Delayed afterdepolarization (DAD)–mediated triggered activity is believed to play an important role in arrhythmias associated with catecholamine-excess, heart failure, and mutations of the ryanodine receptor. DADs are caused by membrane depolarization in response to nonelectrically driven (ie, spontaneous) calcium release from the sarcoplasmic reticulum. Calcium overload caused by enhanced calcium entry is one mechanism for spontaneous calcium release (SCR) from the sarcoplasmic reticulum. Previously, we and others have shown that myocytes near the endocardium and midmyocardium are more prone to elevated diastolic intracellular calcium levels, calcium overload, and thereby triggered activity compared with myocytes near the epicardium. However, preliminary data from our laboratory suggests that only one triggered beat typically occurs from a relatively broad area of diastolic calcium elevation. Therefore, the exact mechanistic relationship between elevated calcium levels and the origin of triggered activity from a specific site is not clear. For example, it is possible that calcium overload occurs at only one unique site, and this is where an SCR event and triggered activity occur. Alternatively, it is possible that calcium overload and SCR events occur at multiple regions simultaneously; however, only the largest SCR event serves as a focus for DAD-mediated triggered activity. Interestingly, polymorphic VT occurred in some experiments when multiple SCR events occurred. In conclusion, multiple, simultaneous SCR events occur over a broad region of relatively slower calcium uptake and elevated diastolic calcium levels. However, SCR events closer to the endocardium have the largest amplitude and earliest onset and are, thereby, more likely to initiate DAD-mediated triggered activity. Finally, multiple SCR events may be a mechanism of polymorphic VT under calcium overload conditions. (Circ Res. 2005;96: 535-542.)

Key Words: intracellular calcium ▪ delayed afterdepolarization ▪ triggered activity ▪ optical mapping ▪ arrhythmias

Cellular Mechanism of Calcium-Mediated Triggered Activity in the Heart

Rodolphe P. Katra, Kenneth R. Laurita

Abstract—Calcium overload due to enhanced calcium entry is a mechanism for spontaneous calcium release (SCR) from the sarcoplasmic reticulum, delayed-afterdepolarizations (DAD), and triggered activity. However, the exact mechanistic relationship between elevated intracellular calcium levels and triggered activity originating from a specific location remains unclear. We hypothesize that under conditions of enhanced calcium entry, elevation of intracellular calcium will result in multiple calcium release events of which only one is more likely to initiate a triggered beat. We used optical mapping of action potentials and ratiometric calcium transients in an electromechanically-uncoupled canine wedge model of enhanced calcium entry, using \( I_{\text{Ca}} \) blockade with \( \beta \)-adrenergic stimulation. Under conditions of enhanced calcium entry, the rate of calcium uptake was faster compared with control conditions; however, during rapid pacing, cytoplasmic calcium elevation at the endocardium was significantly increased (15±4%) compared with control (10±3, \( P<0.04 \)). Rapid pacing induced multiple simultaneous SCR events with largest amplitude and earliest onset near the endocardium compared with the epicardium. Furthermore, SCR events with largest amplitude and earliest onset served as a focus for DAD-mediated triggered activity. Interestingly, polymorphic VT occurred in some experiments when multiple SCR events occurred. In conclusion, multiple, simultaneous SCR events occur over a broad region of relatively slower calcium uptake and elevated diastolic calcium levels. However, SCR events closer to the endocardium have the largest amplitude and earliest onset and are, thereby, more likely to initiate DAD-mediated triggered activity. Finally, multiple SCR events may be a mechanism of polymorphic VT under calcium overload conditions. (Circ Res. 2005;96: 535-542.)

Key Words: intracellular calcium ▪ delayed afterdepolarization ▪ triggered activity ▪ optical mapping ▪ arrhythmias

