Effects of elevated physiological temperatures on sarcoplasmic reticulum function in mechanically skinned muscle fibers of the rat

C. van der Poel and D. G. Stephenson
Department of Zoology, La Trobe University, Victoria, Australia

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Van der Poel C, Stephenson DG. Effects of elevated physiological temperatures on sarcoplasmic reticulum function in mechanically skinned muscle fibers of the rat. Am J Physiol Cell Physiol 293: C133–C141, 2007. First published March 7, 2007; doi:10.1152/ajpcell.00052.2007.—Properties of the sarcoplasmic reticulum (SR) with respect to Ca\textsuperscript{2+} loading and release were measured in mechanically skinned fiber preparations from isolated extensor digitorum longus (EDL) muscles of the rat that were either kept at room temperature (23°C) or exposed to temperatures in the upper physiological range of mammalian skeletal muscle (30 min at 40 or 43°C). The ability of the SR to accumulate Ca\textsuperscript{2+} was significantly reduced by a factor of 1.9–2.1 after the temperature treatments due to a marked increase in SR Ca\textsuperscript{2+} leak, which persisted for at least 3 h after treatment. Results with blockers of Ca\textsuperscript{2+} release channels (ruthenium red) and SR Ca\textsuperscript{2+} pumps [2,5-di-(tert-butyl)-1,4-hydroquinone] indicate that the increased Ca\textsuperscript{2+} leak was not through the SR Ca\textsuperscript{2+} release channel or the SR Ca\textsuperscript{2+} pump, although it is possible that the leak pathway was via oligomerized Ca\textsuperscript{2+} pump molecules. No significant change in the maximum SR Ca\textsuperscript{2+}-ATPase activity was observed after the temperature treatment, although there was a tendency for a decrease in the SR Ca\textsuperscript{2+}-ATPase. The observed changes in SR properties were fully prevented by the superoxide (O\textsubscript{2•}^{-}) scavenger Tiron (20 mM), indicating that the production of O\textsubscript{2•}^{-} at elevated temperatures is responsible for the increase in SR Ca\textsuperscript{2+} leak. Results show that physiologically relevant elevated temperatures (1) induce lasting changes in SR properties with respect to Ca\textsuperscript{2+} handling that contribute to a marked increase in the SR Ca\textsuperscript{2+} leak and, consequently, to the reduction in the average coupling ratio between Ca\textsuperscript{2+} transport and SR Ca\textsuperscript{2+}-ATPase and muscle performance, and (2) that these changes are mediated by temperature-induced O\textsubscript{2•}^{-} production.

skeletal muscle; calcium ion leak; superoxide; skinned fibers

THERE HAVE BEEN MANY STUDIES showing the importance of temperature on the function of the mammalian skeletal muscle (8, 12, 19, 31, 35, 37, 39, 46). In general, as the temperature increases above 37°C, the performance of the skeletal muscle markedly decreases, and there is strong evidence supporting the generation of reactive oxygen species (ROS) as a potential cause for the poor longevity of in vitro skeletal muscle displayed at physiological temperatures (37–43°C) (2, 25, 26, 42, 44, 48).

In a previous study we showed that there was a marked decrease in the maximum Ca\textsuperscript{2+}-activated force responses and a large increase in the amount of superoxide (O\textsubscript{2•}^{-}) released from the isolated extensor digitorum longus (EDL) muscle after the muscles were exposed to temperatures above 40°C, and that the effects were completely preventable in the presence of a permeant O\textsubscript{2•}^{-} scavenger, Tiron (42). This indicated that O\textsubscript{2•}^{-} was responsible for the loss of contractile function at temperatures above 40°C (42). More recently, it has been shown that the increased fatigue rate of in vitro muscle preparations at 37°C was associated with a decrease in the sensitivity of the contractile apparatus to Ca\textsuperscript{2+} and that this effect was markedly attenuated in the presence of Tiron, indicating that O\textsubscript{2•}^{-} did play a role in the increased fatigue rate at 37°C (25, 26). Changes in contractile apparatus have been clearly implicated in the loss of muscle function at physiological temperatures (25, 42); however, other sites involved in excitation-contraction coupling (ECC) could also be affected.

For example, it has long been recognized that proteins in the sarcoplasmic reticulum (SR) are sensitive to ROS (32). ROS and other oxidants have been shown to increase the probability of ryanodine-sensitive Ca\textsuperscript{2+} release channel (RyR) opening and thereby promoting Ca\textsuperscript{2+} release from the SR (1, 16). ROS also have been shown to modulate SR Ca\textsuperscript{2+}-ATPase pump function, with very high ROS concentrations decreasing the reuptake of Ca\textsuperscript{2+} into the SR (7, 21, 36). The present study aimed to characterize SR function of fast-twitch skeletal muscle fibers following exposure of the whole isolated EDL muscle to temperatures in the upper physiological range of temperatures (40–43°C) and determine whether any SR modifications are associated with temperature-induced ROS production. In this study we employed the mechanically skinned fiber preparation, which allows direct access to the SR and the myoplasmic environment while preserving molecular structures and the relationships between SR and the other cellular structures intact. The results show that increasing the temperature of skeletal muscle to the upper physiological range markedly decreases the ability of the SR to load and retain Ca\textsuperscript{2+} through an increased Ca\textsuperscript{2+} leak from the SR and a minor depressing effect on the activity of the SR Ca\textsuperscript{2+} pump. This reduces the coupling ratio between net Ca\textsuperscript{2+} transport into the SR and the SR Ca\textsuperscript{2+}-ATPase. The changes in SR function are most likely due to the temperature-induced increase in O\textsubscript{2•}^{-} production, since the effects are fully prevented in the presence of Tiron. Some of the results presented have been briefly reported previously (43).

METHODS

Animals and muscle dissection. Male Long-Evans hooded rats (16–18 wk old) were killed by halothane overdose in accordance with the procedure approved by La Trobe University Animal Ethics Committee. The EDL muscles were quickly removed, well blotted on filter paper (Whatman no. 1), and then fully immersed in paraffin oil (Ajax Chemicals, Sydney, Australia) at room temperature (23 ± 1°C) contained in a petri dish.

