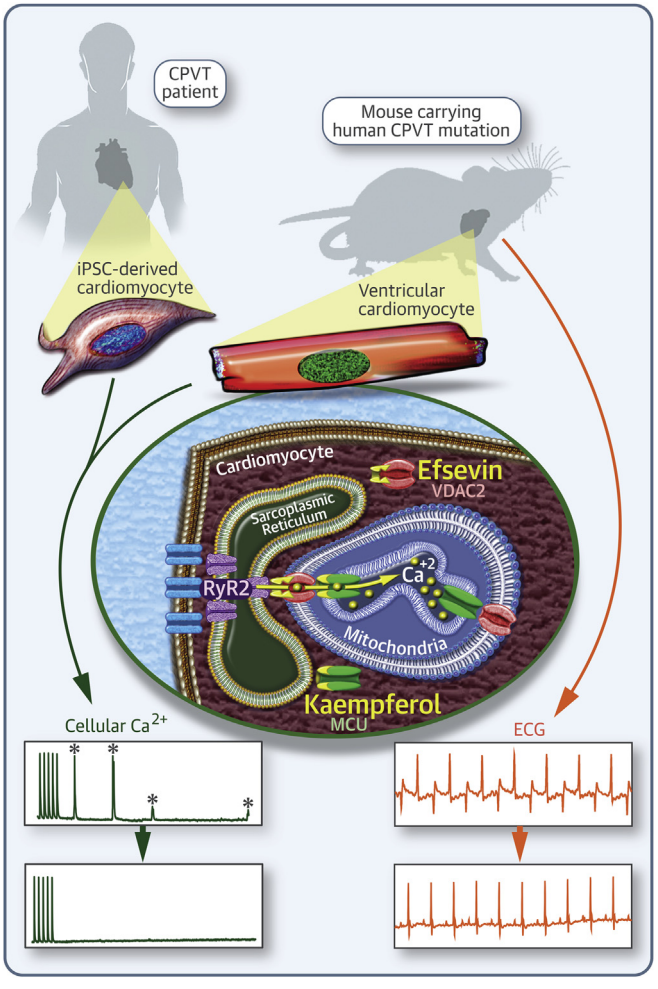
JACC: BASIC TO TRANSLATIONAL SCIENCE
VOL. 2, NO. 6, 2017
ISSN 2452-302X
https://doi.org/10.1016/j.jacbts.2017.06.008

PRECLINICAL RESEARCH

Suppression of Arrhythmia by Enhancing Mitochondrial Ca^{2+} Uptake in Catecholaminergic Ventricular Tachycardia Models

Maria K. Schweitzer, MSc,[a] Fabiola Wilting, MSc,[a] Simon Sedej, PhD,[b] Lisa Dreizehnter, PhD,[c] Nathan J. Dupper, BSc,[d] Qinghai Tian, PhD,[e] Alessandra Moretti, PhD,[c,f] Ilaria My, MD,[c] Ohyun Kwon, PhD,[d] Silvia G. Priori, MD, PhD,[g,h] Karl-Ludwig Laugwitz, MD,[c,f] Ursula Storch, PhD,[a] Peter Lipp, PhD,[e] Andreas Breit, PhD,[a] Michael Mederos y Schnitzler, PhD,[a,f] Thomas Gudermann, MD,[a,f] Johann Schredelseker, PhD[a]

VISUAL ABSTRACT



Schweitzer, M.K. et al. J Am Coll Cardiol Basic Trans Science. 2017;2(6):737–47.

HIGHLIGHTS

- Fast transfer of Ca^{2+} from the sarcoplasmic reticulum into mitochondria in cardiomyocytes can be enhanced by the MiCUps efsevin, targeting the VDAC2, and kaempferol, targeting the MCU.
- Enhancing sarcoplasmic reticulum-to-mitochondria Ca^{2+} transfer with MiCUps suppresses arrhythmogenic Ca^{2+} events and spontaneous action potentials in cardiomyocytes from a mouse model of CPVT.
- In vivo treatment of CPVT mice with MiCUps reduces episodes of ventricular tachycardia after adrenergic stimulation.
- In induced pluripotent stem cell-derived cardiomyocytes from a CPVT patient, both MiCUps reduce arrhythmogenic Ca^{2+} events.
- Our data establish fast mitochondrial Ca^{2+} uptake as a promising candidate structure for pharmacological treatment of human cardiac arrhythmia.

ABBREVIATIONS AND ACRONYMS

CPVT = catecholaminergic polymorphic ventricular tachycardia

epi/caff = epinephrine/caffeine

iPSC = induced pluripotent stem cell

ISO = isoproterenol

MCU = mitochondrial calcium uniporter

MiCUp = mitochondrial calcium uptake enhancer

RyR2 = ryanodine receptor type 2

SR = sarcoplasmic reticulum

VDAC2 = voltage-dependent anion channel type 2

WT = wild type

SUMMARY

Cardiovascular disease-related deaths frequently arise from arrhythmias, but treatment options are limited due to perilous side effects of commonly used antiarrhythmic drugs. Cardiac rhythmicity strongly depends on cardiomyocyte Ca^{2+} handling and prevalent cardiac diseases are causally associated with perturbations in intracellular Ca^{2+} handling. Therefore, intracellular Ca^{2+} transporters are lead candidate structures for novel and safer antiarrhythmic therapies. Mitochondria and mitochondrial Ca^{2+} transport proteins are important regulators of cardiac Ca^{2+} handling. Here, the authors evaluated the potential of pharmacological activation of mitochondrial Ca^{2+} uptake for the treatment of cardiac arrhythmia. To this aim, the authors tested substances that enhance mitochondrial Ca^{2+} uptake for their ability to suppress arrhythmia in a murine model for ryanodine receptor 2 (RyR2)-mediated catecholaminergic polymorphic ventricular tachycardia (CPVT) in vitro and in vivo and in induced pluripotent stem cell-derived cardiomyocytes from a CPVT patient. In freshly isolated cardiomyocytes of $RyR2^{R4496C/WT}$ mice efsevin, a synthetic agonist of the voltage-dependent anion channel 2 (VDAC2) in the outer mitochondrial membrane, prevented the formation of diastolic Ca^{2+} waves and spontaneous action potentials. The antiarrhythmic effect of efsevin was abolished by blockade of the mitochondrial Ca^{2+} uniporter (MCU), but could be reproduced using the natural MCU activator kaempferol. Both mitochondrial Ca^{2+} uptake enhancers (MiCUps), efsevin and kaempferol, significantly reduced episodes of stress-induced ventricular tachycardia in $RyR2^{R4496C/WT}$ mice in vivo and abolished diastolic, arrhythmogenic Ca^{2+} events in human iPSC-derived cardiomyocytes. These results highlight an immediate potential of enhanced mitochondrial Ca^{2+} uptake to suppress arrhythmogenic events in experimental models of CPVT and establish MiCUps as promising pharmacological tools for the treatment and prevention of Ca^{2+}-triggered arrhythmias such as CPVT. (J Am Coll Cardiol Basic Trans Science 2017;2:737–47)

Cardiovascular diseases remain the leading cause of death worldwide (1). Approximately one-half of all cardiovascular deaths are attributed to severe cardiac arrhythmia (2). Cardiac rhythmicity critically depends on precisely regulated Ca^{2+} oscillations in cardiomyocytes (3), and cardiac arrhythmia is frequently associated with perturbations of cellular Ca^{2+} handling (4,5). Most currently used antiarrhythmic drugs target receptors and channels in the cell membrane (sarcolemma) to block the propagation of arrhythmic events along the myocardium but do not restore defective intracellular Ca^{2+} handling. Due to their modulatory effects on the cardiac action potential, these drugs often show pro-arrhythmic side effects. Therefore, intracellular Ca^{2+} transporters are candidate structures for novel and safer antiarrhythmic therapies.

