Alterations in Electrical Activity and Membrane Currents Induced by Intracellular Oxygen-Derived Free Radical Stress in Guinea Pig Ventricular Myocytes

Rita I. Jabr and William C. Cole

Oxygen-derived free radicals (O-Rs) are thought to induce alterations in cardiac electrical activity; however, the underlying membrane ionic currents affected by O-Rs and the mechanisms by which O-Rs induce their effects on ion channels in the heart are not well defined. In this study, we investigated the time-dependent changes in resting membrane potential and action potential configuration and changes in steady-state membrane currents in guinea pig ventricular myocytes after intracellular application of an O-R-generating system. O-Rs were generated from the combination of dihydroxyfumaric acid (3 mM) and FeCl3:ADP (0.05-0.5 mM) added to the pipette solution that was used to record membrane potential and currents via the whole-cell variant of the patch-clamp technique. Intracellular exposure of myocytes to the O-R-generating solution induced three stages of changes: 1) an early depolarization (5–10 mV) and an increase in action potential duration accompanied by a decrease in resting inward rectifying K+ current conductance, 2) delayed afterdepolarizations and triggered activity caused by the activation of transient inward current mediated by Na+-Ca2+ exchange, with failure to repolarize and sustained depolarization between −35 and −20 mV, reflecting the stimulation of nonselective cation current, and 3) a late stage of marked decline in action potential duration, hyperpolarization, and loss of excitability accompanied by activation of the outward current through ATP-sensitive K+ channels. These alterations in electrical activity and membrane currents could be prevented by pretreatment with N-(2-mercaptopropionyl)glycine (500 μM), a scavenger of hydroxyl free radicals. The alterations associated with stages 1 and 2 but not stage 3 were completely abolished on intracellular Ca2+ chelation (5 mM EGTA in the pipette solution) or disruption of sarcoplasmic reticulum Ca2+ handling by ryanodine (10 μM). This study shows that intracellular O-R stress causes specific alterations in membrane ionic currents, leading to changes in resting membrane potential and action potential configuration. Moreover, the data indicate that an elevation in intracellular Ca2+ due to abnormal Ca2+ handling by the sarcoplasmic reticulum is a cause of some of the alterations in membrane currents during O-R stress. (Circulation Research 1993;72:1229–1244)

KEY WORDS • oxygen-derived free radicals • cardiac action potential • inward rectifier current • nonselective cation current • Na+-Ca2+ exchange • whole-cell patch clamp • ventricular myocytes

Oxygen-derived free radicals (O-Rs) are postulated to play a role in the development of myocardial dysfunction during reperfusion after ischemia, leading to a variety of structural and functional changes including altered 1) metabolic activity, 2) ion transport, 3) ionic balance, 4) membrane structure, 5) contractile performance, 6) ultrastructure, and 7) electrical activity marked by severe reflow-induced arrhythmias.1-6 A role for O-Rs in the genesis of reperfusion arrhythmias is suggested by a variety of experimental evidence; e.g., the incidence of reperfusion arrhythmias including premature ventricular complexes (extrasystoles), ventricular tachycardia, and fibrillation in intact preparations of heart (in vivo and in vitro) was reduced when the formation of O-Rs was inhibited with allopurinol.7 Protection was also provided by treatment with scavengers of O-Rs such as superoxide dismutase, mannitol, glutathione, and a-tocopherol.8-11 O-R scavengers were shown to depress arrhythmogenic alterations in electrical activity (including depolarization) and the incidence of arrhythmias and to improve the recovery of action potential (AP) configuration during reperfusion of ischemic right ventricular walls of guinea pigs.12

In an effort to understand how O-R stress may affect electrical activity in the heart, various healthy preparations have been exposed to exogenous O-Rs by inclusion

From the Department of Physiology, Division of Cardiovascular Sciences, St. Boniface Research Centre, University of Manitoba, Winnipeg, Canada. Previously published as preliminary observations in abstract form (J Mol Cell Cardiol 1990;22[suppl II]:P104).

Supported by a grant from the Medical Research Council of Canada. R.I.J. holds a traineeship from the Canadian Heart Foundation, and W.C.C. is a Medical Research Council of Canada Scholar.

Address for correspondence: William C. Cole, PhD, Department of Physiology, Division of Cardiovascular Sciences, St. Boniface Research Centre, 351 Tache Avenue, Winnipeg, Manitoba, Canada, R2H 2A6.

Received March 27, 1991; accepted February 10, 1993.
of an O-R–generating system in the bathing solution.\textsuperscript{13} O-R stress was reported to induce arrhythmias in isolated perfused hearts\textsuperscript{8,14-16} and alterations in electrical activity in various intact, multicellular, and single-myocyte preparations of cardiac muscle.\textsuperscript{13-25} Several changes in activity were described, including a fall in resting membrane potential (RMP), a decline in AP amplitude, a decrease in maximal rate of depolarization, a transient increase in plateau amplitude and AP duration, a marked decrease in AP duration, and early and delayed afterdepolarizations (EADs and DADs, respectively).\textsuperscript{13-25} However, the basis for these alterations in electrical behavior in terms of the underlying changes in membrane ionic currents is poorly defined.

Oxidative stress induced by superfusion of single myocytes with various O-R–generating systems was previously reported to cause a depression in resting K\textsuperscript{+} conductance via the inward rectifying K\textsuperscript{+} current (I\textsubscript{Kr}), an activation of ATP-sensitive K\textsuperscript{+} current (I\textsubscript{KATP}), an increase, a decrease, or no change in calcium (I\textsubscript{Ca}) and delayed rectifier (I\textsubscript{K}) currents, a decrease or no change in sodium current (I\textsubscript{Na}), and a decrease in I\textsubscript{Ks} inactivation.\textsuperscript{13,17,19-27} Recently, Matsuura and Shatocking\textsuperscript{21,22} reported the presence of transient inward current (I\textsubscript{INa}) via forward-mode activity of the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger\textsuperscript{21} and activation of Ca\textsuperscript{2+}-activated steady-state current\textsuperscript{22} during extracellular O-R stress induced by photoliquamation of rose Bengal, which primarily generates singlet oxygen radicals and superoxide anions.\textsuperscript{28}