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Temperature treatment. In brief, using a protocol similar to that described in our previous study (42), we exposed isolated EDL muscles for 30 min at either 40 or 43°C in paraffin oil heated to a set temperature (within ±0.1°C). Note that we have shown previously that O₂ diffuses in the paraffin oil so that the muscles kept in the resting state are not anoxic (42).

After treatment, the muscles were transferred to a petri dish with room temperature paraffin oil, where they remained for the entire experimental period after exposure. With this procedure the temperature in the core of the muscle dropped to room temperature within 2 min (n = 4), as measured by a digital thermometer (Doric 450; Doric Scientific, San Diego, CA). Single muscle fibers were then isolated and mechanically skinned under paraffin oil at different times after temperature treatment.

Fiber skinning and mounting. Single muscle fibers were isolated from as close to the surface of the muscle as possible and mechanically skinned under a dissecting microscope using fine forceps (jeweler’s forceps no. 5) as previously described (37). The skinned fiber was then viewed at high magnification (∼200) on the screen of a television monitor coupled to a Leitz dissecting microscope. The fiber could be twisted in such a way as to permit the measurement of fiber width from different positions in at least four places along its length. The cross-sectional area was calculated by assuming that the fiber was circular with a diameter equivalent to the average width of the fiber.

The skinned fiber was then attached at one end to a piezoresistive force transducer (AME875; SensoNor, Horten, Norway) using braided silk (Deknatel, size 10, 0.2 mm) and at the other end to a pair of forceps fixed to a micromanipulator (11). With the fiber still immersed in paraffin oil, the resting slack length of the fiber was measured with the aid of a Nikon dissecting microscope at ×10 magnification. The fiber was then transferred to a 2-ml Perspex bath containing an equilibrating solution (see Table 1).

Solutions. Solutions were as described previously (37). Table 1 shows the composition of the main solutions used in this study. The pH of these solutions was 7.10 ± 0.03, and unless otherwise stated, they contained (in mM) 126 K⁺, 37 Na⁺, 90 HEPES, 8 ATP, 10 phosphocreatine (PCr), and 1 free Mg²⁺. The release solution contained 30 mM caffeine and low levels (0.02 mM) of ionized Mg²⁺ to facilitate the rapid release of SR Ca²⁺ (13). The maximum Ca²⁺-activating solution contained almost equimolar amounts of Ca²⁺ and EGTA as determined by titration (37) and had an ionized [Ca²⁺] on the order of 3 × 10⁻⁵ M, sufficient for maximal activation of skinned fibers.

Measurement of SR Ca²⁺ movements. In these experiments we used the Ca²⁺-sensitive force response as a Ca²⁺ indicator for measuring Ca²⁺ movements associated with the SR and SR Ca²⁺ content. This endogenously expressed Ca²⁺-sensitive system is ideally suited to measure Ca²⁺ movements associated with the intact SR, since it is permanently located in the vicinity of the SR, can be precisely calibrated in individual fibers for the specific conditions employed, and does not diffuse into or out of the preparation when the surface membrane is removed to gain direct and full access to the myoplasmic side of the intact SR. The relative SR Ca²⁺ content was derived from the area under the caffeine-induced force response. As shown in Fig. 1, the areas under caffeine-induced force responses are directly proportional to the loading times under our conditions and, therefore, to the SR Ca²⁺ content, considering that the rate of SR Ca²⁺ loading should remain constant for conditions used in this study (22). This provides strong support for using the area under the caffeine-induced force response as an index for measuring the relative SR Ca²⁺ content (10, 17, 20, 22). Moreover, the relative SR Ca²⁺ content results obtained from ratios of areas under the caffeine-induced force responses were validated using an independent technique that enables direct measurement of total Ca²⁺ in the SR in absolute rather than in relative units. However, this technique, known as the SR lysing technique within the volume of the fiber (Refs. 9, 13; see also below) can be applied only once to a particular skinned fiber

![Image](http://ajpcell.physiology.org/doi/10.1152/ajpcell.00128.2006)
preparation for measuring its SR Ca\(^{2+}\) content, whereas the caffeine-induced SR Ca\(^{2+}\)-release technique can be applied many times to the same skinned fiber preparation for measuring the relative SR Ca\(^{2+}\) content. The application of these two methods under standard conditions (room temperature, solutions, and protocol for data collection) allowed us to infer changes in the SR Ca\(^{2+}\) content, rate of Ca\(^{2+}\)-leak from the SR, and rate of SR Ca\(^{2+}\) pump as described below.

Measurements of relative SR Ca\(^{2+}\) content using caffeine-induced force responses. The freshly mechanically skinned fibers were initially exposed to an equilibrating solution (50 \(\mu\)M EGTA; pCa 7.1; pCa = -log\(_10\) [Ca\(^{2+}\)] (Table 1) for a 2-min equilibration, where the SR remained loaded with Ca\(^{2+}\) at endogenous levels. The preparation was then exposed to wash 1 solution (0.5 mM EGTA) for 30 s, and rapid SR Ca\(^{2+}\) release was triggered by transferring the preparation into release solution, which contained 30 mM caffeine and 0.02 mM [Mg\(^{2+}\)]\(_{free}\) to facilitate full activation of the RyR/Ca\(^{2+}\) release channels and thorough depletion of SR Ca\(^{2+}\) stores (13). The presence of 0.5 mM EGTA in release solution ensured that the level of Ca\(^{2+}\) during caffeine-induced release did not maximally activate the contractile apparatus, which is necessary to allow quantitative evaluation of the amount of Ca\(^{2+}\) released. Ca\(^{2+}\) release from the SR was estimated from the relative areas under the caffeine-induced force response (Fig. 1; Refs. 10, 20). The fiber was left in the release solution for 2 min to ensure complete SR Ca\(^{2+}\) depletion before being washed for 30 s in wash 2 solution, which was identical in composition to the wash 1 solution but separate to avoid cross contamination of solutions. Thereafter, the SR was reloaded with Ca\(^{2+}\) in load solution (0.2 \(\mu\)M [Ca\(^{2+}\)], pCa 6.7) for various lengths of time (30, 60, and 90 s) before being equilibrated for 30 s in wash 1 solution and subsequently exposed to the release solution. At the end of an experiment each fiber was activated in the maximum Ca\(^{2+}\)-activating solution. Since the areas under the caffeine-induced force responses were proportional to the SR loading times (30, 60, and 90 s) and the relative areas under the caffeine-induced force responses were used to estimate the amount of Ca\(^{2+}\) in the SR (20). Maximum force production is affected by temperature treatment at 40 and 43°C for 30 min, but the sensitivity to Ca\(^{2+}\) is not (42). Therefore, the area under the caffeine-induced curves can be normalized to the corresponding maximum force responses to allow comparison of results obtained from different fibers after different periods of exposure to elevated temperature, which affects the maximum force response (17).

