ARTICLE ADDENDUM

Ca$^{2+}$-activated chloride channel activity during Ca$^{2+}$ alternans in ventricular myocytes

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ABSTRACT

Cardiac alternans, defined beat-to-beat alternations in contraction, action potential (AP) morphology or cytosolic Ca transient (CaT) amplitude, is a high risk indicator for cardiac arrhythmias. We investigated mechanisms of cardiac alternans in single rabbit ventricular myocytes. CaTs were monitored simultaneously with membrane currents or APs recorded with the patch clamp technique. A strong correlation between beat-to-beat alternations of AP morphology and CaT alternans was observed. During CaT alternans application of voltage clamp protocols in form of pre-recorded APs revealed a prominent Ca$^{2+}$-dependent membrane current consisting of a large outward component coinciding with AP phases 1 and 2, followed by an inward current during AP repolarization. Approximately 85% of the initial outward current was blocked by Cl$^{-}$ channel blocker DIDS or lowering external Cl$^{-}$ concentration identifying it as a Ca$^{2+}$-activated Cl$^{-}$ current (ICaCC). The data suggest that ICaCC plays a critical role in shaping beat-to-beat alternations in AP morphology during alternans.

KEYWORDS

action potential; alternans; arrhythmias; calcium; calcium-activated chloride channels; excitation-contraction coupling; heart

Introduction

Cardiac alternans is a recognized risk factor for cardiac arrhythmia$^{1-3}$ and sudden cardiac death.$^{4,5}$ T-wave alternans in the electrocardiogram, corresponding to beat-to-beat alternations in ventricular repolarization, has become a prognostic tool for arrhythmia risk stratification and guidance of antiarrhythmic therapy.$^{6,7}$ At the cellular level cardiac alternans is defined as beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential (AP) duration (APD or electrical alternans) and cytosolic Ca$^{2+}$ transient (CaT) amplitude (Ca$^{2+}$ alternans) at constant stimulation frequency. In cardiac myocytes the beat-to-beat regulation of cytosolic calcium ([Ca$^{2+}$])$_i$ and membrane potential ($V_m$) is bi-directionally coupled and involves complex feedback mechanisms, often mediated by Ca$^{2+}$-dependent membrane conductances that link these 2 parameters. Among Ca$^{2+}$-sensitive channels and transporters Na$^+$/Ca$^{2+}$ exchange (NCX),$^{8,9}$ voltage-gated L-type Ca$^{2+}$ (LCC),$^{8,10}$ Ca$^{2+}$-activated Cl$^{-}$ (CaCCs),$^{11,12}$ small conductance K$^+$ and nonselective cation channels$^{14}$ have been proposed to take part in the development of alternans, however the picture of the specific contribution of any of these conductances to the development of repolarization alternans has remained sketchy. Recently we have demonstrated that CaCCs play a significant role in the development of AP alternans in atria.$^{12}$ This study uses a similar approach to investigate CaT alternans induced beat-to-beat alternations in membrane currents in ventricular tissue.

In summary, our data reveal that during Ca$^{2+}$ alternans complex alternating time-dependent changes in membrane currents occur and both outward and inward current components are observed. Inhibition of ICaCC significantly reduced beat-to-beat alternation in membrane current suggesting a major role of these channels for the generation of APD alternans in rabbit ventricular myocytes.
Methods