Materials and Methods

Experimental Preparation

Experiments were performed in accordance with Public Health Service guidelines for the care and use of laboratory animals.
Mongrel dogs (20 to 25 Kg) (LBL Kennels; Reelsville, Ind) were anesthetized with thiopental sodium (30 mg/Kg intravenous), and hearts were removed by a left lateral thoracotomy and placed in cold (4°C) cardioplegia solution. Transmural wedges of cardiac tissue (average dimensions: 20×10×10 mm) surrounding and parallel to branches of the left anterior descending coronary artery and left circumflex artery were dissected from the left ventricular free wall (n=12). All preparations were taken near the base of the left ventricle and any free running Purkinje fibers were removed from the endocardial surface. The coronary artery of each wedge was cannulated and perfused with oxygenated (95% O2, 5% CO2) Tyrode solution containing (in mmol/L) NaCl 135, NaH2PO4 0.9, MgSO4 0.492, KCl 4.03, dextrose 5.5, CaCl2 1.8, and HEPES 10 (pH 7.40). Perfusion pressure was maintained between 50 to 70 mm Hg by regulating coronary flow. Wedges were stained with the calcium-sensitive indicator indo-1AM dissolved in 1 mL solution of DMSO and Pluronic (20%wt/vol) at a final concentration of 10 μmol/L for 45 minutes at room temperature. The dye-loading period was followed by a 15-minute washout period to remove unhydrolyzed or partially hydrolyzed dye. In addition, a subset of wedges (n=5) was also stained with the voltage-sensitive dye di-4-ANEPPS (15 μmol/L). In all experiments, 10 to 15 μmol/L of Cytochalasin-D was used to ensure that motion artifact, if present, did not influence our result. The absence of motion artifact was confirmed by CCD video imaging and ratiometric bH plots described previously.

The perfused wedge was placed in a Lexan chamber where the transmural surface was gently positioned against an imaging window using a movable piston. To avoid surface cooling and desiccation, the wedge was immersed in the coronary effluent, which was maintained at a temperature equal to the perfusion temperature (37 ± 1°C). The volume-conducted ECG (ECG) was monitored using three silver disk electrodes fixed to the chamber. A fine gauge (0.003-inch diameter) PTFE-coated silver unipolar electrode was inserted into the endocardial surface to stimulate at twice-diastolic threshold current. Physiological stability of the preparation was ensured by monitoring the ECG, coronary pressure, coronary flow, and perfusion temperature continuously throughout each experiment. Preparations remained viable for 2 to 3 hours, but the entire experiment lasted no more than 2 hours. All optical recordings were performed 1 hour after cannulation to allow healing of the cut transmural surface.

**Optical Mapping System**

To determine the amplitude of calcium transients, ratiometric imaging of intracellular calcium was performed. Briefly, excitation light was filtered at 350±10 nm and directed to the preparation. Fluorescent light from the preparation was collected by a tandem lens assembly. A 445-nm dichroic long-pass mirror was positioned in the tandem lens assembly at a 45° angle to transmit all wavelengths above 445 nm to a 16×16 element photodiode array and reflect all wavelengths below 445 nm to another 16×16 element photodiode array. All optical components were aligned with an accuracy of 35 μm. For all experiments before dye loading, background fluorescence was recorded at both emission wavelengths (485 and 405 nm). Ratiometric calcium transients were calculated by dividing the background-subtracted calcium transients at 405 nm by the background-subtracted calcium transients at 485 nm. Although ratiometric calcium transients can be used to accurately compare relative changes in intracellular calcium under certain conditions, absolute calcium levels may be difficult to infer.

To measure intracellular calcium and transmembrane voltage simultaneously, we also used optical mapping techniques described previously. Briefly, in addition to the excitation light used for calcium imaging, light at 514±10 nm was directed to the heart. The longer wavelength calcium emission filter (485 nm) was replaced by a long-pass emission filter (>695 nm) for simultaneous voltage imaging.

All signals recorded from each photodiode and ECG signals were multiplexed and digitized with 12-bit precision at a sampling rate of 1000 Hz per channel. For the present study, an optical magnification of 1.24× was used, resulting in a total mapping field of 14×14 mm with 0.9 mm spatial resolution and 0.8 mm² pixel size. For wedges with a transmural length <14 mm, some edge pixels were omitted from the analysis. To view, digitize, and store the position of the mapping array relative to anatomical features, the dichroic mirror was rotated to reflect an image of the preparation to a CCD video camera.

