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Oxygen Free Radicals and Excitation-Contraction Coupling

JOSHUA I. GOLDHABER and MOHAMMED S. QAYYUM

ABSTRACT

Oxygen free radicals (OFR) contribute to contractile failure, rigor, and calcium (Ca^{2+}) overload in ischemic/reperfused myocardium. Using both multicellular and isolated single-cell preparations, our laboratory has identified two fundamental mechanisms contributing to the deleterious effects of OFR: (i) impaired myocardial metabolism, and (ii) altered myocardial calcium handling. Impaired metabolism leads to activation of metabolically sensitive K^+ currents, which shorten the action potential, thereby decreasing the duration of systole. Ultimately, high-energy phosphate depletion secondary to metabolic failure results in rigor. Altered myocardial Ca^{2+} handling is evidenced by a decrease in Ca^{2+} entry via L-type Ca^{2+} channels [another cause of decreased action potential duration (APD)], a reduction in sarcoplasmic reticulum (SR) Ca^{2+} content, slowed Ca^{2+} uptake in diastole, and increased sodium-calcium exchange (NaCaX) activity. The increase in NaCaX activity may contribute to the early increase in developed tension frequently observed in multicellular preparations exposed to free radicals, as well as the SR depletion occurring early on in voltage-clamped isolated cell preparations. Increased NaCaX activity is likely to be a critical factor underlying the late Ca^{2+} overload that occurs in the setting of increased intracellular Na^+, and which leads to irreversible injury. The extent to which free radical-mediated metabolic inhibition participates in the dysfunction of the L-type Ca^{2+} channel is uncertain. The altered activity of the SR Ca^{2+} pump and NaCaX are more likely caused by direct actions of OFR on these proteins. Antiox. Redox Signal. 2, 55–64.

INTRODUCTION

CONTRACTILE FAILURE is a prominent feature of ischemia/reperfusion in heart muscle, and is thought to be caused in large part by oxygen free radicals (OFR). Aside from loss of cellular membrane integrity, a relatively late phenomenon, numerous mechanisms have been proposed to explain how OFR depress contractile function even in the absence of cell lysis. One suggestion has been that OFR directly modify the behavior of specific cellular calcium (Ca^{2+})-regulating mechanisms that are critical to excitation-contraction (E-C) coupling. Another possibility is that free radicals adversely impact cardiac metabolism, which may also interfere with cellular Ca^{2+} regulation and thus contractility. We have explored these two hypotheses in detail in both multicellular and single-cell preparations. Our results suggest a role for both mechanisms in OFR-induced contractile dysfunction.

STRATEGIES FOR ASSESSING THE EFFECTS OF FREE RADICALS IN EXPERIMENTAL PREPARATIONS

Our general approach has been to apply free radical-generating systems (FRGS) to different

UCLA School of Medicine, Department of Medicine (Cardiology), and the Cardiovascular Research Laboratories, Los Angeles, CA 90095-1679.

cardiac preparations, to assess the effects of OFR on a variety of parameters critical to cardiac function. We have routinely used two different FRGS in our experiments. The first is hydrogen peroxide (H_2O_2), with or without added iron. Although not an oxygen free radical in its own right, H_2O_2 is an oxidizing agent that forms *in vivo* when superoxide anion is dismuted by the endogenous enzyme superoxide dismutase (SOD). H_2O_2 can be broken down subsequently to molecular oxygen and water by endogenous enzymes such as glutathione peroxidase and catalase. However, H_2O_2 may also participate in the generation of the toxic hydroxyl radical ($^{\cdot}OH$) in a reaction with metal ions, particularly iron. The H_2O_2 system is simple to use experimentally, and its generation of hydroxyl radicals is extensively studied in cardiac preparations (for example, see Corretti *et al.*, 1991). We have also used a superoxide anion ($^{\cdot}O_2^-$) generating system, consisting of xanthine in the presence of xanthine oxidase to confirm many of the findings observed with H_2O_2. This latter system has the disadvantage of being difficult to prepare, due to the relative insolubility of xanthine in HEPES-buffered solution. Furthermore, the activity of xanthine oxidase, and the subsequent reaction that produces $^{\cdot}O_2^-$ is difficult to monitor. Nevertheless, the similarity of effects of the two FRGS in our models has been helpful. For further details of these reactions *in vivo* and *in vitro*, please see Goldhaber and Weiss (1992).

EFFECTS OF FRGS ON ELECTROMECHANICAL FUNCTION IN A MULTICELLULAR PREPARATION

A decade ago we described the effects of OFR on the isolated rabbit interventricular septum (Goldhaber *et al.*, 1989). This multicellular preparation is perfused via the septal artery with a Krebs-Ringers bicarbonate-buffered solution, at physiological temperature and pH. Developed tension and positive and negative *dT/dt* can be recorded using a tension transducer, whereas intracellular membrane voltage can be recorded from a single cell in the preparation using floating glass microelectrodes. Lactate efflux is routinely monitored by collecting the effluent, and high-energy phosphates can be sampled in the tissue by freeze clamping. Finally, in some experiments, we measured K^+ efflux using the radioisotope $^{42}K^+$.

