ABSTRACT—To understand the effect of oxygen free radicals on Ca\(^{2+}\)-ATPase, we used sarcoplasmic reticulum (SR) microsomes of canine masseter muscle as a model system in which to explore the effects of oxidation on a biological membrane, and we investigated the effect of hydroxyl radicals (·OH) generated from Fenton's reagent (H\(_2\)O\(_2\)/FeSO\(_4\)). H\(_2\)O\(_2\) (10 mM) alone had no effect on Ca\(^{2+}\)-ATPase activity; in the presence of FeSO\(_4\) (0.2 mM), H\(_2\)O\(_2\) inhibited the enzyme activity. Oxygen free radical species generated from H\(_2\)O\(_2\)/FeSO\(_4\) under the conditions employed in the Ca\(^{2+}\)-ATPase assay were verified by highly sensitive electron spin resonance spectroscopy and the spin-trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) in the absence of SR vesicles; the 1:2:2:1 quartet (\(\Delta\nu = \Delta\delta = 1.49\) mT), characteristic of the DMPO-OH spin adduct, was observed. The Ca\(^{2+}\)-ATPase activity was inversely correlated with the calculated signal intensity of DMPO-OH, which is indicative of the amount of ·OH radical generated. The effect of Fenton's reagent was effectively inhibited by catalase, dimethylsulfoxide, and dimethylthiourea; the effect was also inhibited by sulphydryl (SH) group reducing agents, cysteine and dithiothreitol. The SH group modifying agents, p-chloromercuric benzoate and 5,5-dithiobis(2-nitrobenzoic acid) depressed Ca\(^{2+}\)-ATPase activity; the effects of the SH group modifying agents used were potentiated in the presence of Fenton's reagent. It is suggested that ·OH radical-induced oxidant injury may be caused primarily by modification of the key SH group(s) on the ATPase molecule of masseter muscle SR vesicles.

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Keywords: Hydroxyl radical, Ca\(^{2+}\)-ATPase, Sarcoplasmic reticulum, Masseter muscle, Sulphydryl group
(15), the effect of free radicals on the mechanism responsible for Ca²⁺ homeostasis seems to be of particular interest. Thus, there have been reports of in vitro studies demonstrating inhibition of masseter muscle SR Ca²⁺-ATPase activity (16) and Ca²⁺ uptake velocity (17) by oxygen free radicals generated by the xanthine-xanthine oxidase system, but the direct effects of •OH, the most reactive oxygen species, have not been established fully. The present paper summarizes the results of the first of a series of experiments where we demonstrate that the SH group(s) of the ATPase molecule of SR vesicles are targets of •OH radical attack in Fenton-type reactions.

MATERIALS AND METHODS

Materials

The following pharmacological agents were used: superoxide dismutase (SOD), catalase (CAT), cysteine, dithiothreitol (DTT), and p-chloromercuric benzoate (PCMB) (Sigma, St. Louis, MO, USA); hydrogen peroxide (H₂O₂), and dimethyldithiourea (DMTU) (Aldrich, Milwaukee, WI, USA); FeSO₄, dimethylsulfoxide (DMSO), and 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) (Wako Chemicals, Osaka); and 5,5-dimethyl-pyrroline-N-oxide (DMPO) (Labotec, Tokyo). All these agents were prepared in deionized distilled water. All other reagents were of analytical grade.

