DEBRECENI EGYETEM
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4012 Debrecen Pf. 22.
Intézetigazgató: Prof. Dr. Csernoch László
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ESC First Contact Initiative Grant - Report

Recipient: **Bence Hegyi, MD, PhD**
Host institution: **Department of Pharmacology, School of Medicine, University of California Davis, Davis, California, USA**
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PART 1: Investigation of the mechanisms of triggered activity, especially early afterdepolarisations in isolated ventricular myocytes

Background

One of the main mechanisms leading to life threatening cardiac arrhythmias is the appearance of triggered-activity in the myocardium (1). In the course of that, pathological depolarisations are present, which pathologically excite the neighbouring cardiomyocytes and evoke (trigger) arrhythmia, which becomes subsequently self-established (2). There are two mechanisms of the triggered-activity development: early and delayed afterdepolarisations (EADs and DADs, respectively) (3). These irregular membrane potential changes or oscillations usually related to a normally driven cardiac action potential (AP). EADs appear during the plateau phase of an action potential retarding terminal repolarisation, contrary to DADs, which arise from the resting potential following complete repolarisation (2, 3). While DADs are known to be elicited by spontaneous Ca^{2+} release from the Ca^{2+}-overloaded SR activating Ca^{2+}-dependent inward currents (dominantly Na^{+}/Ca^{2+} exchanger current, I_{NCX}), the mechanism of generation of EADs is still controversial (4). Since EADs are typically evoked by circumstances or drugs that increase the net membrane current during the AP plateau and appear usually at slow pacing frequencies, EADs were explained by reactivation of a potentially regenerative inward current system, e.g. reopening of L-type Ca^{2+} channels (5). This theory - although is suitable to predict many experimental findings related to EADs - fails to explain the proper timing of the membrane potential changes. On the other hand, experimental results have revealed the occurrence of apparently spontaneous Ca^{2+} release events during some types of EADs (4, 6). However, in spite of the intensive research of this field, several questions remained open or

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controversial, including the underlying triggering mechanisms, ionic nature and frequency-dependent properties of afterdepolarisations.

In the present study, therefore, the role of $[Ca^{2+}]_i$ changes in EAD generation was studied using three different EAD models. Dofetilide (inhibitor of the rapid delayer rectifier K^+ current, I_{Kr}), veratridine (activator of late Na^+ current, $I_{Na,L}$) and Bay K8644 (activator of L-type Ca^{2+} current, $I_{Ca,L}$) are equally suitable to evoke EADs, since all of them may increase APD in the required extent. In addition, they cause a comparable elevation of $[Ca^{2+}]_i$. On the other hand, Bay K8644 directly modulates the opening/reopening of L-type Ca^{2+} channels, which is not the case with veratridine and dofetilide. After elaborating the optimal experimental design, $[Ca^{2+}]_i$ was buffered following the development of EADs in each of the above models. This approach allowed to separate the contribution of APD changes and $[Ca^{2+}]_i$ changes to generation of EADs.

Results

1, The I_{Kr} inhibitor dofetilide strongly lengthened the APs in isolated left ventricular cardiomyocytes, which was accompanied by development of EADs in all of the ten cells exposed to 300 nM dofetilide for 10 min (Fig. 1.A). In six of these cells EADs were present continuously, while in four cells the frequency of EAD (which was defined as the percentage of APs where at least 1 EAD could be observed) varied between 20 % and 70 % of the total recording period. All these changes were reverted by superfusion of cell-permeant acetoxymethylester form of the Ca^{2+} chelator BAPTA (BAPTA-AM, 5 μM) for 30 min, although the reversion was not complete. EADs fully disappeared in eight out of the ten cells (Fig. 1.B), and an EAD frequency of only 20-40 % was obtained in the remaining two. The high average value of EAD frequency observed in dofetilide (77±10%) was decreased to 5±4 % in the presence of BAPTA-AM ($p<0.05$, $n=10$).

2, In the presence of $I_{Na,L}$ activator veratridine (300 nM), EAD frequency was lower than observed with dofetilide (Fig. 1C). This could not been overcome even if the concentration of veratridine was elevated up to 1 μM. Although EADs developed in all of the eighteen cells exposed to veratridine, the average frequency of EADs was only 37±6 %, which was reduced by BAPTA-AM superfusion to 22±6 % ($p<0.05$, $n=18$) (Fig. 1D).