The present study describes the alterations in electrical and underlying changes in quasi-steady-state membrane ionic currents in isolated guinea pig ventricular myocytes after intracellular exposure to O-Rs. A generating system based on dihydroxyfumaric acid (DHF) was used. This system is known to generate superoxide, hydroxyl peroxide, and the hydroxyl radical, which are the pertinent reactive oxygen metabolites formed during reperfusion of the ischemic myocardium.\textsuperscript{29,30} In our experiments, the O-R–generating system was added to the pipette solution used for recording membrane potential and ionic currents in the whole-cell configuration of the patch-clamp technique. We chose intracellular application to simulate the effects of endogenous intracellular sources of O-Rs. O-Rs are known to be produced in both compartments by a variety of reactions; extracellular sources include auto-oxidation of catecholamines, neutrophils, and hypoxanthine: xanthine oxidase in some species, and intracellular sources include mitochondrial electron transport and arachidonic acid metabolism.\textsuperscript{31,32} Given the highly reactive nature and extremely short half-life of O-Rs, it is possible that the sites of oxidative damage during intracellular O-R stress may be different from that for O-Rs arising in the extracellular space.\textsuperscript{13}

Materials and Methods

Cell Isolation

Guinea pigs (200–300 g) were anesthetized with CO\textsubscript{2} and then killed by cervical dislocation. Their hearts were quickly removed and placed in well-aerated ice-cold Krebs-Henseleit solution (K-H) containing (mM) NaCl 120, NaHCO\textsubscript{3} 25.0, KCl 4.8, NaH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, glucose 11.0, and CaCl\textsubscript{2} 1.8, pH 7.4, after gassing with 95% O\textsubscript{2}–5% CO\textsubscript{2}. They were then mounted on a Langendorff apparatus and perfused in a retrograde fashion with K-H (37°C) at a constant pressure for a stabilization period of 10–15 minutes. Single ventricular myocytes were isolated by an enzymatic dispersion technique in which hearts were perfused with nominally Ca\textsuperscript{2+}-free K-H for 5 minutes before treatment with collagenase (74.52 units/ml, Worthington Biochemical Corp., Freehold, N.J.) in Ca\textsuperscript{2+}-free K-H for 45 minutes. The right ventricle was then removed, placed in Ca\textsuperscript{2+}-free K-H, and cut into small pieces (2×2 mm), which were incubated in K-H containing collagenase (40.5 units/ml) for 15–30 minutes at 37°C. After a final wash, the tissues were kept in K-H at room temperature, and single myocytes were obtained when needed by gentle trituration.

Whole-Cell Recordings

Isolated guinea pig ventricular myocytes were placed in a recording chamber and superfused with the bath solution at a flow rate of 1.8 ml/min (based on the effects of 9.8 mM [K\textsuperscript{+}], on membrane potential, the time required to effect changes in the bath solution was 10–30 seconds). Only rod-shaped myocytes with clear and distinct striations and obvious marked shortening and relaxation on stimulation were used. Experiments were performed at both 37° and 22°C as indicated in the text and figure descriptions.

The whole-cell configuration of the patch-clamp technique\textsuperscript{33} was used for voltage- and current-clamp recordings with a patch-clamp amplifier (model 8900, Dagan Corp., Minneapolis, Minn.). Patch pipettes were pulled with a P-87 puller (Sutter Instrument Co., Novato, Calif.) and fire-polished to a final resistance of 0.25–0.5 M\textohm when filled with a control pipette solution. The pipette tip was positioned above the cell, and the pipette potential and capacitance were nullified. After the patch membrane was ruptured, the series resistance (1.0–5.0 M\textohm) and cell capacitance were compensated. Membrane voltage and whole-cell currents (filtered at 1 kHz) were recorded directly to hard disk via an analog-to-digital convertor (model TL-1–125 LabMaster, Axon Instruments, Foster City, Calif.) interfaced with an IBM clone computer running PCLAMP software (Axon Instruments). Data analysis was performed with PCLAMP (CLAMPFIT). Since the pipette potentials were nulled in external solution, all current-clamp tracings and voltage-clamp protocols required correction for junction potential. This was accomplished by filling 20 pipettes with standard internal solution. They were then nulled in internal solution, and the difference in potential on immersion in external solution was recorded. The difference was consistently −10 mV, and this value was used to correct all current-clamp data and voltage-clamp protocols.

Solutions

The exogenous free-radical–generating system used to study the effects of O-Rs consisted of 3 mM DHF and FeCl\textsubscript{3}, ADP (0.05–0.5 mM), which were added to the pipette solution (pH readjusted to 7.2 with KOH). The pipette solution contained (mM) potassium gluconate 130, KCl 10.0, MgCl\textsubscript{2} 1.0, Na\textsubscript{2}-ATP 5.0, EGTA 0.1, and HEPES 5.0 (pH 7.2). In all experiments, 0.1 mM EGTA was used unless indicated. The effects of the generating system were compared with those of an internal solution
lacking a generating system or containing FeCl₃·ADP alone.

The control bath solution contained (mM) NaCl 120, NaHCO₃ 3.6, KCl 4.8, NaH₂PO₄ 1.2, MgSO₄ 1.2, glucose 11.0, CaCl₂ 1.8, and HEPES 5, pH 7.4 with NaOH. In solutions in which 50% Na⁺ was replaced, 62.4 mM NaCl in the control bath was replaced with impermeant N-methyl glucamine (NMG) (pH 7.4). In the other experiments, K⁺ was replaced with Cs⁺ in the pipette and bath, or Na⁺ in the bath was completely replaced with equimolar LiCl.

Drugs

Nicardipine (10 μM) was purchased from Research Biochemicals Inc., Natick, Mass. Ryanodine (10 μM) and tetrodotoxin (10 μM) were purchased from Calbiochem Corp., La Jolla, Calif. N-(2-Mercaptopropionyl)-glycine (MPG, 500 μM), glibenclamide (10 μM), NMG (62.4 mM), and all other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Data Analysis

All data were expressed as mean±SEM. Statistical analysis was performed using Student’s t test.

Results

Effect of O-R Stress on Electrical Activity

In the first series of experiments, we monitored the time-dependent changes in electrical activity of isolated guinea pig ventricular myocytes via the whole-cell recording technique in the presence and absence of an O-R-generating system in the pipette solution. The generating system consisted of DHF (3 mM) and FeCl₃·ADP (0.05:0.5 mM). A low concentration of EGTA (0.1 mM) was included in the pipette solution in an attempt to preserve [Ca²⁺], fluctuations and contractions after electrical stimulation. Experiments were conducted at 37°C, resulting in a very rapid progression of changes in electrical activity during exposure to O-Rs. Representative tracings obtained in a single experiment are shown in Figure 1. Similar results were obtained in six other myocytes. The rapid progression of changes did not provide sufficient time to record the underlying alterations in membrane currents by switching from current to voltage clamp and applying command pulses. For this reason, we reduced the temperature to 22°C and compared the changes in activity with those at 37°C. Figure 2 shows results obtained during an experiment at 22°C and illustrates that similar alterations in activity occurred at the two temperatures, although those at 22°C were delayed and slower to develop after achieving whole-cell access. Thus, the alterations in electrical activity at this temperature were similar to those recorded at 37°C. For this reason, the use of the lower temperature to facilitate the switch between recording modes does not compromise the potential relevance of these experiments to in vivo pathophysiological events occurring at 37°C.

