Cytosolic calcium accumulation and delayed repolarization associated with ventricular arrhythmias in a guinea pig model of Andersen-Tawil syndrome

Przemysław B. Radwański, PharmD,*† Rengasayee Veeraraghavan,*‡ Steven Poelzing, PhD*‡

From the *Nora Eccles Harrison Cardiovascular Research and Training Institute, †Department of Pharmacology and Toxicology, and ‡Department of Bioengineering, University of Utah, Salt Lake City, Utah.

BACKGROUND Andersen-Tawil syndrome (ATS1)–associated ventricular arrhythmias are initiated by frequent, hypokalemia-exacerbated, triggered activity. Previous ex vivo studies in drug-induced Andersen-Tawil syndrome (DI-ATS1) models have proposed that arrhythmia propensity in DI-ATS1 derives from cytosolic Ca2+ ([Ca2+]i) accumulation leading to increased triggered activity.

OBJECTIVE The purpose of this study was to test the hypothesis that [Ca2+]i accumulation underlies arrhythmia propensity in DI-ATS1.

METHODS DI-ATS1 was induced in isolated guinea pig ventricles by perfusion of 2 mM KCl Tyrode solution containing 10 mM BaCl2. APD dispersion, underlies arrhythmia propensity during DI-ATS1.

RESULTS APD gradients under all conditions were insufficient for arrhythmia induction by programmed stimulation. However, 38% of DI-ATS1 preparations experienced ventricular tachycardias (VTs), and all preparations experienced a high incidence of premature ventricular complexes (PVCs). Pinacidil decreased APD and APD dispersion and reduced VTs (to 6%), and PVC frequency (by 79.5%). However, PVC frequency remained significantly greater relative to control (0.5% ± 0.3% of DI-ATS1). Importantly, increased arrhythmia propensity during DI-ATS1 was associated with diastolic [Ca2+]i accumulation and increased [Ca2+]i transient amplitudes. Pinacidil partially attenuated the former but did not alter the latter.

CONCLUSION The study data suggest that arrhythmias during DI-ATS1 may be a result of triggered activity secondary to prolonged APD and altered [Ca2+]i cycling and less likely dependent on large epicardial APD gradients forming the substrate for reentry. Therefore, therapies aimed at reducing [Ca2+]i, rather than APD gradients may prove effective in treatment of ATS1.

KEYWORDS Arrhythmia mechanism; Cellular calcium; Mapping; Repolarization; Ventricular arrhythmia

ABBREVIATIONS APD = action potential duration; APD50 = 50% of action potential duration; ATS1 = Anderson-Tawil syndrome; BCL = basic cycle length; Ca2+ = calcium; [Ca2+]i = cytosolic Ca2+; DI-ATS1 = drug-induced Andersen-Tawil syndrome; ECG = electrocardiogram; F405/F485 = calculated ratiometric Ca2+ signal; Ikr = inward rectifier potassium current; QTc = corrected QT; PVC = premature ventricular complex; VT = ventricular tachycardia

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Introduction

Andersen-Tawil syndrome (ATS1) is an inherited channelopathy that results from loss of function of the inward-rectifier K+ current (IKr) secondary to mutations in KCNJ2, the gene that encodes the Kir2.1 channel.1,2 ATS1 is characterized electrophysiologically by a prolonged QT interval (hence its classification as long QT syndrome type 7) and nonsustained ventricular tachycardias (VTs) that often are foreshadowed by frequent triggered activity and occur more frequently during hypokalemia.2,3 Therefore, it has been proposed that arrhythmias in ATS1 may be caused by electrical substrate remodeling,4,5 giving rise to the prolonged QT interval and increased triggered activity frequency. Although heterogeneous action potential duration (APD) prolongation and increased dispersion, both transmural and interventricular, have been reported in experimental models of ATS1,5–7 whether these gradients of repolarization are sufficient for reentry to occur remains unknown.