Mitochondria occupy approximately 30% of the cardiomyocyte volume (6,7) and closely interact with the sarcoplasmic reticulum (SR) to absorb Ca^{2+}, which is released from the SR into the cytosol through cardiac ryanodine receptors (RyR2) (8,9). For that, mitochondria are equipped with a regulated network of Ca^{2+} transporters. While the mitochondrial calcium

From the [a]Walther Straub Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München, Munich, Germany; [b]Department of Cardiology, Medical University of Graz, Graz, Austria; [c]Department of Medicine (Cardiology), Klinikum rechts der Isar, Technische Universität München, Munich, Germany; [d]Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, California; [e]Institute for Molecular Cell Biology, University Medical Center, Saarland University, Homburg/Saar, Germany; [f]Deutsches Zentrum für Herz-Kreislauf-Forschung, Partner Site Munich Heart Alliance, Munich, Germany; [g]Molecular Cardiology, Istituto di ricovero e cura a carattere scientifico (IRCCS) Salvatore Maugeri Foundation, Pavia, Italy; and the [h]Department of Molecular Medicine, University of Pavia, Pavia, Italy. Supported by Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany (SCHR 1471/1-1 to Dr. Schredelseker; TRR152 to Drs. Gudermann, Lipp, Moretti, and Laugwitz), the Friedrich-Baur-Stiftung, Munich, Germany (to Dr. Schredelseker), and U.S. National Institutes of Health (R01GM071779 to Dr. Kwon). Dr. Sedej is supported by the Austrian Science Fund FWF, Vienna, Austria (P27637-B28). Dr. Priori has received research support from Boston Scientific; and is a member of the advisory boards for General Electric and Audentes. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.
All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

Manuscript received April 10, 2017; revised manuscript received June 26, 2017, accepted June 27, 2017.

uniporter (MCU) has been identified as the main route for Ca^{2+} import in the inner mitochondrial membrane (10–12), the voltage-dependent anion channel 2 (VDAC2) in the outer mitochondrial membrane has only recently been shown to be a major part of the Ca^{2+} transport route from the SR into mitochondria (13–15). In addition to the 2 pore-forming channels, the mitochondrial Ca^{2+} uptake complex is regulated by several auxiliary subunits such as the essential MCU regulator EMRE or the mitochondrial Ca^{2+} uptake proteins MICU1 and -2 (16,17). While it is generally accepted that a slow and moderate rise of mitochondrial Ca^{2+} triggers enhanced energy production under higher workload (18), the immediate role of mitochondria in shaping cellular Ca^{2+} signals on a beat-to-beat level remains a matter of debate (19,20). Mitochondria have been demonstrated to be regulators of cardiac rhythmicity (21,22), but their potential to serve as therapeutic targets has not been evaluated so far. The synthetic compound efsevin has been newly identified to activate VDAC2 and to enhance mitochondrial Ca^{2+} uptake (15). Using efsevin, we demonstrated that cardiac rhythmicity in zebrafish embryos can be critically modulated by enhancing mitochondrial Ca^{2+} uptake (15).

This study evaluated the translational potential of pharmacological activation of mitochondrial Ca^{2+} uptake for the prevention and treatment of cardiac arrhythmia. To this end, we used 2 models of catecholaminergic polymorphic ventricular tachycardia (CPVT), exemplifying a Ca^{2+}-triggered arrhythmia. CPVT manifests in early adolescence and is characterized by episodes of life-threatening ventricular tachycardia upon catecholaminergic stimulation after physical exercise or emotional stress. The $RyR2^{R4496C/WT}$ mouse harbors an R4496C mutation in the SR Ca^{2+} release channel RyR2, homologous to the human R4497C mutation, which is associated with CPVT (23) and displays a CPVT phenotype (24). We show that activating mitochondrial Ca^{2+} uptake suppresses arrhythmia in $RyR2^{R4496C/WT}$ cardiomyocytes in vitro and in $RyR^{R4496C/WT}$ mice in vivo. Finally, we demonstrate that activating mitochondrial Ca^{2+} uptake is likewise efficient in blocking arrhythmogenesis in induced pluripotent stem cell (iPSC)-derived cardiomyocytes from a CPVT patient heterozygous for a different RyR2 mutation ($RyR2^{S406L}$).

METHODS

ISOLATION OF CARDIOMYOCYTES. Isolation of ventricular cardiomyocytes from heterozygous knock-in $RyR2^{R4496C/WT}$ mice (24) was performed using a Langendorff perfusion-based enzymatic digestion protocol (25) with minor modifications. Only excitable, rod-shaped, quiescent cells were used for experiments.

HUMAN iPSC-BASED MODEL. iPSCs from a skin biopsy from a CPVT patient carrying the $RyR2^{S406L/WT}$ mutation and from a healthy donor were differentiated into spontaneously beating explants (26,27) and enzymatically dissociated into single cardiomyocytes.

Ca^{2+} IMAGING. Ca^{2+} transients, spontaneous diastolic Ca^{2+} waves, and Ca^{2+} sparks were measured in cardiomyocytes loaded with Fluo-4 acetoxymethyl ester (AM) (Thermo Fisher Scientific, Darmstadt, Germany), using confocal microscopy in line scan mode. Cells were paced by extracellular electrodes at 0.5 Hz.

ELECTROPHYSIOLOGY. Action potentials were recorded in current clamp mode, using the perforated patch-clamp technique. Cells were paced by repetitive, depolarizing intracellular current injections at 0.5 Hz, followed by a 60-s pause to detect potentially proarrhythmic events during this diastolic phase.

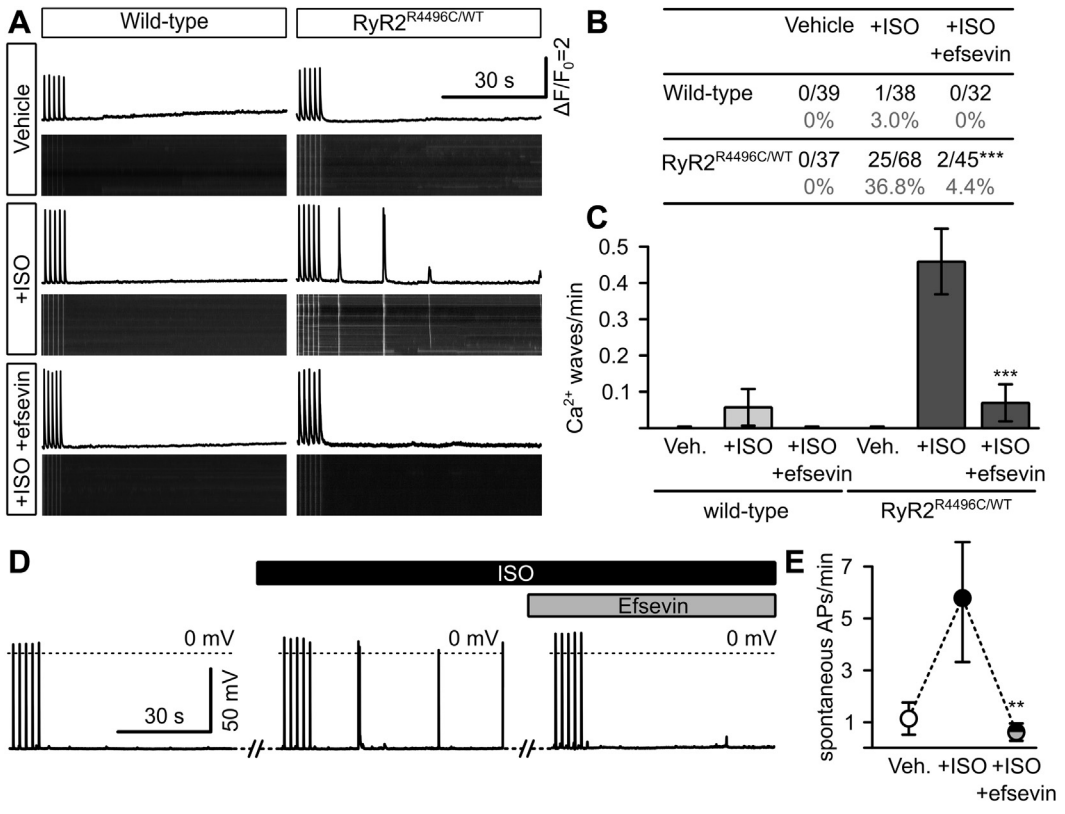
cAMP ACCUMULATION ASSAY. For evaluation of intracellular cAMP levels, cardiomyocytes were labeled with ^{3}H-labeled adenine to measure accumulation of [^{3}H]cAMP over 15 min.