MECHANICAL EFFECTS

When added to the perfusate, both FRGS had reproducible effects on tension (Fig. 1). First

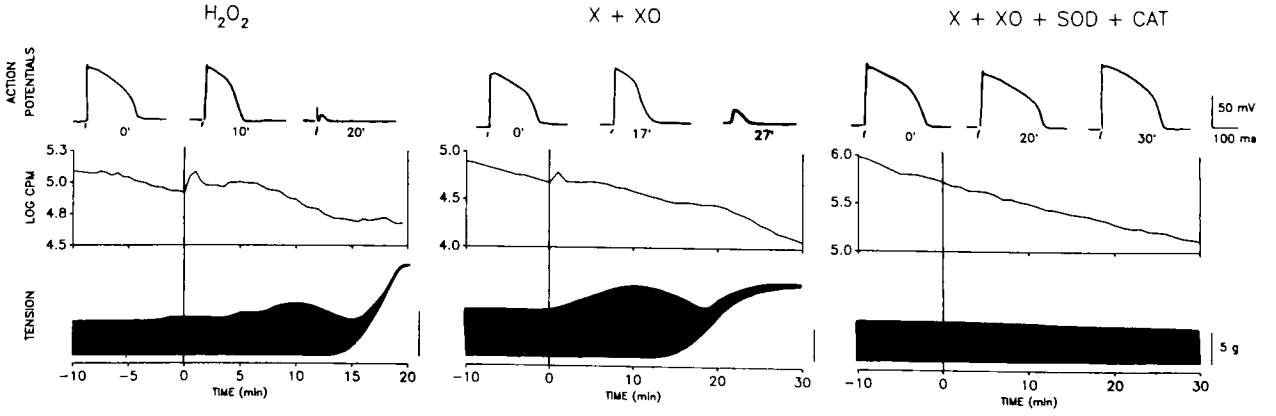

FIG. 1. Effects of two different FGRS on APD, $^{42}K^+$ efflux, and tension in isolated perfused rabbit interventricular septa. Both the $^{\cdot}OH$-generating compound H_2O_2 (1 m*M*) and the $^{\cdot}O_2^-$-generating system xanthine (1 m*M*) + xanthine oxidase (0.01 U/ml) (X + XO) caused an immediate increase in $^{42}K^+$ efflux, followed by an increase in developed tension that peaked at 10 min as the APD decreased. This was followed by continued reduction in APD and eventually increased diastolic tension (rigor) and loss of developed tension. The effects of X + XO could be prevented by using SOD and catalase (CAT), which scavenge $^{\cdot}O_2^-$ and H_2O_2, respectively. (Reprinted by permission from Goldhaber *et al.*, 1989.)

there was a gradual increase in systolic and developed tension (developed tension = systolic tension − diastolic tension), which peaked at 10 min, followed by a decrease. Diastolic tension then began to increase (rigor), resulting in a fall of developed tension. Ultimately, the preparation became inexcitable.

CORRELATION WITH CHANGES IN METABOLISM

The decrease in systolic and developed tension, followed by an increase in diastolic tension, was reminiscent of what had been observed previously in the same preparation during inhibition of glycolytic metabolism using specific metabolic inhibitors (Weiss and Hiltbrand, 1985). We noted other similarities, including the immediate increase in $^{42}K^+$ efflux, the reduction in action potential duration (APD), and the increased lactate production that coincided with the increased diastolic tension. To explore the hypothesis that free radicals were inhibiting metabolism in our preparation, we examined high-energy phosphate levels in freeze-clamped tissue samples. After 2.5 min of exposure to the FRGS, when diastolic tension was normal, there was no significant change in high-energy phosphate levels. However, following 20 min of exposure, at a time when diastolic tension had begun to increase, high-energy phosphate levels were reduced by about 50%. Thus, it appeared that the rigor associated with free radical exposure might be attributable to metabolic inhibition caused by free radicals, evidenced by a decrease in high-energy phosphate levels, and increased lactate production.

SINGLE-CELL APPROACH

We used a second approach to evaluate the effects of OFR on metabolic function. Reductions in the ATP/ADP ratio are known to result in the activation of ATP-sensitive K^+ channels in the sarcolemmal membrane of ventricular myocytes. Thus, opening of these channels can act as a biosensor of metabolic activity in single cardiac myocytes. Therefore, we monitored ATP-sensitive K^+ channel activity using the cell-attached patch clamp technique in enzymatically isolated guinea pig ventricular myocytes. Once patched, the cells were permeabilized at one end using the membrane detergent saponin while bathing in a low-calcium solution that mimicked the cytosolic milieu. The solution also contained ATP. In control experiments, ATP-sensitive K^+ channels opened when ATP was removed from the solution and closed when ATP was returned to the solution. We found that exposure to H_2O_2 had no effect on the ability of ATP to close the channels. Because ATP remained effective at closing the channels, even in the presence of the free radicals, we concluded that there was no direct effect of OFR on the ATP-sensitive K^+ channels themselves, or the channels' ability to interact with ATP.

