THE EFFECT OF FATIGUE ON THE CAFFEINE SENSITIVITY OF SKELETAL MUSCLE SARCOPLASMIC RETICULUM

by

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ABSTRACT

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR. The purpose of this study was to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than that of rested muscle. Following a fatigue bout of electrically evoked tetanic pulses, the functioning of the SR of chemically skinned muscle fibers was determined by the sensitivity of the SR to increasing concentrations of caffeine. Measurements of tension and rate of tension development were made at the maximal Ca^{2+} activated contracture(pCa4.5), the maximal caffeine(25mM) activated contracture and at the caffeine threshold for contraction. All tension and rate values were normalized per cross sectional area and expressed as percents of the maximal calcium activated values. Results of the maximal Ca^{2+} and caffeine data suggest that the both control and fatigue fibers are similar in maximal tension and Ca^{2+} loading characteristics.

While no differences were found between rested or fatigued maximal Ca^{2+} or caffeine contractures, significant difference was found at the caffeine threshold ($p<.05$) with the fatigued muscle tending to

contract at a higher caffeine concentration. This suggests that fatigued muscle is less sensitive to the caffeine stimulus for Ca^{2+} release from the SR.

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CHAPTER I

INTRODUCTION

Fatigue is a term used to describe a variety of conditions resulting in decreased performance over time. Muscular fatigue is characterized as a decrease in the muscle's capacity to perform at a prescribed work intensity. Muscle fatigue has been implicated in decreased worker productivity, decreased athletic performance and as symptomology in various disease states. While the characteristics of this phenomena have been repeatedly studied, the exact mechanisms have yet to be elucidated.

The degree of fatigue exhibited by skeletal muscle can vary greatly, depending on factors such as muscle fiber type, fuel substrate status, work intensity, and work duration. Research on fatigue conducted in intact animals has shown components in the central nervous system (CNS), the neuromuscular junction (NJ), and the muscle cell itself (Bigland-Ritchie & Woods, 1984). This has lead to the differentiation of central and peripheral fatigue. Fatigue sites proximal to the motor end plate (CNS, NJ) have been termed central while sites distal to the NJ have been termed peripheral.

A number of researchers investigating central fatigue processes have shown that maximal force generation in fatigued muscle is the same as electrically elicited force. This suggests that fatigue occurs despite maximal activation (Merton, 1954; Vollestad & Sejersted, 1988; Bigland-Richie, 1986). In addition Bigland-Ritchie and Thomas(1987) used electrostimulation to show that even in muscle that suffered from a great decrement in force generating capacity, the M-wave of the compound electromyogram remained unchanged. This suggests no failure occurs at the neuromuscular junction. Conversely, studies using the diaphragm and others using patients afflicted with the disease myasthenia gravis have implicated a reduction in neuromuscular transmission in fatigue. Despite the evidence for certain cases of fatigue to be centrally related, many researchers feel that the level of sarcolemmal activation remains adequate to sustain maximal activation under most conditions (Vollestad & Sejersted, 1988).

Much research on peripheral fatigue has been centered on the issue of cellular energy depletion. Results compiled in a review by Vollestad and Sejersted (1988) show that intracellular adenosine 5' triphosphate (ATP) depletion on the order of 50% does not affect myosin ATPase activity. This 50% reduction in ATP is far greater than those reported

during fatiguing stimulation (Fitts & Holloszy, 1976). *In vitro* results on skinned muscle fibers suggest that decreases in creatine phosphate (CP) or glycogen concentration have no effect force production (Godt & Nosek, 1989). It is concluded that the reduction in ATP concentration following fatiguing stimulation is not likely to account for the significant reduction in force generating capacity.

The assumption of adequate activation at the level of the sarcolemma with sufficient metabolic energy leaves impaired coupling of the excitation and contraction processes as possible sites of fatigue. A breakdown of the excitation-contraction coupling can occur at a number of sites in the muscle cell. These sites include (1) reduced membrane excitability resulting in impaired transmission of the action potential down the transverse tubule(TT), (2) a reduction in calcium (Ca^{2+}) sensitivity of the contractile elements, (3) a reduction of Ca^{2+} content of the sarcoplasmic reticulum (SR) causing reduced release, (4) and reduced Ca^{2+} release from the SR due to lack of stimuli between the TT and the SR (Westerblad & Lannergren, 1989).

The possibility of reduced membrane excitability has been addressed by several authors. Changes in the resting and action potential have been observed during fatiguing

stimulation. (Bianchi & Narayan, 1982; Westerblad & Lannergren, 1986; Lannergren & Westerblad, 1989). Lannergren and Westerblad (1989) used a high potassium solution to mimic a "fatigued" resting potential in a rested muscle. Results of subsequent stimulation of the fiber action potentials similar to those occurring during fatigue, augmented rather than depressed twitch production. These results suggest that altered resting and action potentials are not the major cause in the fatigue process.

Several investigations have examined increases of intracellular metabolites as possible uncouplers of excitation & contraction. These metabolites are a by-product of enhanced energy production during sustained muscle activity. Intracellular changes such as increased hydrogen ion (H^+) concentration (reduced pH) and increased inorganic phosphate (P_i) have been implicated in the reduction of force generating capacity in skeletal muscle. Results of research conducted by Mainwood and Renaud(1984) investigating the pH effects in whole muscle preparations and Fabiato and Fabiato (1978) on the effects of varying pH on the tension developed by skinned cardiac and skeletal muscle suggest that while decreased pH might play a role in fatigue, it certainly cannot explain the large force decrement exhibited in fatigued muscle.

In contrast to studies suggesting that increases in metabolites affect the contractile machinery of the muscle, Fitts et al.(1982) investigated the hypothesis of decreased sensitivity of the contractile apparatus during fatigue. Results showed that neither fast nor slow myofibrilar Ca^{2+}ATPase activity was affected after intense exercise.

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents (caffeine and potassium) which evoke Ca^{2+} release from the SR. Lannergren and Westerblad (1989) investigated the effect of both these agents on the fatigued muscle cell. While the potassium contracture remained somewhat depressed, the caffeine contracture generated near maximal tension. These results suggest two things, (1) there is impaired TT to SR transmission during fatigue since maximal activation of the TT by potassium results in an impaired contraction, and (2) an adequate amount of Ca^{2+} remains in the SR to cause a maximal activation of the contractile apparatus as indicated by the action of caffeine.

In studies by Allen et al.(1989) and Lee et al. (1991) the intracellular calcium fluxes during fatiguing stimulation were examined. Results indicated a complex response of myoplasmic Ca^{2+} to fatiguing stimulation.