Figure 2 was divided into three panels to indicate that the changes in electrical activity induced by O-R stress occurred in three stages. However, it should be noted that the alterations in activity in our three stages of intracellular O-R stress do not correspond exactly to those observed by Barrington et al.18 during extracellular O-R stress (see “Discussion”). Tracing A in Figure 2 shows a control AP obtained immediately after membrane rupture and before dialysis of the cell interior with the O-R-containing pipette solution. The subsequent tracings (i.e., tracings B–K) were obtained at various time intervals until the myocytes became inexcitable (i.e., there was no AP, regardless of stimulus strength).

The alterations in activity associated with stage 1 consisted of a slow, progressive 5–10-mV depolarization of RMP and an increase in AP duration, especially at 90% of repolarization (APD₉₀). During the initial phase of depolarization, little change or a slight increase in the AP plateau was apparent. However, a subsequent decline in the plateau amplitude was noted, especially when the AP was markedly prolonged (Figure 2, tracings B and C).

The second stage began with the appearance of DADs and low-amplitude oscillations of membrane potential during the plateau. In 10 of 26 myocytes, spontaneous APs were noted as a result of DADs or enhanced automaticity. After the appearance of DADs, the myocytes then failed to repolarize after an AP and showed sustained depolarization between −35 and −20 mV (Figure 2, tracing D). This stage was observed in all but two myocytes exposed to O-Rs. In 15 myocytes, this stage was a transient event, lasting for 0.5–3 minutes before spontaneous recovery of resting potential (Figure 2, tracing E). In nine of 26 myocytes, however, the membrane potential did not repolarize, and the myocytes were observed to shorten significantly (hypercontract) and depolarize to 0 mV. In those myocytes showing repolarization after stage 2, a second period of large

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Time-dependent changes in action potential configuration induced by oxygen-derived free radical (O-R) stress at 37°C. Three tracings (A–C) were recorded from a single myocyte after gaining whole-cell access (tracing A) and after 4.2 (tracing B) and 5.3 (tracing C) minutes of dialysis with pipette solution containing the O-R-generating system at 37°C (stimulation frequency, 0.25 Hz). Note the rapid progression from normal activity in tracing A, through stages of 1) slight depolarization, 2) decline in plateau amplitude and prolongation of action potential duration, and spontaneous activity and failure to repolarize in tracing B, and 3) repolarization and decrease in action potential duration in tracing C.
oscillations in membrane potential, DADs, and spontaneous activity followed (Figure 2, tracings E and F).

The third stage of change was marked by hyperpolarization, decline in AP duration, and loss of excitability. After spontaneous repolarization from $-35$ mV, DADs and oscillations in potential during the plateau disappeared, the myocytes gradually hyperpolarized, and AP duration decreased markedly (Figure 2, tracings H–J). Eventually, the myocytes hyperpolarized to or beyond the value of RMP measured initially on membrane rupture. At this time, all myocytes were inexcitable and would not fire APs even in response to current pulses with strength more than 10–15 times greater than that immediately after whole-cell access (Figure 2, tracing K).

Some variability in the behavior of the myocytes during intracellular O-R stress was evident. However, all changes in activity to inexcitability induced by DHF–FeCl3: ADP were evident within 20 minutes. The average time required for O-R stress to induce stage 1 was calculated on the basis of changes in RMP and APD90 that were greater than 2% and 10%, respectively. Table 1 summarizes the number of myocytes demonstrating each stage of change in electrical activity and the latency for the onset of these stages. A total of 26 myocytes were exposed to the O-R-generating system (in the absence of any other treatments). Ten myocytes demonstrated all three stages, and nine demonstrated stages 1 and 2 but failed to recover from stage 2. Only two myocytes failed to exhibit stage 2, but these myocytes did show stages 1 and 3 at times similar to those found in the previous groups. Thus, all myocytes that demonstrated stage 3 (17 of 26) first demonstrated stage 1 and/or 2. Interestingly, in the five myocytes that failed to exhibit stage 1, the latency to the onset of stages 2 and 3 was reduced. Note that in these myocytes stage 3 also occurred with a reduced latency.

The whole-cell recording technique is well known to cause time-dependent changes in the electrical activity, e.g., a decline in AP duration in the absence of any treatments, presumably as a result of the dilution of intracellular constituents important to membrane channel activity by the pipette solution.34,35 For this reason, we compared the time course and the nature of the changes in myocytes dialyzed with internal solution lacking the O-R-generating system. That the O-R-generating system caused marked changes in electrical activity compared with untreated myocytes or those dialyzed with internal solution containing FeCl3: ADP alone (and no DHF) is evident from a comparison of Figures 2 and 3. Figure 3A shows APs recorded immediately on rupture of the membrane (tracing A) and after 25 minutes (tracing B) in a myocyte dialyzed with control internal solution lacking both DHF and FeCl3: ADP. Neither APD90 (measured as a percentage of control) nor RMP was significantly altered over the first 25 minutes (Figures 3A and 3B). Similar results were obtained with seven other myocytes. Myocytes (n=7) dialyzed with internal solution containing FeCl3: ADP alone (i.e., without DHF) also showed no significant change in APD90 and RMP over 25 minutes (Figures 3C and 3D). These data imply that rundown and/or FeCl3: ADP cannot account for alterations in

Table 1. Summary of the Latency for the Onset of Membrane Electrical Alterations Induced During Intracellular Oxygen-Derived Free Radical Stress

<table>
<thead>
<tr>
<th>Cells</th>
<th>Stage 1 (minutes)</th>
<th>Stage 2 (minutes)</th>
<th>Stage 3 (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=10</td>
<td>3.3±1.1</td>
<td>5.5±1.1</td>
<td>8.4±1.8</td>
</tr>
<tr>
<td>n=9</td>
<td>3.3±0.6</td>
<td>5.4±0.9</td>
<td>...</td>
</tr>
<tr>
<td>n=5</td>
<td>...</td>
<td>2.9±1.0</td>
<td>4.9±0.7</td>
</tr>
<tr>
<td>n=2</td>
<td>3.7</td>
<td>...</td>
<td>9.7</td>
</tr>
<tr>
<td>N=26</td>
<td>21</td>
<td>24</td>
<td>17</td>
</tr>
</tbody>
</table>

n, Number of cells demonstrating a given sequence of stages; N, total of n cells. Values are mean±SEM.
activity observed in myocytes exposed to the complete O-R-generating system.