Under normal conditions, these permeabilized single cells can synthesize ATP when the bath is superfused with the appropriate glycolytic or oxidative substrates. When ATP is generated under these conditions, ATP-sensitive K^+ channels close. If the cells are exposed to inhibitors of ATP synthesis under these conditions, the ATP-sensitive K^+ channels remain open despite the presence of available substrates (Weiss and Lamp, 1987). Using this technique, we were able to compare the efficacy of glycolytic versus mitochondrial substrates for ATP synthesis at closing ATP-sensitive K^+ channels in the setting of exposure to OFR (Fig. 2). We found that neither set of substrates was able to close the ATP-sensitive K^+ channels once the cell had been exposed to a FRGS, suggesting irreversible inhibition of both glycolytic *and* oxidative metabolism by OFR. Therefore, the results in the single cells supported the evidence obtained in the multicellular preparation suggesting that OFR inhibit metabolism in cardiac myocytes.

ELECTROPHYSIOLOGIC CHANGES CAUSED BY FRGS

During continuous recording of the action potential in the rabbit septal preparation (Fig. 1), we observed a steady decrease in APD measured at 90% repolarization (APD_{90}). Because

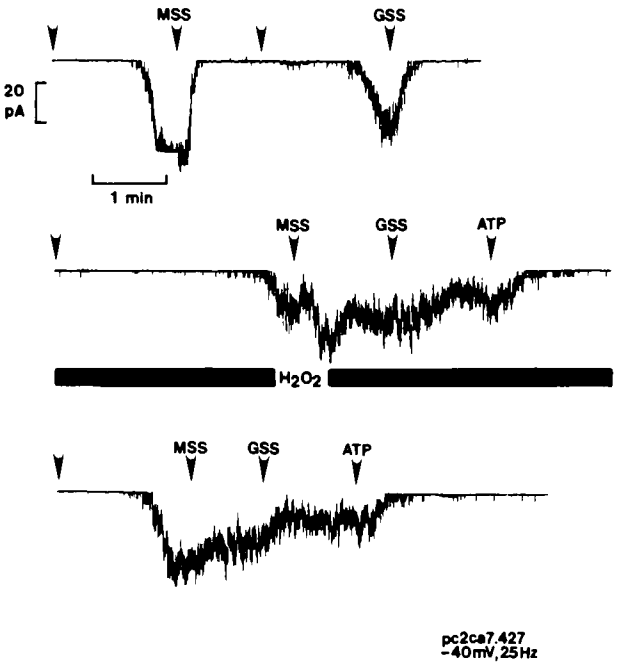

FIG. 2. Effect of H_2O_2 on ability of glycolytic versus mitochondrial metabolic substrates to suppress ATP-sensitive K^+ channels recorded from a cell-attached patch on a permeabilized single guinea pig ventricular myocyte. Inward current is downward, filter setting 50 Hz, and tracings are continuous. The patch electrode was held continuously at +40 mV relative to the bath. One minute following the removal of ATP from the bath (*upper trace, unlabeled arrow*) ATP-sensitive K^+ channels (*downward current deflections*) opened. When individual substrates for mitochondrial oxidative phosphorylation (MSS) or glycolytic metabolism (GSS) were added to the bath (*arrows labeled MSS or GSS*), the ATP-sensitive K^+ channels closed. During (*middle trace*) or after (*bottom trace*) exposure to 1 m*M* H_2O_2, addition of GSS or MSS failed to suppress the channels, although 2 m*M* ATP remained effective. MSS consisted of 2 m*M* pyruvate, glutamate, and creatine, 1 m*M* $K_2H_2PO_4$, and 0.5 m*M* ADP. GSS consisted of 2 m*M* fructose-1,6-diphosphate, 1 m*M* NAD and $K_2H_2PO_4$, and 0.5 m*M* ADP. (Reprinted by permission from Goldhaber *et al.*, 1989.)

we also observed a sudden increase in K^+ efflux at the time we began the infusion of the FRGS, we hypothesized that the decrease in the APD was caused by an increase in K^+ conductance, due at least in part to the opening of ATP-sensitive K^+ channels as described above. A similar decrease in APD caused in part by an increase in K^+ conductance occurs during ischemia, hypoxia, and application of metabolic inhibitors (Goldhaber *et al.*, 1991).

To test this hypothesis more directly, we studied the effects of FRGS on whole-cell current under voltage clamp conditions in isolated guinea pig ventricular myocytes. Both FRGS activated a K^+ conductance typical of the ATP-sensitive K^+ current activated by metabolic inhibition. We also found a decrease in the voltage sensitive L-type calcium current during exposure to FRGS, which is also typical of metabolic inhibition (Goldhaber *et al.*, 1991). Both the increase in K^+ conductance, and the decrease in L-type Ca^{2+} current would be expected to reduce the APD, as it does with metabolic inhibition.