The high frequency and focal nature of bidirectional VTs in ATS1 suggest that triggered activity underlies, at least in part, the observed arrhythmias in ATS1.6 In general, focal arrhythmias have been linked to cytosolic Ca2+ ([Ca2+]i) accumulation.8–10 Indeed, in silico models of ATS1 support the hypothesis that [Ca2+]i accumulation underlies increased triggered activity during partial Ik1 blockade.11,12
Based on ex vivo studies in drug-induced Anderson-Tawil syndrome (DI-ATS1) models, Morita et al. and Poelzing and Veeraraghavan proposed that arrhythmia propensity in ATS1 derives from \([\text{Ca}^{2+}]_i\), accumulation leading to increased triggered activity. However, \([\text{Ca}^{2+}]_i\) accumulation has yet to be demonstrated in an experimental model of ATS1, in part due to limitations in whole-heart \([\text{Ca}^{2+}]_i\) measurement techniques.

Although the development of ratiometric (i.e., dual wavelength) fluorescent \(\text{Ca}^{2+}\) probes has helped minimize artifacts due to inhomogeneities in fluorescence and motion, whole-heart \(\text{Ca}^{2+}\) optical mapping has lacked a calibration procedure that would satisfactorily account for multiple excitation light exposures. Therefore, we designed and validated a ratiometric \(\text{Ca}^{2+}\) optical mapping system capable of simultaneous, quantitative, multisite measurements and use the system here to test the hypothesis that elevated \([\text{Ca}^{2+}]_i\) concomitant with APD prolongation, rather than APD dispersion, underlies arrhythmia propensity during DI-ATS1.

We demonstrate in guinea pig Langendorff-perfused ventricles that gradients of epicardial APD dispersion in DI-ATS1 were insufficient for arrhythmia induction by premature stimuli. However, APD prolongation was associated with increased incidence and severity of spontaneous and rapid pacing induced arrhythmias. Importantly, we demonstrate that this increased arrhythmia incidence is associated with significant diastolic \([\text{Ca}^{2+}]_i\) accumulation. Furthermore, APD abbreviation with the ATP-sensitive potassium channel opener pinacidil alleviated both diastolic \([\text{Ca}^{2+}]_i\) accumulation and the consequent increased arrhythmia burden.

### Methods

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of the University of Utah (Protocol No. 05-07002).

#### Guinea pig Langendorff preparation

Guinea pig ventricles were perfused as Langendorff preparations as previously described. In brief, adult male guinea pig breeders (weight 800–1,000 g) were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally). Their hearts were rapidly excised and the atria removed and perfused as Langendorff preparations (perfusion pressure 55 mmHg) with oxygenated (100% O\(_2\)) Tyrode solution at 36.5°C of the following composition (in mmol/L): CaCl\(_2\) 2, NaCl 140, KCl 4.5, dextrose 10, MgCl\(_2\) 1, and HEPES 10 (pH 7.41).

#### Optical voltage and \(\text{Ca}^{2+}\) mapping

Ratiometric voltage optical mapping was performed as previously described (see Supplemental Methods for more detail).

We developed an optical calcium mapping similar to that developed by Katra et al. Ratiometric \(\text{Ca}^{2+}\) transients were determined by dividing the background-subtracted fluorescence \(\text{Ca}^{2+}\) transients at 405 nm by the background-subtracted fluorescence calcium transients at 485 nm as follows:

\[
\text{Ratio} = \frac{F_{405}}{F_{485}} = \frac{\Delta F_{405} - F_{405,\text{Background}}}{\Delta F_{485} - F_{485,\text{Background}}}
\]

where \(\Delta F = \) actual change in fluorescent amplitude in the cameras after 405 or 485 bandpass filtering, subscript Background = light intensity without Indo-1 dye loading, and \(F_{405}/F_{485} = \) calculated ratiometric \(\text{Ca}^{2+}\) signal.