IN VIVO ARRHYTHMIA TESTING. Drugs were administered to $RyR2^{R4496C/WT}$ mice 8 to 12 weeks of age through osmotic minipumps, and subsequently, electrocardiography recordings were performed under light isoflurane anesthesia, to monitor ventricular tachycardia after bolus injection of epinephrine/caffeine (epi/caff). All animal procedures were performed in accordance with national and European ethical regulations (directive 2010/63/EU) and approved by the responsible government agency (BMWFW-66.010/0012-WF/V/3b/2015).

MITOCHONDRIAL Ca^{2+} UPTAKE. Mitochondrial Ca^{2+} uptake in response to 10 mM caffeine to open RyR2 was measured in Rhod-2 AM-loaded permeabilized HL-1 cardiomyocytes (28) on a fluorescence 96-well plate reader. The Ca^{2+} chelator 1,2-*bis*(*o*-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (1 mM) was used to restrict Ca^{2+} to the low micrometer range around RyR clusters (8,29).

STATISTICAL ANALYSIS. Data are mean ± standard error of the mean. Normality of data was determined by Shapiro-Wilk test, and respective tests for statistical significance were conducted as indicated. Post hoc tests were Dunn's test for Kruskal-Wallis tests and Tukey's multiple comparisons test for ANOVA. Significance for contingency tables was calculated using Fisher's exact test.

FIGURE 1 Efsevin Reduces Diastolic Ca^{2+} Waves and Spontaneous Action Potentials in $RyR2^{R4496C/WT}$ Cardiomyocytes



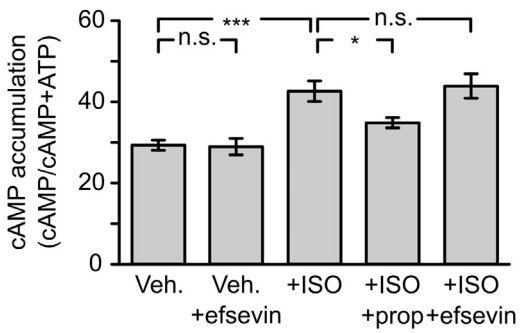
(A) Confocal line scans across the long axis of freshly isolated cardiomyocytes from wild-type and $RyR2^{R4496C/WT}$ mice loaded with Fluo-4 AM to measure intracellular Ca^{2+}. Images show line scans, and graphs depict fluorescence intensity plots. Cells were stimulated at 0.5 Hz for 30 s to reach steady state conditions (last 5 Ca^{2+} transients are shown) before pulsing was stopped and the diastolic phase was recorded for 90 s to screen for spontaneous diastolic Ca^{2+} waves. **(B)** While wild-type cells never showed a significant frequency of spontaneous diastolic Ca^{2+} waves, 25 of 68 $RyR2^{R4496C/WT}$ cardiomyocytes showed waves, which could be significantly reduced to 2 of 45 cells ($p < 0.001$, Fisher's exact test) by addition of 15 μM efsevin. **(C)** Average number of Ca^{2+} waves per minute was significantly enhanced from 0 in vehicle-treated $RyR2^{R4496C/WT}$ cardiomyocytes to 0.46 ± 0.09 waves/min in cells treated with 1 μM ISO ($p < 0.001$, Kruskal-Wallis test). Addition of 15 μM efsevin reduced the number of waves/minute to 0.07 ± 0.05 to levels indistinguishable from untreated cells ($p = 0.578$). **(D)** Patch clamp recordings from $RyR2^{R4496C/WT}$ cardiomyocytes showed an increase in spontaneous action potentials after superfusion with ISO, which were eliminated by addition of 15 μM efsevin. **(E)** Quantitative analysis of patch clamp recordings revealed a significant increase of spontaneous diastolic action potentials (APs) from 1.12 ± 0.62 APs/min under vehicle (Veh.) to 5.76 ± 2.45 APs/min after superfusion with ISO ($p = 0.018$, Friedman test, $n = 15$) and a significant reduction to 0.58 ± 0.34 APs/min when simultaneously treated with ISO and efsevin ($p = 0.011$). **$p < 0.01$; ***$p < 0.001$. ISO = isoproterenol.

Please see the detailed Methods in the Supplemental Appendix.

RESULTS

EFSEVIN REDUCES ARRHYTHMOGENIC Ca^{2+} WAVES IN $RyR2^{R4496C/WT}$ CARDIOMYOCYTES. The triggers for arrhythmia originating from imbalanced cellular Ca^{2+} homeostasis, such as CPVT, are intracellular Ca^{2+} waves during diastole, which arise from an increased SR Ca^{2+} leak through RyR2 (30). We therefore recorded diastolic Ca^{2+} waves in freshly isolated ventricular cardiomyocytes from $RyR2^{R4496C/WT}$ mice and their wild-type (WT) littermates. Under control conditions (vehicle), neither WT cardiomyocytes nor $RyR2^{R4496C/WT}$ cells showed spontaneous Ca^{2+} waves within 90 s after preceding electrical stimulation at 0.5 Hz. However, unlike WT cells, $RyR2^{R4496C/WT}$ cardiomyocytes displayed pronounced spontaneous, diastolic Ca^{2+} waves after stimulation with the catecholamine isoproterenol (ISO) (Figures 1A to 1C). Strikingly, application of 15 μM efsevin significantly reduced the number of cells displaying such Ca^{2+} waves and the average number of Ca^{2+} waves per

FIGURE 2 Efsevin Does Not Affect Beta-Adrenergic Stimulation in $RyR2^{R4496C/WT}$ Cardiomyocytes



cAMP accumulation in freshly isolated $RyR2^{R4496C/WT}$ cardiomyocytes was measured in response to different stimuli. Efsevin had no effect on cellular cAMP levels ($p > 0.999$, Kruskal-Wallis test), whereas ISO induced a significant increase in cellular cAMP ($p < 0.001$). This increase could be blocked using the beta-adrenoreceptor blocker propranolol (prop) ($p = 0.036$), although efsevin had no effect on the ISO-induced increase in cellular cAMP ($p = 0.999$), indicating that efsevin neither activates nor blocks the beta-adrenergic system in cardiomyocytes. *$p < 0.05$; ***$p < 0.001$. Abbreviations as in Figure 1.