Myocyte isolation

Ventricular myocytes were isolated from male New Zealand White rabbits (Harlan Laboratories, Indianapolis, IN, USA). Rabbits were anaesthetized with an intravenous injection of sodium pentobarbital (100 mg/kg) and heparin (1000 UI/kg). Hearts were excised, mounted on a Langendorff apparatus and retrogradely perfused via the aorta. After an initial 5 min perfusion with oxygenated Ca2+-free Tyrode solution (in mM): 140 NaCl, 4 KCl, 2 CaCl2, 1 MgCl2, 10 2,3-Butanedione monoxime (BDM) 1000 UI/l heparin; pH 7.4 with NaOH), the heart was perfused with minimal essential medium Eagle (MEM) solution containing 20 μM Ca2+ and 22.5 μg/ml Liberase TH (Roche Diagnostic Corporation, Indianapolis, IN, USA) for ~20 min at 37°C. The left ventricle was dissected from the heart and minced, filtered and washed in a MEM solution containing 50 μM Ca2+ and 10 mg/ml bovine serum albumin. Isolated cells were washed and kept in MEM solution with 50 μM Ca2+ at room temperature (20–24°C) and were used within 1–8 h after isolation. All animal procedures and protocols were approved by the Institutional Animal Care and Use Committee of Rush University Chicago, and comply with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Solutions and experimental conditions

The standard external Tyrode solution was composed of (in mM): 135 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 Hepes, 10 D-Glucose, 5 Hepes, 10 D-glucose; pH 7.4 with NaOH. All chemicals and reagents were from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. Experiments were performed at room temperature (20–24°C).

Electrophysiological measurements

Electrophysiological signals were recorded from single myocytes in the whole-cell ruptured patch clamp configuration using an Axopatch 200A patch clamp amplifier, the Axon Digidata 1440A interface and pCLAMP 10.2 software (Molecular Devices, Sunnyvale, CA). Current and AP recordings were low-pass filtered at 5 kHz and digitized at 10 kHz.

For AP measurements patch pipettes (1.5–3 MΩ filled with internal solution) were pulled from borosilicate glass capillaries (WPI, Sarasota, FL, USA) with a horizontal puller (P-97; Sutter Instruments, Novato, CA, USA) and filled with internal solution containing (in mM): 130 K+ glutamate, 10 NaCl, 10 KCl, 0.33 MgCl2, 4 MgATP, and 10 Hepes with pH adjusted to 7.3 with KOH. For simultaneous [Ca2+]i measurements 75 μM Fluo-4 pentapotassium salt was added to the internal solution. Internal solutions were filtered through 0.22 μm pore filters. For AP measurements the whole-cell ‘fast’ current clamp mode of the Axopatch 200A was used and APs were evoked by 5 ms stimulation pulses of a magnitude ~1.5–2 times higher than AP activation threshold. Vm measurements were corrected for a junction potential error of ~10 mV. APD was analyzed at 30, 50 and 90% repolarization levels (APD30, APD50 and APD90).

For AP-clamp experiments a voltage command in form of pre-recorded ventricular APs was used. Stimulation frequency was modified by changing diastolic intervals between stimulations (for frequencies <1.3 Hz) or by proportionally changing AP duration and diastolic interval at rates >1.3 Hz. To induce CaT alternans under AP-clamp conditions the stimulation frequency was incrementally increased until alternans developed despite the fact that the voltage command was unchanged from beat to beat. Simultaneously with CaT alternans alternations in membrane current were recorded. The fraction of the membrane current (I_m) that alternated during CaT alternans is referred to here as Ca2+-dependent differential current or Idiff. Idiff is derived as I_{CaT,Large} - I_{CaT,Small}, where I_{CaT,Large} is I_m recorded coinciding with a large amplitude CaT and I_{CaT,Small} is the I_m recorded during the small amplitude CaT during alternans.

For the assessment of the role of I_{CaCC} 2 alternative methods of suppression of Cl− currents were used: 1) application of the Cl− channel blocker 4,4’-Diisothio-cyanatostilbene-2,2’-disulfonic acid (DIDS; Sigma-Aldrich, St. Louis, MO, USA) and 2) low Cl− solution where [Cl−] in the external Tyrode solution was lowered to 20 mM by replacing Cl− with glutamate−. An agar salt bridge (1 M KCl) was used to minimize potential shifts during bath solution changes. In experiments investigating the contribution of K+ conductances, K+ currents were blocked by replacing all K+ ions with Cs+ in both extracellular and intracellular solutions.