Specifically, fatiguing stimulation resulted in (1) decreased SR Ca^{2+} release as determined by an 84% decline in the Ca^{2+} indicator, (2) increases in resting Ca^{2+} levels, and (3) no change in the sensitivity contractile proteins to Ca^{2+}.

STATEMENT OF THE PROBLEM

Since results show that an adequate amount of calcium remains in the SR to cause maximal crossbridge activation and that sensitivity of the contractile apparatus does not appear to be markedly affected in fatigue, two hypothesis arise as possible contributors in the fatigue process. (1) there is less stimulus to release Ca^{2+} from the SR and/or (2) the SR is less sensitive to stimuli which cause the release of Ca^{2+}.

SIGNIFICANCE OF THE STUDY

Because the exact stimulus by which the depolarized TT initiates Ca^{2+} release from the SR is unknown, it is impossible to test for its depletion during fatigue. It is, however, possible to test for a reduced Ca^{2+} release sensitivity of the SR by using agents known to evoke Ca^{2+} release such as caffeine.

Therefore the purpose of this study is to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than is the SR of rested muscle.

RESEARCH HYPOTHESIS

Specifically, this investigation will test the following hypotheses:

H_0 - The concentration of caffeine required to evoke a contracture in fatigued skeletal fibers is not different than that required to release Ca^{2+} from rested SR.

H_0 - The rate of tension development in fatigued fibers is not different than that of rested fibers.

H_0 - The sensitivity of the contractile apparatus to Ca^{2+} in fatigued skeletal muscle is not different than that of rested skeletal muscle.

DELIMITATIONS

The following delimitations were emposed on this study by the investigator:

1. The investigation was limited to amphibian skeletal muscle (semitendinosus) of the species *Rana Pipiens*.
2. The investigation was limited to the use of caffeine as a Ca^{2+} releasing agent.

3. The measurement conditions were limited to the imidazole buffered solution maintained at pH 7.2 and 22°C.

LIMITATIONS

The following limitations of the study were recognized by the investigator:

1. Due to the intense fatigue protocol employed, results are limited to similar protocols.

BASIC ASSUMPTIONS

The following assumptions were made prior to the start of the investigation:

1. All specimens possessed normal anatomy and were free of disease.

DEFINITIONS AND SYMBOLS

Ca^{2+} -Calcium.

TT -Transverse tubules.

SR -Sarcoplasmic reticulm.

dP/dt -Rate of tension rise.

P_o -Maximal tetanic or Ca^{2+} activated tension.

CafIR -dP/dt of the maximal caffeine contracture prior to loading.

CafIT -Tension of the maximal caffeine contracture prior to loading.

CafTR	-dP/dt at the caffeine threshold.
CafTT	-Tension at the caffeine threshold.
CafIIR	-dP/dt of the maximal caffeine contracture post loading.
CafIIT	-Tension of the maximal caffeine contracture post loading.

SUMMARY

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR. These results suggest that an adequate amount of calcium remains in the SR to cause maximal crossbridge activation and that sensitivity of the contractile apparatus does not appear to be markedly affected in fatigue. It has also been shown that during fatigue, the amount of Ca^{2+} released by the SR during stimulation is markedly reduced (Allen et al. 1989, Lee et al. 1990). There appears to be two hypothesis which might explain this lack of Ca^{2+} release from the SR during fatigue. First, there may be less stimulus to release Ca^{2+} from fatigued SR. Second, the fatigued SR may be less sensitive to stimuli which cause the release of Ca^{2+}.

Because the exact stimulus by which the depolarized TT initiates Ca^{2+} release from the SR is unknown, it is impossible to test for its depletion during fatigue. It is, however, possible to test for a reduced Ca^{2+} release sensitivity of the SR by using agents known to evoke Ca^{2+} release such as caffeine. Therefore the purpose of this study was to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than is the SR of rested muscle.

CHAPTER II

REVIEW OF THE LITERATURE

INTRODUCTION

This review explores the concepts of the fatigue process developed by researchers from many scientific disciplines. The result is a review acknowledging many valid theories on the fatigue process and posing further questions to be addressed in the area.

Many data and theories have been generated in an attempt to identify an exact mechanism for the phenomena of skeletal muscle fatigue. The study of this phenomena has brought us closer to understanding components of the fatigue process but the exact mechanisms still elude researchers.

An examination of the anatomical and physiological processes controlling muscle activity reveals many possible sites for failure resulting in skeletal muscle fatigue. The complex pathway between the brain and the actin and myosin interaction encompasses cognitive components, neurological activity, electrophysiological function, cellular ion fluxes and mechanical activity all which have vital roles in muscle control. The complex interaction of these processes has lead to the definition of two broad types of fatigue, central and peripheral. Possible fatigue sites within these areas are

displayed diagrammatically in figure 1.