Effects of O-R Stress on Quasi–Steady-State Membrane Currents

In the second series of experiments, we investigated the changes in membrane ionic currents induced by the O-R-generating system. We could not use conventional step-type voltage-clamp protocols because of the time required to record families of membrane currents over the physiological range of voltages; the changes in electrical activity induced by O-R stress were often very rapid (even at 22°C), so alterations in the magnitude of the whole-cell currents would be expected during the protocols. As an alternative, a ramp protocol that required 8 seconds to obtain a quasi–steady-state current–voltage (I-V) relation for the range of potentials between −130 and +30 or +60 mV (depolarization rate, 20 or 25 mV/sec, respectively) was used. This protocol reflects net steady-state current produced by the myocyte over the range of voltages, which under normal conditions largely reflects I_{K1} at potentials negative to −40 mV. The nature of the current at more positive potentials under control conditions remains to be defined. Some contribution from I_{K1}, the fast and slow components of I_{Ks}, and/or plateau background current may be involved.

Stage 1. The changes in quasi–steady-state currents during O-R stress occurred in three stages at times corresponding to the periods described above for current-clamp data. The first phase of change was marked by a decline in the magnitude of current at potentials near RMP, which accompanied the slight initial depolarization in current clamp (Figure 4). Figures 4A and
Figure 4. Alteration in quasi-steady-state current during stage 1 of oxygen-derived free radical (O-R) stress. Panel A shows representative current-clamp recordings obtained from a single myocyte after gaining cell access (tracing A, control) and after 6.00 minutes of dialysis with O-R-containing pipette solution (tracing B, O-R), when the initial stage of depolarization was observed (stimulation frequency, 0.25 Hz; 22°C). Panel B shows a representative example of the initial change in steady-state current from that observed immediately after gaining cell access (tracing A, control) and at 3.1 minutes (tracing B, O-R), when the slight depolarization was observed. Quasi-steady-state current in this myocyte was evoked by ramping membrane potential between −130 and 0 mV over 6 seconds from a holding potential of −85 mV. The inset figure shows an expanded version of the region of tracings A and B within the box on the current–voltage relation and depicts the decline in steady-state current at voltages negative to −35 mV.

Figure 5. Alteration in chord conductance negative to −40 mV during stage 1 of oxygen-derived free radical (O-R) stress. Panel A: Representative decline in chord conductance [Ipeak/(Vm−Ek), where Ipeak is peak current, Vm is membrane voltage] in a single myocyte (panel A) and the average change in chord conductance (as a percentage of the conductance measured after gaining cell access) in four myocytes exposed to O-Rs versus five myocytes dialyzed with control pipette solution during the first 8 minutes of recording. The average decline in chord conductance by ≈10% over potentials negative to −40 mV (Figure 5B) was similar to the decline in slope conductance measured at the reversal potential in myocytes exposed to O-Rs (i.e., 11.2±1.8% from 76±8 to 67±7 nS [n=4]). The latter change was significantly different (p<0.05) from that observed in untreated myocytes (no DHF in the pipette) (2±3% from 61±8 to 60±7 nS [n=5]). This early depression in Ik1 persisted until masked by changes in other currents.

Stage 2. Figure 6 illustrates the changes in membrane currents observed during the phase of DADs, failure to repolarize, and sustained depolarization; panels A and C show current clamp recordings obtained after gaining cell access and during this second phase, and panels B and D show the corresponding steady-state currents.
Several changes in the currents are apparent: 1) a marked positive shift in reversal potential of the I-V relation, 2) inwardly directed oscillations in current (i.e., $I_t$) recorded after repolarization to the holding potential of $-85 \text{ mV}$, 3) a large negative increase in
holding current (i.e., the \( I_{k} \) was frequently superimposed on a sustained inward current), and 4) inward deflections of membrane currents in the negative slope region of the I-V relation. A magnified version of the \( I_{n} \) is shown in the inset boxes of panels B and D. These oscillations occurred with a frequency similar to the DADs shown in the inset of panel A (i.e., \( \sim 2.0 \text{ Hz} \)). Both the frequency and magnitude of the \( I_{n} \) are similar to those occurring during Ca\(^{2+}\) overload as reported by other investigators.\(^{39-43}\)

To identify the ionic mechanism(s) mediating \( I_{n} \), two sets of experiments were performed. First, we attempted to determine the reversal potential of this current by stepping to a range of potentials between \(-80\) and \(+25 \text{ mV}\) at the end of the ramp (Figure 7A). The oscillatory currents were observed at all potentials, and no distinct reversal potential was apparent between these voltages. Second, the bath solution was exchanged for one in which \([\text{Na}^{+}]_{o}\) was replaced with an equimolar concentration of \( \text{Li}^{+} \) immediately after the onset of stage 2. In this case, the \( I_{n} \) was completely blocked, as shown in Figure 7B, tracing C. Similar results were obtained in seven myocytes. Interestingly, replacement of \([\text{Na}^{+}]_{o}\) with \( \text{Li}^{+} \) did not alter the reversal potential of the current responsible for the shift in the steady-state I-V relation during stage 2 (Figure 7B).

The positive shift in reversal potential of the steady-state current and the inwardly directed increase in holding current were transient phenomena that showed reversibility. Figure 8A illustrates this complete reversibility; the reversal potential and holding current recorded after (tracing C) were the same as those recorded immediately before (tracing A) the shift in reversal potential that occurred during the period of sustained depolarization (tracing B). To determine the O-R stress-activated current responsible for the shift, we calculated the difference current by digital subtraction of the I-V relation recorded before the shift from

**Figure 7.** Blockade of oxygen-derived free radical (O-R) stress-induced transient inward current on 100% \([\text{Na}^{+}]_{o}\), replacement with \( \text{Li}^{+}\). Panel A shows representative current recordings obtained during stage 2, by stepping to different potentials ranging between \(-80\) to \(+25 \text{ mV}\) at the end of the ramp protocol. Note that the direction of the transient inward current was in the inward direction at all potentials and did not demonstrate a reversal potential. Asterisks indicate portion of protocol displayed in current tracings. Panel B shows representative recordings of quasi-steady-state currents obtained from myocytes dialyzed with the O-R-generating system immediately after gaining access (tracing A, control) and during stage 2 (tracing B, O-R). Note the positive shift in steady-state current associated with the transient inward current evoked after stepping back to the holding potential of \(-85 \text{ mV}\). Tracing C was obtained 1 minute after complete \([\text{Na}^{+}]_{o}\), replacement with \( \text{Li}^{+}\).

