Increased susceptibility of aged hearts to ventricular fibrillation during oxidative stress

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Hydrogen peroxide (H2O2) readily promotes early afterdepolarizations (EADs) and triggered activity (TA) in isolated rat and rabbit ventricular myocytes. Here we examined the effects of H2O2 on arrhythmias in intact Langendorff rat and rabbit hearts using dual-membrane voltage and intracellular calcium optical mapping and glass microelectrode recordings. Young adult rat (3–5 mo; N = 25) and rabbit (3–5 mo; N = 6) hearts exhibited no arrhythmias when perfused with H2O2 (0.1–2 mM) for up to 3 h. However, in 33 out of 35 (94%) aged (24–26 mo) rat hearts, 0.1 mM H2O2 caused EAD-mediated TA, leading to ventricular tachycardia (VT) and fibrillation (VF) in 40% of the hearts. Middle-aged rabbit (3–5 yr; normal rabbit life span, 8–12 yr) hearts, in which fibrosis was less extensive (5–35%), developed EADs, TA, VT, and VF in >90% of the hearts. Middle-aged rabbit (3–5 yr; normal rabbit life span, 8–12 yr) hearts, in which fibrosis was less extensive (5–35%), developed EADs, TA, VT, and VF in 40% of the hearts. Although other aging-related factors may also play a role, we hypothesize that reduced cell-to-cell coupling in fibrotic aged hearts promotes oxidative EAD-related arrhythmias by favorably altering source-sink relationships. Computer simulations of two-dimensional (2-D) cardiac tissue incorporating fibrosis supported this interpretation.

MATERIALS AND METHODS

The research protocol was approved by the Institutional Animal Care and Use Committee and followed the guidelines of the American Heart Association.

Langendorff Setup

Male Fisher 344 adult (3–5 mo; N = 25) and aged (24–26 mo; N = 35) rats and adult White New Zealand rabbits (N = 6) ages 3–5 mo and middle aged (N = 10) between ages 3 to 5 yr were used in this study. The exact age of these rabbits could not be ascertained. The aged rats were purchased from the National Institute on Ageing and the rabbits from a private breeder. The hearts of both species were exposed to oxidative stress, fibrosis facilitates the ability of cellular EADs to emerge and generate TA, VT, and VF at the tissue level.
isolated and perfused with oxygenated Tyrode solution at 37 ± 0.5°C in a Langendorff setting as we described previously (22).

**Optical Mapping**

The hearts were stained with RH237 and Rhod-2 AM (Invitrogen Molecular Probes, Carlsbad, CA) for simultaneous dual voltage (V) and intracellular calcium (Ca^{2+}) fluorescent optical imaging, respectively, as described previously (22). Ca^{2+} transient decay rate constant, τ, was determined by a monoexponential fit during the relaxation. Cytochalasin D (5 μmol/l) was added to the perfusate to inhibit motion (9). Single cell action potentials (APs) were recorded with a roving glass microelectrode from left ventricular (LV) epicardial sites showing focal VT as shown by optical voltage activation maps.

**Dynamic AP Duration Restitution**

AP duration (APD) was measured at 90% (APD_{90}) and 50% (APD_{50}) repolarization, and APD restitution curves were determined using a dynamic pacing protocol (22) from the base of the heart. In each heart, the APs were recorded from 3–5 different sites located at the base, mid-, and apical region of the LV anterior surface. Results were pooled since no differences in APD and APD restitution were found among the different sites and between the two aged groups. When VFs were initiated, they were terminated within 1 min of onset by electrical shocks delivered through a pair of 5-cm-long coil electrodes in the tissue bath located at both sides of the isolated hearts.

**Chemical and Pharmacological Interventions**

The isolated hearts were exposed to increasing levels 0.05–2 mM of H_{2}O_{2}. In adult rat and rabbit hearts, raising the H_{2}O_{2} level from 0.01, 0.05, 0.1, 0.2, 1, and 2 mM failed to induce VT/VF in all eight of H_{2}O_{2}. In contrast, however, 0.05 mM H_{2}O_{2} 0.01, 0.05, 0.1, 0.2, 1, and 2 mM failed to induce VT/VF in all eight of H_{2}O_{2}. In adult rat and rabbit hearts, raising the H_{2}O_{2} level from the base, mid-, and apical region of the LV anterior surface. Results were pooled since no differences in APD and APD restitution were found among the different sites and between the two aged groups. When VFs were initiated, they were terminated within 1 min of onset by electrical shocks delivered through a pair of 5-cm-long coil electrodes in the tissue bath located at both sides of the isolated hearts.