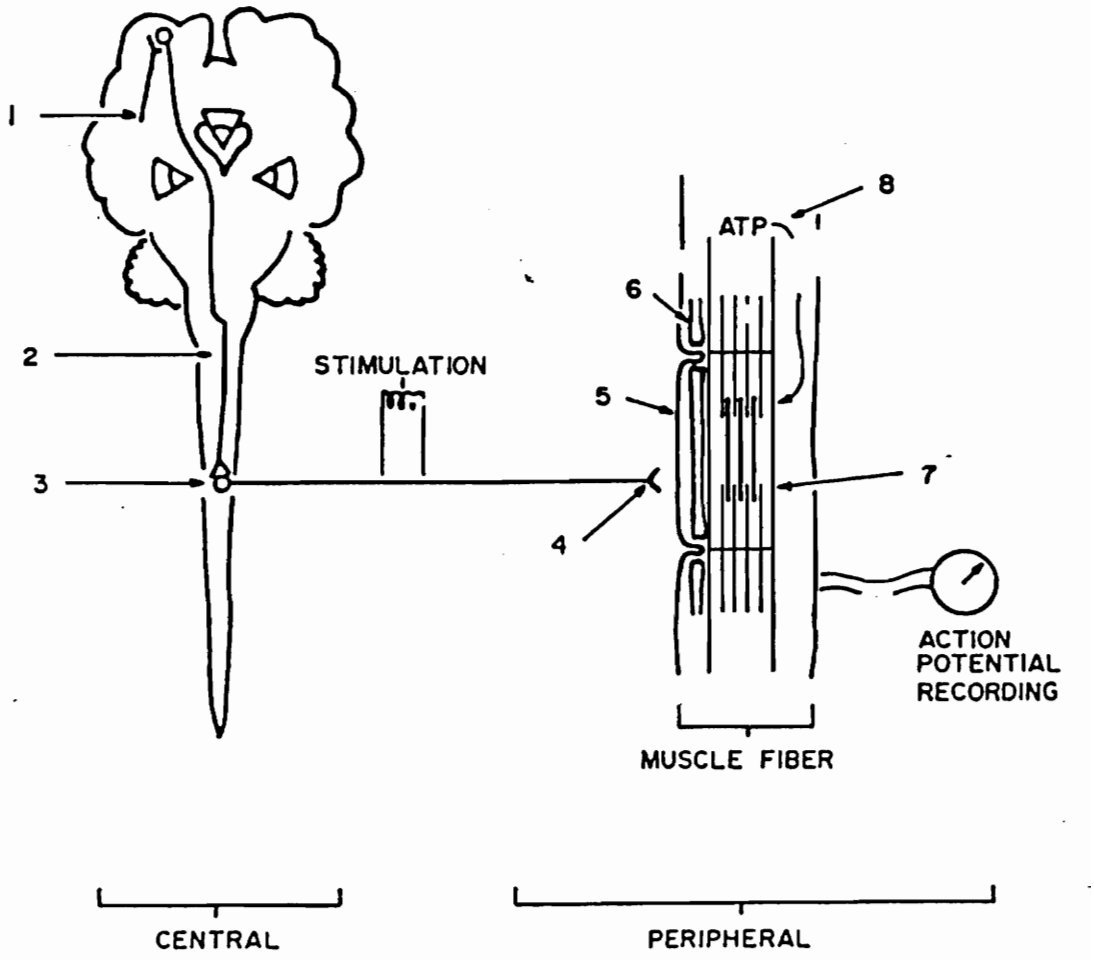

Figure 1. Possible sites of Fatigue: 1-3 Motor neuron excitation. 4. Neuromuscular transmission. 5. Sarcolemma excitation. 6. Excitation-contraction coupling. 7. Contractile mechanism. 8. Cellular energy. (Bigland-Ritchie et al. 1986)

CENTRAL FATIGUE

Central fatigue occurs due to failure in the pathway from the central nervous system (CNS) to the alpha-motor neuron. Failure in the CNS can occur at the higher brain centers, the motor neuron, the feedback mechanisms from the muscle, and the proximal side of the neuromuscular junction. Failure at any one of these sites will ultimately result in the inability to activate the muscle cell.

While central fatigue has been identified in intact animals, (Bigland-Ritchie & Woods, 1984) its overall impact in the fatigue process has been highly debated. Ikai & Stenhaus (1961) have shown that arousal techniques such as hypnosis and sudden, unexpected noises can increase one's maximal voluntary contraction. A number of researchers, however, have shown that maximal force generation in fatigued muscle is the same as electrically elicited force. This suggests that fatigue occurs despite maximal activation by the CNS (Merton, 1954; Vollestad & Sejersted, 1988; Bigland-Richie, 1986). In addition Bigland-Ritchie and Thomas(1987) used electrostimulation to show that even in muscle that suffered from a great decrement in force generating capacity, the M-wave of the compound electromyogram remained unchanged. This suggests that failure does not occur at the neuromuscular junction.

Conversely, studies using the diaphragm and others using patients afflicted with the disease myasthenia gravis have implicated a reduction in neuromuscular transmission in fatigue. Despite the evidence for certain cases of fatigue to be centrally related, many researchers feel that in well motivated subjects under normal conditions, central activation remains adequate to produce a "true" maximal contraction (Vollestad & Sejersted, 1988).

PERIPHERAL FATIGUE

Peripheral fatigue occurs in the complex cellular processes of the muscle fiber. Cell processes can be divided into two areas; cellular energy depletion, and excitation-contraction coupling (ECC) processes. The cellular processes for possible failure are presented diagrammatically in figure 2. These processes include: 1) activation of the motor endplate. 2) generation of a sarcolemmal action potential. 3) propagation of a sarcolemmal action potential. 4) communication between the transverse-tubule (TT) and sarcoplasmic reticulum (SR). 5) Calcium (Ca^{2+}) release from the SR. 6) Ca^{2+} binding affinity on troponin. 7 & 8) Cycling of crossbridges. 9) Ca^{2+} reuptake by the SR.

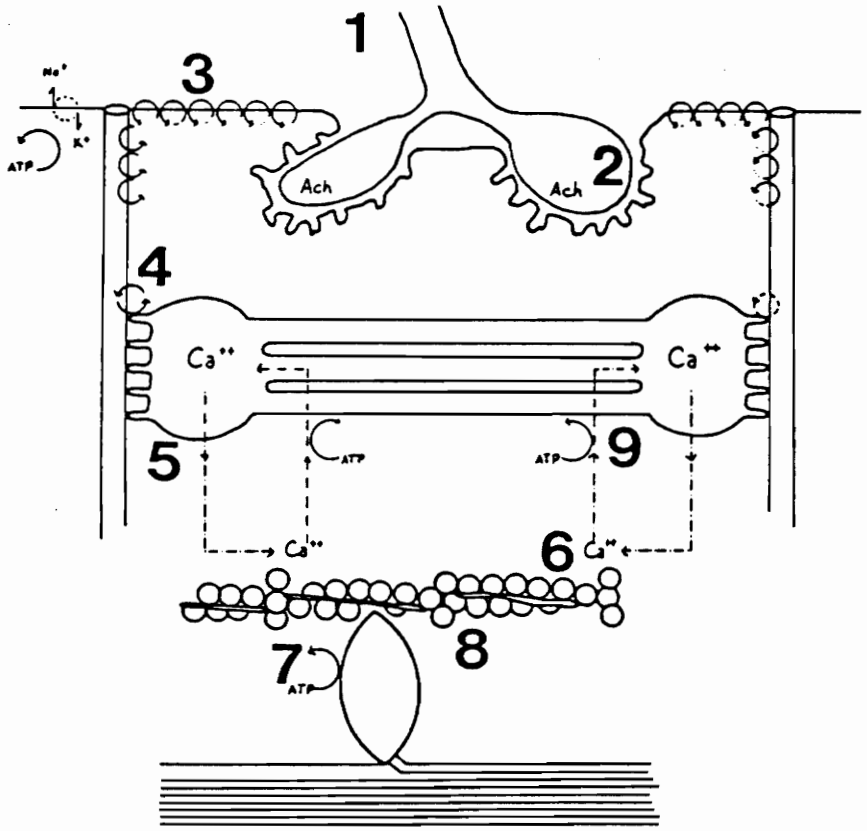

Figure 2. Peripheral Fatigue sites. (Green, 1990)

