Hydrogen Peroxide Modulates Electrophysiological Characteristics of Left Atrial Myocytes

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**Background:** Oxidative stress plays an important role in the pathophysiology of atrial fibrillation (AF). The hydrogen peroxide (H₂O₂) mainly underlies the cellular oxidative stress and free radicals. Left atrium (LA) is the most important AF substrate. However, the effects of H₂O₂ on the action potential (AP) and ionic currents in LA myocytes have not been fully elucidated.

**Methods:** The whole-cell patch clamp was used to investigate the APs and ionic currents of L-type calcium current (I_Ca-L), transient outward currents (I_to), ultra-rapid delayed rectifier potassium current (I_Kur), delayed rectifier potassium currents (I_K), inward rectifier potassium current (I_K1), and sodium-calcium exchanger (NCX) before and after H₂O₂ (100 μM) in isolated rabbit LA myocytes.

**Results:** H₂O₂ (100 μM) shortened by 50% (from 40 ± 7 to 21 ± 5 ms) and 90% the AP duration (from 95 ± 12 to 74 ± 11 ms) in LA myocytes (n = 9), but did not change the resting membrane potentials. The H₂O₂ (100 μM) decreased I_to, but increased I_Kur and I_K. H₂O₂ (100 μM) also reduced the I_Ca-L and the reverse mode NCX. However, H₂O₂ (100 μM) did not change I_K1.

**Conclusions:** H₂O₂ directly modulated the AP morphology and ionic currents in LA myocytes, which may contribute to the genesis of AF in oxidative stress.

**Key Words:** Action potential • Ionic current • Oxidative stress

**INTRODUCTION**

Atrial fibrillation (AF) is the most commonly sustained cardiac arrhythmia in clinical conditions that often cause cardiac dysfunction and even mortality. However, the mechanisms underlying the arrhythmogenesis of AF are not fully elucidated. The oxidative stress has been shown to play a potential role in the pathophysiology of AF. It has been proven that AF increases production of superoxide, which may arise from the effects of the NADPH and xanthine oxidases. Those patients with chronic AF also have increased oxidative modification proteins in the atrium. In addition, anti-oxidant agents have demonstrated their therapeutic potential to treat AF through the prevention of oxidative stress.

The left atrium (LA) plays an important role in the genesis and maintenance of AF. This indicated that the oxidative stress in AF patients could be highly expressed in LA, which manifests the importance of oxidative stress in the electrophysiological characteristics of the LA. Moreover, oxidative stress has been demonstrated to regulate the ionic current in cardiomyocytes. However, the effects of oxidative stress on the action potential (AP) morphology...
and ionic currents in LA have not been fully elucidated. Therefore, the purpose of this study was to investigate the effects of hydrogen peroxide (H₂O₂) on the electrophysiological characteristics of LA myocytes.

**MATERIALS AND METHODS**

**Electrophysiological studies in LA myocytes**

This investigation conformed to those protocols as established in the Guide for the Care and Use of Laboratory Animals. Rabbits (weight 1-2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg kg⁻¹). The LA myocytes were enzymatically dissociated via the same procedure described previously. Tissues of the LA were gently shaken in 5-10 ml of Ca²⁺-free oxygenated Tyrode’s solution until single cardiomyocytes were obtained. The solution for single cardiomyocytes was gradually changed to normal oxygenated Tyrode’s solution. The cells were then allowed to stabilize in the bath for at least 30 min before administration of H₂O₂ (100 μM).

The whole-cell patch clamp was performed by using an Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA). Before the formation of the membrane-seal, tip potentials were zeroed in Tyrode’s solution. Junction potentials between the bath and pipette seal, tip potentials were zeroed in Tyrode’s solution (9 mV) were corrected for AP recordings. The APs and ionic currents were recorded in the current-clamp mode and the voltage-clamp mode, respectively. The APs were elicited through a 1-Hz electrical stimulus in the LA wall. The resting membrane potential (RMP) was measured during the period between the last depolarization and the onset of the subsequent AP. The AP amplitude (APA) was obtained from the RMP to the peak of the AP depolarization. The AP duration at repolarization of 90% and 50% of the amplitude were measured as the APD₉₀ and APD₅₀.

A small hyperpolarizing step from a holding potential of -50 mV to a testing potential of -55 mV for 80 ms was delivered at the beginning of each experiment. The area under the capacitive currents was divided by the applied voltage step to obtain the total cell capacitance. Normally, 60% to 80% series resistance (Rₛ) was electronically compensated. The micropipettes were filled with a solution containing (in mM/L) CsCl 130, MgCl₂ 1, Mg₂ATP 5, HEPES 10, EGTA 10, NaGTP 0.1, and Na₂ phosphocreatine 5, (pH of 7.2 with CsOH) for the L-type calcium current (I_Ca-L) containing (in mM/L) NaCl 20, CsCl 110, MgCl₂ 0.4, CaCl₂ 1.75, TEACl 20, BAPTA 5, glucose 5, Mg₂ATP 5, and HEPES 10, (pH of 7.25 with CsOH) for the Na⁺-Ca²⁺ exchanger (NCX) current; and containing (in mM/L) KCl 20, K aspartate 110, MgCl₂ 1, Mg₂ATP 5, HEPES 10, EGTA 0.5, LiGTP 0.1, and Na₂ phosphocreatine 5, (pH of 7.2 with KOH) for the APs and the potassium currents.

