures treated with 2APB and exposed to this panel of compounds.

Thirdly, small molecules such as SKF-96365 and ML-7 suppress the sporadic ectopy, the high-frequency ectopy, and the fibrillation that 2APB induces in heart muscle.[2,5,6,7] They also interdict voltage-independent calcium signalling.[16] In addition, calmodulin antagonists but not calmodulin-dependent protein kinase II inhibitors suppress 2APB ectopy.[5,6] Our recent data show that bcl-2 inhibitors similarly stifle 2APB ectopy.[7] It would be useful to test if these diverse molecules increase impulse conduction in cultures treated with 2APB or in untreated monolayers, as the restoration of impulse conduction would be required to suppress 2APB ectopy if re-entry were its underlying cause.

Fourthly, 2APB provokes electromechanical ectopy even when it is added to quiescent non-automatic left atria or papillary muscles (e.g. Figure 3B: * and ‡ in ref.6). Since re-entry requires a preceding impulse, how this mechanism for arrhythmia might induce the initial spontaneous depolarization of unpaced muscles treated with 2APB is not apparent to us. Understanding the origin of these primary events which are by definition non-re-entrant will help dissect the molecular mechanism underlying 2APB arrhythmia.

Towards this goal, it is known that 2APB stimulates voltage-independent calcium entry in non-excitable cells through the Orai calcium channels with EC50s identical to those that cause cardiac ectopy.[17] These channels, and the related transient receptor potential proteins, are important regulators of cell calcium signalling. Huo published that 2APB activates calcium entry in isolated myocytes8; we reported that Orai inhibitors suppress 2APB ectopy and that rat left atria and ventricles express Orai1 and Orai3.[2,5,6] Thus the activation of these channels in an excitable cell background may stimulate a novel calcium-linked pathway for automatic arrhythmia, a notion which we have outlined elsewhere in more detail.[5,7]

There are at least three ways that this novel mechanism may be important in arrhythmia. Firstly, intra- and extra-cellular signals including calcium store depletion and inflammatory mediators provoke calcium entry through voltage-independent calcium channels. Thus these channels may provide an unexpected way to couple well-known pathophysiological stimuli to arrhythmia.[18] Secondly, calmodulin-dependent protein kinase II participates in triggered afterdepolarization.[19,20] If our alternate mechanism were to initiate focal atypical automaticity, then calcium signalling could underlie both the triggered and the automatic events that lead to re-entry. Thirdly, the two suggested mechanisms for 2APB ectopy, connexin impairment,1 and aberrant voltage-independent calcium signalling,[2,7] may not be mutually exclusive. That is, calcium entry through voltage-independent calcium channels may offer an unexpected means to suppress connexin activity. Further study of 2APB ectopy may reveal new mechanisms for arrhythmia and identify unforeseen therapeutic targets.

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