Evidence Against the Role of Intracellular Calcium Dynamics in Ventricular Fibrillation

To the Editor:

In our recent publication, we argued that intracellular calcium (Ca) dynamics is not an essential mechanism of the maintenance of ventricular fibrillation (VF). Our argument was based on 2 major observations: (1) Ca transient passively followed the optical action potential along the entire wavelet span, except at the very wavelet tip; and (2) chelating Ca with BAPTA-AM did not change the incidence of wave break (WB) during VF. In their letter to Circulation Research, Ogawa et al raised a concern that our experiments had not shown the effect of BAPTA on Ca dynamics. To support this statement, Ogawa et al referred to their unpublished results in Langendorff-perfused rabbit hearts, where they noted that Ca transient amplitude and contractility were significantly decreased after BAPTA-AM infusion (to 20 μmol/L). Because it was not feasible or at least impractical to calibrate Ca signal in our study, we relied more on the linearity of Rhod-2 fluorescence with respect to calcium concentration. We showed that after 30 minutes of perfusion with 20 μmol/L BAPTA-AM, rapid pacing caused Ca transient alternans and eventual initiation of VF, whereas, after 70 minutes of BAPTA-AM perfusion, Ca transient alternans was no longer present and only monomorphic ventricular tachycardia could be induced.

In response to this critique, we first mention that duration of BAPTA-AM infusion necessary to achieve suppression of Ca transient may vary depending on experimental conditions. The observation reported by Ogawa et al that 30-minute perfusion with BAPTA-AM was not sufficient to achieve the effect cannot be extended to the general case. For example, Marbán et al were able to achieve complete abolishment of Ca transient and contraction after 10 to 20 minutes of BAPTA-AM infusion. Thus, the duration of BAPTA-AM infusion is hardly an adequate or sufficient parameter to assess the drug effect. Some estimation of Ca transient amplitude and/or contractility should be provided to ensure that the effect is indeed present. Unlike the unpublished results referred to by Ogawa et al, our published results included direct assessment of BAPTA-AM effects. Specifically, we showed that the left ventricular developed pressure (LV-devP) and Ca transient amplitude were significantly decreased after BAPTA-AM infusion (to 12.2% and 30.5% of control values, respectively). Note that nonlinearity of Rhod-2 fluorescence with respect to calcium concentration should overestimate the amplitude of residual Ca transient after BAPTA-AM infusion. Because it was not feasible or at least impractical to calibrate Rhod-2 signal in our study, we relied more on LV-devP as an indicator of BAPTA-AM effectiveness. Because, despite 88% decrease in LV-devP, WB incidence remained unchanged, we reasoned that Ca cycling is not a major mechanism of VF maintenance.

One may argue that even this residual level of Ca cycling can still be sufficient to provide the hypothetical destabilizing feedback between the Ca transient and the action potential leading to WB. To make this possibility even less likely, we performed additional experiments focusing on the goal of complete abolishment of Ca transient with BAPTA-AM. It was not an easy task. We could not follow the protocol described by Ogawa et al because, in our experience, longer (>20 minutes) perfusion of porcine hearts with crystalline solutions results in edema, which is itself may lead to slowing and regularization of VF. On the other hand, when BAPTA-AM is infused into the blood circulation, a significant amount of the drug is absorbed by blood cells, preventing efficient delivery to the cardiomyocytes. After some experimentation aimed at enhancing BAPTA-AM delivery, while keeping edema to a minimum, we settled on the following protocol (N = 2): after the initial 10-minute BAPTA-AM infusion, we performed 5 additional short (2- to 3-minute) infusions of BAPTA-AM dissolved in Tyrode’s solution separated by 9- to 10-minute perfusion with blood. This was followed by a final 10-minute BAPTA-AM infusion identical to the first infusion. The entire infusion protocol took ~70 minutes. At the end of this protocol, LV-devP dropped to <5% of control, and Ca transients were virtually undetectable. Still, VF was inducible with rapid pacing, and the movies of the action potential showed multiple WBs. Thus, we reaffirm our position that, in the blood-perfused porcine heart, Ca dynamics is not necessary for VF maintenance. In addition, our new data indicate that Ca dynamics is not necessary for VF induction by rapid pacing.

Sources of Funding

Supported by NIH grant 1RO1HL088444-01A1 and a research grant from the Nora Eccles Treadwell Foundation.

Disclosures

None.

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Circ Res. 2008;102:e103
doi: 10.1161/CIRCRESAHA.108.175901
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/102/9/e103

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