**Figure 8.** Oxygen-derived free radical stress activates a quasi-steady-state current during stage 2. Panel A: Quasi-steady-state current recordings evoked before (tracing A), during (tracing B), and after (tracing C) the phase of failure to repolarize and sustained depolarization in a single myocyte via a ramp protocol (from \(-130\) to \(+30 \text{ mV}\) over 8 seconds from a holding potential of \(-85 \text{ mV}\)). Note that the changes in 1) reversal potential for the steady-state current, 2) current at voltages positive and negative to \(-30 \text{ mV}\), and 3) holding current in tracing B compared with tracing A were completely reversed in tracing C. Panel B: Difference current obtained by digital subtraction of tracing A from tracing B. The reversal potential for the difference current in this myocyte was \(-19.5 \text{ mV}\).
that obtained during the reversal potential shift. The current was largely voltage insensitive and possessed a reversal potential of $-19.5$ mV in this myocyte (Figure 8B). The average reversal potential for the difference current recorded from 16 myocytes was $-19.9 \pm 0.7$ mV. This is not the equilibrium potential corresponding to any single ion under the recording conditions used. However, it is close to the reversal potential of the nonselective cation current of cardiac myocytes that Matsuda identified to be $\text{Ca}^{2+}$ activated. With this in mind, we first altered $K^+$ and $Na^+$ concentrations in the bath when stage 2 was observed. Representative results are shown in Figure 9. Panels A and B show that changing $[K^+]_o$ from 4.8 to 9.6 mM during stage 2 caused a positive shift in reversal potential from $-23.5$ to $-14.8$ mV. The average shift in reversal potential for the difference current calculated from six myocytes was from $-24.4 \pm 2.5$ to $-15.6 \pm 1.7$ mV ($p < 0.05$) or $8.8 \pm 1.7$ mV. In contrast, panels C and D show that changing $[Na^+]_o$ from 124.8 to 62.4 mM by 50% replacement of $Na^+$ with NMG induced a negative shift in the steady-state current and reversal potential of the difference current, respectively. The average shift in reversal potential was from $-19.3 \pm 2.1$ to $-28.5 \pm 2.1$ mV ($n=4, p < 0.01$) or $8.9 \pm 1.1$ mV. Given the effects of $Na^+$ replacement with NMG, we tested for a role of tetrodotoxin-sensitive $Na^+$ channels in the failure to repolarize by applying 10 $\mu$M tetrodotoxin (which was sufficient to block APs and $Na^+$ current under control conditions) to four myocytes after stage 2 occurred. This manipulation failed to change membrane potential, suggesting that tetrodotoxin-sensitive $Na^+$ channels are likely not involved in stage 2 (data not shown).

Finally, to determine whether L-type $\text{Ca}^{2+}$, $Na^+$, and/or $K^+$ channels were contributing to the steady-state difference current, we blocked these conductances with nicardipine (10 $\mu$M), tetrodotoxin (10 $\mu$M), and complete $K^+$ replacement in the pipette and the bath with Cs$^+$. Figure 10 shows that these manipulations failed to prevent activation of a steady-state difference current with a reversal potential of $-18$ mV in this myocyte. The average reversal potential of the difference current calculated from seven myocytes was $-18.3 \pm 3.5$ mV, which was not different from that recorded in the absence of the blockers and Cs$^+$ replacement. Moreover, it is also significant that changing the equilibrium potential for $\text{Cl}^-$ (ECl) in these experiments to $-2.4$ mV from $-60$ mV as in all other experiments had no effect on the reversal potential of the difference current.

Stage 3. The final changes in electrical activity induced by O-R stress included a marked shortening of AP duration, a gradual hyperpolarization, and a loss of excitability as indicated in Figure 11A. Recordings of the quasi-steady-state I-V relation during this period are shown in Figure 11B, which illustrates the gradual
FIGURE 10. Oxygen-derived free radicals (O-Rs) activate the steady-state difference current after the blockade of L-type Ca\(^{2+}\), tetrodotoxin-sensitive Na\(^+\), and all K\(^+\) currents. Left panel shows quasi–steady-state current recordings evoked by a ramp protocol (from \(-130\) to \(+30\) mV over 8 seconds from a holding potential of \(-85\) mV). In this experiment, 10 \(\mu\)M tetrodotoxin and 10 \(\mu\)M nicardpine were added to the bath solution, and \([K^+]_o\) and \([K^+]_i\) were completely replaced with Cs\(^+\). Tracing A was obtained immediately after gaining access from a myocyte dialyzed with 0.1 mM EGTA and the O-R–generating system. Tracings B, C, and D were consecutive recordings obtained when the shift in steady-state was evident. Note the complete reversibility of the changes in steady-state current during O-R stress. Right panel shows the steady-state difference current obtained by digital subtraction of tracing A from tracings B (○), C (○), and D (○). The difference currents had a common reversal potential of \(-18\) mV in this myocyte.

departure from the control recording (tracing A) in successive tracings obtained at 30-second intervals after the first sign of a change in outward current (i.e., at 8.5 minutes in this myocyte). Outward current was enhanced at all potentials positive to \(E_K\) and, when markedly activated, was largely linear between \(-80\) and +20 mV but showed strong inward rectification at potentials positive to the latter voltage. This current resembled the current reported to result from ATP-sensitive K\(^+\) channels (\(I_{\text{KATP}}\)). For this reason, myocytes were exposed to glibenclamide\(^{-46}\) after the outward current was increased to test for the involvement of \(I_{\text{KATP}}\) in stage 3. Figure 12 demonstrates that 3 minutes of exposure to glibenclamide (10 \(\mu\)M) blocked the outward current and restored membrane current to a level similar to that recorded immediately after gaining whole-cell access. A similar result was obtained in three other myocytes. Thus, activation of \(I_{\text{KATP}}\) appears to account for the decline in AP duration, hyperpolarization, and loss of excitability observed in current clamp during O-R stress.