**Histological Analyses**

Percent tissue fibrosis was determined using Masson trichrome stain for collagen as previously described (20). Briefly, with the use of a grid that divided the field of view into 100 squares, the number of collagenous tissue (blue stain) at the 100 intersection points in the grid was scored as 1 (present) or 0 (absent). Results are expressed as the percentage occupied by fibrosis to the total area examined. In few tissue samples, fibroblast receptor discoidin domain receptor 2 was used to test for their fibroblast-to-myocyte ratio (see supplemental online version of this article). In the fibrosis region, fibroblasts were coupled with individual myocytes at densities ranging from 0.5 to 3.

**Computer Simulation Studies**

A 5-cm-by-2.5-cm 2-D tissue with an ellipse fibrosis region of radius 1.8-cm by 0.9-cm in the center was used to simulate the effect of fibroblasts on EAD-triggered focal activity (see supplemental diagram; note: all supplemental material may be found posted with the online version of this article). In the fibrosis region, fibroblasts were coupled with individual myocytes at densities ranging from 0.5 to 3. We modified the rabbit ventricular myocyte model (18) to satisfy the ionic changes in H_{2}O_{2} perfusion by doubling the maximum conductance of sodium/calcium exchange current (I_{NCX}) (6) and peak L-type calcium current (I_{Ca,L}) (39) and by decreasing the Ca^{2+} transient decline rate by 20% (12) and maintaining 0.04 of the peak sodium current (I_{Na}) during the repolarization phase to mimic the late I_{Na} (32, 35, 36).

The membrane voltage (V) of a myocyte (V_{m}) or a fibroblast (V_{f}) in the tissue model is governed by the following:

\[ C \frac{dV}{dt} = -I_{m} + \sum_{k=1}^{n} G_{f,k} (V_{m} - V) \]  

where C is the membrane capacitance of a myocyte (C_{m}) or a fibroblast (C_{f}), I_{m} is the corresponding membrane current (I_{m} or I_{f}), n is the number of coupled neighbors (either myocytes or fibroblasts), and G_{f,k} is the gap junction conductance between a cell (either a myocyte or a fibroblast) and its k^{th} neighbor (either a myocyte or a fibroblast). The size of the myocyte was set to 25 μm × 25 μm × 25 μm, with C_{m} = 125 pF. I_{m} was formulated from the rabbit ventricular model developed by our group (18, 31). The size of the fibroblast was set to 25 μm × 25 μm × 25 μm. For the membrane current of the fibroblast, we mainly used a passive model (15), i.e.,

\[ I_{f} = G_{f} (V - E_{f}) \]

where G_{f} is the membrane conductance and E_{f} is the resting potential of the fibroblast. We set C_{f} = 25 pF (16, 33), G_{f} = 3 nS (15), and E_{f} = −10 mV (14).

To generate the 2-D tissue model, fibroblasts were randomly inserted between myocytes in the fibrotic region, as illustrated in supplemental Fig. 2. To generate this tissue model, we first generated a mesh with a spatial resolution of 25 μm × 25 μm. A fibroblast accounts for one such box, whereas a myocyte accounts for five boxes in a row. Once a fibroblast-to-myocyte ratio (α) is assigned, then the probability of a box being a fibroblast can be determined as α/(α + 5), and a random number is generated to determine whether the present box is a fibroblast or not. If the box is not a fibroblast, then an additional four consecutive boxes in the row are used to form a myocyte (composed of 5 boxes). This process is repeated until all the boxes are assigned as either fibroblasts or myocytes. The gap junction conductance between myocytes was set to 600 nS when two myocytes were coupled end to end and to 1,000 nS when they were fully overlapped side to side. When two myocytes were partially overlapped side to side, the gap junction conductance was proportional to the overlap. This gives rise to a longitudinal conduction velocity of 0.56 m/s and a transverse conduction velocity of 0.13 m/s when no fibroblasts are present. The gap junction conductance between myocyte and fibroblast or between two fibroblasts was set to 5.4 nS (27).