SR preparation

In accordance with our institutional Animal Care Committee guidelines, masseter muscles were taken from healthy, adult dogs during anesthesia with sodium pentobarbital (25 mg/kg, i.v.). SR vesicles were prepared from minced masseter muscle by the method described by Okabe et al. (16), as follows: The muscle was cleaned of fat and connective tissue, minced and then homogenized twice (1 g muscle/4 volumes of 10 mM imidazole buffer, pH 7.0) for 1 min at 4°C in an Excel autohomogenizer (DX-8; Nihon Seiki, Tokyo). The homogenate was centrifuged at 10,000 x g for 20 min. The pellet was rehomogenized in 4 volumes of 10 mM imidazole buffer, pH 7.0, for 1 min at 4°C in an Excel autohomogenizer (DX-8; Nihon Seiki, Tokyo). The homogenate was centrifuged at 10,000 x g for 20 min. The supernatant fractions from this and the previous centrifugation were combined, poured through four layers of cheesecloth, and centrifuged at 12,000 x g for 15 min. The supernatant fraction was filtered through eight layers of cheesecloth and centrifuged at 31,000 x g for 90 min. The pellet from this centrifugation was rehomogenized with a Potter-Elvehjem homogenizer with a teflon pestle, in 1 M KCl, 10 mM imidazole buffer, and then centrifuged at 145,000 x g for 60 min. The SR pellet was rehomogenized in 30% sucrose, 10 mM imidazole (pH 7.0) and stored at −80°C. The protein concentration of this SR preparation was determined by the method of Lowry et al. (18).

Measurement of ATPase activity

Ca²⁺-ATPase activity of SR was determined as the rate at which inorganic phosphate (Pi) was liberated during the incubation. The incubation bath (10 ml) was kept at 27°C and contained 100 mM KCl, 20 mM imidazole buffer (pH 7.0), 10 mM NaN₃, 10 mM potassium oxalate, 5 mM Na₂ATP, 5 mM MgCl₂, and 200 μM CaCl₂. The released phosphate in the filtrate was assayed by a colorimetric method (19). The Ca²⁺-ATPase activity was calculated as the difference in ATPase rate in a bath containing 200 μM Ca²⁺ compared to one containing 0.02 M ethyleneglycol bis (oxyethylene nitrilo) tetraacetic acid (EGTA).

Hydroxyl radicals-generating system

For the •OH-generating system, H₂O₂ plus FeSO₄ were used. A simple mixture of H₂O₂ and an iron (II) salt forms the •OH radicals, as was first observed by Fenton in 1894 (see ref. 20 for a review):

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^- \]

SOD, CAT, and DMSO or DMTU were used to scavenge superoxide anion radical (O₂⁻), H₂O₂, and •OH, respectively. The timed sequence of reagent addition is described under the Results section.

Electron spin resonance (ESR) analysis

The production of oxygen free radicals by Fenton’s reagent (H₂O₂ plus FeSO₄) was verified by ESR spectroscopy. ESR detection of the spin adduct was performed by a JES-RE3X, X-band spectrometer (Jeol, Tokyo) at the following instrument settings: modulation amplitude, 0.25 mT; recording range, 5 mT; recording time, 2 min; time constant, 0.03 sec; microwave power, 8 mW; and magnetic field, 335.6±5 mT. DMPO (15 μl of the original liquid) was used as the spin trap. The desired reaction mixtures (200 μl) were prepared in the reaction bath (27°C) and transferred to a quartz ESR flat cell (130 μl), which was in turn placed in the cavity of the ESR spectrometer. Sequential ESR scans were then started 50 sec after the addition of DMPO to the reaction mixture. To quantitate the DMPO spin adducts detected, the Mn²⁺ standard ESR spectrum (MnO) was obtained.

Statistical analyses

The statistical tests of significance were performed by Student’s t-test or analysis of variance; a value of P < 0.05 was accepted as an indicating a significant difference.
RESULTS

Spin-trapping of hydroxyl radicals and effect on Ca²⁺-ATPase activity

In preliminary experiments, oxygen free radical species generated from H₂O₂ plus FeSO₄ under the conditions employed in the Ca²⁺-ATPase assay studies were verified by highly sensitive ESR spectroscopy and the spin-trap DMPO in the absence of the SR vesicles (Fig. 1). The 1 : 2 : 2 : 1 quartet (the hyperfine splittings were A_N = A_H = 1.49 mT), characteristic of the DMPO-OH spin adduct (21), was observed in the reaction mixture containing H₂O₂ plus FeSO₄ (Fig. 1); H₂O₂ alone did not produce the DMPO-OH adduct (Fig. 1B).

