Oxidative Stress–Induced Afterdepolarizations and Calmodulin Kinase II Signaling

Lai-Hua Xie, Fuhua Chen, Hrayr S. Karagueuzian, James N. Weiss

Abstract—In the heart, oxidative stress caused by exogenous H$_2$O$_2$ has been shown to induce early afterdepolarizations (EADs) and triggered activity by impairing Na current ($I_{\text{Na}}$) inactivation. Because H$_2$O$_2$ activates Ca$^{2+}$/calmodulin kinase (CaMKII), which also impairs $I_{\text{Na}}$ inactivation and promotes EADs, we hypothesized that CaMKII activation may be an important factor in EADs caused by oxidative stress. Using the patch-clamp and intracellular Ca (Cai) imaging in Fluo-4 AM–loaded rabbit ventricular myocytes, we found that exposure to H$_2$O$_2$ (0.2 to 1 mmol/L) for 5 to 15 minutes consistently induced EADs that were suppressed by the $I_{\text{Na}}$ blocker tetrodotoxin (10 μmol/L), as well as the $I_{\text{Ca,L}}$ blocker nifedipine. H$_2$O$_2$ enhanced both peak and late $I_{\text{Ca,L}}$, consistent with CaMKII-mediated facilitation. By prolonging the action potential plateau and increasing Ca influx via $I_{\text{Ca,L}}$, H$_2$O$_2$-induced EADs were also frequently followed by DADs in response to spontaneous (ie, non-$I_{\text{Ca,L}}$-gated) sarcoplasmic reticulum Ca release after repolarization. The CaMKII inhibitor KN-93 (1 μmol/L; n=4), but not its inactive analog KN-92 (1 μmol/L, n=5), prevented H$_2$O$_2$-induced EADs and DADs, and the selective CaMKII peptide inhibitor AIP (autocamtide-2–related inhibitory peptide) (2 μmol/L) significantly delayed their onset. In conclusion, H$_2$O$_2$-induced afterdepolarizations depend on both impaired $I_{\text{Na}}$ inactivation to reduce repolarization reserve and enhancement of $I_{\text{Ca,L}}$ to reverse repolarization, which are both facilitated by CaMKII activation. Our observations support a link between increased oxidative stress, CaMKII activation, and afterdepolarizations as triggers of lethal ventricular arrhythmias in diseased hearts. (Circ Res. 2009;104:79-86.)

Key Words: reactive oxidative species ■ early afterdepolarization ■ triggered activity ■ arrhythmia ■ CaM kinase

Reactive oxygen species (ROS) are generated as natural byproducts of normal oxygen metabolism and play important roles in cell signaling. However, under pathological conditions, such as heart failure and ischemia/reperfusion, ROS levels can become elevated and predispose the heart to arrhythmias. Reactive oxygen species (ROS) are generated as natural byproducts of normal oxygen metabolism and play important roles in cell signaling. However, under pathological conditions, such as heart failure and ischemia/reperfusion, ROS levels can become elevated and predispose the heart to arrhythmias. Oxidative stress induced by exposure to hydrogen peroxide (H$_2$O$_2$) and other agents has been shown to induce early afterdepolarizations (EADs), delayed afterdepolarizations (DADs), and triggered activity (TA), primarily attributed in previous studies to impaired inactivation of the Na current (late $I_{\text{Na}}$). However, H$_2$O$_2$ also affects other ion channels and transporters, including the L-type Ca current ($I_{\text{Ca,L}}$), K currents, ryanodine receptors (RyR), the sarcoplasmic reticulum (SR) Ca pump SERCA2a, and the Na/Ca exchanger ($I_{\text{NCX}}$), which also may influence EADs and TA.

