Spontaneous atrial fibrillation initiated by triggered activity near the pulmonary veins in aged rats subjected to glycolytic inhibition

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Ono N, Hayashi H, Kawase A, Lin S-F, Li H, Weiss JN, Chen P-S, Karagueuzian HS. Spontaneous atrial fibrillation initiated by triggered activity near the pulmonary veins in aged rats subjected to glycolytic inhibition. Am J Physiol Heart Circ Physiol 292: H639–H648, 2007. First published August 18, 2006; doi:10.1152/ajpheart.00445.2006.—Aging and glycolytic inhibition (GI) are known to alter intracellular calcium ion (Ca\textsuperscript{2+}) handling in cardiac myocytes, causing early afterpotentials (EADs) and delayed afterpotentials. We hypothesized that aging and GI interact synergistically in intact hearts to generate EADs and triggered activity leading to atrial fibrillation (AF). We studied isolated and Langendorff-perfused hearts of young (age 3–5 mo, N = 8) and old (age 27–29 mo, N = 14) rats subjected to GI (0 glucose + 10 mmol/l pyruvate). Epicardial atrial activation maps were constructed using optical action potentials, while simultaneously monitoring Ca\textsuperscript{2+} by means of dual-voltage and calcium-sensitive fluorescent dyes. During GI, spontaneous AF occurred in 13 of 14 old but in no young rats. AF was initiated by EAD-induced triggered activity at the left atrial pulmonary vein junction (LA-PVJ). The triggered activity initially propagated as single wave front, but within 1 s degenerated into multiple wavelets. The EADs and triggered activity in the old atria were associated with significantly elevated transient decline and action potential duration were significantly (P < 0.01) prolonged compared with atrial sites 5 mm away from LA-PVJ. During GI and rapid atrial pacing, spatially discordant APD and Ca\textsuperscript{2+} transient alternans developed in the old but not young atria, leading to AF. Atria in old rats had significantly more fibrotic tissue than atria in young rats. We conclude that GI interacts with the aged and fibrotic atria to amplify Ca\textsuperscript{2+} handling abnormalities that facilitate EAD-mediated triggered activity and AF.

MATERIALS AND METHODS

This study protocol was approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center and followed the guidelines of the American Heart Association.

Tissue preparation. We studied 22 Fischer 344 male rats consisting of 8 young (age 3–5 mo old) and 14 old (age 27–29 mo old) rats. The rats were anesthetized, the hearts and lungs were quickly removed, and the aorta was cannulated and perfused with oxygenated Tyrode solution. One pair of bipolar pacing electrodes was sutured halfway between the right atrium and the LA and one pair of bipolar electrodes was attached to the LA. Two widely spaced electrodes were positioned, one on the atrium and the other on the right ventricular apex to record a “pseudo-electrocardiogram,” as our laboratory previously described (17).