3, EADs developed in sixteen out of the twenty one cells exposed to the L-type calcium channel activator Bay K8644 (200 nM), displaying an EAD frequency of 56±9 % (Fig. 1E). In contrast to these results, however, superfusion with BAPTA-AM further increased EAD-frequency to 70±8 % ($p<0.05$, $n=21$) since nineteen cells displayed EADs following BAPTA-AM superfusion (Fig. 1F).

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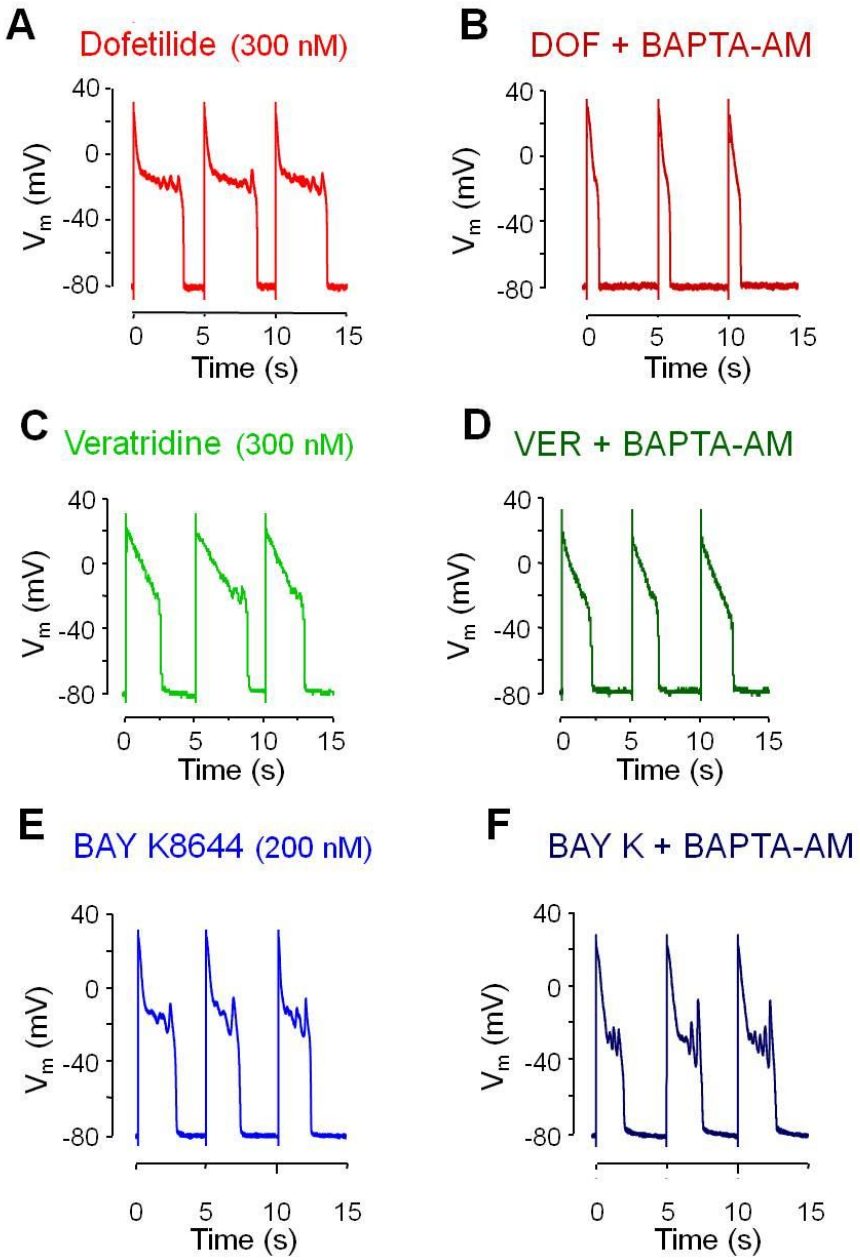

Figure 1. Effect of BAPTA-AM on EAD formation in ventricular myocytes. EADs were evoked by the I_{Kr} blocker dofetilide, the $I_{Na,L}$ activator veratridine and the $I_{Ca,L}$ activator Bay K8644 at 0.2 Hz. Representative action potentials recorded in the presence of 300 nM dofetilide (A), 300 nM veratridine (C) and 200 nM Bay K8644 (E). Each drug was applied for 10 min. After that the myocytes were superfused with 5 μM BAPTA-AM in the presence of dofetilide (B), veratridine (D) and Bay K8644 (E).