The I_Ca-L was measured as an inward current during depolarization from a holding potential of -50 mV to testing potentials ranging from -40 to +60 mV in 10-mV steps for 300 ms at a frequency of 0.1 Hz by means of a perfused patch clamp. The NaCl and KCl in the external solution were replaced by TEACl and CsCl, respectively.

The transient outward potassium current (I_K₁) was studied with a double-pulse protocol. A 30-ms pre-pulse from -80 to -40 mV was used to inactivate the sodium channels, followed by a 300-ms test pulse to +60 mV in 10-mV steps at a frequency of 0.1 Hz. CdCl₂ (200 μM) that was added to the bath solution to inhibit I_Ca-L. I_K₁ was measured as the difference between the peak outward current and steady state current. The ultra-rapid delayed rectifier potassium current (I_Kur) was studied with a double-pulse protocol, consisting of a 100-ms depolarizing pre-pulse to +40 mV from a holding potential of -50 mV, followed by 150-ms voltage steps from -40 to +60 mV in 10 mV increments at room temperature to provide an adequate temporal resolution. The I_Kur was measured as 4-aminopyridine (1 mM) sensitive currents. The delayed rectified outward potassium current (I_Kw) was measured from the peak outward current at the end of 1 s of the depolarization from -40 to +60 in 10-mV steps at a frequency of 0.1 Hz during the infusion of CdCl₂ (200 μM) and 4-aminopyridine (2 mM) in the bath solution. The inward rectifier potassium current (I_K₁) was activated from -40 mV to test potentials ranging from -20 to -120 mV in 10-mV steps for 1 s at a frequency of 0.1 Hz, under the infusion of CdCl₂ (200 μM) and 4-aminopyridine (2 mM) in the bath solution. The amplitudes of the I_K₁ were measured as 1 mM barium-sensitive currents.

The NCX current was elicited by depolarizing pulses between -100 to +100 mV from a holding potential of -40 mV for 300 ms at a frequency of 0.1 Hz. The amplitudes of the NCX current were measured as 10 mM.
nickel-sensitive currents. The external solution (in mM) consisted of NaCl 140, CaCl$_2$ 2, MgCl$_2$ 1, HEPES 5 and glucose 10 with a pH of 7.4, and contained strophanthidin (10 μM), nitrendipine (10 μM) and niflumic acid (100 μM).

**Statistical analysis**

All continuous data are expressed as the mean ± SEM. A paired t-test was used to compare the effects of electric activity of LA myocytes before and after H$_2$O$_2$. A p-value lower than 0.05 was considered statistically significant.

**RESULTS**

**Effects of H$_2$O$_2$ on the morphology of AP in LA myocytes**

As shown in Figure 1, H$_2$O$_2$ (100 μM) significantly shorten APD$_{50}$ by 47%, and APD$_{90}$ by 22% in LA myocytes (n = 9) both in the 1 Hz and 2 Hz pacing modes. However, H$_2$O$_2$ (100 μM) did not change the RMP and APA.

**Effects of H$_2$O$_2$ on ionic currents in LA myocytes**

Figure 2 shows the effects of H$_2$O$_2$ on the $I_{Ca-L}$ in LA myocytes. H$_2$O$_2$ (100 μM) significantly decreased $I_{Ca-L}$ whereas the peak $I_{Ca-L}$ (elicited from -40 to +20 mV) was reduced by H$_2$O$_2$ (100 μM) to an extent of 30% (n = 16).

Figure 3 shows the tracings and I-V relationship of H$_2$O$_2$ on the $I_{to}$ in LA myocytes. H$_2$O$_2$ (100 μM) significantly decreased $I_{to}$, whereas the peak $I_{to}$ currents (elicited from -40 to +60 mV) were reduced by H$_2$O$_2$ (100 μM) to an extent of 45% (n = 24). As shown in Figure 4, the current densities of $I_{Kur}$ (elicited from -20 to +60 mV) were also increased to an extent of 74% after the admin-
administration of H$_2$O$_2$ (n = 12). Besides, the current densities of $I_K$ (elicited from -40 to +60 mV) were increased after the administration of H$_2$O$_2$ by 28% (n = 25, Figure 5). However, H$_2$O$_2$ (100 μM) did not change the $I_K$ (Figure 6).

Furthermore, as shown in Figure 7, H$_2$O$_2$ (100 μM) decreased the reverse mode of nickel-sensitive NCX currents in LA myocytes. The peak reverse mode nickel-sensitive NCX currents (elicited from -40 to +60 mV) were reduced by H$_2$O$_2$ (100 μM) to a significant extent of 34% (n = 22). However, H$_2$O$_2$ (100 μM) did not change the forward mode of nickel-sensitive NCX currents in LA myocytes.