The data of Fig. 2 further document the concentration-related effect of H₂O₂ in the presence of a fixed concentration of FeSO₄ (0.2 mM). The calculated relative signal intensity of the DMPO-OH adduct increased in a concentration-dependent fashion (Fig. 2A). The Ca²⁺-ATPase activity was inversely correlated with the calculated relative signal intensity of the DMPO-OH adduct (Fig. 2B); the responsibility coefficient of 0.98 indicates that there was a high correlation between the produced ·OH radicals and the reduced Ca²⁺-ATPase activity. In deed, as shown in Table 1, the production of the DMPO-OH adduct by H₂O₂ plus FeSO₄ was effectively blunted by ·OH scavengers (DMSO and DMTU) and the H₂O₂ scavenger CAT; the observed effect of H₂O₂ plus FeSO₄ on Ca ²⁺-ATPase activity was inhibited by DMSO, DMTU or CAT. SOD showed no significant protective effect against the effects elicited by H₂O₂ plus FeSO₄ on either the relative signal intensity of the DMPO-OH adduct or Ca²⁺-ATPase activity.

Effects of SH group reducing agents

Cysteine has been shown to protect the Ca²⁺-ATPase of vascular smooth muscle SR from the inhibitory action of oxygen free radicals (22). The results shown in Fig. 3 extend this observation to the SR Ca²⁺-ATPase from skeletal muscle. Figure 3 shows the protecting effect of cysteine against the inhibition of Ca²⁺-ATPase activity by ·OH radicals. Cysteine (0.1 to 10 mM) up to 1.0 mM dose-dependently blocked the inhibition of the Ca²⁺-ATPase by H₂O₂ plus FeSO₄; cysteine alone had no effect on Ca²⁺-ATPase activity (Fig. 3A).

To further describe the effect of ·OH radicals on Ca²⁺-ATPase activity, the effect of DTT, a thiol protective agent, was assessed. The addition of DTT (0.01 to 1.0 mM) up to 0.1 mM produced a dose-dependent protection against the depressed Ca²⁺-ATPase activity induced by ·OH radicals (Fig. 3B). Cysteine and DTT at concentrations of 1.0 mM and 0.1 mM, respectively, significantly affected Ca²⁺-ATPase activity with no effect on the increased relative signal intensity of the DMPO-OH adduct (Fig. 3C).

Effects of SH modifying agents

If oxidative inhibition of the Ca²⁺-ATPase activity results from modification of SH groups, as suggested by the protective effect of reducing agents, the SH modifying agents (SH-binding agents) such as DTNB and PCMB should inhibit Ca²⁺-ATPase activity. When the SR was

