Full Paper

Possible Underlying Mechanism for Hydrogen Peroxide-Induced Electromechanical Suppression in Human Atrial Myocardium

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Abstract. Hydrogen peroxide (H_2O_2) and its metabolites have been shown to exert complex effects on the cardiac muscle during cardiac ischemia/reperfusion. The aim of the present study, by perfusing H_2O_2 or/and different scavengers of oxygen free radicals (OFRs) into the human atrium, is to characterize the electropharmacological effects of H_2O_2 and explore its possible underlying mechanism. Atrial tissues obtained from the heart of 19 patients undergoing corrective cardiac surgery were used. Transmembrane action potentials were recorded using the conventional microelectrode technique, and contraction of atrial fibers was evaluated in normal $[K]_o$ (4 mM) in the absence and presence of tested agents. H_2O_2 (30 μ M–3 mM) had a biphasic effect on the contractile force (an increase, followed by a decrease), reduced the 0-phase depolarizing slope (dV/dt), and prolonged the action potential duration (APD) in a concentration-dependent manner. However, even at a concentration as high as 3 mM, H_2O_2 did not influence diastolic membrane potential (DMP). Pretreatment with *N*-(mercaptopropionyl)-glycine (*N*-MPG), a specific scavenger of the $\cdot OH$ free radical, significantly blocked the 3 mM H_2O_2 -induced electromechanical changes, while the pretreatment with L-methionine (L-M), a specific scavenger of HOCl free radical, did not. Our data suggests that the toxic effects of H_2O_2 are caused mainly through the generation of $\cdot OH$, which is attributed to the electropharmacological inhibitory effects seen in the human atrium.

Keywords: human heart, oxygen free radical, scavenger, electropharmacology, ischemia-reperfusion

Introduction

Accumulation of oxygen free radicals (OFRs) is an important mediator of post ischemia/reperfusion cardiac dysfunction that occurs in some pathological conditions, such as coronary thrombosis, and in certain surgical procedures, such as balloon angioplasty of blood vessels and open-heart surgery with cardioplegic arrest (1, 2). Indeed, it has been found that mammalian hearts exposed to OFRs-generating systems exhibit many features similar to those of ischemia/reperfusion injury, including cellular K^+ loss, action potential duration shortening leading to inexcitability, loss of developed

systolic force, progressive rise in diastolic force (contracture), depressed high-energy phosphate levels, and damage to cellular metabolic machinery (3). Moreover, there is increasing evidence that OFR scavengers, such as catalase and superoxide dismutase, can reduce ischemia- and reperfusion-induced myocardial stunning and arrhythmias (1).

OFRs constitute a class of chemical compounds in which an unpaired electron occupies the outer orbit of the oxygen molecule (4). It has been claimed that hydrogen peroxide (H_2O_2) is one of the most important OFRs, because both itself and its metabolites, such as superoxide ($\cdot O_2^-$) and hydroxyl ($\cdot OH$) radicals, have been shown to play both physiological and pathophysiological roles in a variety of cells (5). For example, it has been found that H_2O_2 can cause lipid peroxidation

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(6), enzyme activation (7), protein oxidation (8), and changes in the concentrations of intracellular ions such as Ca^{2+} (9, 10). In the last decade, the electrophysiological effects of H_2O_2 in animal myocardium have been well explored. It was found that H_2O_2 affects most of the transmembrane currents, including ATP-sensitive K^+ currents (11), Na^+ -inward currents (12), L-type Ca^{2+} currents (13, 14), delayed and inward rectifier K^+ currents (15), and also Na^+ - Ca^{2+} exchange (11). Furthermore, Satoh and Matsui (16) suggested that low concentrations of H_2O_2 (30–100 μM) alter the action potential duration (APD) and contractile force, but not the other electrical parameters in guinea pig cardiac muscles. However, these effects reported in different groups were sometimes contradictory. For example, L-type- Ca^{2+} currents have been reported to be increased in the rabbit sinoatrial node (13), decreased in frog atrial cells (12), or remain unchanged in guinea pig ventricular myocytes (14). In addition, a concentration- and time-dependent action on the contractile force has been observed (16). In contrast, in rat ventricular myocytes, Ward and Giles (17) showed that 50–200 μM H_2O_2 had no significant effect on the action potential, cell shortening, the transient outward K^+ currents, and inwardly rectifying K^+ currents. Therefore, it is likely that these discrepancies arise from both species differences and variations in recording techniques and conditions. However, until now, no related pharmacological studies have been carried out to explore the electromechanical effects of H_2O_2 on the human myocardium.