Cellular Energy Depletion

The issue of cellular energy depletion has been addressed as a possible cause in the fatigue process. During bouts of short- term, high-intensity (anaerobic) exercise, depletion of glycogen, creatine phosphate (PCr), and adenosine 5' triphosphate (ATP) is thought to cause a decrease in muscle performance. ATP hydrolysis is required for cross bridge cycling, crossbridge dissociation, ion pump function, and enzymatic function to resynthesize ATP from energy intermediates. Results compiled in a review by Vollestad and Sejersted (1988) show that intracellular ATP depletion on the order of 50% does not effect myosin ATPase activity. This 50% reduction in ATP has been reported in fatigue induced electrical stimulation of frog skeletal muscle (Nassar-Gentina et al. 1978, Nassar-Gentina et al. 1981). Further reductions in ATP were reported by Nassar-Gentina (1981) following artificially induced caffeine contractures post-fatigue. Also Godt and Nosek (1988) showed that a decrease in ATP concentration did not effect maximal force generation or Ca^{2+} sensitivity of skeletal muscle. Investigations using *In vitro* skinned fiber preparations also suggest that large decreases in creatine phosphate or glycogen concentration have no effect on isometric force production (Godt & Nosek, 1989). Thus the

reduction in ATP concentration following fatiguing stimulation does not appear to account for the significant reduction in force generating capacity of skeletal muscle. The fatigue seems to be due to inhibition of contraction independent of energy status.

Excitation-Contraction Coupling Failure

The assumption of adequate activation at the motor end plate with sufficient metabolic energy leaves impaired coupling of the ECC processes as possible sites of fatigue. A breakdown of ECC can occur in a number of processes in the muscle cell. These include (1) reduced membrane excitability resulting in impaired transmission of the action potential down the TT, (2) reduced sensitivity of the contractile elements, (3) reduced content in the SR causing a reduced release, (4) and reduced release from the SR due to lack of stimuli between the TT and the SR (Westerblad & Lannergren, 1989). Many cellular perturbations occur as a result of intense muscle contraction. Structural changes, functional changes and changes in the intracellular milieu will be discussed as they relate to the above mentioned ECC process.

Electrical Activation The propagation of an action potential from the sarcolemma along the TT and into the central part of the muscle cell is required for the optimal cellular energy utilization and activation of the ECC processes (Edwards. 1986). The possibility of reduced membrane excitability has been addressed by several authors. Reduced membrane excitability would prevent an adequate impulse from being delivered to the TT. Such a situation would result in reduced release of Ca^{2+} from the SR and ultimately decrease force production by the crossbridges. Changes in the resting and action potential have been observed during fatiguing stimulation in isolated muscle. For example, Westerblad and Lannergren (1986) reported an increase in resting membrane potential of skeletal muscle fibers from -90mV to -70mV following fatiguing stimulation. Also reported was a decrease in action potential amplitude with a widening of its shape. This widening suggests a slowing of the conduction velocity. The fatigue state in which changes in the resting and action potentials are manifested can be overcome by either increasing the stimulus intensity or duration (Jones, 1979). To investigate whether these membrane changes cause a decrease in activation in the fatigued fiber, Lannergren and Westerblad (1989) used a high potassium solution to mimic "fatigued" resting and action

potentials in rested muscle, these conditions augmented rather than depressed twitch production. A study by Deleze et al. (1986) measured the action potentials in the TT of amphibian skeletal muscle after the sarcolemma had become unexcitable in a Na^+ free media. These experiments were conducted immediately after exposure to the Na^+ media so that the TT would still contain enough Na^+ for a Na^+ spike. Results of this study by Deleze (1986) suggests that a blocking of the sarcolemmal action potential during fatigue still allows generation of a TT action potential. These results presented above suggest that fatigued induced alterations of sarcolemmal resting and action potentials are probably not a major cause of skeletal muscle fatigue.

Metzger and Fitts (1986) have reported that rat diaphragmatic muscle stimulated at different frequencies resulted in vastly different tetanic fatigue characteristics (21% of P_o at 75 Hz, 71% of P_o at 5 Hz) despite no significant changes in sarcolemmal membrane potential. These results suggest that a site distal to the sarcolemma is responsible for the tension loss in fatigue. Several authors feel that a block of the action potential due to ion accumulation in the TT could occur despite full activation of the sarcolemma (Bigland-Ritchie, 1979; Vollestad and Sejersted, 1988). Adrian and Peachy (1973) have reported

that extracellular sodium (Na^+) decreases and K^+ increases by 0.5 & 0.28 mM respectively during each twitch. The accumulation of extracellular K^+ is reported to be greatest in the TT. The diffusion of the ion out of this space is markedly reduced due to the high surface to volume ratio (Adrian and Peachy, 1973). Increases in extracellular K^+ and intracellular Na^+ are thought to explain the low membrane resistance (Fink, 1976; Grabowski, 1972) and the decrease in driving force for the inward Na^+ current found in fatigued fibers (Vollestad, 1988; Maclaren, 1989). Further, Bianchi and Narayan (1982) suggest that Ca^{2+} accumulation in the TT's may also inhibit the TT action potential.

The hypothesis of ion accumulation in the TT hampering the delivery of an action potential to the fiber interior is plausible. As of yet it has proven difficult to measure the TT action potential during fatigue to support or refute these hypothesis.

Metabolite Accumulation Several investigators have examined increases of intracellular metabolites as possible uncouplers of excitation and contraction. These metabolites are a by-product of enhanced energy production during sustained muscle activity. Intracellular changes such as increased hydrogen (H^+) ion concentration (reduced pH),

increased inorganic phosphate (Pi) and increased in ADP have been implicated in the reduction of force generating capacity in skeletal muscle.

The pH is inversely dependent on H^+ ion concentration. H^+ ions are mainly a byproduct glycolysis. Renaud et al. (1986) investigated pH effects in whole muscle preparations. Whole muscle fatigued to 70-80% of initial tension showed decreases in intracellular pH of 0.3-0.5 pH units. Non-fatigued muscles exposed to CO_2 showed similar reductions in pH; but exhibited only 30% force decrement (Renaud et al. 1986). Several authors investigated the effects of varying pH on the tension developed by skinned cardiac and skeletal muscle (Godt & Nosek. 1989, Metzger & Fitts. 1987, Donaldson & Hermanson. 1978). Fabiato & Fabiato (1978) reported that when pH was reduced from 7.4 to 6.2, in skinned muscle fibers, the tension produced by saturating amounts of Ca^{2+} was decreased by 33% along with a decrease in the $_pCa_{50}$. These results suggest that decreases in pH cause a decrease in maximal force and a decrease in sensitivity of the contractile apparatus to Ca^{2+}.