![Fig. 1. ESR spectra obtained in the absence of H₂O₂ (10 mM) and FeSO₄ (0.2 mM) (A) or presence of H₂O₂ alone (B) and the complete system (C). The ESR spectrum was recorded 50 sec after the addition of the spin-trap DMPO to the reaction mixture. The reaction mixture was the same as that of the Ca²⁺-ATPase assay experiment except that SR vesicles were omitted; DMPO was added 0.5 min after the addition of H₂O₂ alone or the H₂O₂/FeSO₄ system to the reaction bath. When H₂O₂ or the complete system was absent (A), the experiment was performed in a time-matched fashion. Signals appearing at both sides of the ESR spectra correspond to Mn²⁺ (MnO) installed in the ESR cavity as a reference.](image-url)
Fig. 2. Concentration-related effect of H$_2$O$_2$ (5 to 30 mM) in the presence of a fixed concentration of FeSO$_4$ (0.2 mM) on SR Ca$^{2+}$-ATPase activity and relative signal intensity of the DMPO-OH adduct (A) and the relationship between Ca$^{2+}$-ATPase activity and the signal intensity (B). In these experiments, SR was preincubated for 1.0 min prior to initiation of the ATPase reaction. FeSO$_4$ alone or cocktail of H$_2$O$_2$ and FeSO$_4$ was added 0.5 min before beginning the reaction with Ca$^{2+}$, Mg$^{2+}$ and ATP. This sequence assured that the SR was exposed to the FeSO$_4$ or ·OH radical generating system for 0.5 min prior to the start of the reaction. ESR spectra were analyzed under the same experimental conditions as that of the Ca$^{2+}$-ATPase assay except that SR vesicles were omitted; the spin-trap DMPO was added 0.5 min after the addition of H$_2$O$_2$/FeSO$_4$ to the reaction mixture in the presence or absence of the radical scavengers used. When the H$_2$O$_2$/FeSO$_4$ system was absent (none-control), experiments were performed in a time-matched fashion. The relative signal intensity was calculated as described in Fig. 2. All values are means ± S.E.M. (n=4 to 8). *Significantly (P<0.05, **P<0.01) different from the corresponding value of H$_2$O$_2$ alone; † significantly (P<0.05) different from the corresponding value of H$_2$O$_2$/FeSO$_4$.

The timed sequence of additions was designed to ensure exposure of SR vesicles to the H$_2$O$_2$/FeSO$_4$ system at 27°C for 0.5 min before initiation of the reaction as described in Fig. 2, except that H$_2$O$_2$ alone was added 0.5 min before beginning the reaction and scavengers were included. The scavengers used were added before the addition of SR vesicles. When added, the doses were the following: 10 mM H$_2$O$_2$, 0.2 mM FeSO$_4$, 10 µg/ml CAT, 20 µg/ml SOD, 150 mM DMSO and 1 mM DMTU. ESR spectra were analyzed under the same experimental conditions as that of the Ca$^{2+}$-ATPase assay except that SR vesicles were omitted; the spin-trap DMPO was added 0.5 min after the addition of H$_2$O$_2$ alone or H$_2$O$_2$/FeSO$_4$ to the reaction mixture in the presence or absence of the radical scavengers used. When the H$_2$O$_2$/FeSO$_4$ system was absent (none-control), experiments were performed in a time-matched fashion. The relative signal intensity was calculated as described in Fig. 2. All values are means ± S.E.M. (n=4 to 8). *Significantly (P<0.05, **P<0.01) different from the corresponding value of H$_2$O$_2$ alone; † significantly (P<0.05) different from the corresponding value of H$_2$O$_2$/FeSO$_4$.

Table 1. Effect of the ·OH radical-generating system on Ca$^{2+}$-ATPase activity and relative signal intensity of the DMPO-OH signal adduct and effects of radical scavengers

| Treatments                  | Ca$^{2+}$-ATPase activity (µmol Pi/mg·min) | Relative signal intensity       |
|-----------------------------|-------------------------------------------|----------------------------------|
| None                        | 2.10±0.09                                 | 0.48±0.01                        |
| H$_2$O$_2$                  | 1.97±0.09                                 | 0.49±0.01                        |
| H$_2$O$_2$ plus FeSO$_4$    | 1.47±0.13*                                | 4.78±0.10**                      |
| CAT plus H$_2$O$_2$ plus FeSO$_4$ | 1.82±0.11†                              | 3.63±0.08†                       |
| SOD plus H$_2$O$_2$ plus FeSO$_4$ | 1.58±0.07†                              | 4.50±0.17†                       |
| DMSO plus H$_2$O$_2$ plus FeSO$_4$ | 1.92±0.04†                              | 3.84±0.10†                       |
| DMTU plus H$_2$O$_2$ plus FeSO$_4$ | 1.72±0.07†                              | 4.00±0.06†                       |