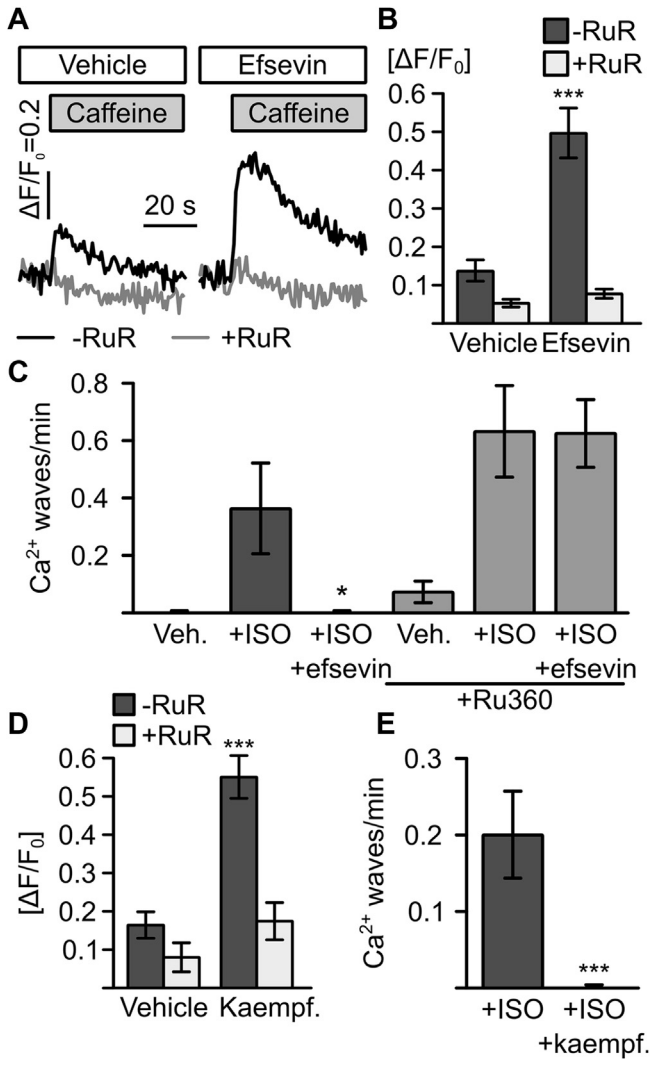
minute in $RyR2^{R4496C/WT}$ cardiomyocytes to levels of unstimulated $RyR2^{R4496C/WT}$ cells and WT cells. Notably, in contrast to previous data from unstimulated cells (15), efsevin did not exert any significant effects on the amplitude and kinetics of electrically evoked Ca^{2+} transients under ISO stimulation (Supplemental Figure 1).

EFSEVIN REDUCES SPONTANEOUS ACTION POTENTIALS IN $RyR2^{R4496C/WT}$ CARDIOMYOCYTES. Cardiac arrhythmia is triggered when Ca^{2+} waves activate the sarcolemmal sodium-calcium exchanger, leading to a transient depolarizing sodium inward current and finally spontaneous action potentials (31), which can propagate along the myocardium. Hence, we recorded spontaneous action potentials in patch-clamped $RyR2^{R4496C/WT}$ cardiomyocytes. We observed a significant increase in the frequency of spontaneous action potentials after cells were superfused with 1 μM ISO. Subsequent treatment with 15 μM efsevin effectively reduced these spontaneous depolarizations to baseline levels (**Figures 1D and 1E**). Notably, efsevin did not exert any effects on the resting membrane potential and amplitude of electrically evoked action potentials under ISO stimulation but caused a significant change of the repolarization phase (Supplemental Figure 1), namely a prolongation of the action potential duration at 50% repolarization (APD50) but not at 90% repolarization (APD90). To evaluate whether this effect seen on the fast, inactivating mouse action potential (32) could lead to QT prolongation in human cells, we recorded action potentials from human iPSC-derived cardiomyocytes in the presence of efsevin and found no changes in APD50 and APD90 compared to vehicle-treated cells (Supplemental Figures 2A and 2B). Furthermore, efsevin did not inhibit hERG channel activity in a heterologous expression system at relevant concentrations (Supplemental Figure 2C).

THE ANTIARRHYTHMIC EFFECT OF EFSEVIN IS MEDIATED BY MITOCHONDRIAL Ca^{2+} UPTAKE. We next investigated the mechanism of efsevin's antiarrhythmic effect. To exclude the possibility that efsevin directly blocks catecholaminergic stimulation by ISO, we measured cAMP accumulation in efsevin-treated $RyR2^{R4496C/WT}$ cardiomyocytes. Stimulation by ISO induced a significant increase in cellular cAMP, which was blocked by the beta-adrenoreceptor blocker propranolol. Addition of efsevin alone did not increase or decrease cellular cAMP concentrations, and addition of efsevin to stimulated cells had no effect on the ISO-induced cAMP increase, indicating that efsevin does not influence beta-adrenergic signaling in cardiomyocytes (**Figure 2**).

Efsevin significantly enhanced transfer of Ca^{2+} from the SR into mitochondria in a Ca^{2+} uptake assay in permeabilized cultured HL-1 cardiomyocytes. Mitochondrial Ca^{2+} was measured after addition of 10 mM caffeine to open RyR2s in the presence of the Ca^{2+} chelator BAPTA to restrict Ca^{2+} released from RyRs to the low micrometer range around RyR clusters (8,29) (**Figure 3A**). The efsevin-sensitive Ca^{2+} transfer between the SR and mitochondria was blocked by addition of the MCU blocker ruthenium red. To test whether the enhanced SR-mitochondria Ca^{2+} transfer was directly responsible for the reduction of diastolic Ca^{2+} wave frequency in $RyR2^{R4496C/WT}$ cardiomyocytes, we assessed whether blocking of mitochondrial Ca^{2+} uptake abolished the antiarrhythmic effect of efsevin. We measured catecholamine-induced Ca^{2+} waves in $RyR2^{R4496C/WT}$ myocytes under simultaneous blockade of mitochondrial Ca^{2+} uptake, using the MCU inhibitor Ru360 (**Figure 3B**). We observed a moderately higher Ca^{2+} wave frequency under all conditions, consistent with the idea that mitochondrial Ca^{2+} uptake prevents Ca^{2+} wave formation. Most strikingly, the ability of efsevin to suppress the ISO-induced increase in Ca^{2+} wave frequency was abolished in the presence of Ru360, indicating that the suppression of diastolic Ca^{2+} waves by efsevin is solely mediated by enhanced mitochondrial Ca^{2+} uptake.

FIGURE 3 Efsevin Acts Through Mitochondrial Ca^{2+} Uptake



(A) Representative recordings of mitochondrial Ca^{2+} in permeabilized HL-1 cardiomyocytes. Superfusion with caffeine induced a rapid uptake of Ca^{2+} into mitochondria **(black trace)**, which was enhanced by 15 μM efsevin and blocked by 10 μM RuR **(gray trace)**. **(B)** Quantification of peak mitochondrial fluorescence showed a significant increase in mitochondrial Ca^{2+} uptake in cells treated with efsevin from $\Delta F/F_0 = 0.14 \pm 0.03$ ($n = 10$) to 0.50 ± 0.07 ($n = 19$, $p < 0.001$, ANOVA). **(C)** 15 μM efsevin reduces spontaneous propagating waves in $RyR2^{R4496C/WT}$ cardiomyocytes under ISO influence from 0.36 ± 0.16 waves/min ($n = 15$) to 0 **(black bars)** ($n = 21$, $p = 0.035$, Kruskal-Wallis test). In the presence of 8 μM of the mitochondrial Ca^{2+} uptake blocker Ru360, vehicle-treated cells showed 0.07 ± 0.04 waves/min ($n = 29$), which increased to 0.63 ± 0.16 under ISO influence ($n = 21$, $p < 0.001$) but could not be blocked with efsevin as shown by an indistinguishable value of 0.62 ± 0.12 waves/min under ISO influence together with efsevin **(gray bars)** ($n = 62$, $p = 0.354$ compared to that with ISO). **(D)** 10 μM of the MCU activator kaempferol significantly increased mitochondrial Ca^{2+} uptake after SR Ca^{2+} release from $\Delta F/F_0 = 0.16 \pm 0.04$ ($n = 9$) to 0.55 ± 0.05 ($n = 10$, $p < 0.001$, ANOVA) Ca^{2+} uptake was inhibited by 50 μM RuR **(gray bars)**. **(E)** Treatment of $RyR2^{R4496C/WT}$ cardiomyocytes with 10 μM kaempferol completely eliminated ISO-induced spontaneous diastolic Ca^{2+} waves from 0.19 ± 0.04 waves/min ($n = 49$) to 0 ($n = 45$; $p < 0.001$, Mann-Whitney *U* test). *$p < 0.05$; ***$p < 0.001$. $\Delta F/F_0$ = change in fluorescence over baseline fluorescence; MCU = mitochondrial Ca^{2+} uniporter; RuR = ruthenium red; SR = sarcoplasmic reticulum.