Quasi–steady-state currents were also recorded from myocytes that were dialyzed with control pipette solution or solution containing only FeCl\(_3\)-ADP (no DHF) to determine the changes in current due to rundown. Figure 13 shows representative recordings obtained on achieving whole-cell access and after extended recording times. Only very slight changes in steady-state currents were evident in both groups over considerably longer recording times than the time required for the alterations occurring in the presence of DHF. Similar results were obtained for four additional myocytes in each group.

Effect of O-R Scavenger on Changes in Electrical Activity and Quasi–Steady-State Currents due to O-R Stress

To provide evidence that alterations in electrical activity and membrane currents were due to O-R stress, we used the membrane-permeant O-R scavenger MPG (500 \(\mu\)M).\(^4\) Pretreatment of myocytes with MPG for at least 30 minutes before exposure to the generating system did not

FIGURE 11. Late changes in action potentials and quasi–steady-state current during stage 3 of oxygen-derived free radical (O-R) stress. Panel A: Action potentials recorded under current clamp from a single myocyte after gaining cell access (tracing A) and subsequently during the phase of decline in action potential duration and hyperpolarization (stimulation frequency, 0.25 Hz; 22°C). Panel B: Quasi–steady-state current recordings obtained sequentially (at 30-second intervals) during stage 3 of O-R stress via a ramp protocol (from \(-130\) to \(+30\) mV over 8 seconds from a holding potential of \(-85\) mV). Tracing A was recorded immediately after obtaining cell access (control), and the remaining tracings were recorded successively after the first sign of a change in outward current.
affect AP configuration or steady-state membrane currents recorded immediately after gaining cell access; however, all three stages of change in electrical activity during O-R stress were prevented. Figure 14 shows representative data from a single experiment. Similar results were obtained from an additional five myocytes. This suggests that the alterations in electrical activity and membrane currents described above were due to O-R stress rather than nonspecific effects of treatment with DHF or FeCl₃:ADP.

Role of Elevated Intracellular Ca²⁺ and Sarcoplasmic Reticulum Ca²⁺ Release in Effects of O-R Stress

We consistently noted that intracellular O-R stress caused marked cell shortening, and since O-Rs are reported to elevate [Ca²⁺], we considered the possibility that alterations in this ion may have played a role in inducing the changes in steady-state membrane currents. To determine whether [Ca²⁺], was involved, we increased the concentration of EGTA in the pipette solution to 5 mM. All myocytes (n=5) that were dialyzed with 5 mM EGTA and the O-R-generating system failed to exhibit the changes in resting membrane potential or AP prolongation associated with stages 1 and 2, but APD₉₀ still shortened because of the activation of the outward current (Figure 15B), with a time delay to onset of shortening of 10.7±2.0 minutes. This absence of depolarization and increase in APD₉₀ during O-R stress is shown in Figure 15A, which plots the average RMP and APD₉₀ as a percentage of control over a period of 16 minutes after achieving access. Since the delay to onset of shortening was not different from that required for stage 3 in myocytes dialyzed with 0.1 EGTA, it seems unlikely that elevated Ca²⁺ is necessary for the initiation of this stage. In light of the inhibition of stages 1 and 2 by Ca²⁺ chelation, we sought to define the source of Ca²⁺. To test for a role of intracellular Ca²⁺ stores, we pretreated myocytes for 30 minutes with
ryanodine at a concentration (10 μM) that is thought to lead to a depletion of sarcoplasmic reticulum (SR) Ca2+ stores before intracellular exposure to O-Rs. Figures 15C and 15D summarize the effects on RMP and APD90 in five myocytes and show a representative example of the increase in outward current associated with the decline in AP duration, respectively. All myocytes pretreated with ryanodine failed to demonstrate stages 1 and 2, but again, AP shortening associated with stage 3 still occurred with a delay of 7.6±2.2 minutes, which was not different from that required for this stage in the absence of ryanodine pretreatment.

**Discussion**

The present study is the first to describe the effects of intracellular O-R stress on electrical activity and quasi-steady-state membrane conductances of mammalian ventricular myocytes. Intracellular application of an O-R-generating system caused three stages of change in electrical activity including 1) an early slight depolarization and AP prolongation, 2) DADs, failure to repolarize, and a sustained marked depolarization, and 3) a marked decline in AP duration, hyperpolarization, and loss of excitability. On the basis of whole-cell voltage-clamp experiments, these alterations in electrical activity appear to involve specific changes in membrane ionic currents carried by Iκ, forward-mode Na+-Ca2+ exchange activity, and nonselective cation and IKATP channels. Moreover, the data indicate an important role for elevated [Ca2+]i due to abnormal Ca2+ handling by the SR in inducing the changes in electrical activity during intracellular O-R stress.

Auto-oxidation of DHF results in the formation of DHF radical (DHF·) and superoxide radical (·O2−).49 These species will subsequently lead to the generation of other reactive metabolites of oxygen, including H2O2, and the hydroxyl radical (·OH). FeCl3:ADP was included in the generating system to enhance the formation of ·OH from H2O2 and ·O2− or the latter directly.49 Thus, this generating system can be expected to produce the species of O-R specifically relevant to ischemia/reperfusion.29,30 However, we found that the changes in electrical activity produced by dialysis with DHF–FeCl3:ADP were inhibited by pretreatment with MPG. Bolli et al4 indicate that this is primarily a scavenger of hydroxyl radical, suggesting that this species of oxygen metabolite may be primarily responsible for the alterations in steady-state membrane currents described in this study.

That O-R stress may produce alterations in electrical activity is apparent from a variety of studies on intact cardiac preparations and isolated myocytes33; however, the specific ionic conductances affected by O-Rs are poorly characterized. We found that guinea pig ventricular myocytes dialyzed with pipette solution containing 0.1 mM EGTA and the O-R-generating system demonstrated three stages of alterations in electrical activity. We concentrated on identifying the changes in steady-state membrane currents in an effort to understand the basis for 1) the slight depolarization during stage 1, 2) the failure to repolarize and sustained depolarization in stage 2, and 3) the hyperpolarization and collapse of AP duration noted during stage 3. Thus, the three stages in this study reflect three periods during which there were distinct changes in steady-state membrane conductances induced by O-R stress. We do not rule out the possibility that intracellular O-R stress may also have affected time-dependent currents as well (particularly Ica and Ik) and that such changes may also contribute to the observed alterations in electrical activity. Further experiments are required to investigate this possibility.