#### Optical action potential and \([\text{Ca}^{2+}]_i\) measurements

Motion was reduced using 7.5 mM 2,3-diacetylmonoxime. Ventricles were stimulated at 1.5 times the stimulation threshold with a unipolar silver wire placed on the basal epicardial right ventricle. Activation time was defined as the time of the maximum first derivative of the action potential as described previously. Repolarization was defined as the time to 95% repolarization from peak voltage amplitude. APD was the time difference between activation and repolarization. APD\(_{50}\) was the time difference between activation and 50% of repolarization. APD dispersion was defined as the difference between epicardial regions with the longest and shortest APD (using 25 spatially contiguous optically mapped sites per region). Relative diastolic \(\text{Ca}^{2+}\) level and \(\text{Ca}^{2+}\) transient amplitude were defined as the minimum diastolic signal before the \(\text{Ca}^{2+}\) transient upstroke and the difference between systolic and diastolic \([\text{Ca}^{2+}]_i\), values, respectively.

#### Drug-induced ATS1

ATS1 was modeled as described previously by perfusion of hypokalemic (2 mM KCl) Tyrode solution containing 10 μM BaCl\(_2\). In this report, drug-induced model of ATS1 will be referred to as DI-ATS1. Pinacidil was always perfused at 15 μM (Sigma Chemical). For most experiments, \(\text{Ca}^{2+}\) transient recordings made during control, DI-ATS1, and DI-ATS1 with pinacidil were made sequentially. In order to rule out involvement of the time-dependent component with respect to changes in \(\text{Ca}^{2+}\) transient recordings, the order of recordings in a subset of preparations was altered so that perfusion of pinacidil during DI-ATS1 directly followed control recordings.

#### Arrhythmia induction

After a 20-beat drive train was delivered to the anterior epicardial surface of the right ventricular base at basic cycle length (BCL) of 400 ms (previously demonstrated as the region with the longest APD), an epicardial premature stimulus (S2) at the left ventricular apex (region with shortest APD) was delivered through the same drive train. The S1-S2 interval was sequentially shortened by 10 ms until refractoriness was reached or an arrhythmia was induced.
Rapid pacing–induced arrhythmias were quantified at the shortest cycle length allowing for 1:1 capture. Volume-conducted electrocardiograms (ECGs) were continuously recorded in a subset of experiments in order to assess arrhythmia burden. QT intervals were corrected for changes in BCL using the formula \( QTc = QT + (1/BCL - 1)^2 \). Ventricular tachycardia (VT) was defined as a run of three or more ventricular beats with a cycle length less than 250 ms. A premature ventricular complex (PVC) was defined as any QRS complex with different morphology that occurred less than 1.5 SD of the intrinsic cycle length. Arrhythmia was defined as any type of VT or PVC. PVC frequency was defined as the number of PVCs per minute. In order to account for interanimal variability, PVC frequency for each animal was normalized to the PVC frequency during DI-ATS1 recording.

**Statistical analysis**

Statistical analysis was performed with two-tailed Student’s t-test for paired and unpaired data. Multiple regression analyses were used to characterize fluorescence Ca\(^{2+}\) signal drift both in vitro and in ex vivo preparations. Fisher exact test was used to test differences in nominal data. \( P < .05 \) was considered significant. All values are reported as mean ± SE unless otherwise noted.

**Results**

**Drug induced-ATS1**

A representative volume-conducted ECG shown in Figure 1A demonstrates QT-interval prolongation by approximately 60 ms during DI-ATS1 relative to control. Additionally, the T wave, which was monophasic under control conditions, was biphasic during DI-ATS1. Over all experiments, QTc during DI-ATS1 (286.7 ± 15.2 ms) was significantly longer relative to control (210.7 ± 5.2 ms, Figure 1B). Underlying the observed QTc prolongation during DI-ATS1 was APD prolongation illustrated by representative optical action potentials shown in Figure 2A. For all experiments, global APD and APD\(_{50}\) were prolonged during DI-ATS1 relative to control (222.5 ± 3.5 ms vs 151.3 ± 1.3 ms, Figure 2B; and 130.1 ± 5.5 ms vs 99.8 ± 6.1 ms, Figure 2D, respectively). Additionally, APD dispersion was greater during DI-ATS1 relative to control (16.9 ± 1.0 ms vs 13.8 ± 1.3 ms, Figure 2C).