EFFECTS OF OFR ON Ca^{2+} HANDLING MECHANISMS AND E-C COUPLING IN CARDIAC MYOCYTES

The decrease in developed tension during exposure to FRGS is consistent with the decrease in the L-type Ca^{2+} current we observed in the single cells. The reduction in Ca^{2+} current (I_{Ca}) could be either a direct consequence of free radical exposure, or perhaps secondary to metabolic inhibition. However, there are several other potential causes of decreased contraction during exposure to OFR. These include depletion of SR Ca^{2+}, decreased E-C coupling "gain" (*i.e.*, amount of Ca^{2+} released from the SR in response to I_{Ca}), and reduced myofilament response to Ca^{2+}. The latter is thought to be the mechanism underlying myocardial stunning (Kusuoka *et al.*, 1987), which can be reproduced in experimental preparations by FRGS (Gao *et al.*, 1996). To study in greater detail the mechanisms underlying the decrease in contractile strength during exposure to OFR, we examined the effects of FRGS on E-C coupling in isolated guinea pig and rabbit ventricular myocytes. In paced guinea pig ventricular myocytes loaded with the membrane-permeable Ca^{2+} indicator indo-1 AM, H_2O_2 briefly increased, then decreased, the amplitude of intracellular Ca^{2+} transients. The changes in the Ca^{2+} transient were mirrored by cell contractions. This response was reminiscent of the increase in systolic tension followed by a decrease observed in the rabbit septum, as described above. Automaticity developed later, followed shortly by inexcitability. Because of our evidence that free radicals inhibit metabolism, we compared the effects of OFR with

metabolic inhibitors. Unlike free radicals, metabolic inhibitors caused rapid inexcitability (likely due to early activation of the ATP-sensitive K^+ current), followed much later by an increase in diastolic intracellular calcium (Goldhaber and Liu, 1994). The different response of the preparation to direct metabolic inhibitors suggests that the effects of free radicals are not mediated solely by their effect on metabolism, implying additional actions of OFR that ultimately result in dramatic alterations of E-C coupling.

To explore the effects of OFR on E-C coupling in greater detail, we used patch-clamped guinea pig and rabbit ventricular myocytes loaded with fura-2 salt to monitor intracellular Ca^{2+} along with membrane current and cell shortening. H_2O_2 reduced the amplitude of I_{Ca}, the Ca^{2+} transient, and active cell shortening (Fig. 3), similar to the effects of combined oxidative and glycolytic inhibition with carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP, 1 μM) and 2-deoxyglucose (2-DG, 10 mM). In patch-clamped cells exposed to OFR, we never observed the transient increase in intracellular Ca^{2+} or cell shortening we had seen in the field-stimulated paced ventricular myocytes loaded with indo-1-AM. The reason for this difference in the patch-clamped cells compared to the paced cells is uncertain, but could be related to changes in the contribution of other Ca^{2+}-handling mechanisms to contraction, *e.g.*, reverse NaCaX or voltage-sensitive Ca^{2+} release mechanisms. Both of these alternative mechanisms would be disabled during voltage clamps from −40 to 0 mV. Another explanation for the discrepancy in the response of the two different preparations may relate to the redox state of the cell, which can influence the gain of E-C coupling (Suzuki *et al.*, 1998).