**Experimental Protocol**

In 12 wedges from 12 animals, HMR1556 (7 μmol/L), a selective L channel blocker, and Isoproterenol (0.2 μmol/L), a β-adrenergic stimulant, were administered simultaneously to enhance calcium entry. This model has been shown previously to be associated with triggered activity in single cells and tissue slices. Ratiometric calcium transients and simultaneous action potentials and calcium transients were recorded at constant baseline pacing (600 ms) and during a momentary (5 second) step increase to 110–200 ms. Recordings were also made during rapid pacing, at the fastest cycle length that would capture the tissue 1-to-1 (100–150 ms, as determined by 10 ms decrements near refractoriness), followed by a halt in pacing to elicit ectopic activity. Before optical recordings were made, all preparations were electrically quiescent, and 2 of 12 wedges demonstrated a slight increase in automaticity due to β-adrenergic stimulation. This increase in automaticity was abolished by rapid pacing.

**Data Analysis**

To quantify the rate of decrease of intracellular calcium to diastolic levels, the decay portion of the calcium transient (from 30% to 100% of the decline phase) was measured by the time constant (τ) of a single exponential fit using an automated Nelder-Mead simplex iterative algorithm (MatLab, Mathworks Inc). When minimum diastolic calcium level alternated from beat-to-beat during rapid pacing, the average diastolic calcium level from 4 consecutive minima was used. To account for differences in baseline diastolic calcium levels, the elevation in diastolic calcium during rapid pacing (ΔCa, diastolic) was measured as the difference in diastolic levels before and at the end of rapid pacing. Action potential depolarization times were determined from the maximum first derivative during the upstroke. Spontaneous calcium release (SCR) events were defined as a spontaneous increase in calcium levels during diastole (ie, non-electrically driven) that exceeded 10% of baseline calcium transient amplitude. Spontaneous calcium release amplitude (SCRamp) was measured as the difference between minimum and maximum calcium levels during the SCR event at the center pixel. SCR onset was calculated as the time of peak SCR level relative to the earliest SCR onset. All measurements were made using automated algorithms with visual inspection by an experienced investigator. Levels of significance were determined using a Student t test, ANOVA, and Fisher exact test where a value of P<0.05 was considered statistically significant.

**Results**

**Transmural Heterogeneity of Calcium Handling Under Conditions of Enhanced Calcium Entry**

Shown in Figure 1A are representative normalized ratiometric calcium transients recorded near the endocardium (Endo) and epicardium (Epi) under conditions of enhanced calcium entry. The decay time constant (τ) of the calcium transient recorded near the endocardium (102 ms) was slower compared with that near the epicardium (78 ms). Over all experiments (Figure 1B), τ was faster, in general, under conditions of enhanced calcium entry (Endo=110±30 ms, Epi=84±25 ms; P<0.002) compared with control conditions (Endo=204±26 ms, Epi=140±13 ms; P<0.001, data not shown). Figure 2A shows ratiometric calcium transients recorded during the last beat of baseline pacing (CL 600 ms)
followed by a period of rapid pacing. During rapid pacing, the apparent increase in mean diastolic intracellular calcium level was higher near the endocardium (51 RU, 8%) compared with the epicardium (42 RU, 7%), and was further increased under conditions of enhanced calcium entry (Endo=71 RU, 13% and Epi=49 RU, 8%). This occurred even though cytosolic calcium removal is faster under conditions of enhanced calcium entry compared with control. Calcium alternans was also greater near the endocardium, especially under conditions of enhanced calcium entry. On average (Figure 2B), pharmacological enhancement of calcium entry caused a significant augmentation of the pacing-induced increase in the mean diastolic ratio near the endocardium (15±4 versus 10±3%, P<0.04), but not the epicardium (8±3 versus 7±2%, P=NS). These data suggest that regions exhibiting relatively slower uptake of intracellular calcium by the SR are more prone to elevated diastolic calcium levels in response to rapid pacing under conditions of enhanced calcium entry.