We next tested if enhancing mitochondrial Ca^{2+} uptake was a general pharmacological approach to suppress arrhythmogenic events or if it is limited to a specific effect of efsevin on VDAC2. To this purpose, we used another activator of mitochondrial Ca^{2+} uptake, the natural plant flavonoid kaempferol, which was reported to directly activate MCU in the inner mitochondrial membrane (33,34). Indeed, kaempferol increased SR-mitochondria Ca^{2+} transfer, comparable to efsevin (**Figure 3D**). To evaluate the antiarrhythmic potential of kaempferol, we measured diastolic Ca^{2+} waves in kaempferol-treated $RyR2^{R4496C/WT}$ cardiomyocytes under ISO to induce catecholaminergic stimulation. Strikingly, 10 μM kaempferol completely eliminated ISO-induced arrhythmogenic Ca^{2+} waves in $RyR2^{R4496C/WT}$ cardiomyocytes (**Figure 3E**).

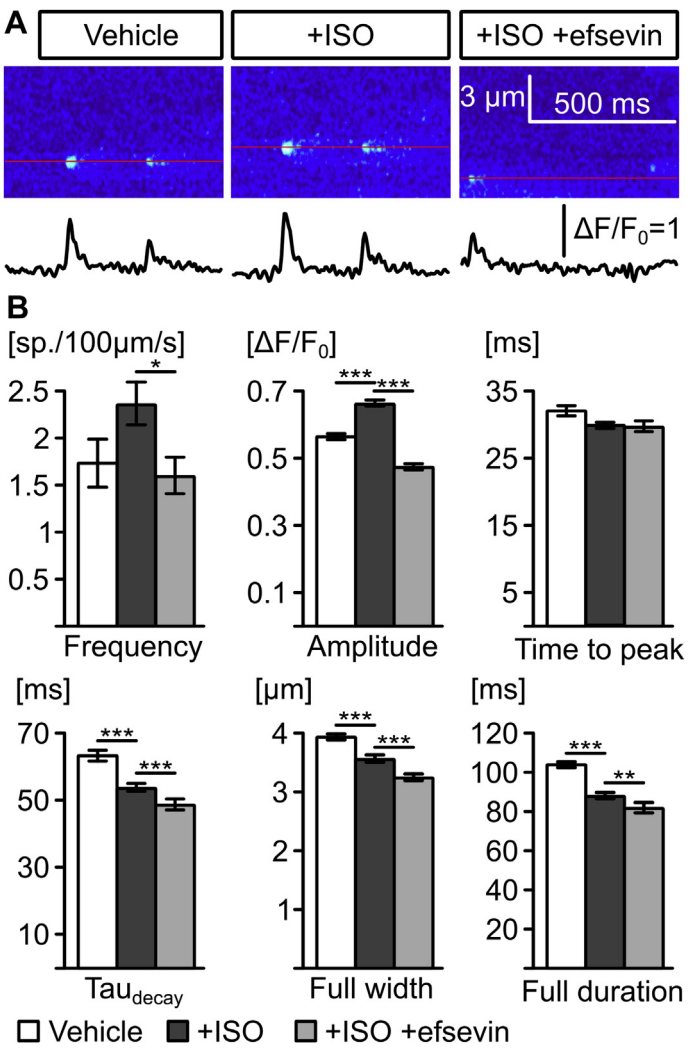
It was previously reported that enhanced mitochondrial Ca^{2+} uptake in cardiomyocytes restricts diffusion of Ca^{2+} inside the cytosol (13) and thereby prevents propagation of cytosolic Ca^{2+} signals under conditions of Ca^{2+} overload (15). Because an enhanced RyR-mediated Ca^{2+} leak was reported to be the mechanism responsible for arrhythmogenesis in CPVT (35–37), we recorded Ca^{2+} sparks from $RyR2^{R4496C/WT}$ cardiomyocytes (**Figure 4**). We observed an increase in Ca^{2+} spark frequency and amplitude after treatment with ISO, consistent with previous work (36), thus explaining the enhanced Ca^{2+} wave frequency under catecholaminergic stimulation. Strikingly, the SR leak in cells treated with ISO together with efsevin was reduced compared to that in cells treated with ISO alone, as indicated by a decrease in Ca^{2+} spark frequency and amplitude. Also, we found removal of cytosolic Ca^{2+} was accelerated under efsevin stimulation, leading to a reduction of full width and full duration of Ca^{2+} sparks. Together, these effects explain the suppressive effect of efsevin on propagating Ca^{2+} waves.

MiCUps REDUCE EPISODES OF STRESS-INDUCED VENTRICULAR TACHYCARDIA IN VIVO. To assess the potency of both of the mitochondrial Ca^{2+} uptake enhancers (MiCUps), efsevin and kaempferol, to suppress arrhythmia in vivo, we administered efsevin and kaempferol to $RyR2^{R4496C/WT}$ mice, using implantable osmotic minipumps. All mice recovered well from surgery, and their behavior was grossly normal. Treated mice showed no signs of discomfort, stress, or abnormal behavior. After 3 days, efsevin and kaempferol showed no effect on electrocardiography (ECG) parameters such as the interval between atrial and ventricular depolarization (PR), the interval between ventricular depolarization and subsequent repolarization (QT), or conduction of ventricular

depolarization (QRS) and basal heart rate (Figures 5A and 5B). To activate their adrenergic response, we injected mice with a bolus of 2 mg/kg epinephrine and 120 mg/kg caffeine (epi/caff). The epi/caff injection induced a significant increase in heart rate in all 3 groups, but no differences were observed between vehicle-treated mice and mice treated with MiCUps. Most strikingly, both of the MiCUps significantly reduced episodes of bidirectional ventricular tachycardia under catecholaminergic stimulation. Injection of epi/caff provoked bidirectional ventricular tachycardia in all vehicle-treated control animals (n = 11) but only in 6 of 10 mice treated with efsevin and 6 of 11 mice treated with kaempferol (Figures 5D and 5E). Comparable results were obtained from mice treated with efsevin for 8 days (Supplemental Figure 3).

MiCUps REDUCE ARRHYTHMOGENIC Ca^{2+} WAVES IN iPSC-DERIVED CARDIOMYOCYTES FROM A CPVT PATIENT. In order to evaluate the translational potential of MiCUps for the treatment of CPVT, we used a human cell-based arrhythmia model for CPVT. Human iPSC-derived cardiomyocytes from a CPVT patient heterozygous for the $RyR2^{S406L}$ mutation associated with CPVT (26) were used to record arrhythmogenic Ca^{2+} waves, whereas cells obtained from a healthy 32-year-old female without history of cardiac disease served as control. In accordance with the CPVT phenotype, where patients show a normal ECG pattern under baseline conditions, only few untreated cells displayed Ca^{2+} waves (Figure 6), whereas beta-adrenergic stimulation induced by ISO led to a significant increase in diastolic Ca^{2+} waves in $RyR2^{S406L/WT}$ cells but did not induce Ca^{2+} waves in control cells. Treatment of $RyR2^{S406L}$ cells with either efsevin or kaempferol significantly reduced the number of cells displaying Ca^{2+} waves and the average frequency of Ca^{2+} waves per minute to baseline levels.

