Action potential shortening prevents atrial calcium alternans

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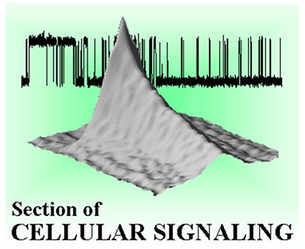

Introduction

Alternans is a risk factor for cardiac arrhythmia, including atrial fibrillation.

At the cellular level alternans manifests as beat-to-beat alternations in contraction strength, action potential duration (APD) and magnitude of the Ca transient (CaT).

Electromechanical and CaT alternans are highly correlated and interplay between membrane potential and intracellular Ca cycling plays a key role in the development and stability of cardiac alternans.

In this study we demonstrate that severity of CaT alternans is strongly affected by the shape of APs and investigate if development of alternans can be prevented by targeted modulation of membrane ion channels that determine AP morphology. The findings that CaT alternans can be controlled or even prevented by pharmacologically modulating AP morphology has important ramifications for arrhythmia prevention and anti-arrhythmic therapy strategies.

Methods

Experiments were performed on freshly isolated **rabbit atrial** cells.

Solutions: External solution (in mM): 135 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, 10 HEPES; pH 7.4 (NaOH). Patch pipette solution (in mM): 130 Kglutamate, 10 KCl, 10 NaCl, 10 HEPES, 0.33 $MgCl_2$, 4 MgATP; pH 7.3 (KOH).

Electrophysiological measurements. Action potentials were recorded in current-clamp mode (**Fig.1**). These recorded APs were used as voltage commands in AP voltage-clamp experiments.

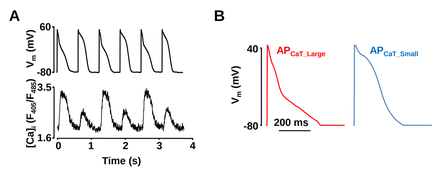

Fig.1 A: Simultaneous recordings of AP and $[Ca]_i$ alternans in current-clamped atrial myocytes. **B:** AP waveforms recorded during large (AP_{CaT_Large}) and small (AP_{CaT_Small}) alternans CaTs were used as voltage commands to pace cells during AP voltage clamp experiments. **C:** During AP-clamp experiments trains of voltage stimuli were applied as same-shape AP_{CaT_Large}, same-shape AP_{CaT_Small} and alternans AP stimulation protocols.

$[Ca]_i$ measurements. Simultaneously with electrophysiological recordings cytosolic $[Ca]_i$ was recorded using Fluo-4 or Indo-1 pentapotassium salts (100 μM) added to the pipette solution. The degree of CaT alternans was quantified as the alternans ratio (AR), defined as AR=1-CaT_{Small}/CaT_{Large}; where CaT_{Small} and CaT_{Large} are amplitudes of small and large CaTs of an alternating pair of CaTs.
For **$[Ca]_{SR}$ measurements** myocytes were loaded with the Ca probe Fluo-5N/AM entrapped in sarcoplasmic reticulum.
All single cell experiments were performed at room temperature (22-24º C).

Whole heart. Atrial electrograms were recorded by placing two electrodes on the left atria in Langendorf perfused isolated rabbit hearts. Electrical stimulation was applied to the right atria and stimulation frequency was gradually increased to induce atrial T-wave (T_a) alternans. Experiments were performed at 37º C.

Statistical significance was evaluated using unpaired and paired Student's *t*-test and differences were considered significant at $p < 0.05$. Summarized data is presented as mean ± SEM.

AP morphology determines threshold and degree of CaT alternans

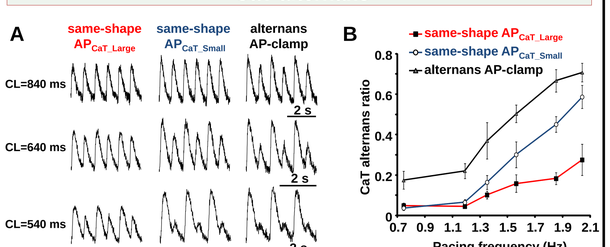

Fig.2 A: CaTs elicited with same-shape AP_{CaT_Large}, same-shape AP_{CaT_Small} and alternans AP-clamp protocols recorded from the same atrial myocyte at different pacing frequencies. **B:** Pacing with same-shape AP_{CaT_Small} waveforms (n=30) enhances the degree of CaT alternans compared to AP_{CaT_Large} stimuli (n=28). CaT alternans ratio is further increased and the pacing threshold is lowered when alternans AP protocol is applied (n=18).