No arrhythmias (defined as one or more premature beats) were induced by premature programmed stimulation under any condition. Furthermore, VTs were neither spontaneous nor inducible under control conditions. However, during DI-ATS1, 38% of preparations experienced spontaneous VTs, and 19% experienced rapid pacing–induced VTs. Some preparations experienced one or more VT type. In total, 0% of control and 8 of 21 DI-ATS1 preparations...
experienced some type of VT. During DI-ATS1, all preparations experienced PVCs. PVC frequency for all experiments was normalized to the PVC frequency during DI-ATS1 (Table 1). PVC frequency during control conditions was significantly lower (by 99.5% ± 0.3%) than during DI-ATS1 alone (100%, Table 1). In total, only 2 of 8 preparations during control experienced any type of arrhythmia, all due to PVCs, relative to 17 of 17 preparations with preparations during DI-ATS1 (Table 1). PVC frequency during control conditions was normalized to the PVC frequency during DI-ATS1 but was significantly different from DI-ATS1 but not significantly different from control. Finally, DI-ATS1 significantly shortened APD to 190.6 ± 0.4 ms (Figure 2B), which was significantly greater relative to control (0.5% ± 0.3%) than during DI-ATS1 alone.

**Validation of ratiometric [Ca^{2+}], mapping**

In order to quantify relative changes in [Ca^{2+}], during ATS1, it was important to validate Ca^{2+} independent drift. The F_{A05}/F_{A85} drift was measured in vitro (see Supplemental Results) and ex vivo in Langendorff-perfused guinea pig ventricles (n = 3), where recordings were made every 5 minutes for 1 hour. Ca^{2+} transients recorded from the same epicardial site at different exposure times demonstrate an upward shift in signal consistent with in vitro observations (Figure 3A, Exp1 black, first exposure; Exp13 gray, last exposure). Both diastolic [Ca^{2+}], and Ca^{2+} transient amplitude were significantly higher during Exp13 relative to Exp1 (Figure 3E). Multiple regression analysis revealed a significant correlation of observed drift with cumulative exposure (parameterized as number of exposures). Specifically, diastolic [Ca^{2+}] increased at a rate of 3.77% (95% confidence interval 3.37–4.17, \( R^2 = 0.79 \)) per exposure (Figure 3B). Importantly, Ca^{2+} transient duration (Figure 4A), QRS duration, and QT interval (see Supplemental Figure 2) were unaffected by the number of exposures.

Mathematically correcting measured transients by subtracting 3.77% per exposure from the ratiometric fluorescence Ca^{2+} signal resulted in a high degree of morphologic correspondence between Ca^{2+} transients recorded several exposures apart (Figure 3C). Over all experiments, mathematical drift correction returned Ca^{2+} transient amplitude and diastolic [Ca^{2+}], after 13 exposures to Exp1 levels (Figures 3D and 3E).

**Validation of Ca^{2+} drift correction**

Under control conditions, rapid pacing (BCL = 200 ms, Exp2, Figure 4) significantly increased drift-corrected diastolic [Ca^{2+}], by 7.6% ± 1.4% (n = 3) relative to baseline pacing (BCL = 400 ms, Exp1) as demonstrated by representative data shown in Figure 4A. Cessation of rapid pacing (BCL = 400, Exp3) returned drift corrected diastolic [Ca^{2+}], to values similar to baseline (BCL = 400, Exp1, 6.7% ± 0.9% decrease). To further validate our ability to measure changes in diastolic [Ca^{2+}], we inhibited SERCA2a with 5 μM cyclopiazonic acid, which significantly increased drift-corrected diastolic [Ca^{2+}], by 15.2% ± 1.5%.
DI-ATS1 alters \([Ca^{2+}]\) handling