DISCUSSION

Although the mechanisms of oxygen free radical injury are not well understood, numerous studies point to the importance of oxygen free radicals in various pathophysiological conditions. Our laboratory has provided evidence that a major target organelle attacked by oxygen free radicals is the portion of the excitation-contraction coupling system that regulates Ca$^{2+}$ delivery (the SR and sarcolemma) to the contractile proteins and not the contractile proteins per se (23–25). SH groups are considered essential for proper functioning of the membrane-bound enzymes (26–28). In this regard, the activity of skeletal muscle SR was reduced by SH reagents, such as N-ethylmaleimide and diamide (27, 28). In the present study, to determine the mechanism of the effect of ·OH radicals responsible for SR Ca$^{2+}$-ATPase interaction, we investigated the ability of the H$_2$O$_2$ plus FeSO$_4$ system to affect the Ca$^{2+}$-ATPase activity, while confirmation of the ·OH radical generation from the H$_2$O$_2$ plus FeSO$_4$ reaction was simultaneously followed in parallel reactions identical in all respects.

The ESR spectra of H$_2$O$_2$ plus FeSO$_4$ reaction revealed reacted with DTNB as well as PCMB, the Ca$^{2+}$-ATPase activity was inhibited; in the presence of the ·OH radical-generating system, the observed effect was significantly potentiated (Fig. 4). In contrast to Ca$^{2+}$-ATPase activity, the increased relative signal intensity of the DMPO-OH spin adduct produced by H$_2$O$_2$ plus FeSO$_4$ was not affected by the SH-binding agents used (Fig. 4).
Fig. 3. Effects of SH group reducing agents, cysteine and DTT, on SR Ca²⁺-ATPase activity and relative signal intensity of the DMPO-OH adduct in the presence of H₂O₂/FeSO₄ system. A and B: Dose-related effects of cysteine (0.1 to 10 mM: A) and DTT (0.01 to 1.0 mM: B) on Ca²⁺-ATPase activity in the presence (○) or absence (●) of the H₂O₂/FeSO₄ system. The conditions of exposure to H₂O₂/FeSO₄ were identical to those described in Fig. 2 and, when added, 10 mM H₂O₂ and 0.2 mM FeSO₄. The timed sequence of addition of cysteine or DTT was the same as that for the radical scavengers described in Table 1; when the H₂O₂/FeSO₄ system was absent, experiments were performed in a time-matched fashion. Data are the average of duplicate determinations on prepared SR vesicles. C: Effects of cysteine (1.0 mM) and DTT (0.1 mM) on Ca²⁺-ATPase activity and relative signal intensity of the DMPO-OH adduct. Reaction conditions were as described in Fig. 2, except that cysteine or DTT was included and, when added, 10 mM H₂O₂ and 0.2 mM FeSO₄. The timed sequence of addition of cysteine or DTT was the same as that for the radical scavengers described in Table 1; when the H₂O₂/FeSO₄ system was absent (none-control), experiments were performed in a time-matched fashion. Each column represents the means (n=4 to 8) and error bar±S.E.M. *Significantly (*P<0.05, **P<0.01) different from the corresponding value of the none-control; †significantly (P<0.05) different from the corresponding value of H₂O₂/FeSO₄.