The electrophysiological features of the three stages of intracellular O-R stress are similar but not identical to those occurring during extracellular O-R stress as described by Barrington et al.18 The following differences should be noted: 1) Stage 1 in both studies was
associated with AP prolongation, but Barrington et al did not observe the slight depolarization described in this study. 2) DADs were observed during stage 2 in both studies, but we did not record EADs, which were frequently present in stage 2 in the study of Barrington et al. 3) Stage 3 in the study of Barrington et al was marked by a loss of excitability due to either a failure to repolarize or marked depolarization. In the present study, we have included failure to repolarize in stage 2 and limited stage 3 to the period of hyperpolarization and decline in AP duration. This was based on 1) the appearance of DADs before and after the myocytes failed to repolarize, 2) the different ionic mechanisms underlying the inexitability during the failure to repolarize and hyperpolarization (see below), and 3) the similar sensitivity of the DADs and failure to repolarize to intracellular Ca\(^{2+}\) chelation and ryanodine pretreatment and the apparent lack of effect of these manipulations on the hyperpolarization and decline in AP duration.

**Stage 1 of O-R Stress**

The first alterations in electrical activity noted during O-R stress were a decline in RMP of 5–10 mV and lengthening in AP duration. We attribute the slight depolarization to reduction in resting K\(^+\) current through I\(_{K1}\). A marked decline in outward current in the negative slope region of the steady-state I-V relation and a decline in chord conductance at all potentials negative to −40 mV were observed in the absence of a significant change in the reversal potential for the quasi-steady-state currents. This implies that the initial change in steady-state current was due to a decline in I\(_{K1}\) in the absence of a change in any other steady-state current(s), e.g., activation of an inward current as was observed during stage 2 of O-R stress. Inhibition of I\(_{K1}\) was also reported to occur in rabbit\(^{22}\) and guinea pig\(^{25,27}\) myocytes in response to extracellular O-R stress. The change in the current apparently arises because of a decrease in the opening probability of the channels rather than a decline in unitary conductance based on cell-attached recordings.\(^{25}\)

Although alterations in I\(_{K1}\) may contribute to changes in the plateau and repolarization phases of the action potential,\(^{30}\) it is also possible that the changes in AP duration in stage 1 may have resulted from alterations in other currents as well. First, the increase in AP plateau amplitude and prolongation of AP duration during early stage 1 may also have involved changes in time-dependent currents, such as I\(_{Na}\) or I\(_{K}\), which were reported to be influenced by extracellular O-R stress.\(^{21,24,26,27}\) Second, during late stage 1 and immediately before the onset of stage 2, the AP plateau phase declined in amplitude when AP duration was very prolonged because of delayed repolarization negative to −30 mV. It is possible that activation of the nonsensitive cation current mediating the failure to repolarize during stage 2 (see stage 2 of O-R stress below) contributed to this change in activity. Activation of nonsensitive current would tend to enhance repolarization positive to, and inhibit repolarization negative to, its reversal potential of −20 mV. Hence, the activation of this current would be expected to cause a concomitant decline in AP plateau potential and delayed repolarization such as were observed during late stage 1. Further experiments are required to determine whether the changes in AP duration during stage 1 reflect a contribution of these other conductances or are due solely to I\(_{K1}\).

**Stage 2 of O-R Stress**

After the initial depolarization, intracellular O-R stress induced DADs and triggered activity as well as failure to repolarize and sustained depolarization at −35 to −20 mV. The presence of DADs in this study is similar to that reported previously in guinea pig papillary muscles,\(^{15}\) ventricular strips,\(^{16}\) and canine,\(^{18}\) rat, and guinea pig\(^{23}\) myocytes during extracellular exposure to O-Rs. We noted that the frequency of DADs and I\(_{K}\) was similar, as previously reported, implying a causal relationship between the inward currents and depolarizations.\(^{30,42}\) The ionic basis of I\(_{K}\) due to Ca\(^{2+}\) overload is controversial; the inward current has been attributed to 1) Ca\(^{2+}\)-activated nonsensitive cation channels and/or 2) forward-mode activity of the Na\(^+-\)Ca\(^{2+}\) exchanger.\(^{41,43}\) A role of Na\(^+-\)Ca\(^{2+}\) exchange current was indicated by sensitivity of the inward current to Li\(^+\) replacement for [Na\(^+\)]. In the presence of a unique reversal potential for the I\(_{K}\) under recording conditions in both [Ca\(^{2+}\)] and [Na\(^+\)] are not exact.\(^{15}\) In contrast, the nonsensitive cation conductance is not sensitive to Li\(^+\) replacement\(^{31}\) and reverses at a voltage approximately halfway between E\(_{K}\) and the equilibrium potential for Na\(^+\), or −20 mV.\(^{45}\) In the present study, we could not identify a reversal potential for the I\(_{K}\) of stage 2 over a voltage range of −80 to +25 mV, and the currents were inhibited when extracellular Na\(^+\) was completely replaced with Li\(^+\). For these reasons, it would appear that the I\(_{K}\) induced by intracellular O-R stress reflects forward-mode Na\(^+-\)Ca\(^{2+}\) exchange activity rather than nonsensitive cation current. It should also be noted that Matsuura and Shattock\(^{21}\) previously reported a similar involvement of exchange current in the I\(_{K}\) occurring during extracellular O-R stress.

The predominant feature of stage 2 was a failure to repolarize after an AP and sustained depolarization to between −35 and −20 mV. The results of our experiments favor the interpretation that activation of an inward current through nonsensitive cation channels accounts for the marked change in membrane potential during stage 2. Neither a decline in seal resistance nor O-R-mediated breakdown of the sarcolemmal integrity appears to be involved, because the changes in holding current and reversal potential of the difference current activated during this stage were completely reversible in 15 myocytes (Table 1), which would not be expected if seal leak or membrane disruption was involved. Moreover, the reversal potential for the difference current evolved during this stage was −19.9±0.7 mV rather than 0 mV, as would be expected for a nonspecific leak conductance. It is also unlikely that the current was due to Cl\(^-\), L-type Ca\(^{2+}\), tetrodotoxin-sensitive Na\(^+\), typical K\(^+\) channels, or Na\(^+-\)Ca\(^{2+}\) exchange current because 1) the reversal potential for the difference current was not affected by changes in E\(_{K}\), 2) membrane potential was unaffected by exposure to tetrodotoxin after failure to repolarize occurred during stage 2, 3) the reversal potential of the difference current induced by O-R stress was unaffected by exposure to nicardipine, tetrodotoxin, and Cs\(^+\), 4) the difference current had a demonstrable reversal potential under conditions in
which [Na\(^+\)] and [Ca\(^{2+}\)] were dynamic, and 5) the current was not affected by Li\(^+\) replacement of Na\(^+\).