**Statistical Analysis**

Differences in the Ca^{2+} transient rate constant and APDs at different pacing cycle lengths (CLs) were determined using repeated-measures two-way ANOVA. Differences among individual means were verified subsequently by Newman-Keuls post hoc tests. Likelihood ratio test was used to determine the significance of site-specific origination of focal activity. P = 0.05 was considered significant. All data are presented as means ± SD.

**RESULTS**

H_{2}O_{2} Fails to Induce EAD-Related Arrhythmias in Young Adult Rat and Rabbit Hearts

Isolated Langendorff rat and rabbit hearts were arterially perfused with Tyrode solution and then exposed to H_{2}O_{2} added directly to the perfusate. All hearts were in regular sinus
rhythm after mounting in a tissue bath. In 25 of 25 hearts isolated from young adult rats, the addition of 0.1 mM H$_2$O$_2$ to the perfusate failed to cause spontaneous ventricular arrhythmias during monitoring for up to 3 h. Raising H$_2$O$_2$ concentration up to 2 mM also failed to induce arrhythmias in eight adult rat hearts studied. Similar results were obtained in six young adult (3–5 mo old) rabbit hearts. These findings are consistent with the hypothesis that EADs are suppressed by cell-to-cell coupling in intact tissue, since H$_2$O$_2$ concentrations that consistently induced EADs and TA in isolated rat and rabbit ventricular myocytes (32, 36, 41) failed to induce EAD-related arrhythmias in all adult rat and rabbit intact hearts studied.

### H$_2$O$_2$ Promotes Spontaneous VF in the Aged Rat and Middle-Aged Rabbit Hearts

Since aging is associated with increased cardiac fibrosis, which disrupts normal cell-to-cell coupling, we examined whether fibrotic aged hearts are more susceptible to EAD-mediated arrhythmias in response to H$_2$O$_2$. We studied aged rats (24–26 mo) and middle-aged rabbits (3–5 yr) since aged rabbits were not available. Histological analysis using Mas"on’s trichrome collagen staining confirmed earlier report (7) of a marked increase in fibrosis in aged rat ventricles compared with those of adults (Fig. 1A), averaging 45 ± 26% of tissue area (Fig. 1B). The distribution of fibrosis was highly heterogeneous, varying between 10% in the right ventricular (RV) to 90% in the LV endocardium (Fig. 1, C–E). Middle-aged rabbit hearts, however, had less extensive fibrosis. Six of the 10 had 18 ± 5% overall fibrosis, whereas the remaining four middle-aged rabbits had ventricular fibrosis averaging 45 ± 16%, ranging from 15% in the RV and mid-LV wall to 40% in the anterior and posterior LV near the base of the heart (Fig. 1G).

In contrast, fibrosis was minimal in adult rat hearts, averaging 4 ± 2.5% of the ventricles and 3 ± 1% in adult rabbit ventricles (P < 0.001 compared with aged rat and middle-aged rabbit ventricles) (Fig. 1, A, B, and G).