FIGURE 4 Efsevin Reduces Intracellular Ca^{2+} Spark Frequency, Amplitude, and Cytosolic Ca^{2+} Clearance Leading to a Restriction of Individual Ca^{2+} Sparks

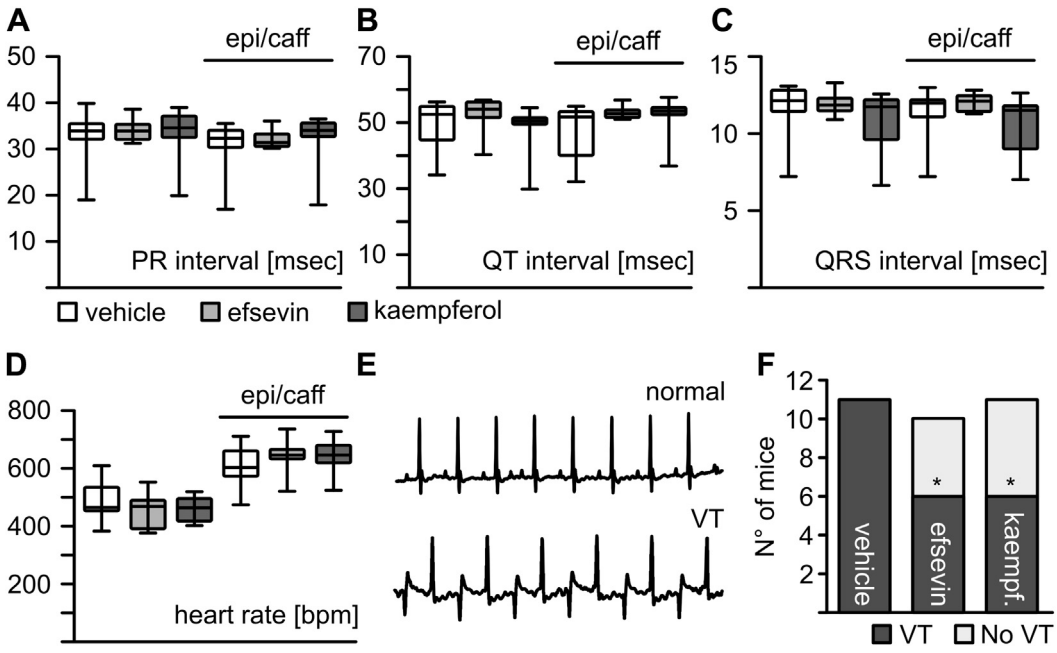


(A) Representative line scan confocal images from $RyR2^{R4496C/WT}$ cardiomyocytes show spontaneous Ca^{2+} sparks. **(B)** Treatment with ISO induced a higher spark frequency and amplitude and accelerated cytosolic Ca^{2+} removal (tau_{decay}) leading to narrower (full width) and shorter (full duration) sparks. Administration of efsevin reduced Ca^{2+} spark frequency ($p = 0.049$, Kruskal Wallis test) and amplitude ($p < 0.001$, Kruskal Wallis test) and limited spatial ($p < 0.001$, Kruskal Wallis test) and temporal ($p = 0.002$, Kruskal Wallis test) expansion of Ca^{2+} sparks. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Abbreviations as in Figure 1.

DISCUSSION

MITOCHONDRIA AS DRUG TARGETS. Mitochondria occupy approximately 30% of the cardiomyocyte volume (6,7). Although their crucial roles in ATP synthesis, regulation of respiratory rate, and apoptosis are well understood, mitochondrial contributions to cardiac Ca^{2+} handling are still under debate. While it is generally accepted that a gradual and moderate rise in mitochondrial Ca^{2+} enhances energy production (18), the role of a low-affinity/high-conductance, fast mitochondrial Ca^{2+} uptake remains unclear. Different experimental approaches suggest an immediate role for this uptake in the regulation of cardiomyocyte bioenergetics (38), contraction (39), and rhythmicity (15,21), but the potential of this rapid mitochondrial Ca^{2+} uptake mechanism to serve as a drug target in cardiovascular disease has not been sufficiently evaluated. It was proposed that inhibition of MCU by Ru360 ameliorates myocardial damage after ischemia reperfusion injury in rats, presumably by inhibiting depolarization of mitochondria and following opening of the mitochondrial permeability transition pore (40).

FIGURE 5 MiCUps Reduce Episodes of Ventricular Tachycardia in $RyR2^{R4496C/WT}$ Mice

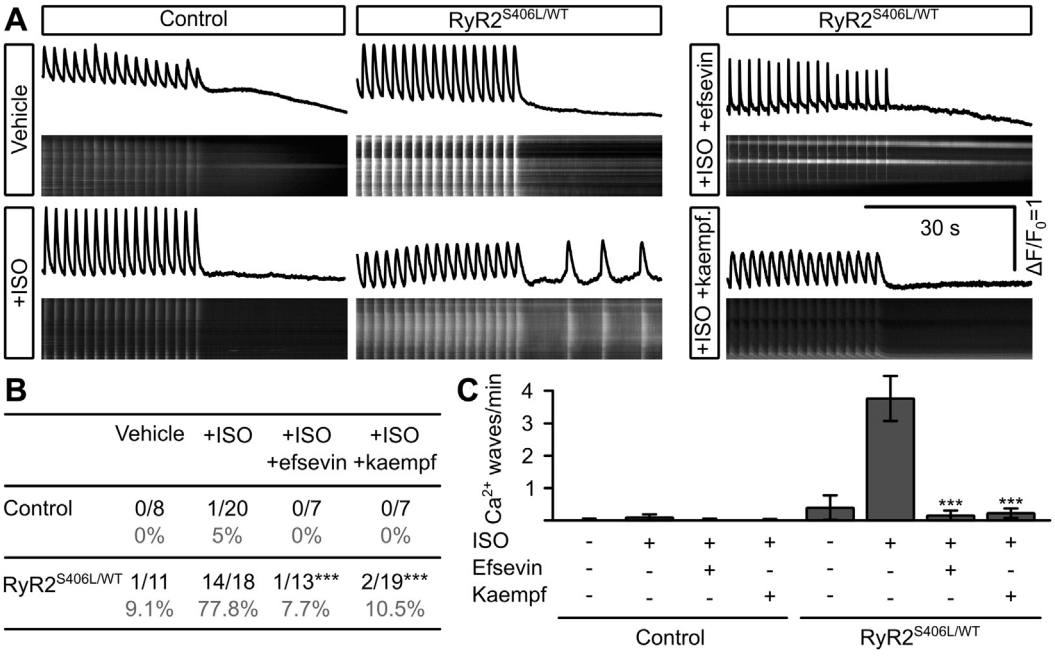


Three-lead surface ECGs from $RyR2^{R4496C/WT}$ mice receiving efsevin or kaempferol through an osmotic minipump are shown. After a baseline ECG was recorded, mice were injected with epi/caff as an adrenergic stimulus. Basic parameters of the ECG like PR **(A)**, QT **(B)**, and QRS **(C)** intervals were comparable between the vehicle-treated group and both of the MiCUp-treated groups at baseline and after epi/caff challenge (Kruskal-Wallis test). **(D)** Heart rate increased upon administration of epi/caff in all 3 groups but remained unaffected by the administration of MiCUps (Kruskal-Wallis test). **(E)** Examples of a normal ECG recording and a recording showing bidirectional ventricular tachycardia. **(F)** Episodes of bidirectional ventricular tachycardia were recorded in 11 of 11 vehicle-treated mice but were significantly reduced to 6 of 10 in mice with efsevin and 6 of 11 in mice with kaempferol ($p = 0.035$, Fisher's exact test). *$p < 0.05$. ECG = electrocardiography; epi/caff = epinephrine/caffeine; MiCUp = mitochondrial calcium uptake enhancer.

However, in healthy myocardium, the role of fast mitochondrial Ca^{2+} uptake remains elusive. Regarding arrhythmia, protective (22) as well as pro-arrhythmic (41) effects of activated mitochondria were discussed. We have recently identified the novel compound efsevin, which binds to the outer mitochondrial membrane VDAC2, enhances mitochondrial Ca^{2+} uptake, and restores rhythmic cardiac contractions in a zebrafish model for Ca^{2+}-induced cardiac arrhythmia (15). Here we show that enhancing mitochondrial Ca^{2+} uptake by using MiCUps efficiently suppresses arrhythmia in a murine and a human model of CPVT in vitro and in vivo. Our results establish pharmacological activation of rapid mitochondrial Ca^{2+} uptake as a novel preventive and therapeutic strategy against CPVT. We have previously shown that efsevin suppresses arrhythmia in cellular models for Ca^{2+} overload induced by high extracellular Ca^{2+} (15). Thus, due to their potent role of suppressing arrhythmogenic Ca^{2+} waves in both Ca^{2+} overload (15) and CPVT, it is conceivable that MiCUps may also be applied in other more common forms of Ca^{2+}-induced cardiac arrhythmias. These include arrhythmias in the setting of heart failure, which are triggered by cellular Ca^{2+} overload or atrial fibrillation, also linked to imbalances in cardiomyocyte Ca^{2+} handling (5). This study serves as a proof-of-principle, holding great promise for additional indications.