Recently, H$_2$O$_2$ has been shown to activate Ca/calmodulin (CaM) kinase (CaMKII) in cardiac myocytes and other cells, by direct oxidation of paired Met residues (Met281/282) in the regulatory domain. It is notable that binding of Ca$^{2+}$/CaM is required to expose the redox sites in the regulatory domain in order for oxidation to persistently activate CaMKII. In addition, ventricular myocytes isolated from transgenic mice overexpressing CaMKIV have been shown to exhibit EADs and TA. Increased endogenous CaMKII activity accompanied EADs and ventricular tachycardia/ventricular fibrillation observed during telemetry in this mouse model. Alterations in $I_{\text{Ca,L}}$ properties have been implicated as a major factor by which CaMKII activation promotes EADs, but a recent study demonstrated that CaMKII activation also increases the late $I_{\text{Na}}$ similar to H$_2$O$_2$. CaMKII inhibition has been shown to suppress EADs and TA. In a genetic heart failure model, for example, CaMKII inhibition prevented EADs and TA and improved mortality. The ability of pharmacological agents such as clofilium to induce EADs in isolated rabbit ventricular myocytes was also suppressed by CaMKII inhibition.

Taken together, this evidence led us to hypothesize that activation of CaMKII by oxidative stress may be an important factor in arrhythmogenic effects of H$_2$O$_2$. Accordingly, we used isolated patch-clamped rabbit ventricular myocytes and
Cai imaging to analyze the ionic mechanism(s) underlying H2O2-induced afterdepolarizations in the absence and presence of CaMKII inhibition. Our findings support a direct link between oxidative stress, CaMKII activation, and the genesis of TA caused by afterdepolarizations.

**Materials and Methods**

**Cell Isolation**

Ventricular myocytes were enzymatically isolated from adult rabbit hearts. Briefly, hearts were removed from adult New Zealand White rabbits (2 to 3 kg) anesthetized with intravenous pentobarbital, and hearts were then perfused retrogradely in Langendorff fashion at 37°C with nominally Ca-free Tyrode’s solution containing ~1.4 mg/mL collagenase (Type XIV, Sigma) for 25 to 30 minutes. After washing out the enzyme solution, the hearts were removed from the perfusion apparatus and swirled in a culture dish. The Ca concentration was slowly increased to 1.8 mmol/L, and the cells were stored at room temperature and used within 8 hours. The use and care of the animals were approved by the Chancellor’s Animal Research Committee at UCLA.

**Intracellular Ca Measurement**

Myocytes were loaded with the Ca indicator Fluo-4 by incubating them for ~30 minutes in bath solution containing 4 μmol/L Fluo-4 AM (Molecular Probes) and 0.016% (wt/wt) pluronic (Molecular Probes), after which the cells were washed and placed in a heated chamber on an inverted microscope. Cai fluorescence was recorded using an Andor Ixon charge-coupled device camera (Andor Technology) operating at ~100 frames per second with a spatial resolution of 512×180 pixels. The fluorescence intensity was recorded in arbitrary units.

**Patch-Clamp Methods**

Myocytes were patch-clamped using the whole-cell configuration of the patch-clamp technique. For action potential (AP) recordings, patch pipettes (resistance, 2 to 4 MΩ) were filled with pipette solution containing (in mmol/L): 110 K-aspartate, 30 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 5 MgATP, 5 creatine phosphate, 0.05 cAMP, pH 7.2 with KOH. In some experiments, the CaMKII inhibitor peptide AIP (autocamtide-2–related inhibitory peptide) (2 to 10 μmol/L) (Biomol or Sigma) was added directly to the pipette solution. The cells were superfused with standard Tyrode’s solution containing (in mmol/L): 136 NaCl, 5.4 KCl, 0.33 Na2PO4, 1.8 CaCl2, 1 MgCl2, 10 glucose and 10 HEPES, pH 7.4 adjusted with NaOH. To isolate IcL, patch pipettes (resistance 2 to 4 MΩ) were filled with pipette solution containing (in mmol/L): 110 Cs-Aspartate, 30 CsCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 5 MgATP, 5 creatine phosphate, 0.05 cAMP, pH 7.2 with KOH, and the cells were superfused with a modified Tyrode’s solution in which KCl was replaced by CsCl. H2O2 (0.2 to 1 mmol/L) was added directly to the bath superfusate. Nifedipine (Sigma) and KN-93 and KN-92 (Biomol) were dissolved in DMSO (Sigma) as stock solution before diluting into the superfusate solution at the final concentration. The maximum DMSO concentration was <1/500 (vol/vol). Chemicals and reagents were purchased from Sigma unless indicated. Voltage or current signals were measured with an Axopatch 200B patch-clamp amplifier controlled by a personal computer using a Digidata 1200 acquisition board driven by pCLAMP 8.0 software (Axon Instruments, Foster City, Calif). Action potentials were elicited with 2-ms, 2- to 4-nA square pulses at pacing cycle lengths (PCLs) of 6 seconds. All experiments were performed at 34 to 36°C. Data are presented as means±SEM. Statistical significance was assessed using unpaired Student’s t tests, with P<0.05 considered significant.