### Arrhythmias in aged rat hearts.

In response to 0.1 mM H$_2$O$_2$, 33 of 35 of the aged rat hearts (94%) developed spontaneous VT/VF after a mean perfusion time of 17.6 ± 7.1 min. As shown in Fig. 2A, VF was preceded by a transient period of VT (mean CL of 70 ± 18 ms), which arose suddenly from regular sinus rhythm with a mean CL of 380 ± 162 ms. Within 2 s, the VT degenerated to sustained VF (CL of 55 ± 16 ms) (Fig. 2A), requiring electrical shock for termination. However, VT/VF reoccurred repeatedly within 2 to 3 min after defibrillation in all 33 hearts, allowing us to capture the onset of multiple VT/VF episodes in each heart. Voltage activation maps showed that in each of the 10 mapped hearts, VT had a focal mechanism that preferentially originated from the base of the LV anterior epicardium in 76% of the episodes (68 out of 89 VT episodes in the 10 mapped hearts). A likelihood ratio test indicated that the origination of the focal VT from the LV base in 76% of the VF episodes was not random but a highly significant (P < 0.001) event. The focal VT propagated as single wavefront from the base of the heart to the apex as shown in the snapshots in Fig. 2B, promoting spatially discordant APD alternans (Fig. 2D), causing localized conduction block (wavebreak) midway between the base and the apex of the heart. After the block, the wavefront continued to propagate laterally past the site of the block, forming a figure-eight-type reentry (Fig. 2B), coinciding in time with the transition from the VT to VF on the ECG (Fig. 2A). In the remaining 21 episodes, the origin and the mechanism of the transient VT preceding the VF could not be determined. In these episodes, a single wavefront entered the mapped region either from the apical site of the LV (14 out 89 episodes, i.e., 18%) or from the left lateral region of the LV (7 out of 89 episodes, i.e., 6%). The effects of H$_2$O$_2$ were reversible. After washout with H$_2$O$_2$-free Tyrode solution for 20 min, spontaneous VT/VF episodes ceased to reoccur for up to 3 h as observed after H$_2$O$_2$ washout.

### Arrhythmias in middle-aged rabbits.

Four of the 10 middle-aged rabbit hearts (40%) with an overall LV fibrosis averaging 45 ± 16% developed similar arrhythmias, culminating in VF after a mean perfusion time of 21 ± 8 min with 0.1 mM H$_2$O$_2$. Figure 3 illustrates an example. As in the case of aged rats, VF was preceded by a transient period of VT (mean CL of 130 ± 20 ms), which arose suddenly from regular sinus rhythm with a mean CL of 460 ± 80 ms. Within 3 s, the VT degenerated to sustained VF (CL of 105 ± 15 ms), requiring electrical shock for termination. However, the reoccurrences of VF after shock-induced termination of VF were less frequent in the middle-aged rabbit hearts compared with aged rats in which repeat spontaneous VF episodes frequently reoccurred. As a result, we could not conduct systematic microelectrode (except 2 episodes of spontaneous VF) and pharmacological studies on VF in the middle-aged rabbit as we did in the aged rats.

### Epicardial EAD-Mediated TA Initiates Spontaneous Focal VT

To gain insight into whether EAD-mediated TA was the cause of focal VT, we studied aged rat hearts exposed to H$_2$O$_2$ in greater detail. After terminating VF with an electrical shock, we then continuously recorded with a roving glass microelectrode transmembrane single cell APs from the LV base (the most frequent site of origin of the focal VT) to capture the onset of new VT/VF episodes. With this approach, we successfully captured the onset of 3–5 episodes of VT/VF in each of the 10 aged rat hearts studied. Figure 4 illustrates an example of a VT episode that precedes the VF. As shown in Fig. 4, the VT was initiated by an EAD-mediated TA that arose from a mean takeoff potential of −51 ± 16 mV (42 cells in 10 hearts). The mean CL of the TA was 66 ± 10 ms and was not significantly different from the mean CL of the VT (70 ± 18 ms). The EAD preceded the QRS complex of a simultaneously recorded ECG by a mean of 8 ± 4 ms and occurred during an isoelectric interval on the ECG as shown in Fig. 4A, indicating the absence of electrical activity elsewhere in the heart during the EAD formation. In two middle-aged rabbits, microelectrode recordings at the onset of spontaneous VF (Fig. 3C) showed a similar pattern of VF initiation as in aged rat hearts (Fig. 4) that arose suddenly during the sinus rhythm by a mechanism suggestive of EAD-mediated TA, causing VT and VF. Figure 5A shows that the EADs arise when Ca$^{2+}$ remains high relative to the diastolic resting level, reflecting a slowed rate of decline of Ca$^{2+}$ (see Table 1). Interestingly, the rapid TA supported by a single wavefront underwent wavebreak and reentry after the emergence of spatially discordant APD and Ca$^{2+}$ alternans (Fig. 5, B and C). This dynamic scenario starting with a focal TA causing VT then progressing to VF after the emergence of spatially discordant APD/Ca$^{2+}$ alternans is compatible with the scenario shown in Figs. 2 and 3.
Fig. 1. Masson trichrome and discoidin domain receptor-2 staining in adult and aged ventricles. A: low-power magnification of the entire cross-sectional view of an adult and aged rat hearts. Notice the increased fibrosis in the aged (blue stain) with almost complete fibrosis at the endocardium (Endo) and diffuse fibrosis of the posterior left ventricle (LV) and the septum. B: overall percent area of fibrosis in adult and aged ventricles. C and D: regional variations of percent fibrosis at 14 different ventricular sites. E: trichrome stain that stains collagen blue. F: discoidin domain receptor 2 that stains the fibroblast in green. G: trichrome stain in adult (left) and middle-aged rabbits (middle and right) in which \( \text{H}_2\text{O}_2 \) induced spontaneous ventricular fibrillation (VF). Ant, anterior; RV, right ventricle; Post, posterior; Epi, epicardium; NS, not significant.
To determine a potential role of epicardial breakthrough activation in the genesis of “focal VT” on the LV epicardial surface, we cryoablated the endocardium and the midmyocardium in five aged rat hearts, sparing only a thin rim of viable epicardium in the LV. As shown in Fig. 6, an exposure of these cryoablated hearts to H2O2 still evoked EADs (total of 13 episodes of EADs in 5 cryoablated hearts with 2 to 3 episodes in each heart) by the surviving epicardial cells at the base of the LV that propagated toward the apex causing VT. These findings indicate that in the LV, the epicardial cells at the base of the rat heart manifest an intrinsic ability to generate EADs, causing focal VT independent of deeper myocardial cells.