Recently, in animal models, both in vivo and in vitro, more and more evidence has demonstrated that the most damaging effects on the heart caused by OFRs seem to be due to $\cdot\text{OH}$ radicals (1, 3, 18, 19), instead of H_2O_2 (4). For example, some studies have found that direct scavengers or inhibitors of $\cdot\text{OH}$ radicals, which have no effect on tissue $\cdot\text{O}_2^-$ and H_2O_2 levels, were as effective as agents that reduced all three species ($\cdot\text{OH}$ and $\cdot\text{O}_2^-$ and H_2O_2) (4). A similar phenomenon has also been found in a study using microspectrofluorimetry to monitor the change in intracellular pH before and after using the OFRs perfusing (20). As yet, however, few pharmacological studies have been carried out on the effects of OFRs and the cardioprotective action of OFR scavengers on the human myocardium.

As H_2O_2 is more stable and thus able to diffuse throughout a greater tissue mass, it subsequently produces other oxygen-derived free radicals such as $\cdot\text{OH}$ and HOCl (4, 21–23). In the present study, H_2O_2 in various concentrations (30 μM –3 mM) was applied to the human atrial myocardium to mimic the electrophysiological effects of OFRs during ischemia and post-reperfusion. Also, to further explore its underlying

mechanism and test the cardioprotective effect of certain potential agents, scavengers of OFRs were applied in the study. Our data suggest that the toxic effects of H_2O_2 are caused mainly through the generation of $\cdot\text{OH}$. Moreover, in light of our findings, the treatment with a more specific and potent scavenger would be a useful approach to reduce the ischemia/reperfusion induced damage.

Materials and Methods

Human atrium tissue

Human atrial tissues were obtained from the hearts of 19 patients (10 females and 9 males) undergoing open-heart surgery for the treatment of a variety of congenital (7 cases, age 29 ± 6 years) and acquired (12 cases, age 59 ± 4 years) heart diseases. Institutional rules for the protection of human subjects were observed. Prior to surgery, informed consent was obtained. Right atrial trabecular tissue, 0.5–1 mm in diameter and 3–5 mm long, was removed, as described previously (24), and immediately immersed in cold bicarbonate-containing Tyrode solution. The preparations were then perfused with oxygenated (95% O_2 , 5% CO_2) Tyrode solution at 37°C. The pH of the solution was adjusted to 7.40 ± 0.02 for the experiments.

Electrophysiological and mechanical experiments

The human atrial myocardia were driven by electrical stimuli of 2-ms duration at a frequency of 1 Hz. Transmembrane potentials were recorded using traditional glass microelectrodes filled with 3 M KCl and the contractile force was measured using a Gould UC2 (Oakland, CA, USA) force transducer, as described previously (25). The electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and a Gould ES 1000 recorder. The action potential amplitude (APA), action potential duration at 50% and 90% repolarization (APD_{50} and APD_{90} , respectively), maximum diastolic potential (MDP), and twitch force were measured as described previously (26).

Chemicals and solutions

All experiments were performed at 37°C in bicarbonate-buffered solutions (equilibrated with 95% O_2 , 5% CO_2) containing 137 mM NaCl, 0.5 mM MgCl_2 , 0.5 mM NaH_2PO_4 , 4 mM KCl, 2.7 mM CaCl_2 , 5.6 mM glucose, 22 mM NaHCO_3 , and 5.6 mM dextrose, the pH being adjusted to 7.4 using 4 N NaOH. pH adjustment of all solutions was performed at 37°C. Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, MO, USA) and Wako (Kyoto). H_2O_2 and antioxidants, *N*-(mercaptopropionyl)-glycine (*N*-MPG)

and L-methionine (L-M) (22, 23), were added as stock solutions (all dissolved in de-ionized water), to the superfusate shortly before use. In those experiments designed to test concentration-dependent effects of H₂O₂, different concentrations of H₂O₂ were added, from lower to higher, to the medium cumulatively. In those experiments designed to test protective effects of antioxidants against H₂O₂-induced responses, the antioxidants (i.e., *N*-MPG and/or L-M) were superfused in the myocardium for 30 min before the 3 mM H₂O₂ was added directly.