The effects of pH on SR function have been investigated by several authors. The pH effects on Ca^{2+} induced Ca^{2+} release from skeletal muscle SR were investigated by Williams and Ward (1991). These authors

showed that reduced pH depresses Ca^{2+} induced Ca^{2+} release from skeletal muscle SR. Other authors using *in vitro* methods have shown that reduced pH depressed both Ca^{2+} uptake and release from the SR (Fabiato & Fabiato. 1978, Mandel et al. 1982).

Increased intracellular concentrations of H^+ increases with the production of lactate (MacLaren. 1989). Mainwood et al. (1986) investigated the hypothesis that since inhibition of tension generation following fatigue is associated with a decreased rate of lactate loss, intracellular lactacidosis may be the cause of this phenomena. Initial studies by these investigators identified that following a fatiguing bout that produced a drop of 0.4 in pH units, corresponding to a lactate load of 18 umol g^{-1} or 33 mM liter of fiber water. Muscles were then exposed to a pH of 6.2 with and without a lactate load of 30 or 40 mM per liter of fiber water. Results suggest that while the pH alone had little effect on the twitch; maximum isometric tension decreased and time to peak tension and half relaxation increased in response to the lactate load. The authors reported that under the conditions investigated only 20-30% of peak tetanic tension suppression could be accounted for by either carbon dioxide (CO_2) or lactacidosis. This explained only some of the 80% tension

loss usually seen after eliciting similar pH and lactate concentrations through electrical stimulation.

Intracellular inorganic phosphate (P_i) occurs as a by-product of the hydrolysis of ATP and of the force generating state between actin and myosin. P_i is reported to be involved in a "low force state" complex (actin-myosin-ADP-P_i)at the beginning of the power stroke, and then released during the "high force" end of the power stroke (Cooke and Pate, 1990). Studies have shown that P_i accumulates as a result of ischemic rest and muscular activity. Maximal voluntary isometric contractions in humans and electrically elicited isometric contractions in the frog sartorius have both yielded a similar 4-fold increase in Pi (MacLaren et al. 1989). Studies conducted in skinned muscle fibers have shown that increased concentrations of P_i inhibit tension development (Brandt et al. 1982, Cooke and Pate, 1985). For example Cooke and Pate (1985) reported that 20mM P_i caused a 30 and 35 percent reduction in tetanic force at pH values of 6.5 and 7.0 respectively.

Increases in ADP concentrations have also been thought to contribute to the fatigue process. Much like P_i, ADP is released during the force generating state in crossbridge cycling. Cooke and Pate (1990) showed that at physiological concentrations of ATP, increasing concentrations of ADP in a

skinned fiber preparation decreased the shortening velocity (Vmax) while increasing isometric tension.

Research on the effect of metabolite accumulation during fatigue can account for approximately 30% of tetanic force reduction. While this is a significant reduction in force, it does not explain the almost 100% reduction in force attainable in both voluntary and involuntary fatigue. This suggests that other factors must also contribute to the fatigue process.

Contractile Apparatus In contrast to studies suggesting that increases in metabolites affect the contractile machinery of the muscle, Fitts et al.(1982) investigated the hypothesis that fatigue directly altered the contractile mechanisms activity to produce force. Results showed that neither fast nor slow myofibrilar sensitivity or ATPase activity was affected after intense exercise. Unfortunately few studies have examined this area of contractile mechanism fatigue and more work is needed to characterize its function during fatigue.

Sarcoplasmic Reticulum The control of tension in the muscle cell is directly due to the regulation of myoplasmic free Ca^{2+} by the SR. Therefore it is plausible that alterations in SR function during fatigue could impair tension development. Byrd et al. (1989) investigated the

effects of SR function in rats after exercise bouts of 20 minutes, 45 minutes or to exhaustion. Results showed that exercise $\geq$ 45 minutes resulted in a depression of the initial rate and maximal uptake of Ca^{2+}, and a decrease in Ca^{2+}ATPase activity. Another study by Byrd et al. (1990) investigated the effect of short-term (5 min.), high-intensity (VO_2max) exercise on the SR function in thoroughbred horses. Results were similar to those reported by Byrd et al (1989) in that the initial rate and maximal uptake of Ca^{2+} by isolated SR fractions were significantly depressed immediately after exercise. The activity of Ca^{2+}ATPase also was found to be depressed. Both of the Ca^{2+} uptake and rate, and the Ca^{2+}ATPase recovered fully within 1 hour. These results suggest that reversible changes in SR function that affect Ca^{2+} kinetics can be elicited by fatiguing exercise.

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR. Caffeine and K^+ both cause Ca^{2+} release from the SR. K^+ acts by depolarizing the TT and causing a secondary release of Ca^{2+} from the SR (Constantin, 1971), whereas caffeine is reported to act directly on the SR to cause Ca^{2+} release (Weber & Herz, 1968). Lannergren and Westerblad (1989)

investigated the effect of caffeine and K^+ on muscle fibers after fatiguing stimulation. In this study, skeletal muscle fibers were fatigued to approximately 40% of original tension. The timecourse of fatigue allowed the determination of fiber types due to fatigue resistance (type 1=easily fatigued, 2=fatigue resistant, 3=very fatigue resistant). Following fatigue all fiber types generated near maximal tension when exposed to a 8mM caffeine solution or a high (190mM) potassium solution. These results suggest that an adequate amount of Ca^{2+} remains in the SR for release providing an adequate stimulus is provided for its release. During recovery, type 1 & 2 fibers displayed post contractile depression (PCD). This condition is characterized by further fatigue during a prolonged recovery period. During PCD a significant difference was reported between caffeine and K^+ contractures. While tetanic stimulation and high K^+ elicited minimal tension; caffeine produced near maximal tension during the PCD state. This suggests that 1) an adequate SR Ca^{2+} store to support full contractile activation and 2) since K^+ acts on the TT and caffeine acts on the SR; failure is occurring in the transmission of a stimuli from the TT to the SR.

In studies by Allen et al.(1989) and Lee et al. (1991), the intracellular Ca^{2+} fluxes during fatiguing stimulation

were examined. In these investigations indicators aequorin and fura2 were used to determine Ca^{2+} concentration in the myoplasm during the contraction. Results indicate a complex response of myoplasmic Ca^{2+} to fatiguing stimulation. Specifically, fatiguing stimulation resulted in (1) decreased SR Ca^{2+} release as determined by an 84% decline in the signal of the Ca^{2+} indicator, (2) increased resting Ca^{2+} levels, and (3) little or no change in the sensitivity contractile proteins to Ca^{2+}. Unfortunately it is unclear if the decreased Ca^{2+} release is a direct effect of fatigue or a secondary effect due to metabolite accumulation.