**Results**

**H2O2-Induced EADs and DADs in Adult Rabbit Ventricular Myocytes**

APs were recorded from isolated rabbit ventricular myocytes using whole-cell current clamp mode. After AP duration (APD) and morphology reached steady state, cells were superfused with 0.2 to 1 mmol/L H2O2. The generation of EADs by H2O2 was highly dependent on the PCL. Typically, at long PCLs (eg, 6 seconds), EADs occurred with every AP, and at short PCLs (eg, 1 seconds), no EADs occurred. In the
intermediate range, EADs occurred irregularly. Therefore, to elicit EADs most reliably, we used a PCL of 6 seconds throughout. EADs appeared after an average exposure time of $6.9 \pm 1.1$ minutes with 0.2 mmol/L H$_2$O$_2$ (n=10) and 6.6 $\pm$ 0.8 minutes with 1 mmol/L H$_2$O$_2$ (n=12). As shown in Figure 1A, EADs could be irregular, single, or multiple with an oscillating membrane potential before repolarization (Figure 1A and 1B). DADs were also observed after prolonged perfusion of H$_2$O$_2$ and occasionally triggered APs (Figure 1B, right). DADs often occurred following a previous AP with an EAD (Figure 1B, middle, and 1C), presumably because the prolonged APD allowed maintained I$_{Ca,L}$ to overload the SR with Ca. Prolonged perfusion (15 to 20 minutes) of H$_2$O$_2$ eventually caused gradual depolarization of the resting membrane potential to less than $-40$ mV, and the cells became inexcitable.

**Ionic Mechanisms of H$_2$O$_2$-Induced EADs and DADs**

We next used pharmacological interventions to analyze the ionic and cellular mechanism(s) underlying H$_2$O$_2$-induced afterdepolarizations. As shown in Figure 2A, H$_2$O$_2$-induced EADs were reversibly suppressed by the selective Na current blocker tetrodotoxin (TTX) (10 $\mu$mol/L), which also shortened APD. Ranolazine (20 $\mu$mol/L), a more selective blocker of late I$_{Na}$, also eliminated H$_2$O$_2$-induced EADs and shortened APD (data not shown), consistent with previous reports by Song et al$^{4,5}$ implicating late I$_{Na}$ as playing a key role. Unlike H$_2$O$_2$, anemone toxin II (ATX), an agent that selectively delays the late phase inactivation of I$_{Na}$, failed to generate frank EADs but did not inhibit I$_{Na}$, producing only small (1- to 2-mV) irregular oscillations during phase 2 of the AP. Note that APD was prolonged by ATX to a greater extent than after H$_2$O$_2$ (Figure 2B), yet no EADs were observed. This finding indicates that activation of late I$_{Na}$ is not, by itself, sufficient to produce EADs under these experimental conditions, implying that other actions of H$_2$O$_2$ are also required.

Figure 2C shows that EAD amplitude after H$_2$O$_2$ depended on its takeoff potential during repolarization, such that the more repolarized the takeoff potential, the larger the EAD amplitude. This relationship parallels the voltage dependence of I$_{Ca,L}$ reactivation and the I$_{Ca,L}$ window current.$^{24,25}$ Consistent with this interpretation, the selective I$_{Ca,L}$ blocker nifedipine (10 $\mu$mol/L) eliminated EADs (Figure 2D), even though APD remained markedly prolonged because of the effect of H$_2$O$_2$ on late I$_{Na}$ (as indicated by shortening of APD with subsequent application of 10 $\mu$mol/L TTX or 20 $\mu$mol/L ranolazine). Taken together, these results suggest that at least two actions of H$_2$O$_2$ are required for EAD formation: activation of late I$_{Na}$ to reduce repolarization reserve (ie, prolong APD) and modification of I$_{Ca,L}$ to enhance its reactivation properties so as to generate the EAD upstroke.