Representative \([Ca^{2+}]\) transients shown in Figure 5A demonstrate that DI-ATS1 shifts ratiometric \([Ca^{2+}]\) transients upward. Diastolic \([Ca^{2+}]\), during DI-ATS1 was significantly greater relative to control by 17.9% ± 1.8% (n = 10, Figure 5B). Additionally, DI-ATS1 significantly elevated \([Ca^{2+}]\) transient amplitude by 18.1% ± 1.3% relative to control (Figures 5A and 5B). Perfusion of pinacidil (15 μM) during DI-ATS1 attenuated the upward shift in diastolic \([Ca^{2+}]\), (Figure 5A). This effect was observed irrespective of the experimental order. For all experiments, DI-ATS1 + Pinacidil reduced diastolic \([Ca^{2+}]\) by 12.7% ± 1.7% (Figure 5C) relative to DI-ATS1 alone; however, diastolic \([Ca^{2+}]\) remained significantly greater relative to control (6.5% ± 2.2%). Furthermore, pinacidil did not reverse the rise in \([Ca^{2+}]\) transient amplitude (Figures 5B and 5C). Specifically, \([Ca^{2+}]\) transient amplitude during DI-ATS1 was not significantly different after pinacidil perfusion; therefore, \([Ca^{2+}]\) transient amplitude during DI-ATS1 + Pinacidil remained significantly greater relative to control (19.4% ± 2.3%).

**Discussion**

Several studies hypothesized that \([Ca^{2+}]\), accumulation concomitant with APD prolongation underlies arrhythmias in ATS1, and DI-ATS1. However, \([Ca^{2+}]\), accumulation had not been demonstrated in whole-heart preparations in part because of methodologic difficulties in quantitative...
Drug-induced Anderson-Tawil syndrome (DI-ATS1) alters \([\text{Ca}^{2+}]\) handling. A: Representative drift-corrected \([\text{Ca}^{2+}]\) transients recorded during control, DI-ATS1, and DI-ATS1 + 15 \(\mu\text{M}\) pinacidil perfusion. During DI-ATS1, \([\text{Ca}^{2+}]\) transients were shifted upward, whereas pinacidil partially reversed that shift. Both DI-ATS1 and DI-ATS1 + pinacidil exhibit greater \([\text{Ca}^{2+}]\) transient amplitude (\(\text{Ca}_{\text{amp}}\)) (horizontal lines) relative to control. B: Summary of \(\Delta\text{Ca}_{\text{amp}}\) (left) and \(\Delta\text{Ca}_{\text{amp}}\) (right) relative to control. DI-ATS1 significantly increased \(\text{Ca}_{\text{amp}}\) and \(\text{Ca}_{\text{amp}}\) relative to control (*\(P < .05\), \(n = 10\)). Pinacidil perfusion (15 \(\mu\text{M}\)) decreased \(\text{Ca}_{\text{amp}}\) relative to DI-ATS1 alone (+*\(P < .05\)). Pinacidil did not completely revert \(\text{Ca}_{\text{amp}}\) to control levels (+*\(P < .05\)). Pinacidil had no effect on \(\text{Ca}_{\text{amp}}\) relative to DI-ATS1 alone.
pinacidil perfusion was lower relative to DI-ATS1 but still was higher relative to control. Therefore, these data suggest that gradients of repolarization in DI-ATS1 are unlikely to be a significant substrate for arrhythmias in this condition.

On the other hand, pinacidil attenuated the rise in APD$_{50}$ due to DI-ATS1 such that APD$_{50}$ during control and DI-ATS1+Pinacidil were not significantly different. Therefore, the observation that arrhythmias were reduced but still present during DI-ATS1+Pinacidil further suggests that these arrhythmias are not correlated to APD$_{50}$ or action potential plateau prolongation.

The two conditions of DI-ATS1 and DI-ATS1+Pinacidil exhibited final repolarization (APD) prolongation and [Ca$^{2+}$]$_i$ accumulation relative to control. These findings suggest two mechanisms that may not necessarily be independent. Specifically, prolongation of final repolarization, as estimated by APD, could also be a substrate for increased triggered activity in DI-ATS1. The hypothesis that APD prolongation leads to recovery from inactivation of L-type calcium channels is not well supported by changes in APD or action potential plateau prolongation.