Statistics

All data are expressed as the mean $\pm$ the standard error of the mean (S.E.M.) for *n* preparations. In the first part of the present study in which the dose-dependent effect of H₂O₂ was examined, data obtained before, during, and after drug treatment was evaluated by one way analysis of variance (one-way ANOVA) to test significance. However, in the second part of the present study in which the cardioprotective effects of antioxidants were tested, statistical analysis was performed by Student's *t* test. A *P* value smaller than 0.05 was regarded as significant.

Results

Electrophysiological and mechanical effects of H₂O₂

The typical experiment shown in Fig. 1 was to examine the effects of H₂O₂ on the electromechanical parameters in the human atrial myocardium. In the whole experiment, different concentrations of H₂O₂ (0.03 – 3 mM) were added directly into the perfused normal Tyrode solution (4 mM [K⁺]_o). Recordings in Fig. 1A are control examples of action potential (AP, upper trace), 0-phase upstroke velocity (*V*_{max}, middle trace) and contractile force (bottom trace). As shown in the original

traces in Fig. 1B, 0.1 and 0.3 mM H₂O₂ had a significant effect on the characteristics of AP, including prolongation of the APD₅₀ (+12% and +21%, respectively) and APD₉₀ (+16% and +27%, respectively) and inhibition of the *V*_{max} (–27% and –30%, respectively). The AP initiation was totally inhibited when the myocardium was treated with 3 mM H₂O₂. Note the dramatic elevation of diastolic membrane potential induced by 3 mM H₂O₂ (see upper panel of Fig. 1D). These H₂O₂-induced inhibitory effects were slowly reversible after about a 1-h washout in drug-free Tyrode solution (Fig. 1E).

Fig. 1. Effects of various concentrations of H₂O₂ (0.03 – 3 mM) on the electrophysiological and mechanical parameters of the human atrial myocardium. The action potential (upper trace), its 0 phase depolarization (middle trace), and the contractile force (lower trace) were recorded (A) in Tyrode solution, (B – D) in the presence of 0.03 (B), 0.1 (C) or 0.3 (D) mM H₂O₂, and (E) after washout in drug-free superfusate.

Table 1. Effects of H₂O₂ on action potential characteristics of human atrial myocardia in Tyrode solution (5% CO₂/HCO₃[–], pH_o = 7.4)

		H ₂ O ₂ (mM)				
	n	Control	0.1	0.3	1.0	3.0
MDP (mV)	9	–74 $\pm$ 4	–70 $\pm$ 5	–64 $\pm$ 5	–59 $\pm$ 9*	–23 $\pm$ 18*
<i>V</i> _{max} (V/s)	9	189 $\pm$ 39	–126 $\pm$ 12*	–115 $\pm$ 14*	–106 $\pm$ 21*	0 $\pm$ 0*
APA (mV)	9	88 $\pm$ 4	78 $\pm$ 7	80 $\pm$ 6	72 $\pm$ 12	0 $\pm$ 0*
APD ₅₀ (ms)	9	188 $\pm$ 14	209 $\pm$ 10	221 $\pm$ 11*	234 $\pm$ 12*	0 $\pm$ 0*
APD ₉₀ (ms)	9	321 $\pm$ 23	354 $\pm$ 26	358 $\pm$ 19*	371 $\pm$ 18*	0 $\pm$ 0*

MDP = maximum diastolic potential, *V*_{max} = maximum upstroke velocity of phase 0 depolarization, APA = action potential amplitude, APD₅₀ = action potential duration at 50% repolarization, APD₉₀ = action potential duration at 90% repolarization. Values are the means $\pm$ S.E.M., *n* = number of preparations. Data were evaluated by one-way analysis of variance (one-way ANOVA), and the Wilcoxon signed rank test was used to test significance. **P* < 0.05, significantly different from the control value in each group.

Similar results of the electrophysiological effects of H_2O_2 in 9 atrial trabecular myocardia are summarized in Table 1.