OPTIMIZED MiCUps. Our work shows that enhancing mitochondrial Ca^{2+} uptake efficiently reduces arrhythmia in experimental models of CPVT. In order to develop MiCUps toward human therapeutics, several steps of optimization must be taken. Candidate compounds need to be optimized to achieve a high affinity MiCUp with low side effects and suitable pharmacokinetics for application in human subjects. We show that the antiarrhythmic effect can be achieved by activation of at least 2 distinct target proteins within the mitochondrial Ca^{2+} uptake complex: efsevin, targeting VDAC2 in the outer mitochondrial membrane, and kaempferol, targeting MCU in the

FIGURE 6 **MiCUps Reduce Spontaneous Ca^{2+} Waves in Human iPSC-derived Cardiomyocytes**



Confocal line scans of iPSC-derived human cardiomyocytes from a CPVT patient ($RyR2^{S406L/WT}$) and a healthy individual are shown (control). **(A)** Confocal line scans across iPSC-derived human cardiomyocytes loaded with Fluo-4 AM to measure intracellular Ca^{2+} and intensity plots show that stimulation with ISO does not induce spontaneous, diastolic Ca^{2+} waves in control cells but induced waves in $RyR2^{S406L/WT}$ cardiomyocytes. These waves can be blocked with either 15 μM efsevin **(upper right panel)** or 10 μM kaempferol **(lower right panel)**. **(B)** Quantitative analysis revealed a significant reduction in the number of $RyR2^{S406L/WT}$ cells showing ISO-induced Ca^{2+} waves upon treatment with MiCUps, to a level comparable to that of unstimulated cells (**asterisks** denote significance compared to $RyR2^{S406L/WT}$ +ISO by Fisher's exact test). **(C)** Quantification of waves/min reveal a significant reduction from 3.78 ± 0.72 waves/min in $RyR2^{S406L/WT}$ cardiomyocytes treated with ISO to levels indistinguishable from control after treatment with efsevin (0.15 ± 0.15; $p < 0.001$ compared to ISO: $p = 0.884$ compared to vehicle, Kruskal-Wallis test) or kaempferol, respectively (0.21 ± 0.14, $p < 0.001$ compared to ISO; $p = 0.980$ compared to vehicle, Kruskal-Wallis test). ***$p < 0.001$. CPVT = catecholaminergic polymorphic ventricular tachycardia; iPSC = induced pluripotent stem cell; other abbreviations as in Figure 1.

inner membrane. Suppression of arrhythmia is thus attributable to enhanced mitochondrial Ca^{2+} uptake and is independent of the molecular target protein within the fast mitochondrial Ca^{2+} uptake complex. Our work thus establishes the entire protein complex as a pharmacological target structure and allows for future optimization of this therapeutic concept through novel compounds and targets. Apart from VDAC2 and MCU, the auxiliary MCU regulators MICU1 and -2, MCUb, EMRE, and MCUR1 (16,17) may serve as future candidate targets.

SIDE EFFECTS. Mitochondrial Ca^{2+} uptake proteins such as VDAC2 and MCU are ubiquitously expressed. Regarding a therapeutic application, it is thus important to evaluate potential side effects of a MiCUp-based therapy. It is important to note that we did not observe any adverse effects of MiCUps in mice treated with efsevin or kaempferol for 3 to 8 consecutive days. Furthermore, kaempferol was previously used in animal experiments, and no adverse effects, even after up to 1 year of treatment or at high doses, were observed (42-44). However, further long-term experiments and large animal studies are needed to further evaluate safety of a MiCUp-based therapy. Although we did not observe changes in cytosolic Ca^{2+} in our experiments, long-term effects of enhanced mitochondrial Ca^{2+} uptake on a potential redistribution of cellular Ca^{2+} in the heart and other organs must be evaluated. Furthermore, because an enhanced mitochondrial Ca^{2+} uptake was observed to activate mitochondrial metabolism and reactive oxygen species production, special focus should be directed toward side effects related to changes in cellular bioenergetics.

Common side effects of actual antiarrhythmic drugs like Na^{+}, K^{+}, and Ca^{2+} channel blockers include

changes in cardiac electrophysiology like deceleration of cardiac de- or repolarization, the latter often expressed as a prolonged QT interval. We observed an effect of efsevin on the repolarization phase of the action potential in mice (Supplemental Figure 1), namely a prolongation of APD50 but not APD90. However, whereas repolarization in mice is carried mainly by fast potassium currents ($I_{to,}$ $I_{K,\ slow}$), the human action potential displays a pronounced plateau phase, and phase 3 repolarization is carried predominantly by the delayed K^+ currents I_{Kr} and I_{ks} (32). To rule out the possibility that the observed prolongation of APD50 by efsevin in mice could be relevant for human therapy, we showed that efsevin does not influence action potential duration in human iPSC-derived cardiomyocytes and does not block hERG activity. It is thus conceivable that efsevin has a direct impact on the fast repolarizing currents in mice but does not influence the human action potential. Most importantly, however, we did not observe effects of MiCUp administration on ECG parameters like PR, QT, and QRS interval and heart rate in mice treated with MiCUps. Because MiCUps target intracellular structures to suppress the generation of ectopic depolarizations and do not influence the cardiac action potential, they might be less prone to severe side effects like, for example, the typical pro-arrhythmic effects observed with class I or III antiarrhythmic drugs. However, the murine repertoire of ion channels governing the cardiac action potential of mice varies from the human one, and additional experiments in other mammalian species are needed to solve this issue.

CONCLUSIONS

Common antiarrhythmic drugs aim at inhibiting expansion of ectopic activity and display perilous side effects. Because major arrhythmias are often associated with imbalanced intracellular Ca^{2+} homeostasis (3–5), intracellular Ca^{2+} transporters are attractive candidates for newer and safer therapies. Here we show that enhancing mitochondrial Ca^{2+} uptake by pharmacological agonists of the mitochondrial Ca^{2+} uptake proteins VDAC2 and MCU efficiently suppresses arrhythmia in a murine and a human model for CPVT. Our data establish MiCUps as attractive compounds for a novel preventive and therapeutic strategy to treat Ca^{2+}-triggered cardiac arrhythmias.

ACKNOWLEDGEMENTS The authors thank Brigitte Mayerhofer for technical assistance and Petra Eigner, Monika Mittermeier, and Clarinda Hofer and the staff of the Institute for Biomedical Research of the Medical University of Graz for animal husbandry.

ADDRESS FOR CORRESPONDENCE: Dr. Johann Schredelseker, Walther Straub Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München Nußbaumstrasse 26, D-80336 München, Germany. E-mail: johann.schredelseker@lmu.de.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Mitochondria regulate cardiac rhythmicity. Pharmacological activation of mitochondrial Ca^{2+} uptake by MiCUps suppresses arrhythmogenesis in murine and human iPSC-based models for catecholaminergic polymorphic ventricular tachycardia.

TRANSLATIONAL OUTLOOK 1: Optimization of compounds and a careful investigation on pharmacokinetics and drug metabolism are needed to develop a potential MiCUp-based human therapy.

TRANSLATIONAL OUTLOOK 2: Additional experiments using models of other Ca^{2+}-triggered arrhythmias could further expand the application range of MiCUps.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics-2016 update. Circulation 2016;133:e38–360.