Dual-voltage and Ca\textsuperscript{2+} transient optical mapping. We used dual-voltage and Ca\textsuperscript{2+} -sensitive fluorescence dyes to map simultaneously optical action potentials and Ca\textsuperscript{2+} transient by means of two charge-coupled device cameras (CA-D1–0128T, Dalsa), as our laboratory previously described in detail (8). Briefly, the hearts were simultaneously stained with rhod-2 AM for calcium and RH237 for voltage imaging. Rhod-2 AM was chosen because of its specificity to cytosolic Ca\textsuperscript{2+} vs. other subcellular organelles, such as mitochondria (7, 11). Epifluorescence was collected simultaneously by the two charge-coupled device cameras through an 715-nm long-pass filter (Nikon) for the voltage image and a 580-nm bandpass filter (Nikon) for calcium imaging. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Transmembrane APs and APD restitution using glass microelectrode. Transmembrane APs were recorded from the LA-PVJ with standard glass microelectrode filled with 3 mol/l KCl in five additional old and five young rats, before and after GI, as our laboratory previously described (30, 33). AP duration to 90% repolarization (APD90) was measured during incremental pacing to construct dynamic APD restitution curves and to determine the effective refractory period (ERP), first at baseline and then 20 min after the onset of GI. Dynamic pacing protocol was initiated starting with a cycle length (CL) of 200 ms, and then the CL was decreased by 10 ms until 1:1 capture was lost. Each pacing CL lasted for 30 s (26). The dynamic pacing protocol was also used to determine the rate dependency of the time constant of Ca^{2+} transient decline (τ). The rate of decline of the Ca^{2+} transient was determined using monoexponential fit to the declining phase of the transient and determining its time constant τ of the decline at each pacing cycle length (PCL). We used the S1S2 pacing protocol to determine the ERP. We used eight consecutive S1 beats at a CL of 200 ms and then S2 was applied, starting with 190-ms coupling interval and 10-ms decrements until 140 ms and then by 2-ms decrements until block. Two to three sites were selected in each of the right atrium and the LA for the ERP measurements. When AF emerged, biphasic electrical shocks (model HVS200, Ventritex, Fig. 1. Selected snapshots of optical action potentials at the onset of spontaneous atrial fibrillation (AF) initiated in an old rat with glycolytic inhibition (GI). A: left atrial (LA) bipolar electrogram (BEG) showing the onset of spontaneous AF with a relatively regular activity, which then becomes irregular (right side). The snapshots of the first six beats (*1 to *6) of the AF shown in A are depicted in the activation map in B (thick arrow pointing downward). The numbers on top right of each frame are milliseconds after the onset of AF. Only a single-activation wave front originating from the LA-pulmonary vein junction (PVJ) at a cycle length (CL) of 50 ms is present. The yellow arrows represent the direction of propagation. The color code depicts red/yellow as depolarization and dark/light blue as repolarization. The asterisks in the snapshots denote the origin of the focal activation, which slightly moves on subsequent beats but remains confined within a 5-mm radius of the LA-PVJ over consecutive beats (asterisks in the schema at the bottom right). LPV and RPV: left and right pulmonary veins, respectively.

Fig. 2. Phase maps of optical action potentials during the onset (regular) and maintained (irregular) periods of the AF shown in Fig. 1. Phase maps in B are constructed over a 104-ms interval of AF shown in A (thick arrow pointing downward). Note the presence of a single wave front during this phase of the AF. Phase maps in C are constructed over a 168-ms interval of AF shown in A (thick arrow pointing downward). White arrows in C indicate the sites of phase singularities (wavebreak points). Multiple phase singularities exist (2–6 wavelets) at different times during AF. The color code bar indicates the phases of the optical actions potentials from $-\pi$ to $+\pi$. 

Sunnyvale, CA) were applied across the atria to terminate the AF. Only in two rats (old GI/H11001) the AF was let to be maintained for up to 20 min before cardioversion to establish the protracted (as opposed to transient, i.e., few seconds) nature of the AF in our model. In all of the remaining episodes, AFs were cardioverted within 5 min of onset to complete the experimental protocol in a timely fashion to prevent potential dye bleaching that might occur with longer (> 2 h) study periods. The overall protocol of our study involved, first, baseline studies during sinus rhythm and during pacing. GI studies were then started with 20 min of monitoring of the rhythm to determine whether spontaneous AF emerges, and then pacing studies were done (after electrical termination of AF, should it occur) to determine ERP, APD restitutional properties, alternans, and τ of the Cai^{2+} transient.

GL. After baseline studies, the hearts were perfused and superfused with Tyrode solution containing 10 mmol/l sodium pyruvate (Sigma) without glucose, an intervention which selectively inhibits glycolysis while maintaining oxidative phosphorylation (20).