**Proposed arrhythmia mechanism in ATS1**

Arrhythmias in ATS1 patients are often preceded by a high PVC incidence, presumably due to triggered activity, and PVC burden in ATS1 is exacerbated by hypokalemia. Importantly, it has been demonstrated that hypokalemia alone leads to [Ca$^{2+}$]$_i$ accumulation and the increased incidence of arrhythmias. Based on these findings, the following mechanism of increased incidence of arrhythmias has been proposed. [Ca$^{2+}$]$_i$ accumulation is associated with increased sarcoplasmic reticular Ca$^{2+}$ loading and an increased propensity for triggered activity, presumably due to spontaneous Ca$^{2+}$ release from the sarcoplasmic reticulum. The resultant spontaneous Ca$^{2+}$ release may lead to depolarization via transient inward currents carried by the forward mode Na$^+$/Ca$^{2+}$ exchanger, facilitating triggered activity. This leads to the hypothesis that [Ca$^{2+}$]$_i$, accumulation, secondary to hypokalemia, underlies triggered activity in ATS1 or DI-ATS1.

Sung et al tested this hypothesis in an in silico study and suggested a role for abnormal [Ca$^{2+}$]$_i$ cycling in ATS1-associated arrhythmias. This finding was indirectly affirmed by Morita et al, who reported that Ca$^{2+}$ channel blockade by verapamil abolished all arrhythmic activity in a canine left ventricular wedge model of DI-ATS1. However, [Ca$^{2+}$]$_i$ accumulation during DI-ATS1 has not been previously demonstrated in an ex vivo intact ventricular model.

**Validation of ratiometric calcium mapping**

In order to assess the effects of DI-ATS1 on [Ca$^{2+}$]$_i$ handling, it is important to characterize any Ca$^{2+}$-independent changes in Indo-1 fluorescence. Despite previous calibration attempts, many reports indicated that incomplete de-esterification of Indo-1/AM along with excitation intensity–dependent photobleaching of Indo-1 affect ratiometric fluorescent Ca$^{2+}$ measurement. Specifically, the fluorescent signals corresponding to bound and unbound Indo-1 (F$_{405}$ and F$_{485}$, respectively) drift toward zero at two different rates, resulting in an apparent decrease in the ratiometric Ca$^{2+}$ signal. In our experimental setup, individual fluorescent Ca$^{2+}$ signals (F$_{405}$ and F$_{485}$) also decreased, yet the ratiometric Ca$^{2+}$ increased (see Supplemental Figure 1A). These seemingly contradictory findings could be attributed to the following experimental differences: the choices of dye of Indo-1 versus ester form of Indo-1 (Indo-1/AM), emission filters, and/or photodetector spectral response. The relative drift rates of the F$_{405}$ and F$_{485}$ signals will dictate whether the ratiometric signal drifts upward or downward.

Future studies that measure [Ca$^{2+}$]$_i$ over time should validate [Ca$^{2+}$]$_i$ response as a function of exposure. The absence of concomitant changes in ECG parameters or Ca$^{2+}$ transient duration (see Supplemental Figure 2) suggests that these changes in fluorescent Ca$^{2+}$ signals may be dye related rather than physiologic in origin. Importantly, mathematical correction of ratiometric fluorescent Ca$^{2+}$ transients by subtracting the observed drift from the signal resulted in a high degree of morphologic correspondence between ratiometric transients recorded several exposures apart (Figure 3C). Finally, the optically measured, drift-corrected diastolic [Ca$^{2+}$]$_i$ increased during rapid pacing and returned to baseline upon cessation of rapid pacing (Figure 4A), which is consistent with previous studies.

Likewise, SERCA2a inhibition by 5 μM cyclopiazonic acid perfusion led to [Ca$^{2+}$]$_i$ accumulation, as previously demonstrated. Therefore, this mathematical drift correction was applied to all subsequent recordings.