On the other hand, the linear I-V relation and reversal potential of \(-19.9\pm0.7\) mV for the difference current activated during stage 2 are similar to that reported by Matsuda, \(-22\pm11\) mV for the voltage-independent nonselective cation current evoked by elevating [Ca\(^{2+}\)]. That intracellular O-R stress leads to the activation of a nonselective cation conductance is supported by the following observations: 1) A 100% increase in [K\(^+\)], (4.8-9.6 mM) or a 50% decrease in [Na\(^+\)], by NMG substitution shifted the reversal potential for the difference current by +8.8±1.7 mV and −8.9±1.1 mV, respectively, values that are compatible with that expected (i.e., +8.83 and −8.83 mV) assuming an equal permeance of K\(^+\) and Na\(^+\) in the channel.\(^{51,52}\) 2) The reversal potential of the difference current activated by O-R stress was unaffected by 100% replacement of [K\(^+\)], and [Na\(^+\)], with Cs\(^+\) and Li\(^+\), respectively, as would be expected for a conductance that was not selective for different cations.\(^{51}\)

**Stage 3 of O-R Stress**

The decline in AP duration and hyperpolarization during the final stage of intracellular O-R stress were the result of the activation of an outward K\(^+\) current that was 1) voltage insensitive negative to +20 mV yet showed strong inward rectification positive to this potential, 2) a “noisy” current suggestive of a large single-channel conductance, and 3) blocked with glibenclamide (10 
\(\mu\)M). These properties are all consistent with those reported for \(I_{\text{KATP}}\).\(^{45}\) Goldhaber et al\(^{17}\) also observed a decline in AP duration during extracellular O-R stress due to activation of \(I_{\text{KATP}}\).

**Mechanism of Alterations in Membrane Ionic Currents During Intracellular O-R Stress**

O-R stress causes oxidation of membrane phospholipids and/or sulfhydryl-containing proteins,\(^{32}\) which could lead to alterations in channel activity and changes in the membrane currents described above. Alternatively, it is possible that other indirect mechanisms are also involved, since ion channels are sensitive to a variety of intracellular factors that might be influenced by O-R stress. For example, intracellular Ca\(^{2+}\) and ATP affect the nonselective cation conductance\(^{44,51,52}\) and \(I_{\text{KATP}}\),\(^{17}\) respectively, and the levels of both factors are known to be altered by O-R stress.\(^{53}\) The results of the present study suggest that intracellular O-R stress indirectly leads to stages 1 and 2 because of elevated [Ca\(^{2+}\)].

**Alterations in Electrical Activity During Extracellular Versus Intracellular O-R Application**

Intracellular O-R application was chosen in this study to simulate the effects of endogenous intracellular sources of O-Rs. Given the highly reactive nature and extremely short half-life of O-Rs, the possibility arises that the sites of oxidative damage during intracellular O-R stress may be different from those for O-Rs in the extracellular space.\(^{13}\) Tarr and Valenzeno\(^{39}\) showed that...
intracellular O-Rs caused a decline but that extracellular O-Rs led to a biphasic increase followed by a decrease in APD in frog atrial myocytes. All previous studies on the effects of exogenous O-Rs on electrical activity and membrane currents in mammalian cardiac myocytes have dealt with the effects of extracellularly applied O-Rs. Although a comparison of intracellular versus extracellular O-R stress is somewhat compromised by different animal species, generating systems, and recording conditions used, two major differences are apparent at this time: 1) EADs were reported to result from extracellular O-R stress in three different studies,18,23,24 but they did not occur during intracellular O-R stress in this study. The mechanism responsible for the EADs is controversial; Cerbai et al22 attributed the EADs to a decline in $I_{Ks}$, whereas Beresewisz and Horackova suggested that they resulted from reduced tetrodotoxin-sensitive Na$^+$ channel inactivation. Therefore, it is possible that the absence of EADs during intracellular O-R stress reflects an influence of O-R stress on a site of the delayed rectifier or Na$^+$ channel not accessible to O-Rs in the intracellular compartment. 2) Although elevated $[Ca^{2+}]_{i}$, appears to be involved in the genesis of changes in electrical activity and membrane currents due to O-Rs in the extracellular as well as the intracellular compartment, the major source of Ca$^{2+}$ may be different. In the present study, intracellular Ca$^{2+}$ chelation completely blocked stages 1 and 2, as was the case for AP prolongation,22 EADs,24 and Ca$^{2+}$-activated steady-state current22 due to extracellular O-R stress. However, ryanodine did not prevent EADs, AP prolongation, and failure to repolarize after an AP23 nor did it inhibit hypercontracture27 during extracellular O-R stress. Both studies concluded that 1) extracellular Ca$^{2+}$ influx was primarily responsible for the elevation in $[Ca^{2+}]_{i}$, 2) increased Na$^+$-Ca$^{2+}$ exchange activity may underlie the increased Ca$^{2+}$ influx, and 3) SR stores were of minor importance. On the basis of our data and these previous studies, it seems possible that O-Rs generated in the extracellular space may not affect SR Ca$^{2+}$ release to the same extent as intracellular O-R stress and that the latter may not alter Na$^+$-Ca$^{2+}$ exchange to increase Ca$^{2+}$ influx. Additional experiments are needed to more fully detail the differences between extracellular and intracellular O-R stress.

Acknowledgment

The authors wish to express their appreciation to Dr. Normand R. Leblanc for many useful discussions during this study.

References

38. Yue DT, Marban E: A novel cardiac potassium channel that is active and conductive at depolarized potentials. Pflugers Arch 1988;413:127–133
51. Ehara T, Noma A, Ono K: Calcium-activated non-selective cation channel in ventricular cells isolated from adult guinea-pig hearts. J Physiol (Lond) 1988;403:117–133
54. Mazzanti M, DiFrancesco D: Intracellular Ca modulates K⁻inward rectification in cardiac myocytes. Pflugers Arch 1989;413:322–324
Alterations in electrical activity and membrane currents induced by intracellular oxygen-derived free radical stress in guinea pig ventricular myocytes.

R I Jabr and W C Cole

doi: 10.1161/01.RES.72.6.1229

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/72/6/1229