Data analysis. Image acquisitions was controlled by custom-designed software based on LabVIEW and the IMAQ Vision toolset (National Instruments). We constructed two kinds of activation maps from the voltage signals: voltage ratio maps, providing snapshots of membrane potential, and voltage phase maps, to determine the number of wavelets by identifying phase singularity points using a time-embedding method (2). We also determined the rate of decline of Cai^{2+} transient using monoexponential fit to compute the rate of the decline. APD_{90} was measured, and the diastolic Cai^{2+} level during an event of interest (i.e., EAD or triggered activity) was expressed as a percentage of the peak systolic Cai^{2+} transient amplitude observed during regular pacing at a CL of 200 ms. During the course of our experiments, it became clear that EADs arose from discrete regions rather than from randomly distributed sites in the mapped region. This led us to hypothesize the presence of regional difference in the APD and the rate of decline of Cai^{2+} transient. Consequently, we compared the APD and the rate of decline of Cai^{2+} transient at the sites of EAD emergence to sites with no EADs. Statistical analyses were performed using ANOVA with Newman-Keuls tests for repeated comparisons and Student’s t-test. The χ^2 test was used to compare AF occurrences in the two groups. P < 0.05 was considered statistically significant, and all data are expressed as means ± SD.

Histological analysis. The weight of the isolated atria was measured, and transmural atrial sections were stained with Masson...
RESULTS

Spontaneous AF. All isolated, perfused hearts from young and old rats were in sinus rhythm after mounting in the tissue bath. The sinus CL was not significantly different in the two groups, both at baseline (279 ± 59 and 298 ± 48 ms, young vs. old) and after GI (297 ± 59 and 340 ± 73 ms, young vs. old) (P > 0.4). However, 15–20 min after the onset of GI, spontaneous AF with a mean CL of 83.5 ± 30.8 ms occurred in 13 out of 14 old rats, but in none of the young rats (0 out of 8) (P < 0.0001). The mean CL of the AF in the 13 rats was determined by measuring 15–20 consecutive beats in the bipolar LA electrogram that displayed relatively high amplitude and discrete spiking during the AF. The onset of AF was characterized by a rapid and a relatively regular activity on the LA bipolar electrograms, with a mean CL of 62 ± 24 ms, which, within 1 s, degenerated into an irregular and complex electrogram morphology (Fig. 1A). While the AF could be sustained for >20 min (2 rats monitored), the AF, however, was terminated with an electrical shocks within 5 min of onset to complete the experiment in a timely fashion. Optical mapping of activation pattern during the onset of AF showed a focal, single wave front originating from the LA-PVJ (Fig. 1B). Atrial activation proceeded via this mechanism for 6–12 beats before the single activation wave front broke up into multiple, independent wavelets (Fig. 2). The number of wavelets during sustained AF ranged between two and six, with a mean of 3.6 ± 1.3 (Fig. 2). This pattern of spontaneous AF was seen in all 13 out of 14 old rats exposed to GI. In four additional rats (2 old and 2 young), 2 h of normal Tyrode perfusion did not induce any arrhythmias, indicating that either isolation and/or potentially time-related changes (“temporal control”) do not promote arrhythmias in our model. In two old rats, after induction of AF with GI, we switched perfusion to normal Tyrode. No AF could emerge after 60 min of normal Tyrode perfusion, indicating reversibility of GI-mediated AF.

EADs and triggered activity. In all atria of old, but not young rats, GI promoted EADs that clustered within 2.5 mm radius around the LA-PVJ (Fig. 3A). No EADs were seen at distances >2.5 mm from the LA-PVJ (Fig. 3A). These EADs led to triggered activity at the LA-PVJ (Fig. 4), which then led to AF in 13 out of 14 hearts (Fig. 4). The emergence of EADs coincided in time with the presence of elevated diastolic Ca\(^{2+}\) levels (Figs. 3 and 4), which was significantly (P < 0.01) greater at the LA-PVJ than at sites located >2.5 mm away from it (71 ± 15 vs. 34 ± 14% of peak systolic Ca\(^{2+}\) transient amplitude) during pacing at a CL of 200 ms. During triggered activity, the diastolic Ca\(^{2+}\) level at the LA-PVJ remained significantly higher than the diastolic Ca\(^{2+}\) level at sites remote (>2.5 mm) from the junction (20 ± 1.9 vs. 6.4 ± 3.1%, P < 0.01) (Figs. 3E and 4). Triggered activity was also preceded by a spontaneous slow rise of diastolic Ca\(^{2+}\) in 9 out of 13 old rats, with GI at the LA-PVJ but not at sites >5 mm away from it (Fig. 4). The mean peak slow rise of Ca\(^{2+}\) just before the onset of Ca\(^{2+}\) transient was 18 ± 6% of the peak systolic Ca\(^{2+}\) transient amplitude (Fig. 4).