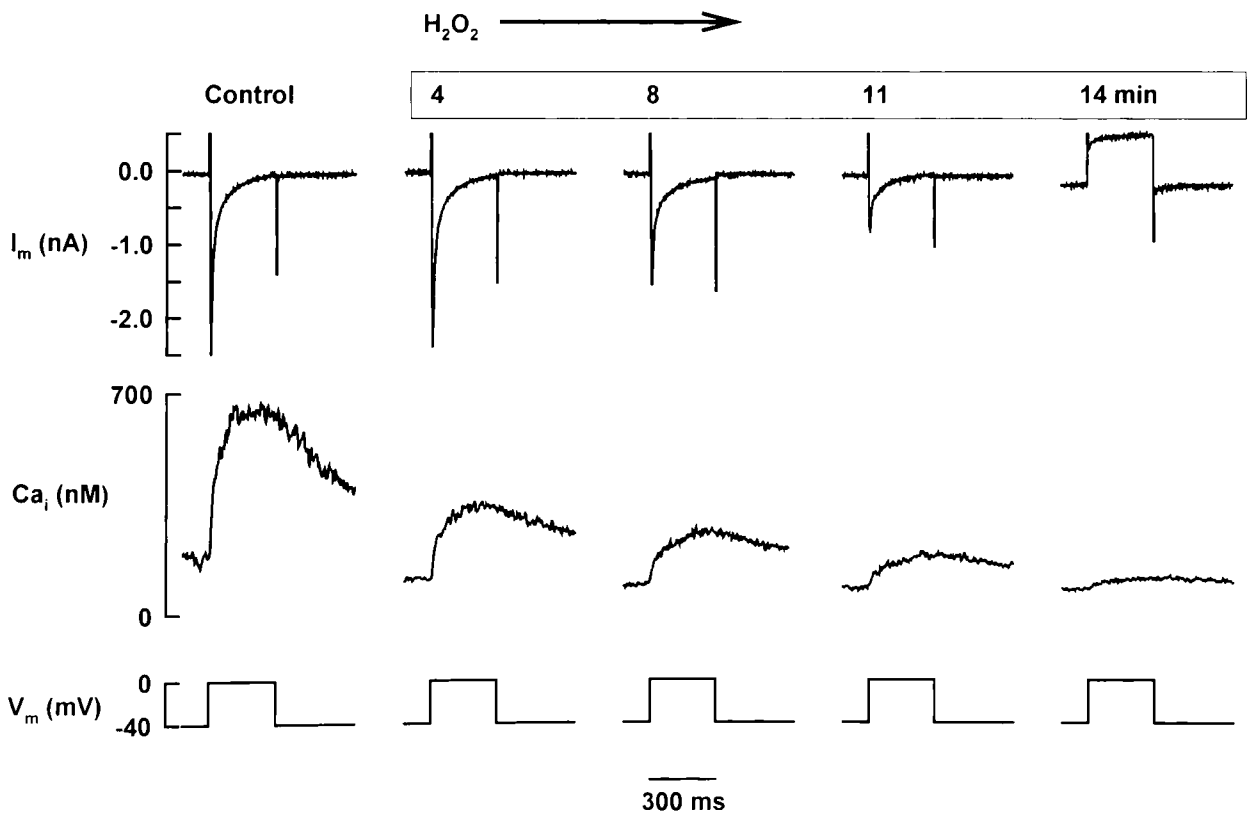

FIG. 3. Effects of H_2O_2 on excitation-contraction (E-C) coupling. Recordings of membrane current (I_m) and calculated intracellular calcium (Ca_i) in a whole-cell patch-clamped rabbit ventricular myocyte loaded with 100 μM Fura 2 salt via the patch electrode and exposed to 1 mM H_2O_2. The cell was voltage clamped once each minute from a holding potential of −40 mV to test potential of 0 mV for 300 msec. Control tracings are displayed in the *far left* panel. Subsequent panels contain tracings after 4-, 8-, 11-, and 14-min exposure to 1 mM H_2O_2, respectively. (Reprinted by permission from Goldhaber *et al.*, 1996.)

In our patch-clamp experiments, we did not routinely add any reducing agents, such as glutathione, to the patch electrode solution. This may have altered the natural redox state of the cell and led to a reduction in the gain of E-C coupling, thereby eliminating any free radical-mediated increases in the extent of Ca^{2+} release or contraction during voltage clamps.

The eventual contractile failure and reduction in Ca^{2+} release we observed in all preparations was consistent with the decrease in the Ca^{2+} current we saw in the patch-clamped cells, and was similar to the effects of direct metabolic inhibition using FCCP and 2-DG. However, we found that H_2O_2 also reduced SR calcium content by 42% when assessed by applying pulses of 5 m*M* caffeine to the cell (Fig. 4). This was unlike direct metabolic inhibitors, which left SR Ca^{2+} stores unaffected. SR Ca^{2+} depletion is expected to result in a further decrease in contractile function during exposure to FRGS.

The mechanism of SR Ca^{2+} depletion is most likely multifactorial. The decrease in Ca^{2+} entry via L-type Ca^{2+} channels by itself could lead to a reduction in SR Ca^{2+} content. But free radicals have also been shown to have a deleterious effect on the SR Ca^{2+} pump (Xu *et al.*, 1997) and to increase SR Ca^{2+} release by the ryanodine receptor (Kawakami and Okabe, 1998). These two mechanisms would certainly aggravate a loss of SR Ca^{2+}. Although we found evidence of a decrease in Ca^{2+} pump activity, reflected in a slowing of the rate of Ca^{2+} transient "relaxation" (Fig. 5), we have not yet evaluated ryanodine receptor activity in our preparations.

SR Ca^{2+} content could also be affected by NaCaX, which competes for Ca^{2+} during relaxation of the Ca^{2+} transient. An increase in Ca^{2+} efflux via NaCaX would tend to deplete SR Ca^{2+}. To assess NaCaX activity, we applied puffs of caffeine to patch-clamped myocytes held at a constant membrane potential of −40

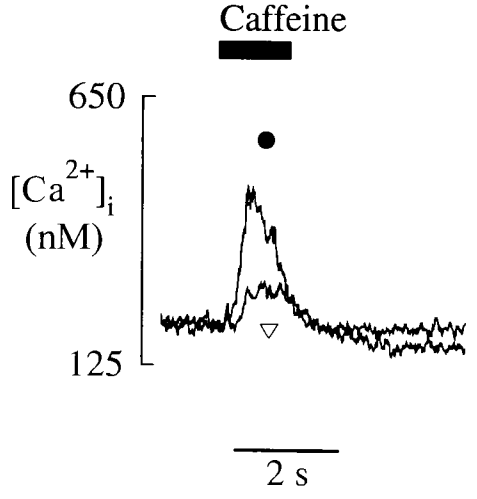

FIG. 4. Fura-2 intracellular calcium ($[Ca^{2+}]_i$) transients in a representative patch-clamped guinea pig ventricular myocyte. Shown are transients before (*solid circle*) and after (*open inverted triangle*) exposure to 1 m*M* H_2O_2, during application of Tyrode's solution containing 5 m*M* caffeine (*solid bar*) while holding the voltage constant at −40 mV. Six 300-msec conditioning pulses from −40 to 0 mV preceded each application of caffeine. Note the reduction in the peak of the $[Ca^{2+}]_i$ transient following administration of H_2O_2. (Modified and reprinted with permission from Goldhaber *et al.*, 1994.)