Whereas the effects of H$_2$O$_2$ on late I$_{Na}$ have been well documented in previous studies,$^{4,5}$ the reported effects of H$_2$O$_2$ on I$_{Ca,L}$ have been variable. Therefore, we examined how H$_2$O$_2$ affected I$_{Ca,L}$ in rabbit ventricular myocytes under our experimental conditions. Figure 3A and 3B shows the time course of both peak I$_{Ca,L}$ and the residual pedestal I$_{Ca,L}$. 

**Figure 2.** The involvement of both I$_{Na}$ and I$_{Ca,L}$ in H$_2$O$_2$-induced EADs. A, The specific I$_{Na}$ blocker TTX (10 $\mu$mol/L) reversibly suppressed EADs and shortened APD. B, ATX (30 nmol/L), a selective activator of persistent I$_{Na}$, prolonged APD but did not generate frank EADs with a distinct upstroke. C, The amplitude of H$_2$O$_2$-induced EADs depended on their takeoff potentials (arrows). D, The I$_{Ca,L}$ blocker nifedipine (Nif) (10 $\mu$mol/L) suppressed the EAD upstroke, although AP plateau remained prolonged, unless TTX (10 $\mu$mol/L) was also added.

**Figure 3.** Enhancement of I$_{Ca,L}$ by H$_2$O$_2$. A, Time course of the peak and residual pedestal (ped) I$_{Ca,L}$ during a 300-ms voltage clamp pulse to 0 mV (voltage protocol shown in B). B, Voltage clamp pulse (above) and superimposed current traces showing I$_{Ca,L}$ before (black) and ~5 minutes after perfusion of 1 mmol/L H$_2$O$_2$ (red). The difference current is shown in the bottom trace. C, Same as in B but with an AP clamp waveform replacing the square voltage clamp pulse.
at the end of a 300-ms voltage clamp to 0 mV in a representative myocyte during exposure to 1 mmol/L $H_2O_2$. Both peak and pedestal $I_{Ca,L}$ increased, from 7.3 to 12.1 pA/pF ($n=5$) and from 0.5 to 3.2 pA/pF ($n=5$), respectively. To examine how these changes affect $I_{Ca,L}$ during the repolarization phase of the AP, we performed AP clamp experiments before and after exposure to 1 mmol/L $H_2O_2$. Figure 3C shows that $H_2O_2$ induced a prominent $I_{Ca,L}$ hump during the late phase of the AP plateau, consistent with the timing of the EAD (compare with Figures 1 and 2). The recordings shown in Figure 3B and 3C represent the nifedipine-sensitive (subtracted) current, indicating that the increase of the inward current (including the hump in the late phase) is mostly attributable to $I_{Ca,L}$, although minor contamination by the Na/Ca exchange current ($I_{NCX}$) cannot be excluded.

The Role of $Ca_2^+$ Cycling

After $H_2O_2$ exposure, we often observed a transient inward current ($I_{ti}$) following repolarization to −80 mV (Figure 4A and inset), consistent with spontaneous SR $Ca$ release as a result of the enhanced $Ca$ influx via $I_{Ca,L}$ causing SR $Ca$ overload. To investigate the relationship to the EAD-induced DADs observed in Figure 1, we simultaneously imaged $Ca_2^+$ in Fluo-4 AM–loaded myocytes (Figure 4B through 4D). During $H_2O_2$-induced EADs, $Ca_2^+$ remained elevated (Figure 4C) or increased (Figure 4D), consistent with additional SR $Ca_2^+$ release attributable to reactivation of $I_{Ca,L}$. The line scan in Figure 4D shows that the rise in $Ca_2^+$ during the second EAD was not spatially homogeneous. This may indicate that $Ca$-induced $Ca$ release from the SR, in addition to $I_{Ca,L}$ enhancement, contributed to EAD formation by enhancing $I_{ti}$ (because $H_2O_2$ has been reported to enhance Na/Ca exchange). Figure 4D documents that after repolarization, a spontaneous $Ca_2^+$ wave originating from the center of the cell and propagating toward both ends was associated with a DAD. This can be attributed to increased SR $Ca$ loading during the preceding AP, which was markedly prolonged by EADs.