**Triggered Activity Under Condition of Enhanced Calcium Entry**

Under conditions of enhanced calcium entry, ectopic activity was observed immediately on termination of rapid pacing. Shown in Figure 3A are membrane potential (V_m) and intracellular calcium (Ca^2+) signals from a representative experiment under conditions of enhanced calcium entry, recorded from a site near the endocardium (baseline pacing, left; rapid pacing, middle) and a site near the epicardium (rapid pacing, right). Immediately after halting rapid pacing, an ectopic beat (asterisk) occurred, followed by a DAD and a SCR ≈250 ms after final repolarization, only at the endocardium. Furthermore, just preceding the ectopic beat, V_m and Ca^2+ levels at the endocardial site slowly increased (arrow) and had a similar initial time course and amplitude compared with the DAD and SCR after the ectopic beat. In contrast, there was no slow increase in V_m and Ca^2+ during baseline pacing (left) or at the epicardial site (right). This is consistent with an ectopic beat triggered by an SCR-mediated DAD at the endocardium. Figure 3B shows the activation pattern of the ectopic beat in Figure 3A. Earliest activation of the ectopic beat occurred near the endocardium (asterisk) at the site of DAD and SCR activity shown in Figure 3A, remote from the pacing site (stimulus symbol). Over all experiments, triggered beats occurred most frequently (P<0.001) closer to the endocardium (Figure 3C), where diastolic calcium levels were highest under conditions of enhanced calcium entry. In several experiments (n=3), optical mapping of voltage and calcium was performed sequentially from the transmural surface and the endocardial surface. When episodes of triggered activity occurred reproducibly at the same rate of rapid pacing with similar ECG morphology, transmural and endocardial contour maps were combined. Contour maps were time-aligned by the pacing artifact and aligned in space using images captured with the CCD camera. Shown in Figure 4 is an example of triggered activity with earliest activation (ie,
breakthrough) occurring on the endocardial surface. In this episode, triggered activity probably originated closer to the endocardial surface rather than the cut surface of the transmural wall. Such episodes of triggered activity were excluded from analysis because the origin was far from the transmural surface. However, these data suggest that triggered activity is not necessarily caused by injury to the cut surface.

**Mechanisms of Calcium-Mediated Triggered Activity and Arrhythmogenesis**

Using ratiometric imaging techniques, we were able to map the amplitude of spontaneous calcium release events. Shown in Figure 5A is a contour map of SCR amplitude in the absence of a triggered beat. Regions of largest calcium release are indicated by lighter shades of gray. Interestingly, we observed multiple simultaneous SCR events in a single recording. In the example shown, two significant release events occurred, one near the endocardium (site a) and a second in the midmyocardium (site c). Figure 5B shows the ECG and calcium transients recorded from sites a, b, c, and d. SCR amplitude and time-to-peak at site a (endocardium) was largest and earliest compared with b and c. No SCR event was observed at site d (epicardium). Furthermore, a much smaller SCR amplitude at site b suggests that the SCR events at a and c are independent. Another interesting finding was that each SCR event did not occur from a single cell but from a region of myocardium comprised of many cells, as indicated by the SCRs occurring over more than one pixel. These data indicate that calcium release does not occur from a single cell but from a large group of cells.

To explain the preferential occurrence of a single ectopic beat near the endocardium in the presence of multiple

**Figure 3.** Triggered activity under conditions of enhanced calcium entry. A, Representative action potentials (Vm) and calcium transients (Ca) recorded during baseline pacing (600 ms) from the endocardium (left) and rapid pacing (180 ms) followed by an abrupt halt in pacing from the same endocardial site (middle) and an epicardial site (right). After cessation of rapid pacing, an ectopic beat is evident (*). In contrast to the epicardium, a delayed-afterdepolarization (DAD) and a spontaneous calcium release (SCR) occurred at the endocardium that did not elicit an ectopic beat. Activation map of the ectopic beat (B) shows its origin (*) close to the endocardium yet away from the site of stimulation (stimulus symbol). Over all experiments (C), triggered activity (TA) occurred more frequently at the endocardium compared with other regions ($P<0.001$).
calcium release events, the timing and amplitude of all calcium release events from each experiment were analyzed. Figure 6A indicates that most SCR events occurred near the endocardium (P<0.001). This pattern closely matches that of triggered activity occurrence (Figure 3C). In addition, SCR events near the endocardium occurred with larger amplitude and earlier onset (Figure 6D) compared with SCR events at other myocardial regions. These data suggest that SCR events (and thereby DADs) are more likely to occur near the endocardium with larger amplitude and earlier onset, all of which may explain why a single triggered beat occurs preferentially near the endocardium.