In regard to the effects of H_2O_2 on the contractile force, Fig. 1 (B and C) showed that 0.1 and 0.3 mM H_2O_2 increased the contractile force (9% and 36%, respectively) and the diastolic resting tension (+2 mg and +5 mg, respectively) in a concentration-dependent manner. On the contrary, 3 mM H_2O_2 totally inhibited contractile force while increasing the diastolic resting tension even more dramatically (Fig. 1D). Like what was found for the electrophysiological effects, H_2O_2 -induced mechanical effects were also slowly but partially reversible after about a 1-h washout in drug-free Tyrode solution (bottom trace of Fig. 1E). The maximum changes in contractile force and diastolic resting tension induced by H_2O_2 (from 0.03–3 mM) in 9 preparations are summarized in the statistic curves of Fig. 2 (filled circles and filled triangles, respectively). Our results clearly demonstrated that H_2O_2 had a biphasic effect on the contractile force; i.e., an increase at lower concentrations, followed by inhibition at higher

Fig. 2. Normalized concentration-response curves showing the percentage change in contractile force (filled circles) and diastolic resting tension of force (filled triangles) versus the logarithm of the H_2O_2 concentration curve for 9 separate experiments similar to that shown in Figs. 1 and 2. The changes in the contractile force and diastolic resting tension of force are each expressed as a percentage change relative to their respective control value before H_2O_2 exposure. Note the phenomenon of increasing force at the lower concentration (0.1 mM), while there is a decreasing force at the higher concentration (3 mM). Data were evaluated by one way analysis of variance (one-way ANOVA), and the Wilcoxon signed rank test was used to test significance. * and # $P < 0.05$, significantly different from the control value in each group.

concentrations, similar to results seen in guinea pig cardiac muscles (16). Note that the phenomenon of a non-significant and larger-variation was seen under the treatment condition of 0.3 and 1 mM H_2O_2 , respectively. This is simply due to the fact that 4/9 samples showed increasing effects ($+14 \pm 18\%$, $n = 4$) while 5/9 samples showed decreasing effects ($-34 \pm 10\%$, $n = 5$) when 0.3 mM H_2O_2 was given. Perfusion with 1 mM H_2O_2 , produced a dramatic increase in contractile force in 3 of 9 preparations ($+42 \pm 17\%$, $n = 3$), while decreasing effects were observed in the remaining 6 samples ($-117 \pm 24\%$, $n = 6$). In other words, the concentration range of 0.3–1 mM were the “turning” concentrations for the biphasic effect on the contractile force. Moreover, it can be seen clearly that the H_2O_2 caused a significant increase in diastolic resting tension only at the largest concentration, i.e., 3 mM.

Electromechanical effects of antioxidants and evaluation of their therapeutic effect against H_2O_2 -induced injury

As H_2O_2 can readily cross the cell membrane and be converted, via the Fenton reaction, to $\cdot OH$ and, possibly, subsequently to $HOCl$, we determined which of these chemical species were involved in the H_2O_2 -induced suppression of the electromechanical parameters of the human atrial myocardium. The tissue was therefore pretreated for 30 min with *N*-MPG (10 mM), a highly potent intracellular scavenger of the $\cdot OH$ free radical, and L-M (1 mM), a specific inhibitor of the $HOCl$ free radical (22, 23), both alone or in combination, before direct addition of 3 mM H_2O_2 . In other words, this experiment could further provide evidence for seeing whether OFRs scavengers could protect human atrial myocardium from the H_2O_2 -induced-inhibitory effects on electromechanical characteristics. Typical experiments were performed to determine the effect of the antioxidants themselves on the electromechanical effects in human atrial trabecular tissues. It was clearly demonstrated that pretreating with L-M (1 mM) itself for 30 min had no significant effect on the electromechanical characteristics (figure not shown, $n = 3$). Similar results of non-significant effects on electromechanical characteristics were found with either *N*-MPG (10 mM) alone or in combination with L-M (1 mM) when added to the human atrial myocardium for 30 min (see Fig. 4B and Fig. 5B, respectively; $n = 3$).

Figure 3B showed that pretreatment with 1 mM L-M alone for 30 min (e.g., Fig. 3A) could not significantly prevent H_2O_2 -induced electromechanical suppression at the 12th min after the addition of 3 mM H_2O_2 . In addition, as shown in Fig. 3C, it was found that the electromechanical parameters were totally inhibited at

Fig. 3. Effects of adding L-methionine together with H₂O₂ on the electrophysiological and mechanical parameters in the human atrial myocardium. Recordings are in control Tyrode solution in the presence of 1 mM L-methionine (A), in the presence of 1 mM L-methionine and 3 mM H₂O₂ (L+H) for 12 (B), 22 (C) min, and after washout in drug-free superfusate (D).