H2O2-Induced Arrhythmias in Aged Rat Hearts Involve Oxidative Activation of CaMKII

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H2O2-Induced Arrhythmias in Aged Rat Hearts Involve Oxidative Activation of CaMKII

To confirm that H2O2-induced arrhythmias in aged rat hearts, like isolated myocytes, are mediated by oxidative stress, we tested the effects of the reducing agent NAC (2 mM) (21). When the reducing agent NAC was administered, either after H2O2 had already induced VF (N = 5; Fig. 7A) or 15 min before H2O2 (N = 5; Fig. 7B), NAC effectively suppressed and prevented, respectively, the VF in all 10 aged hearts studied. The effect of NAC was reversible. Upon washout of the NAC, the VF reemerged after a mean washout time of 18 ± 5 min (Fig. 6).

Since H2O2-induced EADs and TA in isolated rabbit myocytes depend on oxidative activation of CaMKII (5) by H2O2 (1, 41), we tested the efficacy of the specific CaMKII inhibitor KN-93 (1 μM) (1, 35) on H2O2-mediated VF in 10 aged rat hearts. When KN-93 was perfused either 15 min after H2O2 had already induced the VF (N = 5; Fig. 8A) or 15 min before H2O2 exposure (N = 5; Fig. 8B), KN-93 suppressed and prevented VF in all 10 hearts, respectively. The effect of KN-93 was reversible. Upon washout of KN-93, the VF reemerged after a mean 14 ± 4 min as shown in Fig. 7. In contrast, KN-92 (1 μM), the inactive form of the KN-93 (1, 35), failed to prevent or suppress H2O2-mediated EADs and VF in all five aged rat hearts tested.
These findings suggest that the mechanisms by which H$_2$O$_2$ induces EADs and EAD-mediated arrhythmias in intact aged hearts are the same as in isolated myocytes involving oxidative activation of CaMKII (36).

