# New experimental evidence for mechanism of arrhythmogenic membrane potential alternans based on balance of electrogenic $I_{NCX}/I_{Ca}$ currents

Xiaoping Wan, MD, PhD,* Michael Cutler, DO, PhD,* Zhen Song, BS,† Alain Karma, PhD,† Toshio Matsuda, PhD,‡ Akemichi Baba, PhD,‡ David S. Rosenbaum, MD, FHRS*

*From the *Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio, †Physics Department and Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, Massachusetts, and ‡Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.*

**BACKGROUND** Computer simulations have predicted that the balance of various electrogenic sarcolemmal ion currents may control the amplitude and phase of beat-to-beat alternans of membrane potential ($V_m$). However, experimental evidence for the mechanism by which alternans of calcium transients produces alternation of $V_m$ ($V_m$-ALT) is lacking.

**OBJECTIVE** To provide experimental evidence that Ca-to-$V_m$ coupling during alternans is determined by the balanced influence of 2 Ca-sensitive electrogenic sarcolemmal ionic currents: $I_{NCX}$ and $I_{Ca}$.

**METHODS AND RESULTS** $V_m$-ALT and Ca-ALT were measured simultaneously from isolated guinea pig myocytes (n = 41) by using perforated patch and Indo-$1_{AM}$ fluorescence, respectively. There were 3 study groups: (1) control, (2) $I_{NCX}$ predominance created by adenoviral-induced NCX overexpression, and (3) $I_{Ca}$ predominance created by $I_{NCX}$ inhibition (SEA-0400) or enhanced $I_{Ca}$ ($As_2O_3$). During alternans, 14 of 14 control myocytes demonstrated positive Ca-to-$V_m$ coupling, consistent with $I_{NCX}$, but not $I_{Ca}$, as the major electrogenic current in modulating action potential duration. Positive Ca-to-$V_m$ coupling was maintained during $I_{NCX}$ predominance in 8 of 8 experiments with concurrent increase in Ca-to-$V_m$ gain ($P < .05$), reaffirming the role of increased forward-mode electrogenic $I_{NCX}$. Conversely, $I_{Ca}$ predominance produced negative Ca-to-$V_m$ coupling in 14 of 19 myocytes ($P < .05$) and decreased Ca-to-$V_m$ gain compared with control ($P < .05$). Furthermore, computer simulation demonstrated that Ca-to-$V_m$ coupling changes from negative to positive because of a shift from $I_{Ca}$ to $I_{NCX}$ predominance with increasing pacing rate.

**CONCLUSIONS** These data provide the first direct experimental evidence that coupling in phase and magnitude of Ca-ALT to $V_m$-ALT is strongly determined by the relative balance of the prominence of $I_{NCX}$ vs $I_{Ca}$ currents.

**KEYWORDS** Alternans; Repolarization; Action potentials; Intracellular calcium

**ABBREVIATIONS ALT** = alternation; **APD** = action potential duration

(Heart Rhythm 2012;9:1698–1705) 

## Introduction

Microvolt-level T-wave alternans is a sensitive marker of vulnerability to ventricular arrhythmias in patients.[1,2] T-wave alternans of the surface electrocardiogram arises from beat-to-beat alternation of action potential duration (APD) ($V_m$-ALT) at the single-cell level. Under this paradigm, beat-to-beat alternation of the calcium transient (Ca-ALT) causes beat-to-beat alternans in action potential shape and duration ($V_m$-ALT).[3–5] This concept was supported by our previous findings showing a close correspondence between myocytes exhibiting depressed expression or function of calcium cycling proteins and their susceptibility to $V_m$-ALT.[3] Therefore, determining the mechanism by which electrogenic ionic currents transform Ca-ALT to $V_m$-ALT is critical to understanding how cardiac alternans promotes electrophysiological heterogeneities and cardiac arrhythmias. Previously, computer simulations have predicted that the balance of various electrogenic sarcolemmal ion currents may control the amplitude and phase of beat-to-beat alternans of membrane potential.[5–8] However, to our knowledge, these theoretical predictions have not been tested experimentally. We hypothesized that Ca-to-$V_m$ coupling during alternans (ie, the relationship between alternating calcium transients and the corresponding phase and amplitude of action potential alternans) is determined by the balanced influence of 2 Ca-sensitive electrogenic sarcolemmal ionic currents: $I_{NCX}$ and $I_{Ca.}$ This hypothesis is based on established sensitivity of these currents to cytoplasmic cal-

This study was supported by a grant from the National Institutes of Health (grant no. RO1-HL54807 to Dr Rosenbaum). Zhen Song also acknowledges support of an AHA predoctoral fellowship. David S. Rosenbaum is deceased. **Address for reprint requests and correspondence:** Dr Xiaoping Wan, PhD, MD, Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Rammelkamp, 6th Fl, #643, 2500 MetroHealth Dr, Cleveland, OH 44109. E-mail address: xwan@metrohealth.org.

1547-5271/$ -see front matter  http://dx.doi.org/10.1016/j.hrthm.2012.06.031

cium concentration. During Ca-ALT, a large calcium release is expected to promote forward-mode $I_{NCX}$, hence prolonging the APD, whereas this will be opposed by calcium-induced inactivation of $I_{Ca}$, which shortens the APD. Therefore, the relative predominance of each current would determine how Ca-ALT is coupled with respect to gain and phase to $V_m$-ALT. We used complementary and selective approaches to modify $I_{NCX}$ or $I_{Ca}$ function and hence to examine Ca-to-$V_m$ coupling sign and Ca-to-$V_m$ gain under conditions of $I_{NCX}$ vs $I_{Ca}$ predominance. Our data supported the hypothesis that Ca-to-$V_m$ coupling is determined by a competing balance of $I_{NCX}$ (positive Ca-to-$V_m$ coupling) and $I_{Ca}$ (negative Ca-to-$V_m$ coupling) and demonstrated that $I_{NCX}$ is the major electrogenic mechanisms of $V_m$-ALT. These findings also have implications for disease states in which the balance of ion channel expression is altered.