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PART 2: Acquirement of new experimental techniques

During performing research work in the host institution, I could learn and acquire new experimental techniques (namely the measurement of intracellular Ca^{2+} concentration and Ca^{2+}-releasing events with confocal microscopy in combination with electrophysiological techniques, analysis of single myocytes contraction in a three-dimensional elastic matrix (7, 8)), as well as to broaden the knowledge regarding the function of cardiac ion channels and signalling pathways during the discussions and seminars in the Department of Pharmacology, University of California Davis. Furthermore, I had opportunity to use and test the new superresolution microscopy technique (stimulated emission depletion – STED) in comparison to the 'conventional' confocal microscopy imaging (Fig. 2).

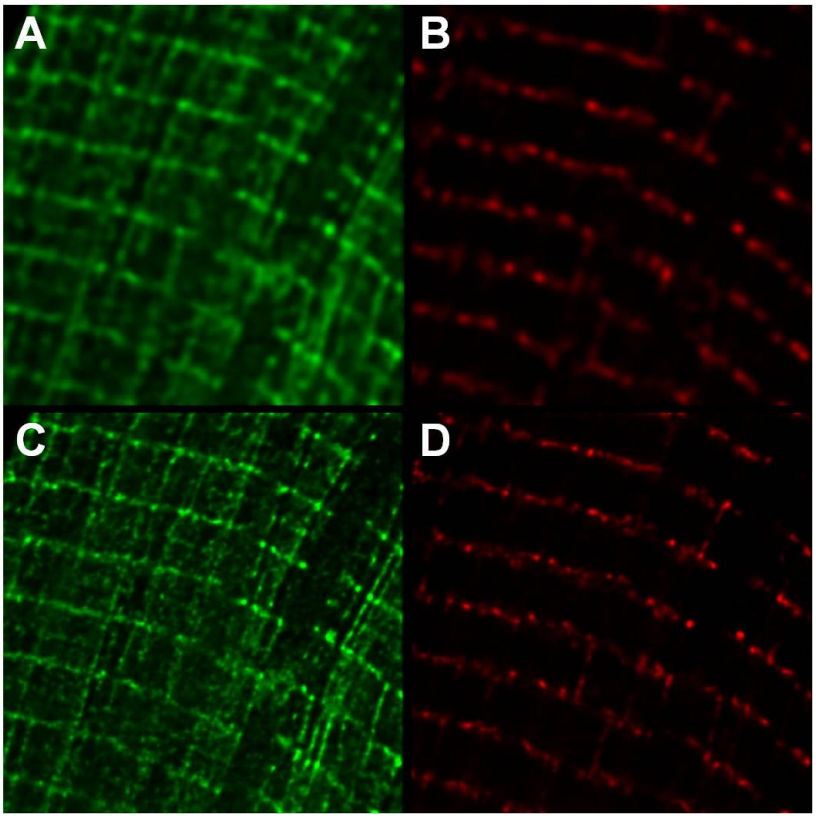

Figure 2. Confocal and superresolution images of Ca^{2+}-calmodulin kinase II (CaMKII) and ryanodine receptor (RyR) clusters in a ventricular myocyte. A-B: Representative 'conventional' confocal microscopy images of CaMKIIδ (green) and RyRs (red). C-D: Superresolution (Stimulated emission depletion – STED) microscopy images of the same region by using Leica TCS SP8 STED 3X.

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I would like to thank the ESC Council for providing me the ESC First Contact Initative Grant and also to express my very great appreciation to Dr. Ye Chen-Izu, Dr. Leighton T. Izu and Prof. Dr. Donald M. Bers for their great help and continuous support. I really enjoyed the open-minded atmosphere in the Cardiac Signaling Lab and the extensive discussions with them. To conclude, I was very impressed with the multidisciplinary approach to study the complex behaviour of the dynamic electro-mechano-chemo transduction and Ca^{2+} signalling systems that control cardiac function.
Finally, the decision was made at the end of my stay to join the team of the Cardiac Signaling Lab as a postdoctoral scholar. It will contribute to develop my skills in imaging and molecular techniques and combined with my research background on the physiology of cardiac ion channels, we hope that it will favour to the identification of novel therapeutic targets and approaches in the treatment of cardiac arrhythmias.

Yours sincerely,

Bence Hegyi M.D., Ph.D.

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