Two-Dimensional Computer Simulations Support Fibrosis as a Mechanism Promoting EAD-Mediated Arrhythmias

To investigate whether reduced cell-to-cell coupling due to fibrosis is sufficient to account for the emergence of EADs and TA in aged hearts exposed to oxidative stress, we performed computer simulations in 2-D cardiac tissue in which the ratio of fibroblasts to ventricular myocytes coupling was varied (supplemental Fig. 1) using a rabbit ventricular AP model (18). Longitudinal coupling between myocytes had a gap junction conductance of 600 nS, and H$_2$O$_2$-mediated EADs were simulated by doubling the maximum conductance of $I_{\text{NCX}}$ (6, 17) and peak $I_{\text{Ca-L}}$ (39, 41), decreasing the $C_{ai}$ transient decline rate by 20% (12) and by maintaining 0.04 of the peak $I_{\text{Na}}$ during the repolarization phase to mimic the late $I_{\text{Na}}$ (32, 35, 36). As shown in Fig. 9, when the fibroblast-to-myocyte ratio was in an intermediate range of 1.3–1.5 (25–30% by area), EADs were of sufficient amplitude to propagate into the surrounding tissue, causing repetitive TA (Fig. 9B).

These findings agreed well with the histological analysis in aged rat hearts when we compared the percent tissue fibrosis at the site of frequent origination of EADs and TA (i.e., the LV epicardial base) to 13 other LV and RV sites. As shown in Fig. 1C, the site of frequent EAD origination had an intermediate percent fibrosis (mean of 28%) compared with RV (4%) and to mid- and apical LV anterior epicardium (10–20%) and posterior LV epicardial, endocardial, and septal regions (40–90%) (Fig. 1, D and E). Fibrosis was characterized both by increased collagen deposits (Fig. 1, E and G) and increased fibroblast density as shown by discoidin domain receptor 2 staining of fibroblast receptors (3) (Fig. 1F).

Electrophysiological Differences Between Young Adult and Aged Rat Hearts

Although the computer simulations (Fig. 9) support increased fibrosis in aged hearts as a plausible explanation for their increased susceptibility to H$_2$O$_2$-induced arrhythmias, aging-related changes in electrophysiological and calcium cycling properties at the myocyte level may also be important. In comparing young adult versus aged rat hearts, however, we found that H$_2$O$_2$ had no significant effect on APD$_{90}$, APD$_{50}$, or the maximum APD restitution slope, both before and after...
exposure to H$_2$O$_2$ (see supplemental Table). On the other hand, some differences in Cai$^{2+}$/H$_{11001}$ cycling between young adult and aged rat hearts were noted. The mean rate constant $\tau$ of the Cai$^{2+}$ transient decline (fit to a single exponential) was significantly longer in aged compared with young adult ventricles ($P < 0.05$), both before and after H$_2$O$_2$ exposure (Table 1). Furthermore, there was greater regional heterogeneity in Cai$^{2+}$/H$_{11001}$ handling in the aged than in the young adult rat hearts, with the slowest $\tau$ clustering at the base of the LV compared with mid- and apical sites (Table 1).

**DISCUSSION**

The major finding of this study is that when aged hearts are exposed to a level of H$_2$O$_2$-induced oxidative stress that is benign in young adult hearts, they are highly susceptible EAD-mediated arrhythmias and VF. The high reproducibility of spontaneous VT/VF in aged rat hearts makes this model potentially valuable for studying VF episodes arising abruptly during otherwise stable sinus rhythm without prior heart rate acceleration and/or visible T-wave alternans of the sinus beats (19). This study also highlights the influence of cell-to-cell coupling on the ability of EADs to form and cause arrhythmias in tissue. Isolated young adult rat and rabbit ventricular myocytes readily develop EADs and TA in response to 0.1 mM H$_2$O$_2$ (32, 36, 41). However, young adult rat and rabbit hearts exhibited no arrhythmias under the same conditions. This discrepancy supports the supposition that cell-to-cell coupling is a potent mechanism suppressing EAD formation in tissue by creating a source-sink mismatch that prevents local EAD currents generated by a small group of myocytes from reversing repolarization when they are electrotonically coupled to a large group of adjacent normally repolarizing myocytes.

To explore the effects of cell-to-cell coupling on EAD suppression, we performed computer simulations of 2-D cardiac tissue in which normal myocyte-to-myocyte coupling was disrupted by inserting fibroblasts into the tissue. With too few fibroblasts, EADs and TA were suppressed by cell-to-cell coupling, and with too many fibroblasts, EADs occurred but were unable to propagate. With an intermediate myocyte-to-fibroblast ratio, however, EADs both formed and successfully propagated into the surrounding tissue. These findings agreed well with the experimental results in aged rat hearts in which EADs and TA typically arose from regions with intermediate levels of fibrosis (30–40%), usually at the base of the LV epicardium. Importantly, our experiments in cyroablated aged rat hearts demonstrated that EADs were generated in the LV epicardium itself, rather than arising from the underlying tissue or the His-Purkinje system.