In several experiments (n=3), ectopic activity and SCR events occurred reproducibly from the same location across the transmural wall, under conditions of enhanced calcium entry. Shown in Figure 7 are contour maps of τ, ΔCa_{dias}, SCR_{amp}, and activation time of the ectopic beat from one such experiment. The map of τ (Figure 7A) reveals a distinct region of slow decay of the calcium transient near the endocardium (white spot). During rapid pacing, mean diastolic calcium levels (Figure 7B) were, in general, greater near the endocardium but particularly high around the same region of slow τ. After rapid pacing (Figure 7C), two SCR events occurred; however, the SCR with earliest onset and largest amplitude (asterisk) occurred where τ and ΔCa_{dias} were slowest and largest, respectively. Finally, from the same preparation, when an ectopic beat occurred, the site of earliest activation occurred where the largest and earliest SCR event occurred (Figure 7D). These data suggest that the mechanism of ectopic activity in this model is calcium overload where calcium uptake kinetics are slowest.

It is possible that multiple regions of calcium release events may produce ectopic beats originating from several regions. Figure 8A shows a representative example of polymorphic ventricular tachycardia under conditions of enhanced calcium entry. On termination of rapid pacing (S1), multiple ectopic beats were observed (V1 to V4). The first beat (V1) is likely to be triggered activity occurring near the transmural surface, similar to that shown in Figures 3 and 7. V2 is preceded by a relatively long isoelectric interval (≈350 ms) that also suggests triggered activity. The remaining beats (V3 and V4) may or may not be triggered activity. The exact mechanisms are unknown because the activation sequence from deeper layers could not be determined. These data suggest that multiple SCR events may be a mechanism of polymorphic tachycardia in the intact heart.

**Discussion**

In this study, we investigated the cellular mechanisms of calcium-mediated triggered activity under conditions of enhanced calcium entry in an intact cardiac preparation. We found spontaneous calcium release (SCR) events occurring over broad regions of relatively slower calcium uptake and elevated diastolic calcium levels. Interestingly, we observe multiple SCR events occurring across the transmural wall; however, they occurred more frequently and had largest
amplitude and earliest onset near the endocardium. These SCR events are also responsible for DAD-mediated triggered activity and may represent an important mechanism of arrhythmogenesis associated with abnormal calcium cycling.

**Heterogeneity of Calcium Handling Under Condition of Enhanced Calcium Entry**

We have shown previously that under normal conditions, myocytes near the endocardium exhibit a slow intracellular calcium decay ($\tau$) and elevated diastolic calcium levels during rapid pacing. In the present study, using ratiometric optical mapping techniques, we were able to compare calcium transients over long time periods (ie, across interventions) and across experiments. Under conditions of enhanced calcium entry, $\tau$ was faster, in general, compared with control conditions, presumably due to phospholamban phosphorylation. However, as under control conditions, $\tau$ near the endocardium was still relatively slower (ie, larger) than $\tau$ near the epicardium. We have previously shown in the guinea pig that Isoproterenol increases the rate of calcium uptake overall, but it does not change the base to apex gradient of $\tau$ on the epicardial surface of the heart. Interestingly, although uptake was faster overall under conditions of enhanced calcium entry, mean diastolic calcium level during rapid pacing was greater. This may reflect increased calcium entry through L-type calcium channels.

**Mechanism of Triggered Activity and DADs**

Under conditions of enhanced calcium entry, we and others have observed triggered activity after brief periods of rapid pacing. The triggered activity we observed is most likely due to DADs, considering the following. Near the origin of triggered activity, action potentials were preceded by a slow depolarization in membrane potential: the hallmark of a DAD-mediated beat. Such slow depolarization was absent during baseline pacing and at sites remote to the origin of triggered activity. The amplitude of DADs we measured reached $\approx 25$ mV (based on a 100 mV action potential and $-80$ mV resting potential), which is sufficient to activate some sodium channels and trigger an action potential. DADs of comparable amplitude (7 to 15 mV) were measured in vivo and in single cells. Finally, DAD activity was always accompanied by a simultaneous SCR event, suggesting that DADs were mediated by spontaneous calcium release.