SR Ca\(^{2+}\) leak experiments. To estimate the percentage of Ca\(^{2+}\) lost from the SR due to the passive leak of Ca\(^{2+}\) over a 60-s time interval, we used the following experimental protocol. The fiber was loaded for 90 s in load solution (pCa 6.7) and then washed in wash 1 solution for 30 s before the SR Ca\(^{2+}\) content was released in release solution (control). Thereafter, the preparation was washed in the wash 2 solution before the SR was reloaded with Ca\(^{2+}\) for 90 s in load solution and transferred to the leak solution for 90 s (test) to give a 60-s extra leak period at pCa 8.3 (with 0.5 mM EGTA) compared with control. After the leak period, the remaining SR Ca\(^{2+}\) was released in release solution, giving the test force response that is necessary for the estimation of the relative amount of SR Ca\(^{2+}\) lost in 60 s. The control (30 s in wash 1/leak solution) was then repeated, and the area under the test response was divided by the average of the areas under the caffeine-induced force responses in the controls before and after the test response. This ratio represents the fraction of the initial Ca\(^{2+}\) in the SR after the 60-s leak period, and therefore, the percentage of Ca\(^{2+}\) leaked from the SR over 60 s can be calculated from the following expression (22):

\[
\%\text{leaked SR Ca}^{2+} = \frac{100(1 - \text{test response area})}{\text{average control response area}}
\]  

TBQ experiments. SR Ca\(^{2+}\) leak was also measured under conditions in which the SR Ca\(^{2+}\) pump was blocked with 20 \(\mu\)M 2,5-di(tert-butyl)-1,4-hydroquinone (TBQ) (3). TBQ stock solution was obtained by dissolving TBQ in pure DMSO (dimethyl-d$_2$sulfoxide) at a concentration of 20 mM. The wash 2 and leak solutions contained 20 \(\mu\)M TBQ, and the corresponding control wash 2 and leak solutions contained the same concentration of DMSO as the wash solution with TBQ. In all TBQ experiments, solutions were made in double the required volume, mixed, and split into two equal parts, thus ensuring the identical free [Ca\(^{2+}\)] in both sets of solutions. First, the fiber was depleted of endogenous Ca\(^{2+}\), as described above, and then loaded with Ca\(^{2+}\) for 90 s in load solution. The fiber was then placed in leak solution without TBQ for 60 s, dipped in paraffin oil for 10 s [to remove TBQ from the preparation (3)] and in wash 1 solution for 10 s, dipped back in paraffin oil for 10 s, and washed in wash 1 solution for 15 s before being depleted of Ca\(^{2+}\) in release solution. The cycle was then repeated with TBQ only in wash 2 and leak solutions. The paraffin oil treatment following exposure to TBQ was important for removing TBQ from the skinned fiber before SR Ca\(^{2+}\) release (3).

The area under the caffeine-induced force response was compared with the first response after 90 s of load (control performed before TBQ protocol), with the difference in size due to the Ca\(^{2+}\) lost from the SR during the leak period in the presence and absence of TBQ. In this case, the SR Ca\(^{2+}\) leak was calculated from the following expression:

\[
\text{SR Ca}^{2+}\text{leak} \% = \frac{100(1 - \text{test response area/\control response area})}{\text{control response area}}
\]  

Ruthenium red experiments. SR Ca\(^{2+}\) leak was also measured under conditions in which the RyRs were blocked with 5 \(\mu\)M ruthenium red (RR) (23) to determine whether the RyRs are involved in the temperature-induced effects on SR function. RR stock solution (1 mM) was obtained by dissolving RR in leak solution. Leak experiments were performed as described for TBQ experiments, except TBQ was replaced with 5 \(\mu\)M RR and the preparation was not moved to paraffin oil after RR exposure in the leak solution.

Measurement of total SR Ca\(^{2+}\) content. The total amount of Ca\(^{2+}\) in the SR was measured in some preparations from control and 43°C-treated muscles using a procedure that has been described comprehensively elsewhere (9, 13). In brief, the skinned fiber was loaded for 30 s and then equilibrated for 20 s in a solution containing a known concentration of BAPTA (a fast Ca\(^{2+}\) buffer with well-known Ca\(^{2+}\)-binding properties) and then lysing all membranous compartments within the fiber by exposing the fiber to an emulsion of Triton X-100 (10% vol/vol) in paraffin oil (TX oil). The Ca\(^{2+}\) released from compartments upon lysing rapidly binds to the known amount of BAPTA within the fiber and to other sites, predominantly troponin C, which are unaffected by Triton X-100, causing a submaximal force response. Once a force plateau was reached, the fiber was returned to a wash solution, and then the fiber was exposed to heavily Ca\(^{2+}\)-buffered solutions of known [Ca\(^{2+}\)] made by mixing the maximum Ca\(^{2+}\)-activating solution with the relaxing solution (see Table 1) in various proportions. Once a steady force was reached in the respective solution, the preparation was placed in the paraffin oil/Triton X-100 to mimic the conditions encountered during SR lysis, and the [Ca\(^{2+}\)] reached in the preparation following SR lysis ([Ca\(^{2+}\)]\(_{SR}\)) was estimated by comparing the force response under these conditions with the force response obtained following SR lysis. The [Ca\(^{2+}\)]\(_{SR}\) in the SR before SR lysis was then calculated from the value of [Ca\(^{2+}\)]\(_{SR}\) as previously described (9, 13).