Summary

The degree of fatigue exhibited in skeletal muscle can vary greatly depending on factors such as muscle fiber type, fuel substrate status, work intensity, and work duration. Despite the implication of central factors in fatigue, it is thought that in well motivated subjects under normal conditions, adequate central activation is provided to the fiber for normal function.

Research on energy depletion has shown that reductions in ATP and intermediate substrates to levels found in severely fatigued fibers did not affect force generation suggesting that fatigue is independent of energy status.

Studies have shown alterations in membrane potentials during fatigue. The alterations in both action and resting potentials do occur in fatigue but do not depress force production. Other studies on ion accumulation in the TT show no depressant effect on the activation of the TT.

The effects of metabolic by-products (ADP, P_i, H^+, Lactate) on force generation have been able to account for approximately 30% of tension decline. While this is significantly less than the >80% tension decline seen in fatigue, these by-products do seem to contribute in the fatigue process.

Studies have shown that changes in SR function could contribute to the fatigue process. It has been reported that fatigued skeletal muscle could elicit near maximal tension if exposed to agents such as caffeine which cause Ca^{2+} release from the SR. This suggest that enough Ca^{2+} remains in the SR for a full contraction and if provided an adequate stimulus, the SR will release this Ca^{2+} store. Results have also shown a reversible decrease in the initial rate and maximal uptake of Ca^{2+} immediately after high intensity exercise. Other studies have reported a complex response of myoplasmic Ca^{2+} during the fatigue process. These results show a relationship between the tension reduction and decrease in Ca^{2+} release from the SR and support previous

literature that suggests that the sensitivity of the contractile proteins to Ca^{2+} is not changed during fatigue.

These results show reversible changes in SR function that could play a major role in the fatigue process. Since Ca^{2+} flux from the SR ultimately regulates tension generation, these changes in SR function are important to understand. Hopefully, future investigation of the mechanisms which cause this SR disfunction will enable us to further elucidate causes of skeletal muscle fatigue.

Chapter III

JOURNAL MANUSCRIPT

CAFFEINE SENSITIVITY OF FATIGUED, SKINNED SKELETAL MUSCLE FIBERS

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ABSTRACT

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR. The purpose of this study was to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than that of rested muscle. Following a fatigue bout of electrically evoked tetanic pulses, the functioning of the SR of chemically skinned muscle fibers was determined by the sensitivity of the SR to increasing concentrations of caffeine. Measurements of tension and rate of tension development were made at the maximal Ca^{2+} activated contracture(pCa4.5), the maximal caffeine(25mM) activated contracture and at the caffeine threshold for contraction. All tension and rate values were normalized per cross sectional area and expressed as percents of the maximal calcium activated values. Results of the maximal Ca^{2+} and caffeine data suggest that the both control and fatigue fibers are similar in maximal tension and Ca^{2+} loading characteristics.

While no differences were found between rested or fatigued maximal Ca^{2+} or caffeine contractures, significant difference was found at the caffeine

threshold ($p<.05$) with the fatigued muscle tending to contract at a higher caffeine concentration. This suggests that fatigued muscle is less sensitive to the caffeine stimulus for Ca^{2+} release from the SR..

INTRODUCTION

The control of tension in the muscle cell is due primarily to the regulation of intracellular free Ca^{2+} concentration by the SR. Therefore it is plausible that alterations in SR function during fatigue could impair tension development. Byrd et al. (1989) showed that the initial rate and maximal uptake of Ca^{2+} by isolated SR fractions were significantly depressed immediately after fatiguing exercise with full recovery within one hour. Results suggest that reversible changes in SR function that affect Ca^{2+} kinetics can be elicited by fatiguing exercise.

Other studies by Allen et al.(1989) and Lee et al. (1991) have investigated the intracellular calcium fluxes during fatiguing stimulation. In these investigations Ca^{2+} indicators aequorin and fura2 were used to determine Ca^{2+} concentration in the myoplasm during the contraction. Results indicate a decreased SR Ca^{2+} release as determined by an 84% decline in the signal of the Ca^{2+} indicator, increases in resting Ca^{2+} levels, and little or no change in the sensitivity contractile proteins to Ca^{2+}.

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR.

These results suggest that an adequate amount of calcium remains in the SR to cause maximal crossbridge activation and that sensitivity of the contractile apparatus does not appear to be markedly affected in fatigue. It has also been shown that during fatigue, the amount of Ca^{2+} released by the SR during stimulation is markedly reduced (Allen et al. 1989, Lee et al. 1990). There appears to be two hypothesis which might explain this lack of Ca^{2+} release from the SR during fatigue. First, there may be less stimulus to cause Ca^{2+} release from fatigued SR. Second, the fatigued SR may be less sensitive to stimuli which cause the release of Ca^{2+}.

Because the exact stimulus by which the depolarized TT initiates Ca^{2+} release from the SR is unknown, it is not possible to test for its depletion. It is however, possible to test for the sensitivity of the SR Ca^{2+} release mechanism by using an agent known to cause Ca^{2+} release from the SR such as caffeine. The purpose of this study was to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than is the SR of rested muscle.

METHODOLOGY

Experimental Solutions

For whole muscle preparations a normal Ringer's solution (NR) containing (mM): NaCl (115), KCl (2.5), Ca_2Cl (1.8), Na_2HPO_4 (0.85) NaH_2PO_4 (2.15) and 0.1 mg/ml D-tubocurarine was used.

All skinned fiber solutions were made with deionized water and contained 20mM imidazole as a pH buffer, with the final pH adjusted to 7.0 with KOH. Sufficient potassium methanosulfate was also added to all solutions to maintain an ionic strength of 180 mM. The standard relaxing solution (G10) also contained (mM), $MgSO_4$ (4.0), Na_2ATP (4.0), ethylene glycol-bis(Beta-aminoethyl ether)-N,N,N',N',-tetra acetic acid (EGTA, 10). An additional relaxing solution was prepared (G2) as above except that the EGTA concentration was reduced to 2mM. Caffeine solutions of 0-10 mM (1 mM increments) and 25 mM were made by adding appropriate amounts caffeine stock (100mM) to the G2 solution. The loading and activating solutions were prepared as G10 except that $CaSO_4$ was added to obtain pCa 6.3 and 4.5 (-log free[Ca^{2+}]), respectively. The amount of $CaSO_4$ added to obtain each pCa solution was calculated using apparent stability constants adjusted for pH 7.0 and 20°C. (Fabiato, 1988).