Cytosolic [Ca2+]i measurements

[Ca2+]i was monitored simultaneously with APs or I_m. For [Ca2+]i measurements cells were loaded
with the fluorescent probe Fluo-4 pentapotassium salt (75 μM) via the patch pipette (Life Technologies, Grand Island, NY). Fluo-4 fluorescence was excited at 485 nm with a Xe arc lamp and [Ca\(^{2+}\)]\(_i\)-dependent Fluo-4 signals were collected at 515 nm using a photomultiplier tube. Background-subtracted fluorescence emission signals (F) were normalized to resting fluorescence (F\(_0\)) recorded under steady-state conditions at the beginning of an experiment, and changes of [Ca\(^{2+}\)]\(_i\) are presented as changes of F/F\(_0\). Data recording and digitization were achieved using the Axon Digidata 1440A interface and pCLAMP 10.2 software. Fluorescence signals were low-pass filtered at 50 Hz.

Ca\(^{2+}\) alternans was induced by incrementally increasing the pacing frequency until stable alternans was observed. The degree of Ca\(^{2+}\) increasing the pacing frequency until stable alternans and CaT alternans, AP and [Ca\(^{2+}\)]\(_i\) dynamics were performed in voltage-clamped ventricular myocytes. For AP-clamp experiments a voltage stimulus in form of a presynaptic stimulation pulse and at the peak of the CaT. The amplitude of a CaT was measured as the difference in F/F\(_0\) measured immediately before the stimulation pulse and at the peak of the CaT.

### Data analysis and presentation

Results are presented as individual observations or as mean ± SEM, and n represents the number of individual cells. Statistical significance was evaluated using unpaired Student’s t-test and differences were considered significant at p<0.05.

### Results

#### CaT and AP alternans are correlated

To investigate the interplay between electrical (AP) and CaT alternans, AP and [Ca\(^{2+}\)]\(_i\) dynamics were monitored simultaneously in current-clamped rabbit ventricular myocytes. An increase in pacing frequency led to the concurrent onset of beat-to-beat alternations in both CaT amplitude and AP morphology. Figure 1 shows a representative example of simultaneous recordings of APs and CaTs obtained from the same single ventricular myocyte paced at 0.5 Hz and 1 Hz. At 0.5 Hz alternans was absent (Fig. 1Aa), whereas at 1 Hz beat-to-beat alternans of both CaT amplitude and AP shape was observed (Fig. 1Ba). APs of 2 consecutive beats in cells without CaT alternans (mean AR = 0.03 ± 0.01) were essentially identical (Fig. 1Ab), thus the mean APD ratio was ~1 at all repolarization levels analyzed (n = 7; Fig. 1Ac), indicating that there is no apparent beat-to-beat variation in APD. However when CaT alternans was elicited a significant alternation in beat-to-beat AP morphology was observed (Fig. 1Bb). Summary data obtained from ventricular myocytes with CaT AR > 0.5 (mean AR = 0.77 ± 0.04, n = 6) showed APD\(_{\text{CaT_Small}}\)/APD\(_{\text{CaT_Large}}\) ratios of 1.40 ± 0.09, 1.02 ± 0.04 and 0.88 ± 0.03 for 30, 50 and 90% AP repolarization, respectively (n = 6; Fig. 1Bc).