Estimation of Ca\(^{2+}\)-ATPase activity. Measurements of the SR Ca\(^{2+}\)-ATPase activity were performed by combining and modifying two techniques. The first technique is a sensitive spectrophotometric technique that uses molybdate for measuring inorganic phosphate (P) (6, 28). With this technique, mechanically skinned muscle fiber segments are placed in 2-\(\mu\)l droplets of solutions similar in composition to the relaxing and maximally activating solutions in Table 1 but in which 10 mM PCr is replaced by 8 mM ATP (total ATP, 16 mM). The omission of PCr from these solutions is because PCr breaks
down at acidic pH, which is a step in the spectrophotometric method. Fiber ATPase activity can be measured from the extent of Pi production, taking into account the duration of the incubation and the volume of the fiber segment. To measure the amount of Pi, produced by the fiber over a known period of time, we diluted a 1-μl sample of the incubation medium with 50 μl of H2O and 100 μl of a 1:1 mixture of 1% ammonium molybdate and 6% ascorbic acid in 1 M HCl. After 4 min, the reaction was quenched with 150 μl of 2% sodium citrate, 2% sodium metasemisulphite, and 2% acetic acid in double-distilled water.

The reaction mixture was then left for 20 min at room temperature for the reaction to be completed, after which 700 μl of the reaction mixture was added to bring the final volume to 1 ml. Absorbance was finally read at 850 nm, and the Pi production for individual fibers was calculated from measured absorbances and the standard curve covering the range 0–5 mM Pi in a solution similar to relaxing solution but in which all ATP and PCr were replaced by 18 mM HDTA.

The second technique involved N-benzyl-β-toluene sulfonamide (BTS) (47). BTS very strongly inhibits myofibrillar ATPase activity by preventing force generation and cross-bridge ATP utilization at micromolar levels without interfering with SR Ca2+-release and ATPase activity (47). By combining the two techniques and including the use of caffeine, it was possible to isolate the SR Ca2+-ATPase activity as described below:

Fresh mechanically skinned muscle fibers were placed under paraffin oil and had their length and diameter measurements recorded at ×125 magnification to determine their volume. Using a piece of silk tied to one end, we transferred the fibers from paraffin oil to a 2-μl droplet of relaxing solution without PCr and containing 16 mM ATP (IPi solution; pH > 9) and allowed it to equilibrate for 5 min. The skinned fiber preparation was then shifted for 20 min to a 2-μl droplet of maximally activating solution without PCr and containing 16 mM ATP and 10 mM caffeine (IPi solution; pH ~ 4.5) to activate all skeletal muscle ATPase activities. Caffeine was added to open the RyRs and prevent feedback inhibition, thus allowing the SR Ca2+ pump to remain maximally activated. The fiber segment was allowed to shorten, because it has been shown that myofibrillar ATPase activity in rat EDL muscles remains near maximal at sarcromere lengths <2.7 μm (38). The resultant amount of Pi produced was measured spectrophotometrically with the rate of Pi production per unit time and fiber volume used as an indicator of total ATPase activity of mechanically skinned muscle fibers.

The preparation was then moved for 5 min into a IPi solution containing 30 μM BTS to block all myofibrillar ATPase activity (47) and was then placed in IIP, solution (2 μl) containing 30 μM BTS for 20 min. This gave a measure of the ATPase activity of all fiber ATPases minus the myofibrillar ATPase of the contractile apparatus. The fiber was then placed again in the IPi solution containing 30 μM BTS and 20 μM TBQ for 5 min. As described above, TBQ is a potent SR pump blocker (3). By blocking the SR pump and effectively stopping SR Ca2+-ATPase activity, the amount of Pi produced in the IIP solution containing both BTS and TBQ gives a measure of the background ATPase activity that includes ATPases such as the Na+ / K+-ATPase. By using the three ATPase measurements made on the same fiber segment (1) total fiber ATPase, (2) total fiber ATPase − myofibrillar ATPase, and (3) total fiber ATPase − PCr − myofibrillar ATPase − SR Ca2+-ATPase], it was possible to calculate the levels of SR Ca2+-ATPase activity (in Pi production per second per fiber volume) from the last two measurements.

Data analysis. Results are expressed as means ± SE. Curve fitting and statistical analyses were performed using the scientific analysis program GraphPad Prism (GraphPad Software, San Diego, CA). Statistical significance was tested at P < 0.05 using t-tests and one-way ANOVA (followed by Bonferroni post hoc test) as appropriate.

RESULTS

Effects of elevated temperature on SR Ca2+ accumulation. To determine whether the ability of the SR to accumulate Ca2+ is affected by exposure to elevated temperatures, the SR of mechanically skinned fibers from control and temperature treated EDL muscles of the rat was loaded with Ca2+ at pCa 6.7 in the SR Ca2+ loading solution for 30, 60, and 90 s. After each loading period, the accumulated Ca2+ in the SR was released in the release solution containing 30 mM caffeine and 0.02 mM Mg2+, and the areas under the caffeine-induced force responses were measured and then divided by the maximum Ca2+-activated force (Fmax) measured in the same preparation to allow comparisons to be made among fibers (see METHODS).

A series of representative force traces for mechanically skinned fibers from control muscles is shown in Fig. 1A, and the average data from 10 fibers are shown in Fig. 1B. The data points are well fitted by a straight line, and the rate of SR Ca2+ accumulation in control fibers as calculated from the slope of the data presented in Fig. 1B was 0.026 ± 0.004 Fmax, indicating that the amount of Ca2+ loaded by the SR in 1 s produced, on average, a force response that had an area of 2.6% Fmax’s. Under our standard loading conditions for 30 s, the area under the caffeine-induced force response corresponded to 230.8 ± 33.4% area when the SR was loaded at endogenous level (n = 10), indicating that under our conditions the SR would load to endogenous level in ~15 s. Further considering that endogenous SR Ca2+ in EDL fibers is ~1.2 mmol per liter of fiber volume (13), the rate of SR Ca2+ accumulation in control fibers corresponds to 0.092 mmol/l of fiber volume per second at pCa 6.7.