Although the disruption of normal cell-to-cell coupling by fibrosis provides a plausible explanation for the increased susceptibility of aged hearts to EADs and EAD-mediated arrhythmias, we cannot exclude the possibility that other aspects of aging-related remodeling (e.g., electrical, Cai$^{2+}$ cycling, and gap junction remodeling) also make important contributions. Nevertheless, isolated myocytes from young adult rat (or rabbit) ventricular myocytes developed frank EADs when exposed to H$_2$O$_2$ (32, 36, 41), even though intact young adult hearts never developed EADs, TA, or VT/VF when perfused with the same or even 20-fold higher H$_2$O$_2$ concentrations for up to 3 h. These findings indicate that young adult myocytes are clearly susceptible to H$_2$O$_2$-induced EADs, but something in the tissue environment suppresses their emergence. Our findings support a critical
The role of partial cellular uncoupling in EAD formation at the tissue level caused by increased interstitial tissue fibrosis. The mechanisms by which H$_2$O$_2$ induced EADs in isolated young adult ventricular myocytes and aged hearts seem to be identical, since both were suppressed by CaMKII inhibitors and antioxidants (41), consistent with the oxidative activation of CaMKII as the underlying molecular mechanism (40). It might be speculated that intact young adult hearts are less susceptible to H$_2$O$_2$-induced EADs because they have greater antioxidant capacity than aged hearts (8). However, this is unlikely since increasing perfusate H$_2$O$_2$ concentration 20-fold (2 mM H$_2$O$_2$) still failed to induce EADs, TA, VT, and VF in young adult rat hearts. Young adult and aged rat hearts also exhibited no differences in APD or APD restitution during regular pacing at different pacing CLs, either before or after oxidative stress. On the other hand, the rate of Cai$^{2+}$/H$_{11001}$ transient decline was significantly slower in aged than young adult ventricles, especially after H$_2$O$_2$, which directly inhibits sarc(endo)plasm reticulum calcium-ATPase 2a (28). We can-

Fig. 5. Simultaneous microelectrode (Me) and dual optical AP (O-AP)-intracellular calcium (O-Cai$^{2+}$/H$_{11001}$) map at the onset of VT/VF in an aged rat heart exposed to H$_2$O$_2$ (0.1 mmol/l). A: single cell APs recorded with glass microelectrode along with simultaneous optical AP-Cai$^{2+}$/H$_{11001}$ signals recorded within 1 mm of the microelectrode. Notice that the cellular EAD arises when Cai$^{2+}$ is about 80% of the peak systolic calcium transient amplitude, reflecting a slowed decline rate of Cai$^{2+}$. The Cai$^{2+}$ remains above the resting level (35%) during the TA, leading to VF. B: simultaneous 10 voltage and Cai$^{2+}$ optical signals recorded from base to apex (shown in the snapshots in C) during the transition from VT supported by a single wavefront to wavebreak causing figure-eight reentry (C; beat 1 to beat 2) after the emergence of spatially discordant APD and Cai$^{2+}$ alternans.

Table 1. Intracellular calcium decline rate constant ($\tau$) in aged and adult rat hearts

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Values are means ± SD; N, number of rats. Repeated-measures ANOVA comparison of intracellular calcium decline rate at different left ventricular (LV) sites during pacing at a cycle length of 200 ms are shown. *P < 0.05, significant difference between adult and aged hearts at all LV sites both at baseline and after H$_2$O$_2$; †P < 0.01, significant regional differences exist in the aged hearts between the base and the mid- and the apex of LV both at baseline and after H$_2$O$_2$. No differences are seen between mid- and apical sites at baseline and after H$_2$O$_2$ in the aged hearts. NS, not significant.

role of partial cellular uncoupling in EAD formation at the tissue level caused by increased interstitial tissue fibrosis.