AP morphology determines SR Ca load and kinetics of L-type Ca current

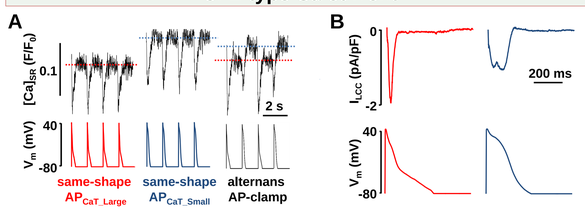

Fig.3 A: Diastolic $[Ca]_{SR}$ is elevated when myocytes are paced with same-shape AP_{CaT_Small} protocol. Alternans AP protocol results in beat-to-beat oscillations in diastolic $[Ca]_{SR}$. Traces were recorded from the same Fluo-5N/AM loaded myocyte. **B:** Representative traces of L-type Ca current (I_{LCC}) elicited with AP_{CaT_Large} and AP_{CaT_Small} voltage commands from the same atrial myocyte paced at 0.7 Hz (no CaT alternans). *Kanaporis & Blatter, JMCC 2017*

Suppression of Ca-activated chloride channels inhibits APD alternans, prolongs AP, but does not eliminate CaT alternans

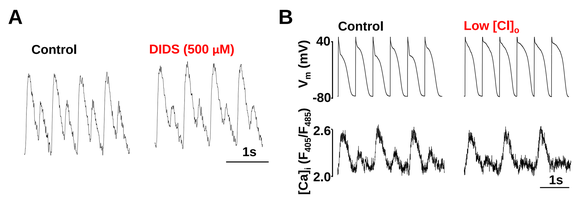

Fig.4 A: CaT alternans recorded in control and in the presence of Cl channel blocker DIDS in field stimulated atrial myocyte. **B:** AP and $[Ca]_i$ traces observed in current-clamped atrial myocytes in control and after the elimination of Ca-activated Cl currents by lowering $[Cl]_o$ to 20mM. Suppression of Cl channels leads to prolongation of AP duration and reduces beat-to-beat alternation in AP morphology, but has little effect on the degree of CaT alternans.

APD is shortened in the presence of K channel agonists NS1643 and ML277

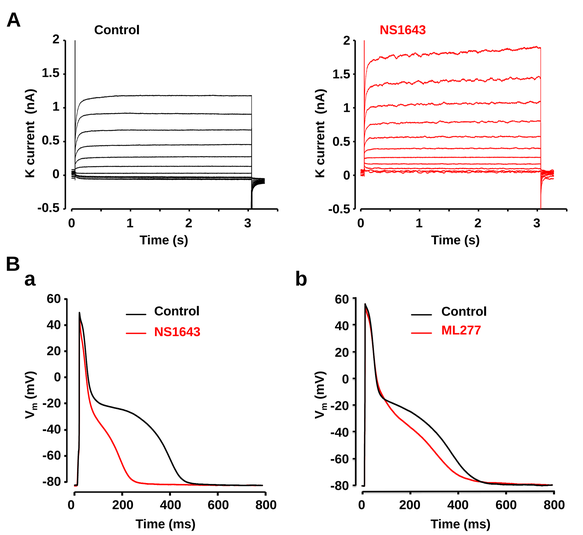

Fig.5 A: K currents recorded in control and in presence of 20 μM NS1643. **B:** Effect of NS1643 (20 μM), agonist of hERG K (K_v11.1) channels (**a**), and ML277 (500 nM), agonist of slow delayed rectifier K (K_v7.1) channels (**b**), on action potential morphology in atrial cells paced at 1Hz and exhibiting no CaT alternans.

K channel agonists reduce degree of CaT alternans in field stimulated atrial myocytes

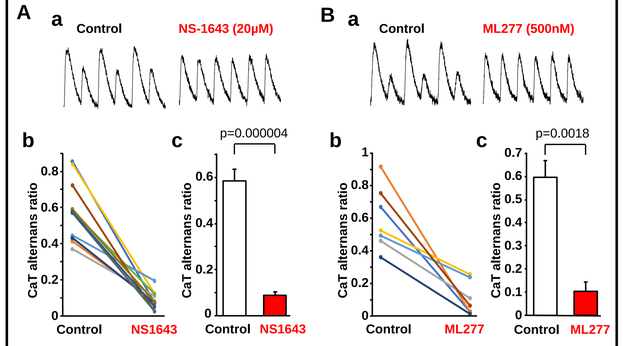

Fig.6 Activation of hERG K channels (K_v11.1) **(A)** and slow delayed rectifier K channels (K_v7.1) **(B)** reduces degree of CaT alternans in field stimulated atrial myocytes. $[Ca]_i$ traces before and after the application of K channels agonists are shown in panels **a.** Panels **b:** CaT alternans ratio in individual cells. Panels **c:** mean CaT AR in control and after application of K channels agonists.