The average data for SR loading in fibers taken from muscles within 30 min after exposure for 30 min to 40 and 43°C are shown in Fig. 2 together with the average results for SR loading in control fibers. After exposure to 40 and 43°C for 30 min, there was a statistically significant decrease (1-way ANOVA, P < 0.01) in the apparent rate of SR Ca2+ accumulation to 0.0136 ± 0.0007 Fmax (P < 0.05) and 0.0123 ±
0.0005 $F_{\text{max}}$ ($P < 0.01$) respectively, compared with controls. This indicates that the amount of $Ca^{2+}$ loaded by the SR in 1 s produced a force response that had an area of only $1.36 \pm 0.07$ and $1.23 \pm 0.05\% F_{\text{max}}$ in fibers from muscles treated at 40 and $43^\circ C$, respectively. This decrease in the apparent rate of $Ca^{2+}$ accumulation shows that the SR was not able to load, hold, and/or subsequently release as much $Ca^{2+}$ after exposure to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$. Importantly, these changes in SR properties persisted for 3 h following treatment at elevated temperatures to 40 and $43^\circ C$.

The maximum relative rate of force development upon exposure to caffeine and 0.02 mM $Mg^{2+}$ before and after the temperature treatments expressed as peak caffeine-induced force response ($F_{\text{caffe}}$) was not significantly different ($P > 0.5$, 1-way ANOVA). Rates of rise (expressed as $F_{\text{caffe}}$) were $1.15 \pm 0.12$ ($n = 6$), $1.23 \pm 0.18$ ($n = 5$), and $1.27 \pm 0.23$ ($n = 5$) for control, $40^\circ C$, and $43^\circ C$, respectively, indicating that temperature treatment does not affect the time course of the caffeine-induced SR $Ca^{2+}$ release when the RyRs are maximally activated. Therefore, this points out that the reduction in the average caffeine-induced force response after temperature treatment may be due to reduced $Ca^{2+}$ in the SR and not to changes in the RyR properties.

To confirm that the SR $Ca^{2+}$ content was actually reduced after exposure to elevated temperature, we employed a method comprehensively described elsewhere (9, 13) that permits the measurement of SR $[Ca^{2+}]_r$ in absolute rather than relative units. Using this method (see METHODS), we found that the SR $Ca^{2+}$ content of fibers (expressed in mmol $[Ca^{2+}]_r$ per liter of fiber) from control muscles following a 30-s loading period was $2.26 \pm 0.23$ mmol/l fiber ($n = 5$), whereas fibers from muscles that had undergone exposure to $40^\circ C$ for 30 min contained only $1.10 \pm 0.08$ mmol/l fiber ($n = 5$) SR $Ca^{2+}$. The ratio between the average value of the SR $Ca^{2+}$ content in fibers from muscles treated to $40^\circ C$ and that from control muscles (0.49) is very close to the ratio between the average rate of SR $Ca^{2+}$ accumulation in fibers from muscles treated to $40^\circ C$ and that from control muscles (0.52) determined using caffeine responses. These results show that, indeed, less $Ca^{2+}$ was present in the SR after muscles were exposed for 30 min to $40^\circ C$ and fully support the use of the area under caffeine-induced responses to estimate relative content of SR $Ca^{2+}$.

**Effect of elevated temperature on leak rate.** One of the ways in which SR $Ca^{2+}$ accumulation rates can be affected is by an increase in SR $Ca^{2+}$ leak rates. To determine whether the decrease in the rate of SR $Ca^{2+}$ accumulation was due to an increase in SR $Ca^{2+}$ leak, we placed fibers in leak solution that contained 0.5 mM EGTA (pCa 8.3) to chelate $Ca^{2+}$ that leaked out of the SR, therefore reducing the likelihood of any $Ca^{2+}$ being recycled back into the SR by the pump.

Figure 3 shows the amount of $Ca^{2+}$ lost from the SR after a 60-s leak at pCa $> 8$ (see METHODS). Control fibers lost 22.4 ± $0.12$ ($n = 6$), $1.23 \pm 0.18$ ($n = 5$), and $1.27 \pm 0.23$ ($n = 5$) for control, $40^\circ C$, and $43^\circ C$, respectively. Therefore, it appears the SR loading properties do not recover with time after the return of the muscles to room temperature following temperature treatment at 40 and $43^\circ C$. This is unlike the ability of the contractile apparatus to develop force, which gradually recovers after temperature treatment at 40 and $43^\circ C$ as shown in our previous work (42).

The loss of SR $Ca^{2+}$ after exposure to elevated temperature may be due to reduced $Ca^{2+}$ in the SR and not to changes in the RyR properties.

To confirm that the SR $Ca^{2+}$ content was actually reduced after exposure to elevated temperature, we employed a method comprehensively described elsewhere (9, 13) that permits the measurement of SR $[Ca^{2+}]_r$ in absolute rather than relative units. Using this method (see METHODS), we found that the SR $Ca^{2+}$ content of fibers (expressed in mmol $[Ca^{2+}]_r$ per liter of fiber) from control muscles following a 30-s loading period was $2.26 \pm 0.23$ mmol/l fiber ($n = 5$), whereas fibers from muscles that had undergone exposure to $40^\circ C$ for 30 min contained only $1.10 \pm 0.08$ mmol/l fiber ($n = 5$) SR $Ca^{2+}$. The ratio between the average value of the SR $Ca^{2+}$ content in fibers from muscles treated to $40^\circ C$ and that from control muscles (0.49) is very close to the ratio between the average rate of SR $Ca^{2+}$ accumulation in fibers from muscles treated to $40^\circ C$ and that from control muscles (0.52) determined using caffeine responses. These results show that, indeed, less $Ca^{2+}$ was present in the SR after muscles were exposed for 30 min to $40^\circ C$ and fully support the use of the area under caffeine-induced responses to estimate relative content of SR $Ca^{2+}$.