the 22nd min after the addition of 3 mM H₂O₂. A similar result has been found in another experiment (–100% on contractile force at 30th min after adding H₂O₂). However, note that the 3 mM H₂O₂-induced depolarized resting potential and increased diastolic resting force were abolished by treatment with 1 mM L-M, as shown in Fig. 3C (compare to that of Fig. 1D). Our results, therefore, suggest that 1 mM L-M has, at least, very little but not significantly cardioprotective effects against H₂O₂-induced inhibition in the human atrial myocardium (compare to that of Fig. 1). The underlying mechanism for this waits further study. On the contrary, 10 mM *N*-MPG significantly inhibited the H₂O₂-induced electromechanical suppression, such as APD prolongation ($-3 \pm 10\%$ and $9 \pm 12\%$ for APD₅₀ and APD₉₀, respectively, $n = 3$; $P > 0.05$) and contractile force suppression, as shown in Fig. 4 ($n = 3$, $P > 0.05$). A similar cardioprotective effect was seen using the combination of 10 mM L-M and 1 mM *N*-MPG in three atrial tissues (Fig. 5, $P > 0.05$). In other words, *N*-MPG (10 mM) could afford a significant cardioprotective effect against perfusion with H₂O₂ (3 mM for 30 min) (Figs. 4C and 5C, compare with Fig. 1D).

Discussion

Electromechanical suppression caused by H₂O₂

Using the conventional microelectrode technique, we have, for the first time, demonstrated that H₂O₂ (30 μ M–3 mM) causes marked electromechanical inhibition in the human atrial myocardium (Fig. 1). This effect was

Fig. 4. Effect of adding *N*-MPG together with H₂O₂ on the electrophysiological and mechanical parameters [action potential (upper trace), its 0 phase depolarization (middle trace), and the contractile force (lower trace)] in the presence of a free radical generating system in the human atrial myocardium. Recordings are control (A), in the presence of *N*-MPG (N) for 30 min (B), in the presence of *N*-MPG and 3 mM H₂O₂ (N+H) for 30 min (C), after washout in drug-free superfusate (D).

Fig. 5. Effect of adding *N*-MPG together with L-methionine and H₂O₂ on the electrophysiological and mechanical parameters [action potential (upper trace), its 0 phase depolarization (middle trace), and the contractile force (lower trace)] in the presence of a free radical generating system in the human atrial myocardium. Recordings are in control Tyrode solution (A), in the presence of *N*-MPG plus L-methionine (N+L) for 30 min (B), in the presence of *N*-MPG plus L-methionine and 3 mM H₂O₂ (N+L+H) for 30 min (C), after washout in drug-free superfusate (D).

concentration-dependent (Fig. 1). The underlying mechanism for the H₂O₂-induced suppression is probably the intracellular production of $\cdot$ OH after H₂O₂ influx (i.e., via the Fenton reaction). This conclusion is strongly supported by the observation that the 3 mM H₂O₂-induced electromechanical changes was blocked by the specific potent $\cdot$ OH scavenger, *N*-MPG (Figs. 4 and 5), but not by the HOCl scavenger, L-M (Fig. 3). Similar observations have been reported in animal models (20). These results indicate that the intracellular concentration of HOCl produced by H₂O₂ entry makes very little contribution to the H₂O₂-induced electromechanical changes in the human atrial trabecular muscle.

In addition, our data showed that the effects of H₂O₂

on electrophysiological parameters, such as the APD, 0 phase depolarization, and resting membrane potential, are concentration-dependent (Fig. 1, Fig. 2, and Table 1). In particular, the total inhibitory effect induced by 3 mM H_2O_2 suggests that 3 mM, at least in our condition, is the toxic concentration of H_2O_2 in the function of human atrial trabecular tissue. Moreover, our results show that H_2O_2 has a biphasic and concentration-dependent effect on the contractile force in the human atrial trabecular muscle (filled circles in Fig. 2). Moreover, it can be seen clearly from Fig. 2 that the diastolic resting tension (filled triangles in Fig. 2) increased significantly only when the largest concentration, i.e., 3 mM, of H_2O_2 was added. Therefore, whether the increased diastolic resting tension is the underlying mechanism for the "biphasic" phenomenon seen in contractile force waits for further study. Similar results have been obtained in guinea pig heart muscles (16). The induced biphasic response, i.e., the initial increase in contractile force followed by inhibition, and the increased diastolic resting tension are strong support for the hypothesis that oxidant injury plays a critical role in the development of ischemia/reperfusion-induced cardiac injury, such as that due to stunning and arrhythmia. A similar biphasic effect on contractile force in the guinea pig ventricular myocytes has been reported (27). They demonstrated a potent stimulatory effect of H_2O_2 on the L-type Ca current as the underlying mechanism for the increase in contractile force, while an activation effect took place on the adenosine A_1 receptor to attenuate for the contractile force. However, whether other factors may contribute to cardiac stunning, such as depolarized transmembrane potential, NO (28), cytokines (29) and prostaglandins (30), requires further investigation.