**DI-ATS1 alters [Ca$^{2+}$]$_i$ handling**

DI-ATS1 was associated with a significant rise in [Ca$^{2+}$]$_i$ as reflected in both diastolic [Ca$^{2+}$]$_i$, and Ca$^{2+}$ transient amplitude and an increased incidence of ventricular arrhythmias. These data are the first direct evidence in an intact ventricular preparation for [Ca$^{2+}$]$_i$ accumulation during DI-ATS1. Furthermore, these data are consistent with the theoretical mechanisms proposed for arrhythmias in ATS1. More generally, the finding that [Ca$^{2+}$]$_i$ accumulation concomitant with APD prolongation is related to increased arrhythmia propensity is consistent with previous studies using alternative methods of [Ca$^{2+}$]$_i$ loading to increase the incidence of triggered activity. Further studies are necessary to elucidate the relationship among the extent of [Ca$^{2+}$]$_i$, accumulation, APD prolongation, and the origin of triggered activity during DI-ATS1.
Study limitations
Although APD gradients in guinea pig (present study) or canine\textsuperscript{5} were not associated with increased arrhythmia propensity, APD distribution and heterogeneity are known to vary among animal models.\textsuperscript{33,34} The nature of electrophysiologic remodeling induced by chronic functional \(I_{K1}\) down-regulation, as occurs in patients with ATS1, remains unclear.\textsuperscript{5,7,11,17} Furthermore, it is well appreciated that pharmacologic models of cardiac disease should be interpreted cautiously due to the acute nature of the study as well as the specificity of the intervention.\textsuperscript{35}

Conclusion
This study suggests that arrhythmias during DI-ATS1 may be the result of triggered activity secondary to prolonged APD and altered \([Ca^{2+}]_i\) cycling and less likely dependent on large gradients of repolarization acting as a substrate for reentrant arrhythmias. Therefore, ameliorating myocyte \([Ca^{2+}]_i\) load may prove a more effective therapeutic goal in ATS1 compared to decreasing APD gradients.

Appendix
Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hjrthm.2010.03.044.

References
Supplemental Figure 1  In Vitro Characterization of Ca^{2+} Fluorescence Drift

A) Representative fluorescent Ca^{2+} signals (gray – F_{405}, black – F_{485}) of a vial containing 0.1 μM [Ca^{2+}] and 0.1 μM Indo-1 during the first (Exp 1) and last exposures (Exp 7). In this example during Exp 1, F_{485} decreased faster relative to F_{405} (0.080% [95% CI, 0.085 – 0.075] per second vs 0.024% [95% CI, 0.027 – 0.020] per second, respectively). After 7 exposures (Exp 7), both F_{405} and F_{485} decreased relative to the first recording (Exp 1).

B) Representative ratiometric fluorescent Ca^{2+} signals corresponding to the F_{405} and F_{485} in Figure 3A. The drift in F_{405} and F_{485} during Exp 1 translated into a rise in F_{405}/F_{485} within the recording (0.023% [95% CI, 0.006 – 0.039] per 1 second). After 7 exposures (Exp 7) ratiometric fluorescent Ca^{2+} signals was enhanced relative to Exp 1.

C) Multiple regression analysis of drift measurements pooled from all experiments (n = 57) conducted at different exposure frequencies. The drift demonstrated a significant correlation with the number of exposures with rate of 5.98% (95% CI 5.52–6.44), R^2 = 0.63 increase in ratiometric Ca^{2+} fluorescence per exposure.

Supplemental Figure 2  Effect of time on Ca_{DUR} and ECG parameters

A) Representative volume-conducted ECGs recorded under control conditions to assess Ca^{2+}-independent changes in indo-1 fluorescence. No changes in the ECG morphology were observed between the first (Exp 1 – black) and last recordings (Exp 13 – grey).

B) Summary data (n = 3) depicting no significant difference in QRS, QTc, and Ca^{2+} transients duration (Ca_{DUR}) between the first (Exp 1 – black) and last recordings (Exp 13 – grey).