The presence of 5 $\mu$M RR (22.35 ± 4.56% control compared with 31.55 ± 6.46% with RR, n = 8) when a 10.22 ± 0.33 s for control fibers, 0.0081 ± 0.022 s for fibers from muscles exposed to $40^\circ C$ for 30 min, and 0.015 ± 0.002 s for fibers from muscles exposed to $43^\circ C$ for 30 min ($P < 0.001$, 1-way ANOVA). Although there was a highly significant statistical difference between control and $43^\circ C$ treatment data ($P < 0.01$, post hoc Bonferroni multiple comparison test), there was no statistically significant difference between the 40 and $43^\circ C$ treatment results ($P > 0.05$).

**Effects of ruthenium red on SR $Ca^{2+}$ leak.** The RyR channel blocker RR was used to establish whether the increased SR $Ca^{2+}$ leak after exposure to elevated temperature was via the RyR or some other pathway. Figure 4 shows the results of experiments in which preparations were loaded for 90 s, followed by a 60-s leak and then release of the remaining $Ca^{2+}$ in the SR using release solution. There was no statistically significant difference in $Ca^{2+}$ leak between control fibers without RR and control fibers with 5 $\mu$M RR (22.35 ± 4.56% control compared with 31.55 ± 6.46% with RR, n = 8) when a significant fraction of RyRs was blocked under our conditions (23). The presence of 5 $\mu$M RR also did not significantly change the SR $Ca^{2+}$ lost after 60 s in fibers from muscles exposed to $40^\circ C$ (41.9 ± 5.5% vs. 38.6 ± 8.6%, n = 4) or $43^\circ C$ (61.7 ± 6.3% vs. 68.2 ± 14.7%, n = 5), indicating that RR did not affect the elevated temperature-induced increase in SR $Ca^{2+}$ leak. Since RR does not reduce the elevated temperature-induced SR $Ca^{2+}$ leak, the elevated temperature induced SR $Ca^{2+}$ leak must be via some other pathway than through the RyRs. The lack of RR effect on the area under the caffeine-induced force response even though some RyRs would be expected to stay blocked when the preparation was exposed to...
with 10 mM caffeine to maximally activate all fiber ATPases. The myofibrillar ATPase activity was found to be 62.29 ± 4.94% (n = 4) of total ATPase, whereas the SR Ca<sup>2+</sup>-ATPase was 33.73 ± 6.43% (n = 4) of total ATPase with the background ATPases such as Na<sup>+</sup>/K<sup>+</sup>-ATPase producing 7.15 ± 2.61% (n = 4) of total ATPase in the presence of Ca<sup>2+</sup>. This is similar to that previously found in fast-twitch fiber types from human muscle (67, 25, and 8%, respectively) (41).

The average SR Ca<sup>2+</sup>-ATPase activity (P<sub>i</sub> production in mmol per liter of fiber per second) for control fibers was 0.26 ± 0.05 (n = 4), and the summary of results with control fibers and fibers from muscles treated to either 40 or 43°C for 30 min is shown in Fig. 5. Although there is a clear trend for a decrease in SR Ca<sup>2+</sup>-ATPase activity in fibers treated to either 40 or 43°C for 30 min compared with controls, the decrease did not reach statistical significance (P > 0.1, 1-way ANOVA).

SR Ca<sup>2+</sup> accumulation after corrections for SR Ca<sup>2+</sup> leak. To determine whether the decreased rate in SR Ca<sup>2+</sup> accumulation could be explained by the marked increase of SR Ca<sup>2+</sup> leak, we first corrected the data presented in Fig. 2 for the Ca<sup>2+</sup> that had leaked during the 30-s wash in the wash 1 solution before the caffeine-induced SR Ca<sup>2+</sup> release. This was done by multiplying the results in Fig. 2 by the factor exp (30 s × k), where k is the average leak rate for control fibers [0.0042 ± (1 ± 0.24) s<sup>−1</sup>] and fibers from muscles treated to 40°C [0.0081 ± (1 ± 0.28) s<sup>−1</sup>] and 43°C [0.015 ± (1 ± 0.17) s<sup>−1</sup>] measured above. It was then assumed that during loading, the rate of the SR Ca<sup>2+</sup>-pump (A<sub>i</sub>) remained constant while Ca<sup>2+</sup> leaked from the SR with the rate k mentioned above such that the rate of SR Ca<sup>2+</sup> accumulation (dSR Ca<sup>2+</sup>/dt) is described by Eq. 3:

\[
\text{dSR Ca}^{2+}/\text{dt} = A - k[\text{SR Ca}^{2+}]
\]

The solution of this differential equation is Eq. 4:

\[
[\text{Ca}^{2+}]_{\text{SR}(t)} = (A/k) \times [1 - \exp(-kt)]
\]

where [Ca<sup>2+</sup>]<sub>SR</sub> refers to the SR Ca<sup>2+</sup> content at time t after the start of loading. Equation 4 was then fitted to the data points for control fibers and fibers from muscles treated to 40 and 43°C as shown in Fig. 4 using the k values indicated above, yielding the following values for the SR Ca<sup>2+</sup> uptake rate A of 0.033 ± 0.010, 0.019 ± 0.005, and 0.028 ± 0.005 F<sub>max</sub> for control fibers and fibers from muscles treated to 40 and 43°C.

Effects of elevated temperature on SR Ca<sup>2+</sup>-ATPase pump activity. To find out whether the elevated temperature also affects the SR Cr<sup>2+</sup>-ATPase pump activity, we measured the SR Ca<sup>2+</sup>-ATPase activity spectrophotometrically from the production of P<sub>i</sub> in skinned fiber segments from freshly skinned mechanically skinned muscle fibers that were maximally activated by Ca<sup>2+</sup> as described in METHODS. To determine the ATPase activity of all ATPases in a mechanically skinned fiber, we placed the fiber in an activating solution of pCa 4.5

![Fig. 4. Effect of ruthenium red (RR) and 2,5-di tert-butyl)-1,4-hydroquinone (TBQ) on SR Ca<sup>2+</sup> leak. Ca<sup>2+</sup> loss from the SR after a standard 90-s loading period followed by a 60-s leak period in the presence of 0.5 mM EGTA in the leak solution with and without 5 μM RR or 20 μM TBQ (%SR Ca<sup>2+</sup> leak; see METHODS) was determined after various temperature treatments [40°C for 30 min (RR and TBQ, n = 4) and 43°C for 30 min (RR, n = 5; TBQ, n = 4)]. Control values represent 8 fibers in the presence of RR and 5 fibers in the presence of TBQ. There were no statistically significant differences among any sets of results in the absence or presence of RR for any temperature treatment. The presence of TBQ did not significantly decrease or increase the SR Ca<sup>2+</sup> leak in fibers exposed to 40 or 43°C for 30 min.