Isolated Muscle/Skinned Fiber Preparations

Experiments were performed using skeletal muscle obtained from male grass frogs Rana Pipiens obtained from Carolina Biological Supply and stored under refrigeration prior to use. After a cold-induced torpor, frogs were killed by decapitation and both semitendinosus muscles were dissected free (tendon to tendon). Paired muscles were randomized for either control or fatigue experiments. In both cases muscles were stored in aerated NR until use.

For the fatigue experiment, one muscle from each animal was tied at each tendon with surgical suture (5-0) and placed in the muscle chamber. One end of the suture was fixed to a stationary post and the other to a calibrated Harvard isometric tension transducer. The bath was filled NR solution (20°C) and bubbled with room air. The muscle was then adjusted to a length that produced maximal tetanic tension. Fatigue was induced by electrical field stimulation provided by a Grass S48 stimulator coupled to a SIU5 stimulus isolation unit. Pulses were delivered across platinum wire electrodes positioned on opposite ends of the muscle. A 5 minute protocol employing supramaximal 100msec, 100hz square wave pulses delivered at 2 per second was used to induce

fatigue. Control muscles were tied with sutures, stretched to optimal length and incubated quietly in NR until use.

In both cases the muscle was placed into G10 solution and fiber bundles (1-5 fibers) dissected free. Fatigue muscle were dissected and used immediately following the fatigue treatment. Muscle bundles were then chemically skinned for 20 minutes in G10 (20°C) containing saponin(20 ug/ml). The 20 minute skinning protocol allows for permeablization of the sarcolemma without damage to the SR (Endo and Iino, 1980).

Mechanical Measurements

Following the 20 minute skinning period, fiber bundles were placed in G10 and dissected to single fibers. Segments of skinned fiber(2-5mm) were then suspended horizontally between a pair of jewelers forceps one fixed to a micromanipulator and one fixed to a calibrated Grass FT-03 isometric tension transducer. Force output was amplified by a low level DC amplifier (Grass P122, 0-30Hz)and displayed for permanent record on a flat bed strip chart recorder (BD8, Kipp & Zoen). Fiber length was adjusted to 130% of slack length.

The amount of Ca^{2+} stored in and released from the SR was determined indirectly by the caffeine contracture

method (Endo, 1977). Solutions were poured into 400ul wells milled in a plexiglass block so that a convex bubble of solution remained above the surface. Fibers were positioned just above the surface of the block and introduced to different solutions by sliding the block until the fiber was submerged in solution bubble.

Measurement Protocol

The SR of the skinned fiber was first depleted of Ca^{2+} by exposure to 25mM caffeine. The SR was then loaded by exposing the fiber to the loading solution for 2 or 4 minutes. Following loading, the fibers were exposed to G10 to remove any free Ca^{2+}. Fibers were then exposed to increasing caffeine concentrations until a concentration was reached which caused tension development. This concentration was termed the threshold concentration. 25 mM Caffeine was again applied and the fiber was again loaded for either 2 or 4 minutes. Following this loading period a 25 mM caffeine contracture was evoked. Each experiment was bracketed by a max Ca^{2+} contracture(pCa4.5) to adjust for fiber rundown. All measurements were made at pH 7.0 and 20^{0}C. Fatigued fibers were only used if measurements were completed within 45 minutes of the fatigue protocol.

Data Reduction

Following the experiment, the smallest and largest fiber diameter was measured by calibrated micrometer (Reichert-Jung) to determine cross-sectional area (CSA). Peak tension values and the rates of tension increase were then normalized for CSA. All rate and tension values were also expressed as a percent of the maximal Ca^{2+} activated tension to normalize the data between fibers.

The size of the Ca^{2+} contracture served as a measure the amount of Ca^{2+} stored in the SR (Endo, 1977). Similar tensions suggested that the amount of Ca^{2+} released from the SR was similar in both conditions. The rate measurements were calculated to elucidate any differences in the rate of Ca^{2+} release from the SR (Salvati & Volpe, 1988). Data between the 2 and 4 minute loading periods were compared to assure maximal loading of the SR.

Statistical Analysis

Preliminary studies suggested that while the caffeine sensitivity of muscle fibers between muscles is somewhat variable, the sensitivity of muscles taken from a single frog was not. Therefore, analyses of variance adjusted for repeated measures was used to examine difference in the parameters measured between rested and fatigued

muscles. Significance was established at the $p<.05$ level. All means are expressed as $\pm$ S.E.M

RESULTS

Initially data were collected (n=4) to determine the effectiveness of the fatigue protocol. Data showed that the protocol caused tension reduction to 1.8 $\pm$ 0.2 % of initial tension (P_0). The half-time of recovery ($t_{1/2}$) was 66.0$\pm$4.7 min and recovery was complete within 3.0 hr of fatiguing stimulation.

Figure 1a shows a typical tracing of maximal Ca^{2+} activated contractures between control and fatigue conditions. Results show no significant difference between the maximal Ca^{2+} activated tension (190.68$\pm$29.02, 207.79$\pm$38.87, $p>.05$) or rate (92.66$\pm$15.61, 109.26$\pm$19.88, $p>.05$) measurements between the control and fatigue treatments respectively (n=11).

Statistical analysis also revealed no differences in the maximal caffeine activated tension and rate of tension rise between the control and fatigue treatments following either the 2 or 4 minute loading periods (Table 1). Representative maximal caffeine contractures between the control and fatigue experiments are shown in figure 1b.

The caffeine threshold concentration was analyzed within each loading period for differences between treatment condition. Figure 2 shows typical tracings of two fibers exposed to increasing levels of caffeine following either control or fatigue conditions. These data were collected following a 2 minute loading period. As can be seen, the fatigued fiber contracted at a higher caffeine concentration.