**DISCUSSION**

Oxidative stress plays a vital role in the pathophysiology of a wide range of cardiovascular diseases, including AF.$^6,14$ H$_2$O$_2$ may mediate the pathological process of the ischemia, heart failure, inflammation, and renin-angiotensin or adrenergic activations, which are recognized to increase the occurrence of AF as well.$^7,15,16$ Oxidative stress not only can induce atrial remodeling, but also may alter atrial electrophysiology.$^{15,17}$ Additionally, several studies have investigated the electrophysiological variation after the exposure of free radicals and oxidative stress to result in a reduction of the resting membrane potential, action potential amplitude and an augmentation of the automaticity as similar as our study.$^{18,19}$ Since H$_2$O$_2$ shortened the AP duration to a great extent in the cardiomyocytes of the LA, it could ex-
pand the dispersion of the AP duration in the substrate of LA to facilitate the genesis of reentry circuits, which is one of the arrhythmogenic mechanisms. Although, the concentration (100 μM) of H$_2$O$_2$ used in this study had been assumed to be clinically relevant, which demonstrated a generally pathophysiological phenomenon in the atrial electrophysiology, the single dose of H$_2$O$_2$ limited the opportunity to examine its dose-dependent effects.

In the study, we found that H$_2$O$_2$ at a pathophysiological concentration significantly modified the function of the potassium and Ca$^{2+}$ regulatory channels in left atrial myocytes. Our data showed that oxidative stress induced a dominantly decreased level in $I_{Ca,L}$ and $I_{K}$ but not in $I_{K1}$. The mechanism of reduced potassium currents by oxidative stress is unclear and inconsistent. It is possibly influenced by inadequate buffering of the calcium channels and restricting the cell membrane of cardiomyocytes. The $I_{K1}$ in this study was significantly diminished over 40% after the administration of H$_2$O$_2$. This result resembled what in some studies is related to chronic human AF and rapid pacing heart failure model. Moreover, the increased density of $I_{K}$ current could be the main character to downgrade the level of APD$_{50}$ and APD$_{90}$ in our study. On the other hand, Liu et al. reported that the direct inhibition of the $I_{Kur}$ could prolong the action potential period to prevent atrial fibrillation. It explained the effect of increased current densities of...
IKur to reduce the action potential period in our study and possibly induce atrial arrhythmia in the oxidative stress of atrial myocytes. Previous study has shown that oxidative stress could induce a Ca\(^{2+}\)-overload by H\(_2\)O\(_2\) that disorganized the regulation protein of calcium channel or lipid oxidation of the cell membrane.\(^{26}\) That is consistent with our results of reducing I\(_{Ca-L}\) and NCX current density. In addition, H\(_2\)O\(_2\) enhanced the intracellular Ca\(^{2+}\) release by increasing the opening probability of ryanodine receptors.\(^{27,28}\) Those effects may demonstrate the arrhythmogenic effects of H\(_2\)O\(_2\), but previous studies reported that the reactive oxygen species (ROS) revealed controversial in I\(_{Ca-L}\) and the mechanism was unidentified.\(^{29-31}\) Nonetheless, the manifestation of the LA myocytes in this study received a high level of ROS could mimic acute oxidant cardiac injury, which was similar with those in the study from Fearon et al.\(^{29}\) Therefore, it was reasonable to assume that oxidative stress has a high arrhythmogenic potential for inducing AF through enhancing the atrial automaticity and the triggered activity with Ca\(^{2+}\) overload. Moreover, higher expression of the intracellular Ca\(^{2+}\) overload and Ca\(^{2+}\) release by the influence of H\(_2\)O\(_2\) may increase the contractility of atrial myocytes in our previous findings.\(^{15,32}\) In chronic AF, sustained oxidative stress created the conditions that favor reduced I\(_{Ca-L}\) entry and increased I\(_{Ca-L}\) efflux via forward-mode operation of the NCX by strengthening the manifestation of the NCX protein and attenuating the performance of the I\(_{Ca-L}\) channel.\(^{22,23}\) However, Qin et al. reported that H\(_2\)O\(_2\)-mediated oxidative stress could impair calcium handling of the cardiomyocytes through decreasing sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) activity.\(^{33}\) In our study, oxidative stress reduced the currents at the reverse mode of the NCX and I\(_{Ca-L}\) but remained Ca\(^{2+}\) overloaded probably owing to the increased calcium in the cytoplasm through the opening of ryanodine receptors and decreased SERCA activity by H\(_2\)O\(_2\).

Anti-oxidants have been indicated to have an anti-arrhythmic potential in previous research indicating that ascorbic acid might decrease the occurrence of post-operative AF and modify the electrical remodeling in rapid atrial pacing model. Hence, the comprehensive cognition of oxidative stress induced by sepsis, heart failure, and the activation of the renin-angiotensin or adrenergic systems in the pathogenesis of AF indeed may provide the possible therapy for this obsessive arrhythmia. Furthermore, our study brought out a newly probable mechanism of oxidative stress in the electrophysiological characteristics of atrial myocytes to trigger AF.

**CONCLUSIONS**

H\(_2\)O\(_2\) directly modulated the AP morphology and ionic currents in LA myocytes, which may contribute to the genesis of AF in oxidative stress.
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