![Fig. 5. Effect of elevated temperature on SR ATPase activity. The SR ATPase activities of fibers from muscles exposed to 40°C for 30 min (n = 3 fibers) or 43°C for 30 min (n = 3 fibers) were not significantly different from the SR ATPase activity of control fibers (n = 4).]
respectively (Figs. 4 and 5). The corrected SR Ca\(^{2+}\) uptake rates in fibers from muscles treated at 40 and 43°C were not significantly different from those in control fibers (P > 0.1, 1-way ANOVA), although they showed the same trend of being somewhat smaller than for control fibers. This indicates that the major effect of muscle exposure to 40 and 43°C is a marked increase in the leak rate of Ca\(^{2+}\) from the SR.

**Effect of elevated temperature treatment in the presence of 20 mM Tiron on SR Ca\(^{2+}\) loading and SR Ca\(^{2+}\) leak rates.** Under conditions similar to those used in this study, we have shown that exposure to elevated temperature markedly increased the rate of O\(_2\)\(^{•−}\) production (42). Therefore, it was of interest to determine whether the temperature-induced changes observed on the SR function were influenced by temperature-induced O\(_2\)\(^{•−}\) production. This was achieved by application of the membrane-permeable O\(_2\)\(^{•−}\) scavenger Tiron (42).

Without Tiron present during temperature treatment, the SR Ca\(^{2+}\) leak (after 90 s) after exposure to 40 and 43°C was 38.6 ± 8.6 and 59.4 ± 6.3% SR Ca\(^{2+}\) content, respectively. Upon the addition of 20 mM Tiron to the muscles before and during temperature treatment, it was observed that the Ca\(^{2+}\) lost during the 60-s leak period significantly decreased to 13.9 ± 6.3 and 8.8 ± 6.2% of the total SR Ca\(^{2+}\) after treatment at 40 and 43°C (Fig. 6), respectively. Note that the presence of Tiron did not have a significant effect on fibers from control muscles (Fig. 6). Tiron also prevented the decrease in the rate of SR Ca\(^{2+}\) accumulation after temperature treatment. Thus, in the presence of Tiron, the SR Ca\(^{2+}\) accumulation rates of fibers from muscles treated at 40 and 43°C were not significantly different from controls (Fig. 7). The results clearly demonstrate that pretreatment with 20 mM Tiron reduces the temperature-induced changes on SR function and suggest that the observed temperature-induced effects are to a large extent related to the production of O\(_2\)\(^{•−}\).

**DISCUSSION**

**Temperature effects on the SR.** The results show that exposure of mammalian skeletal muscle to temperatures in the upper physiological range (as defined in Refs. 4, 14, 34) significantly reduces the ability of the SR to accumulate Ca\(^{2+}\), seemingly via an increase in the SR Ca\(^{2+}\) leak without markedly affecting the maximum SR Ca\(^{2+}\)-ATPase. The SR Ca\(^{2+}\) leak rate at 23°C is ∼5 μmol·fiber\(^{−1}\)·s\(^{−1}\) if one considers the leak rate constant of 0.0042 s\(^{−1}\) measured in this study and an endogenous SR Ca\(^{2+}\) level of 1.2 mmol per liter of fiber (13). To cope with this leak, the SR Ca\(^{2+}\) pump will consume 2.5 μmol ATP·fiber\(^{−1}\)·s\(^{−1}\), considering that 2 Ca\(^{2+}\) molecules are transported for each ATP molecule hydrolyzed by the pump. This corresponds to 0.96% maximal SR ATPase rate measured in this study or to 0.32% of maximal fiber ATPase. Furthermore, assuming that ∼40–50 kJ are liberated as heat per molecule of ATP hydrolyzed under conditions prevalent in the resting EDL muscle fiber (22) and that the efficiency of producing ATP by oxidative phosphorylation is ∼40%, the heat associated with SR ATPase necessary to cope with the SR Ca\(^{2+}\) leak under resting conditions amounts to ∼0.25–0.3 mW/g EDL muscle, which corresponds to ∼15% of the resting heat measured in the rat EDL muscle at 27°C (5). The increased Ca\(^{2+}\) leak observed in fibers exposed to 40 and 43°C will translate to an increased SR ATPase corresponding to 0.62 and 1.14% maximal fiber ATPase, which would make a significant contribution to the fiber resting heat.

**Fig. 6.** Effect of exposure to elevated temperature on SR Ca\(^{2+}\) leak rate in the presence of 20 mM Tiron. Ca\(^{2+}\) leak loss from the SR after a standard 90-s loading period followed by a 60-s leak period (%leaked SR Ca\(^{2+}\); see Eq. 1 in METHODS) was determined under control conditions and after elevated temperature treatment at 40 and 43°C for 30 min in the absence and presence of 20 mM Tiron. There were no statistically significant differences between results obtained on muscle fibers from control muscles and from muscles treated in the presence of Tiron (P > 0.3, 1-way ANOVA). Data are means ± SE; n = 15, 3, and 4 for control, 40°C, and 43°C, respectively.