Microelectrode recording, EADs, and triggered activity. To further characterize EADs during GI, we made standard glass microelectrode recordings in five additional old and five young rats before and after GI. No optical mapping was performed in these series of experiments. All rats were initially in sinus rhythm and showed no afterpotentials at baseline (Fig. 5A). However, after GI, spontaneous AF occurred in all five old rats, but in none of the young rats. GI promoted EADs and short runs of rapid, repetitive atrial activity at CLs of 65 ± 11 ms (Fig. 5, B–D). EADs eventually led to triggered activity, causing AF (Fig. 5E). The voltage range over which EADs arose varied between −40 and −70 mV (mean of −55 ± 8 mV). No EADs or triggered activity occurred in any of the young atria, either before or after GI.

Rate of decline of Ca\(^{2+}\) transient. The mean τ of the Ca\(^{2+}\) transient decline in the mapped field was significantly longer in old compared with young rats, both before and after GI (P < 0.01) (Fig. 6A). The difference in τ between the two groups after GI further increased as the pacing CL was progressively decreased from 200-ms PCL to 100 ms (Fig. 6C). Significant regional differences in τ were also observed in the old but not in the young atria after GI. The slowest rates of Ca\(^{2+}\) removal clustered within 2.5-mm radius around the LA-PVJ (77 ± 11 ms within 2.5 mm of the LA-PVJ vs. 50 ± 9 ms at LA sites >2.5 mm away from the junction) (P < 0.001) (Fig. 6B). For
the detection of regional differences in $\tau$, up to 20 sites at each of the LA-PVJ (within 2.5 mm of LA-PVJ) and at sites >2.5 mm from the junction were measured and analyzed in each of the 14 old rats before and after GI.

APD and Ca$^{2+}$ transient alternans. APD and Ca$^{2+}$ alternans were induced by rapid atrial pacing in both young and old atria. At a pacing CL of 100 ms, the alternans ratio (defined as $1 - S/L$, where $S$ is the small Ca$^{2+}$ transient or short APD, and $L$ is the large Ca$^{2+}$ transient or long APD during alternans) was significantly larger in old compared with young atra, both before (0.17 ± 0.04 vs. 0.02 ± 0.02, $P < 0.001$ for APD$_{90}$, and 0.13 ± 0.06 vs. 0.01 ± 0.01, $P < 0.05$ for Ca$^{2+}$ transient, respectively) and after GI (0.31 ± 0.14 vs. 0.09 ± 0.11, $P < 0.005$ for APD$_{90}$, and 0.31 ± 0.14 vs. 0.15 ± 0.14, $P < 0.05$ for Ca$^{2+}$ transient, respectively) (Fig. 7, A and B). Moreover, after GI in old but not in young atria, the APD and Ca$^{2+}$ transient alternans at a mean pacing CL of 120 ± 10 ms became spatially discordant (Fig. 7D). However, as previously seen in the ventricular tissue (7, 36), atrial electromechanical alternans remained concordant, i.e., the longer APD is associated with the higher Ca$^{2+}$ transient amplitude, and the shorter APD is associated with the smaller Ca$^{2+}$ transient amplitude (Fig. 7D).