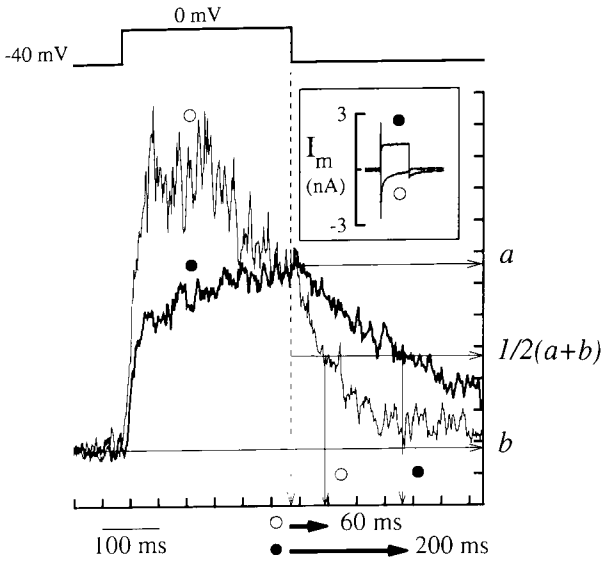

FIG. 5. Effect of H_2O_2 on the half-time for return of the intracellular Ca^{2+} transient to baseline following a voltage clamp. The upper panel shows the voltage protocol and the lower panel the fluorescence transients from a patch-clamped guinea-pig ventricular myocyte loaded with Fura-2 salt under control conditions (open circles) and after exposure to 1 m*M* H_2O_2 (solid circles). The Ca^{2+} transients were scaled to arbitrary units so that they could be superimposed at the onset of repolarization (line *a*) and at baseline (line *b*). The half-time for return of the Ca^{2+} transient to baseline was determined by subtracting the time at repolarization from the time at which the Ca^{2+} transient crossed the half-way point, *i.e.*, the time at which Ca^{2+} had returned half-way from *a* to *b*. In this example, the half-time of the control transient was 60 msec, and the half-time of the transient following exposure to H_2O_2 was 200 msec. The inset shows the corresponding membrane current tracings for the two conditions. (Reprinted by permission from Goldhaber *et al.*, 1994.)

mV. Caffeine triggers Ca^{2+} release from the SR, which in turn leads to Ca^{2+} extrusion and Na^+ entry by the NaCaXer, and thus an inward NaCaX current (Callewaert *et al.*, 1989). We found an absolute increase in integrated exchanger current during exposure to free radicals, despite the reduction in SR Ca^{2+} content (Fig. 6). To confirm the identity of this current, we demonstrated that it could be blocked by Ni, or by substituting Cs^+ for Na^+ (the Ca^{2+}-activated nonselective current can pass Cs^+, ruling out the nonselective current as an alternative). We also confirmed the dependence of this inward current on SR Ca^{2+}, since depleting SR Ca^{2+} using the SR Ca^{2+} pump blocker Thapsigargin (200 n*M*) eliminated the inward current observed upon application of caffeine (Goldhaber, 1996). Thus, our results suggest a significant increase in NaCaX current during exposure to free radicals, even as SR Ca^{2+} and L-type Ca^{2+} current are decreasing. In the patch-clamped preparation, with stable intracellular Na^+, the consequence of slowed Ca^{2+} uptake by the SR together with stimulated NaCaX is further depletion of SR Ca^{2+}.

The increase in NaCaX activity has important implications for E-C coupling. For example, it could explain why developed tension increased early after exposure to free radicals in the rabbit septum as well as in field-stimulated myocytes. Although the L-type Ca^{2+} current is the major source of trigger Ca^{2+} during a normal action potential, additional Ca^{2+} influx may occur via reverse NaCaX, when the depolarized membrane voltage favors Ca^{2+} entry via this electrogenic mechanism. Ca^{2+} entry via reverse NaCaX is further augmented by the entry of Na^+ into the subsarcolemmal space during the rapid upstroke of the action potential (LeBlanc and Hume, 1990). An increase in the contribution of trigger Ca^{2+} entering via reverse NaCaX could occur as a result of OFR-mediated stimulation of the exchanger. This would lead to an increase in Ca^{2+} release by the SR, as well as an increase in the extent of contraction. More importantly, an increase in the activity of NaCaX would also be expected to increase Ca^{2+} entry and lead to profound Ca^{2+} overload (and irreversible injury) during ischemia/reperfusion, when intracellular Na^+ concentrations are markedly increased (Pike *et al.*, 1990).