**Fig. 7.** Effect of Tiron on the rate of SR Ca\(^{2+}\) accumulation. SR Ca\(^{2+}\) accumulation rates were determined in skinned fibers dissected within 30 min after elevated temperature treatment in the presence of 20 mM Tiron. Data points for all fibers were normalized to the maximum Ca\(^{2+}\)-activated force of that particular fiber: • 40°C for 30 min (n = 3 muscles and 3 fibers at each point); ○, 43°C for 30 min (n = 5 muscles and 5 fibers at each point); and ■, control fibers. Data points were fitted by linear regression; R\(^2\) > 0.99. There were no significant differences among the various sets of data points (P > 0.4, 1-way ANOVA).
[Ca$^{2+}$] could cause uncoupling of Ca$^{2+}$ release from t-system depolarization (18, 45) and result in a reduced force response. Together with the marked temperature-induced depression on the force response (42), these results could explain at least in part the poor longevity of the isolated muscle at temperatures in the range 40–43°C. Interestingly, the decrease in SR Ca$^{2+}$ accumulation was not associated with a marked decrease in SR ATPase activity.

Results with RR clearly show that the increased SR Ca$^{2+}$ leak is not a result of Ca$^{2+}$ leak through the Ca$^{2+}$ release channels, since the increased leak was not reduced by RR. Thus the SR Ca$^{2+}$ leak must either be through another pathway in the SR membrane, such as the ryanodine-insensitive Ca$^{2+}$ channel (29), or via the SR Ca$^{2+}$ pump, which can act as a Ca$^{2+}$ carrier across the SR membrane through a process known as “slippage” (22). Another potential cause of the observed increase SR Ca$^{2+}$ leak and no significant change in SR Ca$^{2+}$ pump activity is oligomerization of Ca$^{2+}$-ATPase molecules in the SR membrane, which has been observed after exposure to high temperature (45°C) (14). The authors of this study (14) provide evidence that heat treatment induces protein pump oligomerization, causing the Ca$^{2+}$-ATPase molecules to rearrange themselves in the SR membrane to form channels and allow Ca$^{2+}$ to leak out of the SR without an effect on the apparent activity of the SR Ca$^{2+}$-ATPase. In this oligomerization process, different sites on the SR Ca$^{2+}$ pump are exposed to the myoplasmic environment (7, 36). The lack of TBQ effect on the temperature-induced Ca$^{2+}$ leak does not necessarily indicate that the leak is not via the SR Ca$^{2+}$-ATPase, since TBQ may act normally but cannot block the leak pores created between oligomerized pump complexes.

Mechanism of temperature-induced effects on SR function.

We have previously shown that under the conditions of these experiments, large amounts of O$_2^-$ are produced in the isolated skeletal muscle preparations when the preparations are exposed to elevated temperatures (42, 44). In the present study we have shown that application of 20 mM Tiron, a potent cell-permeant O$_2^-$ scavenger, to the muscle before and during elevated temperature treatment was able to prevent the temperature-induced increase in SR Ca$^{2+}$ leak, which in turn allowed mechanically skinned muscle fibers to accumulate Ca$^{2+}$ at the same rate as control muscle fibers. This indicates that the production of O$_2^-$ at elevated temperatures is directly responsible for the increased SR Ca$^{2+}$ leak and decreased SR Ca$^{2+}$ loading efficiency.

The Ca$^{2+}$ release channels are potentially the most redox-sensitive component of the SR with the RyR1 isoform having ~50% of its 101 cysteines in a reduced state in the resting muscle (24, 40). Therefore, one would have expected that they would be most sensitive to increased O$_2^-$ production. However, it is important to point out that channel activity is only affected when the number of thiols per RyR1 subunit decreases below 38. Furthermore, channel activity increases reversibly as the number of thiols per RyR1 subunit is reduced to ~ 23, but more extensive oxidation to less than ~13 thiols per subunit causes irreversible channel inactivation (40). Thus it is not easy to predict what effect different oxidants have on the Ca$^{2+}$ release channels. For example, at 150 mmHg partial pressure of oxygen (the conditions of the current study), low concentrations (0.2 mM) of 3-morpholinosydnonimine (SIN-1), which generates NO and O$_2^-$ in a 1:1 ratio, increased Ca$^{2+}$ channel opening (40), thus providing an explanation as to why so many studies have inferred that the leak associated with oxidation of the SR is due to redox modification of the Ca$^{2+}$ release channel. In contrast, higher production of NO and O$_2^-$ from 1 mM SIN-1 caused inhibition of channel opening (40). This could explain why in our current study there is not an increase in SR Ca$^{2+}$ leak through the Ca$^{2+}$ release channel, since exposure to elevated temperature produces concentrations of oxidants that do not cause channel opening. Nevertheless, it is quite possible that other properties of the Ca$^{2+}$ release channels are affected that may also play a role in the depression of the force response to electrical stimulation (30).

The possibility that the temperature-induced Ca$^{2+}$-leak pathway is via the pump protein that becomes oligomerized during the temperature treatment is supported by the fact that oligomerization of the pump protein at higher temperatures exposes thiol groups on the SR Ca$^{2+}$ pump (7, 36) that can be oxidized by O$_2^-$ or other ROS derived from O$_2^-.$ Once oxidized, the oligomerization process is unlikely to be reversed upon returning the preparation to room temperature where the properties of the SR are tested.

In summary, we found that incubation at elevated temperature results in a decrease in SR Ca$^{2+}$ accumulation and an increase in the leak rate of Ca$^{2+}$ from the SR that is not through the Ca$^{2+}$ release channel but is potentially through the SR Ca$^{2+}$ pump. The net effect of these changes would be an increase in the myoplasmic [Ca$^{2+}$] that causes uncoupling of the Ca$^{2+}$ release channels from the activation of the voltage sensors in the t-system (18, 45) and a gradual depletion of the SR Ca$^{2+}$. This result is both novel and interesting, because it provides new information about SR regulation and the mechanisms of rundown in isolated muscle preparations at physiological temperatures. These temperature-induced effects on the SR together with other temperature-induced effects on the contractile apparatus and muscle excitability reported earlier by us (42, 44) could be prevented by the potent O$_2^-$ scavenger Tiron, demonstrating an intriguing link between elevations of skeletal muscle temperature above normal physiological temperature and an associated increase in muscle derived oxidants. Together, these observations provide a deeper understanding of the complex relationships between various pathophysiological conditions that cause increased O$_2^-$ production, such as fever, reperfusion injury, and various diseases.

GRANTS

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