a DMPO-OH signal (Fig. 1). The calculated relative signal intensity of the DMPO-OH adduct, which is indicative of the amount of ·OH radical generated, was negatively correlated with Ca²⁺-ATPase activity (Fig. 2). The depressed Ca²⁺-ATPase activity induced by ·OH radicals was protected by CAT, DMSO or DMTU; in contrast, the increased relative signal intensity of the DMPO-OH adduct produced by the H₂O₂ plus FeSO₄ reaction was simultaneously blunted by CAT, DMSO or DMTU (Table 1). ·OH radical generation can be regulated by H₂O₂ via the Fenton reaction; therefore, the observed effect of CAT is due to the scavenging H₂O₂. H₂O₂ at the concentration of 10 mM had no effect on Ca²⁺-ATPase activity; thus, the only role of H₂O₂ would still be through the Fenton reaction producing ·OH radicals in our system. These results support the view that ·OH radicals inhibit the SR Ca²⁺-ATPase. Why CAT, DMSO or DMTU only partially (but not completely) protected against the decreased signal intensity of the DMPO-OH adduct but nearly afforded complete protection against the depressed Ca²⁺-ATPase activity caused by ·OH radicals (Table 1) is unclear. One possible explanation is that Ca²⁺-ATPase may possess refractoriness towards a low concentration of the generated ·OH radicals, possibly at <4.00 relative signal intensity of DMPO-OH.

Several lines of evidence are consistent with inhibition of Ca²⁺-ATPase resulting from the oxidation of SH groups [the Ca²⁺-ATPase contains 95% of the total SH groups in the SR (29)]. First, SH-reducing agents, cysteine and DTT, showed protective effects against the
Fig. 4. Effects of SH modifying agents, DTNB and PCMB on SR Ca\(^{2+}\)-ATPase activity and relative signal intensity of the DMPO-OH adduct in the presence or absence of the H\(_2\)O\(_2\)/FeSO\(_4\) system. Reaction conditions were as described in Fig. 2, except that DTNB (0.1 mM) or PCMB (0.01 mM) was included and, when added, 10 mM H\(_2\)O\(_2\) and 0.2 mM of FeSO\(_4\). The timed sequence of addition of DTNB or PCMB was the same as that for the radical scavengers described in Table 1; when H\(_2\)O\(_2\)/FeSO\(_4\) system was absent (none-control, DTNB alone and PCMB alone), experiments were performed in a time-matched fashion. Each column represents the mean (n=4 to 8) and error bar±S.E.M. *,** Significantly (*P<0.05, **P<0.01) different from the corresponding value of none-control; † significantly (P<0.05) different from the corresponding value of H\(_2\)O\(_2\)/FeSO\(_4\).

Depression in Ca\(^{2+}\)-ATPase activity by ·OH radicals (Fig. 3). Two possibilities concerning the mechanisms of the protective effect of cysteine and DTT on Ca\(^{2+}\)-ATPase activity can be considered. The first may be a reduction of the SH groups of the enzyme protein that were previously oxidized by oxygen free radical reactions. The second possibility may be a direct reaction of cysteine and DTT with oxygen free radicals because both cysteine and DTT contain SH groups in their molecules (30, 31). However, at the concentrations used here, cysteine or DTT did not affect the observed increase in relative signal intensity of the DMPO-OH adduct by ·OH radicals (Fig. 3), and thus its action is not likely to be due to a oxygen free radical scavenging activity.

Second, the dependence of enzyme activity on the existence of SH groups is indicated by the ability of an SH-binding agent, DTNB and PCMB, to depress Ca\(^{2+}\)-ATPase activity. It is known that DTNB and PCMB modify the enzyme by combining irreversibly with SH groups in the ATPase molecule (32).

Third, the effect of DTNB and PCMB on Ca\(^{2+}\)-ATPase activity was markedly potentiated in the presence of an ·OH radical-generating system (Fig. 4), indicating that modification of an SH group(s) near the binding site of DTNB or PCMB may be involved in the action of ·OH radicals.

It is known that in the presence of free radicals, the SH group of glutathione can be modified to the sulfonate (33). Sulfonate formation in our experimental system is suggested by the irreversibility of the reaction. It is equally possible that the SH group undergoes some other modification that is irreversible. In our system, the depression of Ca\(^{2+}\)-ATPase activity caused by DTNB or PCMB could not be reversed by DTT (data not shown). DTT and other SH reducing agents may protect the enzyme by forming rapidly exchangeable intermediates and thus making the key SH group in the ATPase unavailable for such a modification. This is the possible mechanism by which SH reducing agents commonly protect SH groups present on proteins. DTNB and PCMB modify the ATPase by combining irreversibly (cross-linking formation) with the key SH group(s) in the ATPase molecule (34, 35).