The relationship between intracellular pH and $\cdot\text{OH}$

Many cellular mechanisms are sensitive to changes in the intracellular pH (pH_i). These include enzyme activity (31), control of the cell volume (32), the regulation of cellular growth and differentiation (33), the kinetic properties of K^+ and Ca^{2+} channels (34), and cell contractility (35). During myocardial ischemia, the pH_i is substantially lowered (36). Moreover, during post-ischemia reperfusion, rapid recovery of the pH_i is observed (37). These pH_i disturbances have been claimed to be responsible for the reversible contractile dysfunction and malignant ventricular arrhythmias seen in cardiac myocytes and in other cell types (37, 38). In cultured rat cardiac myoblasts, 100 μM H_2O_2 induces a marked decrease in the pH_i (20). Similar results are seen in cerebellar astrocytes and C6 glioma cells (23). These large pH_i reductions are suggested to be compati-

ble with the production of intracellular $\cdot\text{OH}$ as a result of H_2O_2 oxidation, i.e., via the Fenton reaction, since the acidosis is completely inhibited by two different potent membrane-crossing $\cdot\text{OH}$ scavengers (23). Therefore, one of the possible underlying mechanisms of $\cdot\text{OH}$ on the observed changes in electromechanical parameters is the dramatic changes in pH_i .

pH is a major modulator of cardiac function

In regard to the effect of pH disturbances on the contraction of the cardiac muscle, it was reported as early as in the 1880s that reducing extracellular pH (pH_o) caused a positive inotropic effect on cardiac muscle tension. Increasing pH_o caused a positive inotropic effect (39). A similar result was found in skinned cardiac fibers (40). These inhibitory effects on contraction appear to be due mainly to an inhibitory effect of H^+ ions on the Ca^{2+} sensitivity of the myofibrillar apparatus (40, 41). This can occur through H^+ ions decreasing binding of Ca^{2+} to troponin-C (42) and through H^+ ions directly inhibiting myofibrillar cross-bridge cycling (40, 41). In contrast, in experiments on an isolated mammalian papillary muscle, it was demonstrated that intracellular acidosis can sometimes produce an increase rather than a decrease in contraction, particularly when pH_o is kept at 7.40 (35). Similarly, in rat tabeculae, Orchard (43) found that acidosis can increase systolic Ca^{2+}_i . This variability in the contractile response to intracellular acidosis is probably due to the effect of acidosis on intracellular Na^+ concentration, as developed-tension in cardiac tissue is steeply dependent upon Na^+_i (44). The inotropic response of cardiac tissue to acidosis is therefore modulated by the negative inotropic effect of H^+_i in combination with changes in Na^+_i leading to changes in Ca^{2+}_i . In other words, the biphasic responses on contractile force induced by H_2O_2 or/and $\cdot\text{OH}$ found in the present study are compatible with the results found in the previous studies shown above. In addition, it is well documented that the membrane transport systems of myocardial cells are also affected by pH_i disturbance. For example, the kinetic properties of Na^+ , Ca^{2+} , inward rectifying K^+ channels, the Ca^{2+} -activated K^+ channel, and the acetylcholine receptor were all reported to be reversibly blocked by acidic pH_i (3, 16, 20, 45). However, whether H_2O_2 and/or $\cdot\text{OH}$ -induced changes in the parameters of action potential in the human atrial myocardium were mainly due to its effect on the transmembrane ion currents awaits further study.

Clinical relevance

In terms of clinical implications, the results of our present study support the idea that the underlying mechanism by which OFRs induce cardiac dysfunction

is most probably the intracellular production of $\cdot\text{OH}$. This further affects the normal cardiac functions such as 0-phase upstroke velocity of action potential, maximum diastolic potential, contractile force, and rhythm. Thus, the development of a more specific and potent $\cdot\text{OH}$ scavenger would be one potential approach to preventing certain pathological conditions, especially those involving oxidative challenge.

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