Although we saw an increase in the magnitude of the absolute exchanger current in response to SR Ca^{2+} release by caffeine, it is difficult to assess rigorously the effects of free radicals on the exchanger while SR Ca^{2+} is changing (since the extent of Ca^{2+} release has a major influence on the magnitude of the exchanger current). To avoid this problem,

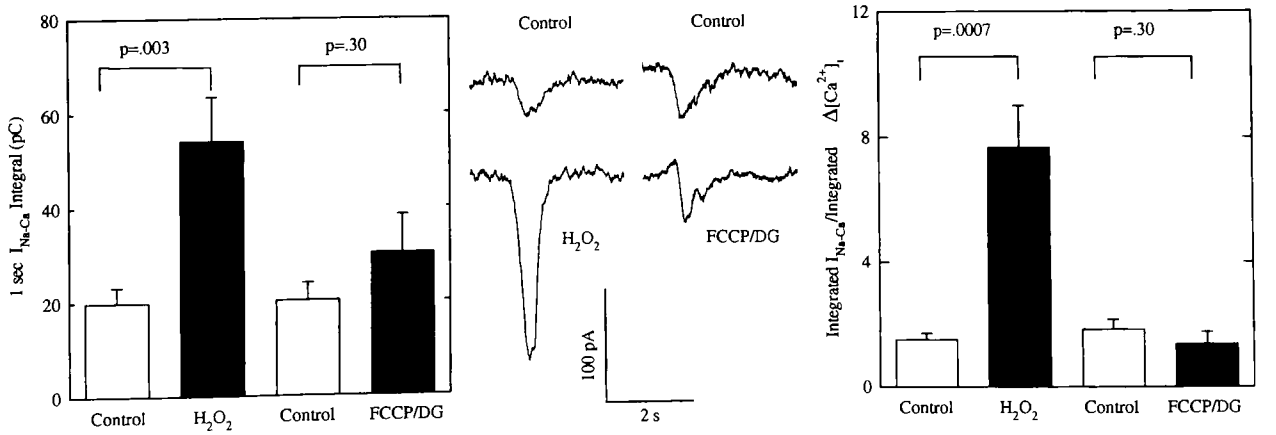

FIG. 6. Effects of H_2O_2 versus metabolic inhibitors on NaCaX currents. The lefthand graph shows 1-sec current integrals of the inward NaCaX current during a caffeine-induced Ca^{2+} transient under control conditions and after exposure to either 1 m*M* H_2O_2 (13 cells) or FCCP-DG (6 cells). Representative caffeine-induced NaCaX currents from two myocytes are shown in the middle panel. In the right-hand graph, the integrated NaCaX current was normalized to the integral of the caffeine-induced Ca^{2+} transient, showing that H_2O_2 markedly increased the relative amplitude of the NaCaX current whereas metabolic inhibition had no significant effect. Myocytes were held at −40 mV throughout and caffeine applied with a rapid solution exchanger for 650 msec. (Reprinted by permission from Goldhaber *et al.*, 1994.)

NaCaX can be assessed by depolarizing cells to positive potentials and then evaluating the magnitude of the Ni^+-sensitive outward current (with K^+ currents blocked). Recently, we have used this approach to test the effects of a hydroxyl radical generating system, H_2O_2 plus Fe^{3+}, on NaCaX outward current, and cell shortening, at +80 mV. Consistent with our previous results, we found a significant increase in outward current at +80 mV, which coincided with an increase in the extent of cell shortening. These two findings likely reflect an increase in Ca^{2+} entry via reverse NaCaX due to enhancement of this protein's transport function. It is unlikely that an increase in myofilament Ca^{2+} sensitivity was responsible for the increase in the extent of contraction we observed, because oxygen free radicals *decrease* the response of myofilaments to calcium (Gao *et al.*, 1996).

SUMMARY

Oxygen free radicals contribute to contractile failure, rigor, and Ca^{2+} overload in ischemic/reperfused myocardium. Our lab has identified two fundamental mechanisms underlying these effects: (i) impaired myocardial metabolism, and (ii) altered myocardial Ca^{2+} handling. These effects are summarized in Fig. 7. Impaired metabolism leads to (i) activation of metabolically sensitive K^+ currents, (ii) shortening of the action potential (and therefore reduced time in systole), and (iii) rigor. Altered myocardial Ca^{2+} handling is evidenced by (i) a reduction in Ca^{2+} entry via L-type Ca^{2+} channels (another cause of decreased APD), (ii) a reduction in SR Ca^{2+} content, (iii) slowed Ca^{2+} uptake in diastole, and (iv) increased NaCaX activity. Whether the reduction of I_{Ca} is caused by a direct effect of free radicals on the Ca^{2+}