K channels agonists shorten APD and reduce degree of CaT and APD alternans in current-clamped atrial myocytes

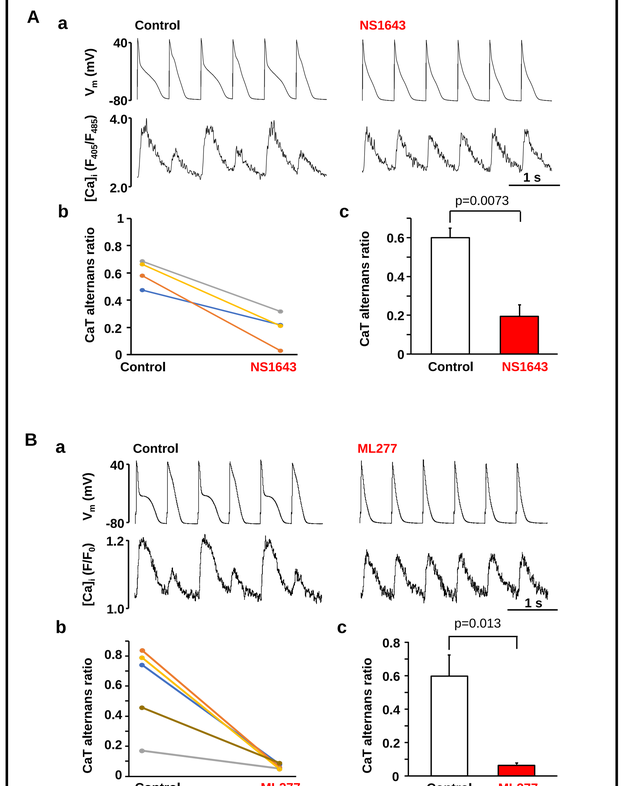

Fig.7 Effect of K channel agonists NS1643 (**A**) and ML277 (**B**) on CaT alternans in current-clamped atrial myocytes. Action potential and $[Ca]_i$ traces observed before and after application of NS1643 or ML277 are shown in panels **a**. Panels **b**: CaT alternans ratio in individual cells in control and after the application of K channels agonists. Panels **c**: mean CaT alternans ratio in control and in presence of K channel agonists

NS1643 and ML277 have no effect on CaT alternans in voltage-clamped atrial myocytes

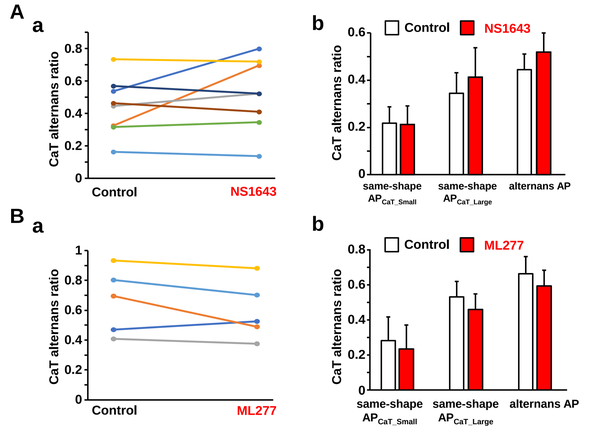

Fig.8 Application of NS1643 (**A**) and ML277 (**B**) has no effect on the degree of CaT alternans in AP voltage-clamped atrial myocytes, demonstrating that the effect of these compounds is caused by shortening of APD. Panels **a**: CaT alternans ratio before and after application of agonists of K channels observed during alternans-AP stimulation protocol. Panels **b**: mean CaT alternans ratios in the absence and presence of the drugs during same-shape AP_{CaT_Small}, same-shape AP_{CaT_Large} and alternans-AP protocol. In all groups mean CaT alternans ratios are not statistically different.

Shortening of AP suppresses atrial alternans at whole heart level

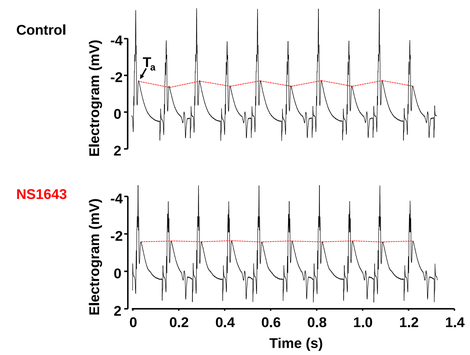

Fig.9 Application of NS1643 (20 μM) abolishes atrial T-wave (T_a) alternans in Langendorf perfused isolated rabbit hearts.

Summary

- Both disturbance of Ca signaling and AP dynamics play an important role in development of cardiac alternans
- AP morphology contributes to CaT alternans by modulating: (a) SR Ca load and (b) kinetics of L-type Ca currents.
- Occurrence and severity of CaT alternans can be modulated by pharmacological interventions targeting ion channels that shape the morphology of action potential: K channel agonists reduce APD and abolish CaT and APD alternans.

Acknowledgments

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