### Effect of CaT alternans on net membrane current

We\(^{12,15}\) and others\(^{16}\) have previously demonstrated that beat-to-beat alternations in AP morphology is not obligatory for CaT alternans to occur and CaT alternans can be observed in voltage-clamped myocytes where V\(_m\) is kept constant on every beat. These observations suggest that alternation in AP morphology might result from beat-to-beat differences in intracellular Ca\(^{2+}\) release, a notion that is well accepted in the field.\(^{17-20}\) To determine how membrane currents are affected by beat-to-beat alternations of [Ca\(^{2+}\)]\(_i\), simultaneous measurements of [Ca\(^{2+}\)]\(_i\) and I\(_m\) were performed in voltage-clamped ventricular myocytes. For AP-clamp experiments a voltage stimulus in form of a pre-recorded ventricular AP was applied at various stimulation rates. In cells without CaT alternans the I\(_m\) profile remained the same on every beat (Fig. 2A). At higher pacing rates, however CaT alternans was induced and simultaneously beat-to-beat alternations in I\(_m\) were observed (Fig. 2B). Since the membrane voltage was fixed and AP shapes remained constant during the course of the entire experiment the only parameter that changed on a beat-to-beat basis was [Ca\(^{2+}\)]\(_i\). Consequently the observed alternations of I\(_m\) could not be attributed to beat-to-beat differences in AP morphology and it was assumed that changes in I\(_m\) were primarily driven by beat-to-beat differences of [Ca\(^{2+}\)]\(_i\).\(^{12}\)
The difference between $I_m$ recorded simultaneously with large and small CaTs during alternans was defined as differential current $I_{\text{diff}}$. $I_{\text{diff}}$ exhibited 2 characteristic peaks consisting of a large outward current component ($I_{\text{outward}}$), followed by a transient small amplitude inward current ($I_{\text{inward}}$) (Fig. 2C). The overlay of $I_{\text{diff}}$ and the AP-clamp voltage command (Fig. 2C) demonstrates that the outward current peak occurred around APD30 and is consistent with above observation that beat-to-beat changes in APD during CaT alternans was largest at the APD30 level compared to APD50 and APD90. The peak amplitude of $I_{\text{outward}}$, normalized to cell membrane capacitance, ranged from 0.7 to 3.7 pA/pF with a mean current density of $1.69 \pm 0.24$ pA/pF (n=18; Fig. 2D). The mean amplitude of peak $I_{\text{inward}}$ was $-0.20 \pm 0.02$ pA/pF (Fig. 2D; range: $-0.06$ to $-0.37$ pA/pF).

**Ca$^{2+}$-activated chloride channels sustain $I_{\text{diff}}$ in ventricular myocytes**

Recently we established that Ca$^{2+}$-activated Cl$^{-}$ currents play a decisive role in the generation of APD alternans in rabbit atrial myocytes. Therefore we applied the same experimental approach to ventricular myocytes to identify the ionic nature of $I_{\text{diff}}$. Pilot experiments demonstrated that suppression of K$^{+}$ currents by replacing intra- and extracellular K$^{+}$ with Cs$^{+}$ had no significant effect on $I_{\text{diff}}$ parameters (data not shown) and therefore indicating that K$^{+}$ currents play only a minor role (if any) in sustaining $I_{\text{diff}}$. Next we suppressed Cl$^{-}$ currents with the Cl$^{-}$ channel inhibitor DIDS (500 $\mu$M) or by exchanging the normal bath solution (containing 147 mM Cl$^{-}$) with a low Cl$^{-}$ solution (20 mM Cl$^{-}$) to determine a potential contribution of $I_{\text{CaCC}}$ to $I_{\text{diff}}$ (Fig. 3). Both methods
of Cl− current suppression had significant effects on $I_{\text{diff}}$ consisting of a pronounced inhibition of the outward component with a much lesser effect on the inward current (Fig. 3D). In the presence of 500 µM DIDS peak amplitude of $I_{\text{outward}}$ decreased by ~85% to $0.24 \pm 0.06$ pA/pF ($n = 5$, mean CaT AR = $0.76 \pm 0.03$), i.e. significantly smaller than in control ($1.69 \pm 0.24$ pA/pF; $p < 0.001$). A similar effect was observed when myocytes were exposed to the low Cl− solution. In this case peak amplitude of $I_{\text{outward}}$ decreased to $0.21 \pm 0.09$ pA/pF ($n = 5$, $p < 0.001$, mean CaT AR = $0.86 \pm 0.03$). Inhibition of $I_{\text{CaCC}}$ had no apparent effect on intracellular Ca2+ signaling in AP voltage-clamped myocytes and no significant difference in CaT AR in control, DIDS and low Cl− was observed. Figure 3B,C also shows that after Cl− current suppression the remaining $I_{\text{diff}}$ still exhibited an outward as well as an inward component, which is most likely sustained by other Ca2+-regulated ion currents ($I_{\text{LCC}}, I_{\text{NCX}}$), or reflects an incomplete inhibition of $I_{\text{CaCC}}$. Nonetheless, the data clearly show that $I_{\text{diff}}$ in rabbit ventricle is largely carried by $I_{\text{CaCC}}$.

