Hypokalemia promotes late phase 3 early afterdepolarization and recurrent ventricular fibrillation during isoproterenol infusion in Langendorff perfused rabbit ventricles

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BACKGROUND Hypokalemia and sympathetic activation are commonly associated with electrical storm (ES) in normal and diseased hearts. The mechanisms remain unclear.

OBJECTIVE The purpose of this study was to test the hypothesis that late phase 3 early afterdepolarization (EAD) induced by I_{KATP} activation underlies the mechanisms of ES during isoproterenol infusion and hypokalemia.

METHODS Intracellular calcium (Ca_i) and membrane voltage were optically mapped in 32 Langendorff-perfused normal rabbit hearts.

RESULTS Repeated episodes of electrically induced ventricular fibrillation (VF) at baseline did not result in spontaneous VF (SVF). During isoproterenol infusion, SVF occurred in 1 of 15 hearts (7%) studied in normal extracellular potassium ($[K^+]_o$, 4.5 mmol/L), 3 of 8 hearts (38%) in 2.0 mmol/L $[K^+]_o$, 9 of 10 hearts (90%) in 1.5 mmol/L $[K^+]_o$, and 7 of 7 hearts (100%) in 1.0 mmol/L $[K^+]_o$ ($P < .001$). Optical mapping showed that isoproterenol and hypokalemia enhanced Ca_i transient duration (Ca_iTD) and heterogeneously shortened action potential duration (APD) after defibrillation, leading to late phase 3 EAD and SVF. I_{KATP} blocker (glibenclamide, 5 μmol/L) reversed the post-defibrillation APD shortening and suppressed recurrent SVF in all hearts studied despite no evidence of ischemia. Nifedipine reliably prevented recurrent VF when given before, but not after, the development of VF. I_{Kr} blocker (E-4031) and small-conductance calcium-activated potassium channel blocker (apamin) failed to prevent recurrent SVF.

CONCLUSION Beta-adrenergic stimulation and concomitant hypokalemia could cause nonischemic activation of I_{KATP}, heterogeneous APD shortening, and prolongation of Ca_iTD to provoke late phase 3 EAD, triggered activity, and recurrent SVF. I_{KATP} inhibition may be useful in managing ES during resistant hypokalemia.

KEYWORDS Ventricular fibrillation; Electrical storm; Hypokalemia; Afterdepolarization; Intracellular calcium; ATP-sensitive potassium channels

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **Ca_i** = intracellular calcium; **Ca_iTD** = duration of Ca_i transient; **EAD** = early afterdepolarization; **ES** = electrical storm; **I_{CFTR}** = cystic fibrosis transmembrane regulator chloride current; **I_{KAS}** = small conductance calcium activated potassium current; **I_{KATP}** = ATP-sensitive potassium current; **I_{Kr}** = rapid component of delayed rectifier potassium current; **$[K^+]_o$** = extracellular potassium concentration; **SVF** = spontaneous ventricular fibrillation; **VF** = ventricular fibrillation; **V_m** = membrane voltage; **VT** = ventricular tachycardia

(Heart Rhythm 2014;11:697–706)

This manuscript was processed by a guest editor. This study was supported in part by National Institutes of Health Grants P01 HL78931, R01 71140, and R21HL106554; Grant-in-Aid for Scientific Research 24591076 from the Ministry of Education, Culture, Sports, Science and Technology of Japan to Dr. Maruyama; a Heart Rhythm Society Fellowship in Cardiac Pacing and Electrophysiology to Dr. Shen; a Medtronic-Zipes endowment to Dr. Chen; and the Indiana University Health–Indiana University School of Medicine Strategic Research Initiative. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. **Address reprint requests and correspondence:** Dr. Peng-Sheng Chen, Krannert Institute of Cardiology and the Division of Cardiology, Department of Medicine, Indiana University School of Medicine, 1800 N. Capitol Ave, E475, Indianapolis, IN 46202. E-mail address: chenpp@iupui.edu.

Introduction

Electrical storm (ES) describes a clinical condition characterized by multiple spontaneous ventricular fibrillation (SVF) episodes that necessitate repeated defibrillation. Although ES usually occurs in patients with serious organic heart diseases, it is not rare in patients with structurally normal hearts and genetic arrhythmias such as the Brugada and early repolarization syndromes. It is known that hypokalemia is a common trigger of ES.[1,2] Bansch et al[3] reported that the recurrence risk of ES was highest in cardiomyopathic

1547-5271/$-see front matter http://dx.doi.org/10.1016/j.hrthm.2013.12.032

patients with hypokalemia and associated conditions such as diarrhea and vomiting. Hypokalemia is also associated with ES in structurally normal hearts, and correction of hypokalemia can prevent the recurrent ventricular fibrillation (VF).[4,5] However, because rapid and safe correction of serum potassium often is difficult, additional drug therapy often is necessary to manage ES. Sympathetic blockade, although not always effective, is commonly used in the management of ES.[6] This clinical practice is consistent with the observation that sympathetic nerve activity is a direct and immediate trigger of ventricular tachycardia (VT) and ventricular fibrillation (VF).[6] Hypokalemia alone does not enhance VF inducibility in normal dogs.[7] However, sympathetic stimulation with isoproterenol and concomitant hypokalemia can induce VF in a rat model.[8] Massive reactive sympathetic activation[9] and the frequent use of epinephrine during fibrillation–defibrillation episodes[10] also may induce hypokalemia in patients with ES because beta-adrenergic stimulation itself can elicit hypokalemia.[11] We have shown that heart failure in rabbits up-regulates a small-conductance calcium-activated potassium current (I_{KAS}) and promotes late phase 3 early afterdepolarization (EAD) leading to ES.[12,13] However, as far as we know, there are no animal models of ES in ventricles without chronic structural remodeling.