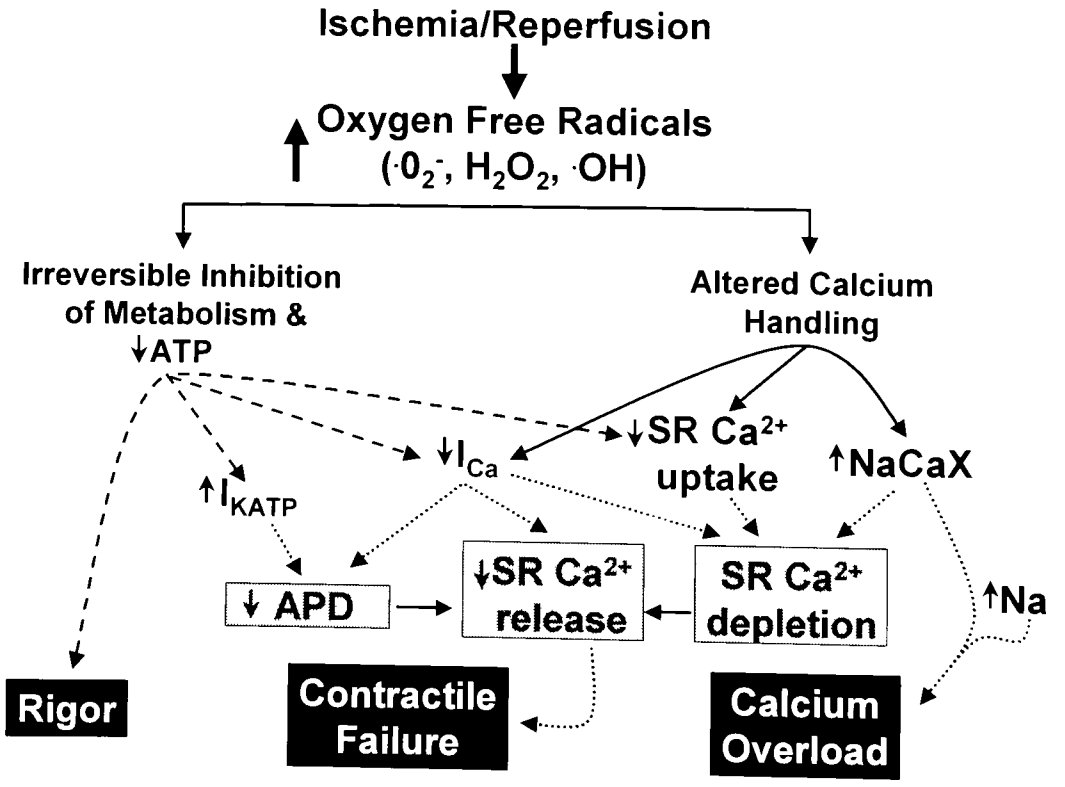

FIG. 7. Scheme depicting the dual effects of oxygen free radicals on myocardial metabolism and Ca^{2+} handling mechanisms in patch-clamped ventricular myocytes. See text for details.

channel, or whether the effect is mediated by OFR-induced metabolic inhibition, is uncertain. The decrease in SR Ca^{2+} uptake, and the increase in NaCaX are clearly not adequately explained by metabolic inhibition alone, because direct metabolic inhibitors do not deplete SR Ca^{2+} and have no effect on the exchanger (Goldhaber *et al.*, 1991; Goldhaber and Liu, 1994). The increase in NaCaX activity may explain both the early increase in developed tension in multicellular preparations exposed to free radicals, as well as the Ca^{2+} overload that occurs in the setting of ischemia/reperfusion when intracellular Na^+ is increased. The dual effects of free radicals on cardiac metabolism and Ca^{2+} handling have profound implications for E-C coupling. However, one must place these effects in the context of other reports, which have shown important effects of free radicals on contractile proteins and other cellular ion channels and transporters, including the ryanodine receptor and the Na^+ pump. Decreased myofilament Ca^{2+} responsiveness, in particular, is certainly a major cause of OFR-mediated reductions in contractile force generation (Gao *et al.*, 1996; Murphy *et al.*, 2000). The huge variety of cellular functions altered by OFR challenges the ability of cells to survive ischemia/reperfusion, and may explain the disappointing results of clinical trials attempting to use free radical scavengers to limit ischemiareperfusion injury.

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ABBREVIATIONS

ADP, Adenosine diphosphate; APD, action potential duration; APD_{90}, action potential duration at 90% repolarization; ATP, adenosine triphosphate; Ca^{2+}, calcium; 2-DG, 2-deoxyglucose; E-C, excitation-contraction; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; FRGS, free radical generating systems; H_2O_2, hydrogen peroxide; I_{ca}, calcium current; K^+, potassium; Na^+, sodium; NaCaX, sodium-calcium exchange; OFR, oxygen free radicals; $^{\cdot}O_2^-$, superoxide anion; $^{\cdot}OH$, hydroxyl radical; SOD, superoxide dismutase; SR, sarcoplasmic reticulum.

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Address reprint requests to:
Dr. Joshua I. Goldhaber
UCLA School of Medicine
Division of Cardiology, 47-123 CHS
10833 LeConte Avenue
Los Angeles, CA 90095-1679

E-mail: jgoldhaber@mednet.ucla.edu

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