Interestingly, we also found that SCR and DAD events occurred from a group of cells over several pixels, suggesting that these events do not occur in just a single cell. In fact, if DADs and SCRs had occurred only in a single cell, it would be unlikely that our optical mapping system could detect them. However, the exact size (ie, number of cells) at the site of origin is unknown because its location may be slightly below the transmural surface. Nevertheless, from a biophysical stand point, our finding makes sense because to electrically capture and stimulate well-coupled myocardium, a large “source” is required. Therefore, our data are consistent with the notion of a spatially large afterdepolarization, potentially explaining why DAD-mediated triggered activity can occur in well-coupled myocardium.

**Mechanism of SCR Events**

In this study and others, triggered activity occurs preferentially near the endocardium. Our data suggests that under conditions of enhanced calcium entry, mean elevated diastolic calcium levels and calcium overload (as with digitalis and Ouabain) occurred preferentially near the endocardium where the rate of calcium uptake was relatively slower compared with the epicardium. Furthermore, SCRs near the endocardium had the largest amplitude, greatest occurrence rate, and earliest time of onset. In cases of triggered activity originating consistently from one site ($n=3$), we observed a distinct region of large $\tau$ (slower uptake), elevated diastolic calcium level, corresponding to the “strongest” (ie, largest and earliest) SCR and origin of triggered activity. Taken together, these findings suggest a causal relationship between regions of slower calcium uptake, diastolic calcium elevation, and the location of triggered activity. It is possible that local calcium alternans is a mechanism for increased diastolic calcium levels and SCR activity in this model. However, although calcium alternans was greater near the endocardium, it did not correlate with mean elevated diastolic calcium levels and SCR activity (data not shown).

The exact mechanism by which elevated calcium initiates an SCR event remains unclear; it could be due to elevated cytoplasmic calcium, increased SR calcium, or both. Our data suggest that elevated cytoplasmic levels...
directly trigger SCR events. However, we did not measure SR calcium content and cannot completely rule out the contribution of SR calcium load. It is also unknown why we observed a relatively large (several pixels) region of calcium release. It is possible that overload and, hence, an SCR event occurs simultaneously from many cells. Alternatively, an SCR can occur from a single cell, which then initiates a calcium wave that diffuses from cell-to-cell across gap junctions, similar to calcium waves in Purkinje cell aggregates. Further studies are needed to elucidate the exact mechanism of SCR events in intact tissue. We do know, however, that in our model, SCR are needed to elucidate the exact mechanism of SCR events in this study.

Aventis Pharma for their generous donation of HMR1556 for predoctoral fellowship 0415213B (to R.P.K.). The authors thank HL68877 and American Heart Association, Ohio Valley Affiliate. This work was supported by National Institutes of Health grant and may represent a mechanism of TdP based on multiple morphic ventricular tachycardia occurred in one third of all activations and could explain the occurrence of polymorphic ventricular tachycardia. Ultimately, this study implicates for calcium-mediated arrhythmogenesis associated with atrial fibrillation, heart failure, mutations of calcium regulatory proteins, and long QT syndrome.

Multiple Simultaneous Calcium Release Events

One interesting finding was the presence of multiple simultaneous SCR events, confirming our hypothesis and suggesting that ectopic activity may originate from different locations and could explain the occurrence of polymorphic ventricular tachycardia. In our study, nonsustained polymorphic ventricular tachycardia occurred in one third of all preparations and exhibited multifocal activity. Theoretically, such activity could be sustained and occur more frequently in an intact heart where the ventricular mass is much greater, and may represent a mechanism of TdP based on multiple ectopic foci. Nonetheless, our findings suggest a mechanistic link between abnormal calcium homeostasis and the onset of polymorphic ventricular tachycardia. Ultimately, this study may help in devising better pharmacological or gene therapies for calcium-mediated arrhythmogenesis associated with atrial fibrillation, heart failure, mutations of calcium regulatory proteins, and long QT syndrome.

Acknowledgments

This work was supported by National Institutes of Health grant HL68877 and American Heart Association, Ohio Valley Affiliate predoctoral fellowship 0415213B (to R.P.K.). The authors thank Aventis Pharma for their generous donation of HMR1556 for this study.

References


Cellular Mechanism of Calcium-Mediated Triggered Activity in the Heart
Rodolphe P. Katra and Kenneth R. Laurita

Circ Res. 2005;96:535-542; originally published online February 17, 2005;
doi: 10.1161/01.RES.0000159387.00749.3c
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/96/5/535

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org//subscriptions/