Pacing-induced spatially discordant alternans in the old rats with GI also led to AF, either during the pacing (9 out of 14) or immediately after the end of the pacing (5 out of 14). The mean atrial PCL that induced AF in the old rats with GI was 130 ± 20 ms. Pacing at similar CLs did not induce AF in young rats. These results are consistent with our laboratory’s previous experimental (17) and simulation studies (39) that showed that heterogeneous (but not homogenous) partial and completed uncoupling in a two-dimensional excitable medium promotes alternans.

Effects of GI on ERP and APD restitution. ERP was determined at two to three left and right atrial sites, at baseline and 15–20 min after the onset of GI during pacing at a CL of 200 ms. At baseline, the mean ERP of pooled RA and LA sites (no difference was seen between RA and LA) was 49 ± 20 ms and 47 ± 25 ms in the young and old atria, respectively. After GI, while the mean ERP tended to increase in the young and old rats (63 ± 15 and 69 ± 38 ms, respectively), these differences, however, were not statistically significant ($P > 0.50$). GI had no significant effect on the APD in young rats, both at the LA-PVJ (73 ± 6 vs. 75 ± 7 ms, $P = 0.05$) and in the LA (67 ± 9 vs. 68 ± 9 ms, $P = 0.02$) (Fig. 8). However, in the old rats, the APD was significantly ($P < 0.01$) longer within 2.5-mm radius of the LA-PVJ than at LA sites >2.5 mm, both before and after GI (Fig. 8). GI in the old rats had no significant effect at LA sites >2.5 mm away from the LA-PVJ (Fig. 8). GI had no effect on the maximum slope of the APD restitution curve in the old (0.41 ± 0.3 vs. 0.42 ± 0.19) and young (0.56 ± 0.24 vs. 0.36 ± 0.09) rats within a 2.5-mm radius of the PV-LAJ and at LA sites >2.5 mm away from the junction.

Body and atrial weights. There were no significant differences in the body weight between the young (337 ± 47 g) and the old (359 ± 45 g) rats ($P > 0.20$). The atrial weight, however, was significantly ($P < 0.001$) greater in the old (0.74 ± 0.15 g) compared with young (0.27 ± 0.03 g) rats.

Interstitial fibrosis. The percent area occupied by interstitial fibrosis relative to the total atrial area was significantly higher in the old compared with young atria (13.5 ± 11.9 vs. 1.2 ± 0.7, $P < 0.001$) (Fig. 9), consistent with our laboratory’s previous report (17).

DISCUSSION

Novel findings. The novel finding of this study is that the stress of GI in old, but not young, rat hearts leads to a high incidence of spontaneous AF related to triggered activity arising in the vicinity of the LA-PVJ, as is often the case in human AF (4, 5, 16, 21). Our findings show that, with GI, the LV-PVJ in old (but not young) atria become very susceptible to the development of EADs and triggered activity at CLs ~60 ms. This focal triggered activity then paces the atria rapidly enough
to induce discordant alternans, creating a dynamic substrate for wavebreak (34, 50) and AF. Our findings also implicate differences in SCR Cai^{2+}/H11001 handling and APD between the LA-PVJ and the LA in the old but not young rats, potentially predisposing the LA-PVJ in the old rats to the development of EADs and triggered activity. The relatively larger atrial mass in the aged rats might also contribute to the longer life span of AF in the old rats after GI (17). Taken together, these data indicate

Fig. 6. Time constants of Cai^{2+} decline (τ) in young and old rats before and after GI. A: monoeponential fit of the decline phase of the Cai^{2+} (gray line). Mean τ of the mapped field is longer in the old compared with young rats, both before and after GI (A). B: significant regional differences in τ were seen between LA-PVJ (within 2.5-mm radius of the junction) and more distant (>5 mm) LA sites in the old but not young rats (solid symbols are old rats and open symbols are young rats). C: note a significant increase of τ was in the old but not young rats as the pacing CL (PCL) decreases from 200 to 100 ms, both before and after GI. Bars on the curves indicate SD of the mean from 14 old rats and 8 young rats.