Consistent with this interpretation, it has been reported that the SH content of SR declined with Ca\(^{2+}\)-ATPase activity in photooxidized SR (36) and in SR oxidized by o-iodosobenzoic acid or KMnO\(_4\) (37). Wolff and Dean (13), however, have reported formation of aggregates for bovine serum albumin subjected in vitro to ·OH attack, and this effect could not be reversed by SH reducing agents. Robert et al. (38) also have found that ·OH can induce an alteration of Ca\(^{2+}\)-activated Mg\(^{2+}\)-ATPase on cardiac myofibrils with no oxidation of protein SH groups. As regards to the redox state of myofibrillar SH groups, a different effect has been described with the O\(_2^−\) (39, 40). This radical was found to cause an oxidation of protein.
thiols. We do not suggest that SH oxidation is the only oxidizing modification of the Ca\(^{2+}\)-ATPase, but rather that the primary source of inhibition appears to be SH oxidation. In light of the structural and functional similarities of the Ca\(^{2+}\)-ATPase from skeletal muscle SR with the Ca\(^{2+}\)-ATPase in liver endoplasmic reticulum (41), smooth and cardiac muscle (42), and platelets (43), the postulation that several types of oxidative stress appear to inhibit the SR Ca\(^{2+}\)-ATPase by the common mechanism of the SH group modification may be relevant to redox regulation of these Ca\(^{2+}\)-transporting enzymes. Also, the plasma membrane Ca\(^{2+}\)-ATPase may have a similar sensitivity to oxidative stress since it is inhibited by the SH-binding agent diamide and is stimulated by reducing agents (44).

It is well known that the oxygen free radicals can react with membrane lipids, proteins, and nucleic acids (31). The unsaturated bonds of membrane cholesterol and fatty acids can readily react with oxygen free radicals and undergo peroxidation (31). The lipid peroxidation of the membrane lipids can be seen to affect the membrane-bound enzyme activities by changing the lipid microenvironment of the membrane-bound enzyme proteins, by forming cross-links between protein and phospholipids of the membrane or by oxidizing SH groups of the membrane-bound enzyme proteins (11). Arkhipenko et al. (45) reported that the Ca\(^{2+}\)-ATPase of skeletal muscle SR is inhibited by lipid peroxide levels in excess of 16 nmol malondialdehyde/mg protein. In terms of the physiologic function of the SR, the Ca\(^{2+}\)-ATPase may be inhibited before such high levels of lipid peroxides are formed. The postulation that oxidative damage to the SR is independent of the extent of lipid oxidation is consistent with previous reports examining SR oxidation. Restall et al. (36) reported no significant change in the fatty acid composition of SR after photooxidation, although the Ca\(^{2+}\)-ATPase activity was reduced by 50%. Okabe et al. (46) found that oxidative inhibition of the Ca\(^{2+}\)-ATPase of cardiac SR by the O\(_2^-\) and/or \(\cdot\)OH was lipid independent. Nonetheless, SH groups of Ca\(^{2+}\)-ATPase may be modified by oxygen free radicals because of either direct action or via lipid peroxidation; and on the basis of the data presented in this study, it can be concluded that \(\cdot\)OH radical-induced oxidant-injury may be caused primarily by modifying a key SH group(s) on the Ca\(^{2+}\)-ATPase molecule of masster muscle SR vesicles. This finding emphasizes the possible importance of the role of SR in the mechanisms of oxygen free radical injury to the muscle cells during various pathophysiological conditions, and inhibition of the Ca\(^{2+}\)-ATPase by oxidative stress is relevant to models of cellular dysfunction in which toxicity may be caused by a rise in intracellular Ca\(^{2+}\).

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