To test the importance of hypokalemia and sympathetic activation in the development of ES, we aimed to develop a model of ES in normal rabbit ventricles in the presence of hypokalemia during isoproterenol infusion. This animal model was then used to test the hypothesis that late phase 3 EAD is important in the recurrence of SVF after defibrillation, similar to that occurring after electrical shocks in normal canine atria.[14] We then tested a third hypothesis that glibenclamide, an I_{KATP} blocker, is effective in preventing ES in this model.

Methods

The details of experimental methods and protocol are available in the Online Supplementary Data. The isolated rabbit hearts (n = 32) were perfused to maintain a perfusion pressure >70 mmHg throughout the study. Intracellular calcium (Ca_i) and membrane voltage (V_m) were simultaneously mapped using optical mapping techniques as described previously.[12]

VF was electrically induced and allowed to persist for 3 minutes under maintained coronary perfusion, followed by defibrillation. The set of 3-minute VF and 1-minute observational period was repeated up to 5 times unless SVF occurred. VF induction and defibrillation were repeated in control and during isoproterenol infusion (0.3 μmol/L) to determine the role of beta-adrenergic stimulation in action potential duration (APD) shortening after VF episodes (n = 5). Thereafter, we tested various levels of $[K^+]_o$ (4.5, 2.0, 1.5, and 1.0 mmol/L) during beta-adrenergic stimulation (n = 17). In another 10 hearts, pharmacologic interventions were applied to identify the mechanism of post-defibrillation APD shortening during beta-adrenergic stimulation at the normal $[K^+]_o$ level. APD was measured at 50% (APD_{50}) and 80% (APD_{80}) repolarization. Continuous variables are expressed as mean ± SEM. $P \leq .05$ was considered significant.

Results

Model of ES in normal ventricles

We successfully developed a model of ES in these normal ventricles. We defined ES as multiple (≥3) consecutive episodes of SVF recurrences after initial successful defibrillation. In control, no SVF was observed with 5 attempts of induced VF–defibrillation episodes. During isoproterenol infusion, SVF occurred in 1 of 15 hearts (7%) studied in normal $[K^+]_o$ (4.5 mmol/L), 3 of 8 hearts (38%) in 2.0 mmol/L $[K^+]_o$, 9 of 10 hearts (90%) in 1.5 mmol/L $[K^+]_o$, and 7 of 7 hearts (100%) in 1.0 mmol/L $[K^+]_o$ ($P < .001$). The SVF episodes evolved into ES in 0 of 15 hearts (0%), 1 of 8 hearts (13%), 8 of 10 hearts (80%), and 6 of 7 hearts (86%) studied in the $[K^+]_o$ of 4.5 mmol/L, 2.0 mmol/L, 1.5 mmol/L, and 1.0 mmol/L, respectively ($P < .001$). ES developed after episodes 2.7 ± 1.1 of electrically induced VF. In the majority of ES (13/15 hearts [87%]), VTs (cycle length 345 ± 10 ms) were observed between the SVF episodes. Figure 1A shows a typical example of ES observed under isoproterenol infusion in 1.0 mmol/L $[K^+]_o$. First, a pacing-induced VF was successfully defibrillated. VT followed the successful defibrillation, and then SVF occurred. Despite multiple attempts of defibrillation, all subsequent shocks seemed to fail to defibrillate the SVF on pseudo-ECG. However, optical recordings revealed that each shock actually terminated the SVF successfully, followed by immediate recurrences of SVF. A large difference between APD and Ca_i transient duration (Ca_iTD) was noted during ES. Initiation of SVF was associated with a short-coupled ventricular ectopy (Figure 1B). The coupling interval of the ectopic beat that initiated SVF was significantly shorter than that of the ectopic beat not initiating SVF (150 ± 5 ms vs 239 ± 6 ms, $P < .001$). There was no apparent QRS or T wave alternans during VT before transition to SVF. Repeated defibrillation (5 ± 2 successful defibrillation, shock intensity 177 ± 6 V) alone terminated ES in only 2 hearts (14%).

Post-defibrillation VT (Online Supplementary Figure 1) was observed only during isoproterenol infusion and more frequently with a lower $[K^+]_o$ (VT incidence: 17%, 33%, 60%, and 71% at $[K^+]_o$ of 4.5, 2.0, 1.5, and 1.0 mmol/L, respectively). Spontaneous Ca_i elevations preceded the VT beats, and the VT was effectively suppressed by 10 μmol/L nifedipine but not by ventricular overdrive pacing.

Post-defibrillation action potential triangulation during beta-adrenergic stimulation

In control, APD was modestly shortened after defibrillation of 3-minute electrically induced VF, which likely is because of the dependence of APD on the entire activation history