To further investigate the role of $Ca_2^+$ cycling in $H_2O_2$-induced afterdepolarizations, we examined the effects of pre-loading myocytes with BAPTA-AM to buffer changes in $Ca_2^+$ (Figure 5A). We also pretreated myocytes with thapsigargin (0.2 μmol/L) and ryanodine (10 μmol/L) to suppress SR $Ca$ cycling (Figure 5B). Both interventions completely prevented EADs and DADs during exposure to 1 mmol/L $H_2O_2$ (Figure 5), indicating the intact $Ca_2^+$ cycling plays a critical role. However,
APD remained prolonged under these conditions, probably because of reduced Ca-induced inactivation of $I_{\text{Ca,L}}$ when the intracellular Ca transient was suppressed.26

The Role of CaMKII Signaling

Similar to the effects of H$_2$O$_2$, Ca$^{2+}$-dependent CaMKII activation has been shown to modify $I_{\text{Na}}$, enhance $I_{\text{Ca,L}}$, and promote EADs.19–21 CaMKII could either be activated by Ca$_i$, resulting from the effects of H$_2$O$_2$ on Ca$_i$-cycling proteins, or directly by oxidative stress, as shown recently in cardiac myocytes.16,18 The Ca$_i$ dependence is still consistent H$_2$O$_2$-mediated activation of CaMKII, because persistent activation of CaMKII by oxidation requires Ca-CaM binding to CaMKII to expose its redox sites.18 We examined the effects of CaMKII inhibition to test these possibilities. Pretreatment with the CaMK inhibitor KN-93 (1 μmol/L) prevented the emergence of EADs during exposure to H$_2$O$_2$ (1 mmol/L for up to 20 to 30 minutes) in 4 myocytes (Figure 6A), compared to nonpretreated myocytes in which H$_2$O$_2$ (1 mmol/L) consistently induced EADs within 5 to 10 minutes of H$_2$O$_2$ exposure (Figures 1A and 6B). In addition, KN-93 also suppressed EADs after they were induced by H$_2$O$_2$ perfusion (Figure 6B).

Because both KN-93 and KN-92 have nonspecific effects, such as blockade of Ca and K currents,22,27,28 we also examined the effects of the selective CaMKII inhibitor peptide AIP (2 μmol/L). In 8 myocytes, AIP added to patch pipette dialyzing the cytoplasm delayed the appearance of EADs during exposure to 200 μmol/L H$_2$O$_2$, from 6.6 ± 0.8 (n=10) to 13.9 ± 2.7 minutes (Figure 6C) (P<0.05). Moreover, AIP dialysis also prolonged APD in a concentration-dependent manner, presumably because of nonspecific peptide effects, such that higher AIP concentrations were ineffective at preventing H$_2$O$_2$-induced EADs.

Discussion

In the present study, we investigated the mechanisms by which oxidative stress caused by exposure to H$_2$O$_2$ induces afterdepolarization and TA in rabbit ventricular myocytes. The novel findings are as follows: (1) $I_{\text{Ca,L}}$ modification, in addition to $I_{\text{Na}}$ modification, plays a crucial role in EAD generation by H$_2$O$_2$; (2) CaMKII activation, either directly via oxidative stress or indirectly via elevated Ca$_i$ is critical for these effects; (3) by enhancing SR Ca loading and promoting spontaneous Ca$_i$ waves, H$_2$O$_2$-induced EADs also cause DADs, providing a direct link between these 2 types of afterdepolarizations, an effect that compounds the arrhythmogenic potential of oxidative stress.