Fig. 7. Discordant optical V (black signals) and Cai^{2+} (gray signals) alternans during pacing at a CL of 120 ms in an old rat GI (D). Note that, at baseline, no alternans is present in either young (A) or old (C) rats. However, after GI discordant APDs and Cai^{2+}, transient amplitude emerge in the old (D) but not the young (B) rat. Long APD and high-amplitude Cai^{2+} transient (L) at the LA-PVJ are associated with short APDs and smaller amplitude Cai^{2+} transient (S) at the LA (discordant alternans). The electromechanical alternans remains concordant during discordant APD-Cai^{2+} alternans. Note that the APA also alternates, an event that might result from activation arising from less repolarized membrane potential during the long APD, causing a smaller APA due to less recovery of sodium current from inactivation.
that alteration of Ca\textsuperscript{2+} dynamics and APD caused by GI interacts synergistically with fibrotic structural changes associated with aging to promote EAD-mediated triggered activity and sustained AF.

Ca\textsuperscript{2+} and the mechanism of EAD and triggered activity. EADs occur when the balance of net membrane current during repolarization reverses from outward to inward. In the present study, the EADs occurred when the diastolic Ca\textsuperscript{2+} levels were still elevated (Fig. 4). Elevated Ca\textsuperscript{2+} has been shown to promote EADs in the atria (3), PVs (35), ventricular myocytes (43), and in this study. EADs and EAD-mediated triggered activity clustered within 2.5-mm radius of the LA-PVJ where the rate of Ca\textsuperscript{2+} removal was the slowest, the APD longer, and the diastolic Ca\textsuperscript{2+} levels elevated compared with more distant LA sites. Perhaps the elevated Ca\textsuperscript{2+} explains the relatively longer APD at the junction via the activation of the Na/Ca exchanger that promotes a net inward current, causing APD prolongation (43). We do not know why the LA-PVJ manifests a different Ca\textsuperscript{2+} handling pattern than other LA sites. However, this observation is consistent with previous reports showing rapid focal activity originating from the PV and LA-PVJ (4, 6, 16, 21). The ionic mechanisms of GI-induced EADs in the old rats remain to be defined. These EADs arose over a

Fig. 8. Bar graphs of APD to 90% repolarization in old and young rats before and after GI. In the old rats, the APD was longer within 2.5-mm radius of the LA-PVJ than in areas $>2.5$ mm away from the junction, both before and after GI. In the young rats, no regional differences in the APD were seen, and GI exerted no effect on the APD in the young rats.

Fig. 9. Histological sections of trichrome staining at the LA-PVJ (A and C) and LA sites (B and D) in a young (A and B) and an old rat (C and D). Note the increased interstitial fibrosis (blue stain) in the old rat, causing separation of myocardial bundles and myocytes (stained red) in C and D. Epi, epicardium.
relatively broad range of membrane voltages that spanned between −40 and −70 mV (mean −55 ± 8 mV). Previous work suggests that Ca<sup>2+</sup>-sensitive inward current, such as the Na/Ca exchanger current, can produce EADs (43). We tested the effects of elevating external calcium from 1.35 to 4.08 mM in the old and young rats and were able to promote EADs, triggered activity, and AF in the old (5 out of 5) but none (0 out of 5) in the young rats (data not shown). These data suggest a key role for an elevated Ca<sup>2+</sup> in causing EADs. The ability of the small depolarizing EADs to successfully reach threshold and propagate as triggered activity may have been facilitated in the aged atria by increased interstitial fibrosis, which promotes partial electrical uncoupling (42) and reduces the “sink” effect by neighboring atrial cells, allowing EADs to emerge (19). Small depolarizing currents in well-coupled cells may not succeed in reaching threshold due to the electrical load of adjacent cells (19). The importance of increased atrial fibrosis in AF has been demonstrated in transgenic mice (44), in canine atria with heart failure (28), or partial gap junctional uncoupling with heptanol (33), and in aged atria (17). However, in none of these studies was spontaneous AF initiated. We propose that the additional stress imposed by GI may be necessary to provide the two necessary components for the initiation of spontaneous AF: partial uncoupling and elevated diastolic Ca<sup>2+</sup> to promote EAD and triggered activity leading to AF.