2. Mehra R. Global public health problem of sudden cardiac death. J Electrocardiol 2007;40:S118–22.

3. Bers DM. Calcium and cardiac rhythms: physiological and pathophysiological. Circ Res 2002;90:14–7.

4. Choi B-R, Burton F, Salama G. Cytosolic Ca^{2+} triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. J Physiol 2002;543:615–31.

5. Greiser M, Lederer WJ, Schotten U. Alterations of atrial Ca^{2+} handling as cause and consequence of atrial fibrillation. Cardiovasc Res 2011;89:722–33.

6. Barth E, Stämmler G, Speiser B, Schaper J. Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. J Mol Cell Cardiol 1992;24:669–81.

7. Kim H-D, Kim CH, Rah Bb-J, Chung H-I, Shim T-S. Quantitative study on the relation between structural and functional properties of the hearts from three different mammals. Anat Rec 1994;238:199–206.

8. Szalai G, Csordás G, Hantash BM, Thomas AP, Hajnóczky G. Calcium signal transmission between ryanodine receptors and mitochondria. J Biol Chem 2000;275:15305–13.

9. Dorn GW, Maack C. SR and mitochondria: calcium cross-talk between kissing cousins. J Mol Cell Cardiol 2013;55:42–9.

10. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. Nature 2004;427:360–4.

11. Baughman JM, Perocchi F, Girgis HS, et al. Integrative genomics identifies MCU

as an essential component of the mitochondrial calcium uniporter. Nature 2011;476:341–5.

12. De Stefani D, Raffaello A, Teardo E, Szabò I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 2011;476:336–40.

13. Subedi KP, Kim J-C, Kang M, Son M-J, Kim Y-S, Woo S-H. Voltage-dependent anion channel 2 modulates resting Ca^{2+} sparks, but not action potential-induced Ca^{2+} signaling in cardiac myocytes. Cell Calcium 2011;49:136–43.

14. Min CK, Yeom DR, Lee K-E, et al. Coupling of ryanodine receptor 2 and voltage-dependent anion channel 2 is essential for Ca^{2+} transfer from the sarcoplasmic reticulum to the mitochondria in the heart. Biochem J 2012;447:371–9.

15. Shimizu H, Schredelseker J, Huang J, et al. Mitochondrial Ca^{2+} uptake by the voltage-dependent anion channel 2 regulates cardiac rhythmicity. Elife 2015;4.

16. Jhun BS, Mishra J, Monaco S, et al. The mitochondrial Ca^{2+} uniporter: regulation by auxiliary subunits and signal transduction pathways. Am J Physiol Cell Physiol 2016;311:C67–80.

17. Marchi S, Pinton P. The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. J Physiol 2014;592:829–39.

18. Brandes R, Bers DM. Intracellular Ca^{2+} increases the mitochondrial NADH concentration during elevated work in intact cardiac muscle. Circ Res 1997;80:82–7.

19. Williams GSB, Boyman L, Chikando AC, Khairallah RJ, Lederer WJ. Mitochondrial calcium uptake. Proc Natl Acad Sci U S A 2013;110:10479–86.

20. O'Rourke B, Blatter LA. Mitochondrial Ca^{2+} uptake: tortoise or hare? J Mol Cell Cardiol 2009;46:767–74.

21. Seguchi H, Ritter M, Shizukuishi M, et al. Propagation of Ca^{2+} release in cardiac myocytes: role of mitochondria. Cell Calcium 2005;38:1–9.

22. Zhao Z, Gordan R, Wen H, Fefelova N, Zang W-J, Xie L-H. Modulation of intracellular calcium waves and triggered activities by mitochondrial ca flux in mouse cardiomyocytes. PLoS One 2013;8:e80574.

23. Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 2001;103:196–200.

24. Cerrone M, Colombi B, Santoro M, et al. Bidirectional ventricular tachycardia and fibrillation elicited in a knock-in mouse model carrier of a mutation in the cardiac ryanodine receptor. Circ Res 2005;96:e77–82.

25. O'Connell TD, Rodrigo MC, Simpson PC. Isolation and culture of adult mouse cardiac myocytes. Methods Mol Biol 2007;357:271–96.

26. Jung CB, Moretti A, Mederos y Schnitzler M, et al. Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. EMBO Mol Med 2012;4:180–91.

27. Moretti A, Bellin M, Welling A, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 2010;363:1397–409.

28. Claycomb WC, Lanson NA, Stallworth BS, et al. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc Natl Acad Sci U S A 1998;95:2979–84.

29. Sharma VK, Ramesh V, Franzini-Armstrong C, Sheu SS. Transport of Ca^{2+} from sarcoplasmic reticulum to mitochondria in rat ventricular myocytes. J Bioenerg Biomembr 2000;32:97–104.

30. Paavola J, Viitasalo M, Laitinen-Forsblom PJ, et al. Mutant ryanodine receptors in catecholaminergic polymorphic ventricular tachycardia generate delayed afterdepolarizations due to increased propensity to Ca^{2+} waves. Eur Heart J 2007;28:1135–42.

31. Allen DG, Eisner DA, Orchard CH. Characterization of oscillations of intracellular calcium concentration in ferret ventricular muscle. J Physiol 1984;352:113–28.

32. Nerbonne JM, Nichols CG, Schwarz TL, Escande D. Genetic manipulation of cardiac K^+ channel function in mice: what have we learned, and where do we go from here? Circ Res 2001;89:944–56.

33. Montero M, Lobatón CD, Hernández-Sanmiguel E, et al. Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids. Biochem J 2004;384:19–24.

34. Vay L, Hernández-Sanmiguel E, Santo-Domingo J, et al. Modulation of Ca^{2+} release and Ca^{2+} oscillations in HeLa cells and fibroblasts by mitochondrial Ca^{2+} uniporter stimulation. J Physiol 2007;580:39–49.

35. Jiang D, Xiao B, Zhang L, Chen SRW. Enhanced basal activity of a cardiac Ca^{2+} release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. Circ Res 2002;91:218–25.

36. Fernández-Velasco M, Rueda A, Rizzi N, et al. Increased Ca^{2+} sensitivity of the ryanodine receptor mutant $RyR2^{R4496C}$ underlies catecholaminergic polymorphic ventricular tachycardia. Circ Res 2009;104:201–9.

37. Sedej S, Heinzel FR, Walther S, et al. Na^+-dependent SR Ca^{2+} overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. Cardiovasc Res 2010;87:50–9.

38. Tomar D, Dong Z, Shanmughapriya S, et al. MCUR1 Is a scaffold factor for the MCU complex function and promotes mitochondrial bioenergetics. Cell Rep 2016;15:1673–85.

39. Drago I, De Stefani D, Rizzuto R, Pozzan T. Mitochondrial Ca^{2+} uptake contributes to buffering cytoplasmic Ca^{2+} peaks in cardiomyocytes. Proc Natl Acad Sci U S A 2012;109:12986–91.

40. García-Rivas G, de J, Carvajal K, Correa F, Zazueta C. Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic functional recovery in rats in vivo. Br J Pharmacol 2006;149:829–37.

41. Xie W, Santulli G, Reiken SR, et al. Mitochondrial oxidative stress promotes atrial fibrillation. Sci Rep 2015;5:11427.

42. Song H, Bao J, Wei Y, et al. Kaempferol inhibits gastric cancer tumor growth: An in vitro and in vivo study. Oncol Rep 2014;33:868–74.

43. Montero M, de la Fuente S, Fonteriz RI, Moreno A, Alvarez J. Effects of long-term feeding of the polyphenols resveratrol and kaempferol in obese mice. PLoS One 2014;9:e112825.

44. Shih T-Y, Young T-H, Lee H-S, Hsieh C-B, Hu OY-P. Protective effects of kaempferol on isoniazid- and rifampicin-induced hepatotoxicity. AAPS J 2013;15:753–62.

KEY WORDS CPVT, MCU, MiCUp, mitochondria, RyR2, VDAC2

APPENDIX For expanded Methods section as well as supplemental figures, please see the online version of this article.