Ionic Mechanisms of EADs and DADs Induced by H$_2$O$_2$

Two conditions are required to generate an EAD. First, repolarization reserve must be reduced during phases 2 and 3
attributed to CaMKII activation, because, as shown in Figure 6A, H$_2$O$_2$ failed to prolong APD significantly or cause EADs when CaMKII was blocked by KN-93.

Oxidative stress also modulates the properties of other Ca$_v$-cycling proteins, including ryanodine receptors$^{11,12}$ and SERCA2a,$^{13}$ which could potentially promote EAD genesis by modulating SR Ca release during repolarization. Indeed, some recent studies have shown that EADs and DADs can share a common mechanism under Ca$_v$ overload conditions.$^{29-31}$ Non-$I_{Ca,L}$-gated SR Ca$^{2+}$ release during repolarization has been proposed to contribute to EAD genesis by activating Ca-sensitive inward currents,$^{29}$ but it is difficult to unequivocally distinguish from SR Ca release triggered by reactivated L-type Ca channels.

It has been reported that voltage can directly activate SR Ca$^{2+}$ release in cardiac myocytes, which is enhanced by cAMP.$^{32,33}$ However, EADs were readily induced by H$_2$O$_2$ using cAMP-free pipette solution (data not shown; see also Song et al$^{19}$), excluding an essential role of a cAMP-sensitive voltage-activated Ca release in EAD generation.

**Figure 7.** Failure of KN-92, an inactive analog of KN-93, to prevent H$_2$O$_2$-induced EADs. A, Same protocol as in Figure 6A but with KN-92 initially in place of KN-93. KN-92 failed to prevent EADs during exposure to 1 mmol/L H$_2$O$_2$, which were subsequently partially suppressed by KN-93. APs at points a (control), b (~2 minutes after KN-92), c (KN-92+H$_2$O$_2$), and d (KN-93+H$_2$O$_2$) are shown below. B, Same as in A but applying BAPTA-AM to suppress EADs after KN-92+H$_2$O$_2$.

of the AP, by an increase in inward current, a decrease in outward current, or both. Second, once repolarization reserve has been compromised, reactivation of $I_{Ca,L}$ window current, and/or additional SR Ca release augmenting Ca-sensitive inward currents such as $I_{NCX}$, must be sufficiently powerful to reverse repolarization and generate the EAD upstroke. In ventricular muscle, inward currents influencing repolarization reserve include $I_{Na}$, $I_{Ca,L}$, and $I_{NCX}$; whereas outward currents include the rapid and slow delayed rectifier K$^+$ currents ($I_{Kr}$ and $I_{Ks}$), the transient outward current ($I_{to}$), and the inward rectifier potassium current ($I_{IK}$). Previous studies analyzing the mechanisms of H$_2$O$_2$-induced EADs have implicated impaired $I_{Na}$ inactivation as the primary mechanism reducing repolarization reserve.$^{4,5}$ However, H$_2$O$_2$ has also been reported to affect other currents and transporters. In the present study, we show that in addition to modification of $I_{Na}$, modification of $I_{Ca,L}$ by H$_2$O$_2$ plays a key role in EAD genesis. Not only did H$_2$O$_2$ substantially increase the peak amplitude of $I_{Ca,L}$, but it also impaired $I_{Ca,L}$ inactivation (increase of pedestal current as shown in Figure 3A and 3B). The consequence was a large increase in the late phase of $I_{Ca,L}$, as seen during the AP clamp in Figure 3C, which was not successfully overcome by outward currents, accounting for the genesis of the EAD. The experiments using multiple ion channel blockers (Figure 2) showed that despite prolonging APD, $I_{Na}$ modification was not sufficiently induced to generate EADs when $I_{Ca,L}$ was blocked. Both $I_{Na}$ and $I_{Ca,L}$ modification could be
suppressed EAD formation by H₂O₂ generally supports a common mechanism. On the other hand, both KN-93 and KN-92 have nonspecific effects, including substantial block of I_{Ca,L}²⁷ which is critical in EAD formation. However, the selective CaMKII peptide inhibitor AIP also significantly delayed the onset of EADs during exposure to 0.2 mmol/L H₂O₂. Theoretically, AIP should be equally effective at preventing CaMKII activation by oxidative stress as by elevated Ca, because both activation modes require initial interaction of the regulatory domain of CaMKII with Ca-CaM complexes to expose the redox and autophosphorylation sites on the catalytic domain of CaMKII.¹⁸ This also explains why BAPTA-AM prevents EADs during H₂O₂ exposure, by preventing Ca from rising sufficiently for Ca-CaM to interact with CaMKII and trigger the subsequent persistent activation by redox modification.