Rate of Ca<sup>2+</sup> transient decline, EAD, and triggered activity. The clustering of EADs and triggered activity in the old rats at the LA-PVJ may be explained by the slower rate of decline of Ca<sup>2+</sup> transient at this site. Glycolysis has been implicated as a preferential energy source for SERCA2a in the SR (51) and sarcotubular functions (32, 49). Since SERCA2a activity decreases in the senescent rats (41), additional inhibition by GI may alter Ca<sup>2+</sup> handling to a sufficient degree to enhance diastolic Ca<sup>2+</sup> elevation, causing EADs. We observed spontaneous and slow rise in diastolic Ca<sup>2+</sup> in old rats, with GI preceding EAD-mediated triggered activity. We do not know the mechanism of this slow, spontaneous rise of diastolic Ca<sup>2+</sup>; however, a recent study by Zima et al. (52) suggests that pyruvate directly increases SR calcium content, which then facilitates spontaneous calcium release (13). Alternatively, other sources of Ca<sup>2+</sup>, such as mitochondria, could also be important (10). Finally, it is also possible that EADs may also arise in the absence of diastolic elevation of Ca<sup>2+</sup> seen in some of the EAD episodes in previous (45) and in the present study.

Discordant alternans and wavebreak. The emergence of discordant APD and Ca<sup>2+</sup> alternans during rapid rates of activation, caused either by rapid pacing or triggered activity, provides a dynamic substrate for wavebreaks, causing a transition from single to multiple wavelets (34, 50). Rapid activation rates during multiple wavelet AF could, in turn, perpetuate elevation of diastolic Ca<sup>2+</sup> (positive feedback), promoting EAD-mediated triggered activity and perpetuation of the AF (3). The absence of steep APD restitution in old rats with GI suggests altered Ca<sup>2+</sup> dynamics as an alternate potential mechanism of APD/Ca<sup>2+</sup> alternans (13, 14, 50). Moreover, two-dimensional simulation studies have shown that heterogeneous partial and complete cellular uncoupling, as might occur in the case of increased interstitial fibrosis, facilitated the emergence of alternans that was independent of electrical restitution (39).

Limitations. It may be argued that EADs result from an electronic current arising from underlying activation masqueraded as EADs. However, we did not detect any evidence of such activation in the simultaneous bipolar electrograms (recorded outside the mapped region), making this explanation unlikely. It may also be argued that the optically recorded EADs may result from motion artifact. However, the clustering of EADs at some (LA-PVJ) but not other sites, and perhaps more importantly the demonstration of EADs with single-cell glass microelectrode recordings, refute motion artifact as a cause of the EADs. A potential role of the uncoupler in our studies can also be refuted, as we have used low concentration (2.5 μM) of CytoD to decrease motion, which has no significant effect on rat atrial APD (Miyauchi Y and Karagueuzian HS, unpublished observations). Finally, it is possible that some of the triggers of AF may also be caused by reentry (53) rather than by triggered activity.

Potential clinical impact. Human paroxysmal AF commonly involves triggers at the LA-PVJ (4, 5, 16). Because of the high degree of reproducibility of our model and its amenability to detailed analysis at the molecular, cellular, and tissue levels, this preparation potentially represents a very useful model relevant to human AF. While the clinical relevance of GI may be questioned, we point out that replacement of glucose with pyruvate is a relatively “mild” form of GI, which may occur under a variety of diseased cardiac conditions (12, 24, 31), including cardiac hypertrophy and the presence of free radicals, which increases with aging, causing SERCA2a inhibition (9). Finally, the rapid yet regular activity at the onset of AF, which resembles atrial flutter, offers a new insight into the mechanism of the commonly observed conversion of atrial flutter to AF in human patients (27, 46).

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GRANTS

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