The mechanisms by which H$_2$O$_2$ induced EADs in isolated young adult ventricular myocytes and aged hearts seem to be identical, since both were suppressed by CaMKII inhibitors and antioxidants (41), consistent with the oxidative activation of CaMKII as the underlying molecular mechanism (40). It might be speculated that intact young adult hearts are less susceptible to H$_2$O$_2$-induced EADs because they have greater antioxidant capacity than aged hearts (8). However, this is unlikely since increasing perfusate H$_2$O$_2$ concentration 20-fold (2 mM H$_2$O$_2$) still failed to induce EADs, TA, VT, and VF in young adult rat hearts. Young adult and aged rat hearts also exhibited no differences in APD or APD restitution during regular pacing at different pacing CLs, either before or after oxidative stress. On the other hand, the rate of Cai$^{2+}$ transient decline was significantly slower in aged than young adult ventricles, especially after H$_2$O$_2$, which directly inhibits sarc(endo)plasm reticulum calcium-ATPase 2a (28). We can-
not exclude that this effect promoted EADs by elevating $\mathrm{Ca}^{2+}$ during repolarization (23, 34). It may also have enhanced the susceptibility of aged hearts to $\mathrm{Ca}^{2+}$ and APD alternans (30), since depressed sarcoplasmic reticulum $\mathrm{Ca}^{2+}$ uptake is known to promote $\mathrm{Ca}^{2+}$ alternans (25, 37). Once APD alternans has developed, fibrosis is known to promote its transition to spatially discordant APD alternans (26). The combined effects of these two factors is likely to account for the experimental

Fig. 6. Cryoablation of mid- and endocardial structures in an aged rat heart and the effect of 0.1 mM $\mathrm{H}_2\mathrm{O}_2$. A: simultaneous microelectrode (top) and ECG (bottom) recordings in a heart subjected to cryoablation of the endo- and midmyocardial structures (whitish area in D). EADs, TA causing VF, are still present after the cryoablation procedure. C: optical snapshots of two EADs (1 and 2 in B) causing focal activation that arises from the base of the surviving thin LV epicardial rim. Snapshots in C show activation time starting with 0 ms (arbitrary) with the second EAD (EAD2) arising 68 ms after the first EAD (EAD1). Only a thin rim of epicardial tissue survives the ablation procedure as indicated by the reddish area in this triphenyltetrazolium chloride-stained cross-sectional view of the LV (D). Me, the site of microelectrode recording shown in A and B; Ant, Sep, Lat, and Post, anterior, septal, lateral, and posterior wall of the LV, respectively. Notice that the VF in this cryoablated heart terminates spontaneously within 1 min of initiation consistent with tissue mass reduction (Ref. 13).

Fig. 7. Suppression and prevention of $\mathrm{H}_2\mathrm{O}_2$-induced EAD and VF in 2 aged rat hearts by $\mathrm{N}$-acetylcysteine (NAC; 2 mM). A and B: simultaneous microelectrode and ECG recordings, showing NAC-induced suppression of EAD-mediated VF and its reemergence 16 min after NAC washout (WO). B: prevention of VF by NAC pretreatment and the emergence of VF 25 min after NAC WO.
observation that during focal VT, spatially discordant APD alternans preceded the degeneration of VT to VF.

Potential Clinical Impact

The clinical implications of our findings can be summarized as follows: VF is the most common cause of sudden cardiac death and prematurely claims the lives of ~300,000 persons every year in the United States (29). Many known triggers of oxidative stress, such as aging (11), heart failure (10), and ischemia-reperfusion (2), are linked to an increased risk of VF. Diffuse tissue fibrosis is also a common feature of diverse heart abnormalities, including heart failure (24), myocardial ische-
mi/a/infarction, and cardiomyopathy (38). Decreasing oxidative stress and preventing and/or reversing tissue fibrosis in these cardiac conditions may therefore be a promising novel strategy to reduce VF risk and possibly AF risk as well (4). The aged heart model could contribute to the understanding of VF triggers in patients that do not manifest visible T-wave alternans and/or heart rate acceleration before the onset of the VF episode.

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DISCLOSURES

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