Based on these observations, the role of CaMKII activation in H₂O₂-induced EADs can be summarized as follows: (1) CaMKII activation by H₂O₂ contributes to both impaired I_{Na} inactivation and I_{Ca,L} facilitation; (2) Both factors enhance cellular Ca loading by prolonging APD and increasing Ca influx via I_{Ca,L}, promoting further CaMKII activation via Ca-CaM; (3) KN-93 synergistically suppresses EADs by preventing CaMKII activation by H₂O₂ and reducing Ca influx by directly blocking I_{Ca,L}. Without concomitantly inhibiting CaMKII activation, partial block of I_{Ca,L} by KN-92 is not sufficient to suppress EAD formation; and (4) direct redox modification of ion channel proteins may also contribute, because selective CaMKII inhibition with AIP delayed the onset of, but did not entirely suppress, H₂O₂-induced EADs. We do not have a ready explanation for why AIP showed less effectiveness than KN-93 in our present study. KN-93 also blocks the L-type Ca current on which EAD showed less effectiveness than KN-93 in our present study. Both KN-93 and KN-92 have nonspecific effects, including substantial block of I_{Ca,L} which reduced repolarization reserve, because it modestly prolonged APD.

EAD-Associated DADs

Associations between EADs and DADs have been noted previously,⁶,³¹,³⁹,⁴⁰ but the underlying mechanisms have not been analyzed in detail. For example, in atrial myocytes, Song et al⁴⁰ found that ATX-induced EADs were also associated with DADs. They postulated that DADs were attributable to excessive Ca loading as a result of APD prolongation by EADs, leading to spontaneous SR Ca release following repolarization. Here, we directly documented this postulated mechanism for H₂O₂-induced EADs by using optical Ca₄ imaging to detect the spontaneous Ca₄ wave inducing a transient inward current causing the DAD (Figure 4). It is also conceivable that oxidative stress directly sensitizes the SR to non-I_{Ca,L}-gated SR Ca²⁺ release through direct effects on Ca₄-cycling proteins such as RyR,¹¹,¹² but this remains to be established. In either case, our findings suggest that both EADs and EAD-induced DADs may both contribute to TA during oxidative stress, amplifying the arrhythmogenic consequences.

Clinical Implications

Increased oxidative stress is believed to be an important factor predisposing the diseased heart to lethal arrhythmias. Here, we have shown how oxidative stress caused by exogenous H₂O₂ predisposes the heart to both EADs and DADs, and we have demonstrated a link to CaMKII activation, which is also involved in other aspects of heart failure.⁴¹,⁴² Other free radical sources have also been reported to produce similar results, i.e., inducing EADs, DADs, and TAs.⁵ Although it is still under debate, H₂O₂ levels up to ≈35 μmol/L have been reported in human blood plasma.⁴³ It is also well known that ROS levels can increase during ischemia and reperfusion by as much as 100-fold⁴⁴ and also with age by 7.5-fold.⁴⁵ Because ROS are short-lived radicals, it is conceivable that the local concentrations near sites of production are considerably higher than circulating concentrations. Therefore, the concentrations (100 to 200 μmol/L) used in most of our experiments are reasonable approximations of the pathophysiologically relevant range. Targeting CaMKII signaling may have therapeutic potential to prevent arrhythmias in the failing heart, although negative inotropic actions⁴⁶ may limit the use of CaMKII inhibitors.

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Disclosures

None.

References

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