**Discussion**

This study investigated the mechanisms how beat-to-beat alternation in intracellular Ca2+ release translates into AP repolarization alternans in ventricular myocytes. We demonstrated that CaT and APD alternans occurred simultaneously and CaT alternans led to a beat-to-beat alternation in membrane current even under conditions where the AP shape was kept constant on every beat. Pharmacological inhibition of Cl− channels with DIDS or low extracellular [Cl−]
demonstrated that the observed beat-to-beat alternations of Im could be attributed largely to CaCC activity, however CaCC inhibition had no effect on the magnitude and kinetics of CaTs and the degree of CaT alternans.

Interaction between \([\text{Ca}^{2+}]_i\) and Vm plays a key role in the development of cardiac alternans

Electrical (APD), mechanical and \(\text{Ca}^{2+}\) alternans are temporally and spatially correlated.\(^{12,15,21-23}\) It is well established that numerous feedback mechanisms govern the bi-directional interplay between Vm and \([\text{Ca}^{2+}]_i\) in cardiac myocytes. Vm directly determines the activity of \(\text{Ca}^{2+}\) fluxes and \(\text{Ca}^{2+}\) handling mechanisms that are voltage-dependent (Vm→[Ca\(^{2+}\)]\(_i\) coupling), while on the other hand various ion channels and transporters that determine Vm are regulated by intracellular \(\text{Ca}^{2+}\) itself ([Ca\(^{2+}\)]\(_i\)→Vm coupling). It is generally agreed that bi-directional coupling between these parameters underlies the development of cardiac alternans,\(^{18,19}\) however it has remained a matter of debate which one of the 2 parameters, when its regulation becomes disturbed, is the primary cause of cardiac alternans. While computational studies have generated arguments in favor of both parameters ([Ca\(^{2+}\)]\(_i\) or Vm),\(^{9,24-26}\) experimental studies have provided growing evidence that alternans are initiated by disturbances of intracellular \(\text{Ca}^{2+}\) handling.\(^{15-17,27,28}\)

Major support for this hypothesis came from the demonstration that CaT alternans can be elicited in voltage-clamped ventricular myocytes even when beat-to-beat changes of Vm were kept constant.\(^{15,16}\) Recently we demonstrated that the same principle also applies to atrial myocytes.\(^{12,15}\) The current study is consistent with these previously reported observations by showing that pronounced CaT alternans was observed despite the fact that Vm was clamped with a command voltage in form of a ventricular AP-waveform that was constant from beat-to-beat (Figs. 2 and 3), i.e., in the absence of APD alternans. These results demonstrate that the strong correlative link between CaT and AP morphology (Fig. 1) can be broken and provide strong evidence that instabilities of \(\text{Ca}^{2+}\)
handling can act as the primary cause for alternans. In addition, it was shown that suppression of intracellular Ca\(^{2+}\) release could abolish AP alternans\(^{15,17}\) providing further support for the notion that the primary cause of cardiac alternans resides in a disturbance of intracellular Ca\(^{2+}\) signaling. SR Ca\(^{2+}\) load,\(^{27,29-31}\) refractoriness of SR Ca\(^{2+}\) release\(^{25,28,32,33}\) and the Ca\(^{2+}\)-dependent modulation of LCCs and NCX that in turn affects AP morphology\(^{8,18,34,35}\) were proposed as underlying mechanisms. While activity of LCCs and NCX affect both [Ca\(^{2+}\)]\(_i\) and V\(_m\), other membrane currents such as Ca\(^{2+}\)-activated K\(^+\) channels,\(^{13,36,37}\) members of the non-selective transient receptor potential melastatin channel family (e.g. TRPM4,\(^{38,39}\) TRPM5\(^{40}\)) and CaCCs,\(^{11,41}\) are also regulated by Ca\(^{2+}\) and thus might play a role in determining AP morphology. However the specific role of these channels in the development cardiac alternans is little investigated and remains elusive. Our data obtained from the AP-clamp experiments in cells exhibiting CaT alternans demonstrated complex changes in membrane current (defined here as I\(_{diff}\)) with a large, fast and well-defined outward current component that was followed by a significantly slower inward component of considerably smaller amplitude (Fig. 2C, D). We identified the large outward component during the early phase of the AP to be sustained by I\(_{CaCC}\) (Fig. 3). This result is strikingly similar to our previous demonstration that CaCCs play a major role for AP alternans in atrial myocytes.\(^{12}\) In atrial myocytes we have additionally demonstrated that AP alternans can be abolished by the suppression of Cl\(^{-}\) channels while CaT alternans essentially remain unchanged.\(^{12}\)

**Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the heart and their role in arrhythmias**

CaCCs are expressed in various tissues and are involved in numerous and diverse cellular functions such as Cl\(^{-}\) secretion in epithelia, regulation of smooth muscle contraction, blood pressure control or neuronal excitability.\(^{42}\) In the heart CaCCs have been described in various species including mouse,\(^{43}\) rabbit,\(^{12,41,44}\) ferret,\(^{45}\) sheep,\(^{46}\) dog\(^{47}\) and pig.\(^{48}\) During excitation activation of CaCCs together with K\(^+\) channels results in a transient outward current (I\(_{to}\)) that plays an important role in phase 1 repolarization of the AP.\(^{41,48-50}\) The complete understanding of CaCC function is hampered by the fact that the molecular nature of CaCCs in cardiac tissues has not been identified yet. Members of the bestrophin protein family and anoctamin-1 (Ano-1, also known as transmembrane member 16A, TMEM16A) have been proposed as putative candidates.\(^{51}\) Expression of at least 3 bestrophin family members (Best1, Best2 and Best3)\(^{52,53}\) and Ano-1\(^{54}\) was demonstrated in mouse heart. However, the functional significance of these 2 protein families in the heart is unclear.

Little is known about the role of CaCCs for the generation of cardiac arrhythmias. In cardiomyocytes the equilibrium potential for Cl\(^{-}\) is \(\approx -50\) mV and therefore I\(_{CaCC}\) can be both depolarizing and hyperpolarizing depending on the actual membrane potential level. During excitation, when cardiomyocytes are depolarized and [Ca\(^{2+}\)]\(_i\) is increased due to Ca\(^{2+}\)-induced Ca\(^{2+}\) release, Cl\(^{-}\) influx through CaCCs significantly contributes to I\(_{to}\) resulting in early repolarization of AP phase 1.\(^{41,48}\) However at negative potentials near the resting membrane potential I\(_{CaCC}\) would act as a depolarizing current.\(^{55}\) Furthermore at resting membrane potentials a contribution of both NCX and CaCC currents to an inward current leading to delayed afterdepolarizations (DADs) was demonstrated in canine and sheep ventricular myocytes.\(^{46,56}\) as well as in sheep Purkinje fibers.\(^{46}\) Suppression of I\(_{CaCC}\) alone prevented DADs from reaching the threshold for triggering of APs.\(^{46}\) The role of CaCCs for the generation of arrhythmias may become more relevant under pathological conditions where normal Ca\(^{2+}\) handling and homeostasis are disturbed and diastolic [Ca\(^{2+}\)]\(_i\) is elevated. In addition, a significant upregulation of the expression of Ano-1 at both molecular and functional levels during myocardial ischemia\(^{54}\) and a decrease of I\(_{CaCC}\) in heart failure\(^{57}\) have been suggested, lending further support to the notion that I\(_{CaCC}\) could play a more prominent role in cardiac disease.