## Methods

### Study design

Myocytes were divided into 3 groups to investigate the competing balanced influence of 2 Ca-sensitive electrogenic sarcolemmal ionic currents—$I_{NCX}$ and $I_{Ca}$—on Ca-to-$V_m$ coupling during alternans: (1) control, (2) $I_{NCX}$ predominance, and (3) $I_{Ca}$ predominance. $I_{NCX}$ predominance was achieved by in vivo NCX gene transfer by using a modified cross-clamping method.[9] Western blot from in vitro NCX overexpression showed that NCX protein expression was indeed increased by (3.8 ± 2.9)-fold compared with control (n = 3), and previously Ranu et al[10] demonstrated that the overexpression of NCX increases $I_{NCX,}$ with no change in $I_{Ca}$. $I_{Ca}$ predominance was achieved by $I_{NCX}$ inhibition or $I_{Ca}$ enhancement. One micrometer of the selective $I_{NCX}$ inhibitor SEA0400 (Taisho Pharmaceutical Co, Ltd, Saitama, Japan) was used to achieve 80% $I_{NCX}$ inhibition with no change in $I_{Ca}$.[11,12] $I_{Ca}$ was increased with $As_2O_3$.[13] Myocytes were studied after 4 hours of incubation with 3 $\mu$M of $As_2O_3$. The procedure increased $I_{Ca}$ density by about 100% with no significant effect on $I_{NCX}$ under our experimental condition (see supplemental data).

### $V_m$-ALT and Ca-ALT recordings

As described in the supplementary material, $V_m$-ALT and Ca-ALT were measured simultaneously from isolated guinea pig myocytes (n = 41) by using perforated patch and Indo-$1_{AM}$ fluorescence, respectively. All experiments were performed at 32°C. $V_m$-ALT amplitude was measured by calculating the ratio of the difference in $APD_{90}$ to the average $APD_{90}$ for 2 consecutive beats. Ca-ALT amplitude was measured by calculating the ratio of the difference in Ca transient amplitude to the average Ca transient amplitude for 2 consecutive beats. Ca-to-$V_m$ coupling was determined from the coincident phase of $V_m$-ALT to Ca-ALT (ie, positive vs negative Ca-to-$V_m$ coupling), and Ca-to-$V_m$ gain was calculated as the ratio of $V_m$-ALT to Ca-ALT.

### $I_{Ca}$ recordings

$I_{Ca}$ were elicited from a holding potential of 40 mV with depolarizing voltage pulses to 0 mV for 140 ms. Stimulation protocol, solutions, and temperature were the same as for V-ALT recordings.

### Computer simulations

Computer simulations were performed by using a guinea pig ventricular myocyte model that was constructed by combining mathematical formulations of selected sarcolemmal currents from Luo and Rudy[14] with a mathematical model of Ca handling from Mahajan et al[15] (see Supplementary Material for details) that produces CaT alternans. To study how the pacing rate and the balance of $I_{NCX}$ and $I_{Ca}$ influence Ca-to-$V_m$ gain, we performed simulations for the control case and $I_{Ca}$ predominance. The latter case was simulated by decreasing the maximum strength of $I_{NCX}$ by 80%. Simulations were also carried out by using the rabbit ventricular myocyte model of Mahajan et al[15] to demonstrate the robustness of electrogenic mechanisms underlying Ca-to-$V_m$ coupling in different mammalian species (shown in the Supplemental Material).

### Statistical analysis

Statistical analysis of data was performed by using Sigmastat (SPSS, Inc, Chicago, IL). Statistical differences were assessed with 1-way analysis of variance. A $P$ value of $<.05$ was considered statistically significant. Results were expressed as mean ± standard error of the mean.

## Results

### Effect of $I_{NCX}$ vs $I_{Ca}$ predominance on Ca-to-$V_m$ coupling during alternans

To examine the influence of $I_{NCX}$ and $I_{Ca}$ on Ca-to-$V_m$ coupling during alternans, action potentials (V) and calcium transients (Ca) alternans were simultaneously recorded as shown in Figure 1. In the control myocyte (top left), Ca-to-$V_m$ coupling was positive; that is, large Ca transient amplitude was coupled with a long APD, whereas small Ca transient amplitude was coupled with a short APD on subsequent beat, consistent with $I_{NCX}$, but not $I_{Ca}$, as the major electrogenic current. Consistent with this observation, with NCX overexpression (top right) positive Ca-to-$V_m$ coupling was maintained with a concurrent increase in Ca-to-$V_m$ gain; that is, the ratio of $V_m$-ALT to Ca-ALT was larger than in control myocyte (0.62 vs 0.2 in these examples), confirming increased forward-mode electrogenic $I_{NCX}$. In contrast, $I_{Ca}$ predominance induced by inhibiting $I_{NCX}$ with SEA0400 (1 $\mu$M) (bottom left) or increasing $I_{Ca}$ with $As_2O_3$ (bottom right) produced negative Ca-to-$V_m$ coupling at 240 beats/min; that is, small Ca transient amplitude was coupled with a long APD, whereas large Ca transient amplitude was coupled with a short APD on subsequent beat. The gain is small and negative (0.13 and 0.09, respectively, in these examples). These results are summarized in Table 1. All control myocytes (14 of 14) demonstrated positive Ca-to-$V_m$ coupling, consistent with $I_{NCX}$, but not $I_{Ca}$ as the major electrogenic current. Positive Ca-to-$V_m$ coupling was