Here we propose a new mechanism by which CaCCs contribute to the development of cardiac arrhythmias. It was shown that AP repolarization alternans, commonly observed preceding arrhythmic episodes, plays a major role in generation of a proarrhythmic substrate and therefore facilitates re-entry phenomena leading to sustained cardiac arrhythmias.\(^{1,4}\) For the first time the contribution of CaCCs to the development of AP alternans was suggested by Guo et al.\(^{11}\) who speculated on a possible role of I\(_{CaCC}\) for the occurrence of T-wave alternans in a left-ventricular rabbit hypertrophy
model, however in this study ICaCC was not correlated with intracellular Ca2+ dynamics. Recently we have shown that CaCCs play a major role in the APD development in atria12 and here we demonstrate that ICaCC also is involved in AP alternans in ventricle. While our data strongly support the notion that cardiac alternans is primarily driven by disturbances in Ca2+ cycling, [Ca2+]i-induced alternation in AP morphology is also expected to feed back on intracellular Ca2+ handling. Changes in Ito and associated alterations in AP morphology were shown to have profound effects on the magnitude and kinetics of ICaCC resulting in corresponding changes of sarcoplasmic reticulum (SR) Ca2+ release and contractility.58,59 In addition, reduced Ito was attributed to the risk of inherited ventricular arrhythmias and sudden cardiac death in dogs.60 Slower AP repolarization was shown to result in reduced LCC10,59,61 that leads to less efficient CaT triggering. In addition, prolongation of the AP was suggested to increase SR Ca2+ load.62,63 Therefore AP alternans might contribute to beat-to-beat variations in [Ca2+]SR, a mechanism that has been proposed to underlie CaT alternans.27,29–31 In this case AP prolongation during the small CaT would result in higher [Ca2+]SR available for the next Ca2+ release. Consequently, one could expect that beat-to-beat alternation of ICaCC would further contribute to the development of CaT alternans by 2 mechanisms: a) beat-to-beat changes in CaT triggering efficiency and b) beat-to-beat variation in SR Ca2+ load.

We demonstrate that ICaCC is a major contributor to AP repolarization alternans not only in atrial12 but likely also in ventricular cells. However ICaCC densities observed in ventricular myocytes are significantly (~3-fold) smaller than in atrial cells12 using identical methods. The expression patterns of CaCCs in cardiac tissues are unknown due to unresolved molecular identity of these channels. However, our observation concurs with a previous report of much higher ICaCC densities in atrial cells than in ventricular myocytes,41 indicating that indeed there are functional differences in CaCCs activity in ventricle and atrium. Therefore the observation that rabbit atrial myocytes exhibit a higher degree of beat-to-beat alternation in APD than ventricular myocytes15 can be at least partially explained by higher ICaCC density in atrial myocytes. The higher degree of AP alternans in atrial myocytes might contribute to the higher susceptibility of atria to arrhythmias.

Summary and conclusions

CaT alternans in ventricle are accompanied by large beat-to-beat alternations in ICaCC. Activation of ICaCC leads to the shortening of the AP (mostly around APD30 level), whereas the late small inward component induced by the activity of other Ca2+-dependent currents is likely responsible for a slight prolongation of APD90. The identification of ICaCC as a key contributor to alternans opens the possibility of specifically targeting this channel therapeutically to prevent cardiac alternans and potentially cardiac arrhythmias.

Abbreviations

- Ano-1 anoctamin-1
- AP action potential
- APD action potential duration
- APD30 30% repolarization level
- APD50 50% repolarization level
- APD90 90% repolarization level
- AR alternans ratio
- [Ca2+]i cytosolic Ca2+ concentration
- [Ca2+]SR sarcoplasmic reticulum Ca2+ concentration
- CaCC Ca2+-activated Cl− channels
- [Cl−]− Cl− concentration
- CaT Ca2+ transient
- DIDS 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid
- IcaCC Ca2+-activated Cl− current
- Idiff difference current recorded during large and small amplitude alternans CaTs
- Im membrane current
- Ito transient outward current
- LCC L-type Ca2+ channel
- NCX Na+/Ca2+ exchanger
- SR sarcoplasmic reticulum
- Vm membrane potential

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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