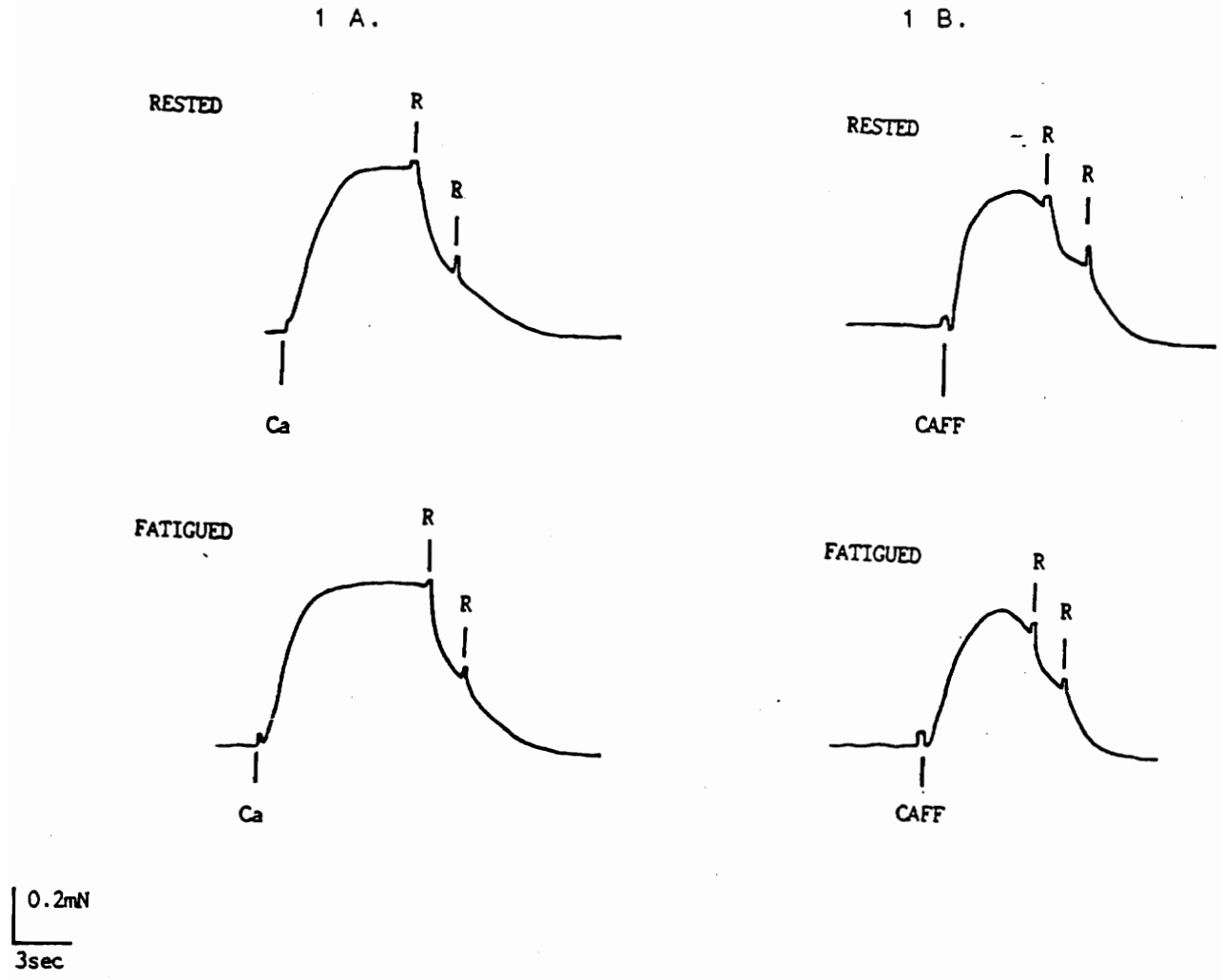

Figure 1. A) Representitive Maximal (pCa4.5) Calcium Contracture.
B) Representitive Maximal (25mM) Caffeine Contractures.

Table 1

25mM Caffeine Contracture

	Control	Fatigue
2 min. load (n=6)		
P_o	85.22 ± 5.68	90.87 ± 3.56
dP/dt	148.21 ± 19.07	131.91 ± 22.24
4 min. load (n=5)		
P_o	91.31 ± 9.57	79.22 ± 4.73
dP/dt	141.15 ± 12.64	114.17 ± 34.37

Values expressed as % of max.Ca^{2+} (pCa 4.5) activated force.

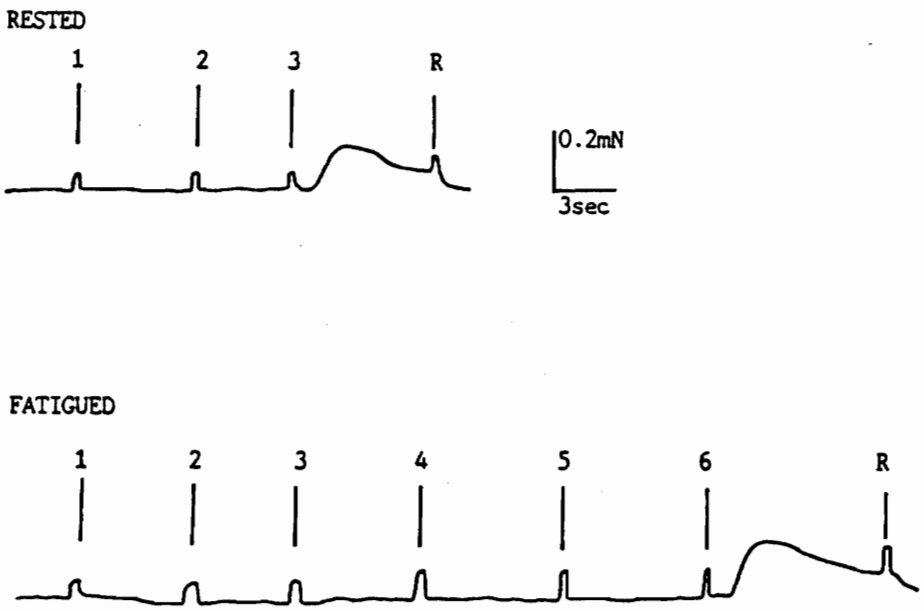

Figure 2. Representitive tracings of fibers at the threshold for caffeine contraction.

The results presented in Table 2 show significant differences between the conditions in both two and four minute loading periods with the fatigued fibers being less sensitive to the caffeine stimulus. Figure 3 shows the percent of the total number of fibers in each experimental condition which contract at each caffeine concentration. Because the results between the loading conditions were not different, data for this figure was pooled for ease of presentation.

The rate and tension measurements at the caffeine threshold concentration were analyzed to determine differences between control and fatigue treatments in the 2 and 4 minute loading conditions. Results show no significant differences exist between rate and or tension in the treatment groups for either the 2 or 4 min loading condition respectively (Table 2). These results are not surprising due to the large variability in rate and tension measurements found within and between fibers at the caffeine threshold.

Table 2

Contraction at Caffeine Threshold

	Control	Fatigue
2 min. load (n=6)		
P_o	30.19 ± 7.13	26.30 ± 5.68
dP/dt	45.30 ± 8.33	30.30 ± 3.56
Conc.	3.75 ± 0.73	6 ± 0.45 *
4 min. load (n=5)		
P_o	51.46 ± 7.27	39.34 ± 6.81
dP/dt	65.29 ± 36.71	52.28 ± 12.18
Conc.	2.8 ± 0.37	4.4 ± 0.24 *

Values expressed as % of max Ca^{2+}(pCa 4.5) contraction, * $p<.05$ between rest and fatigue

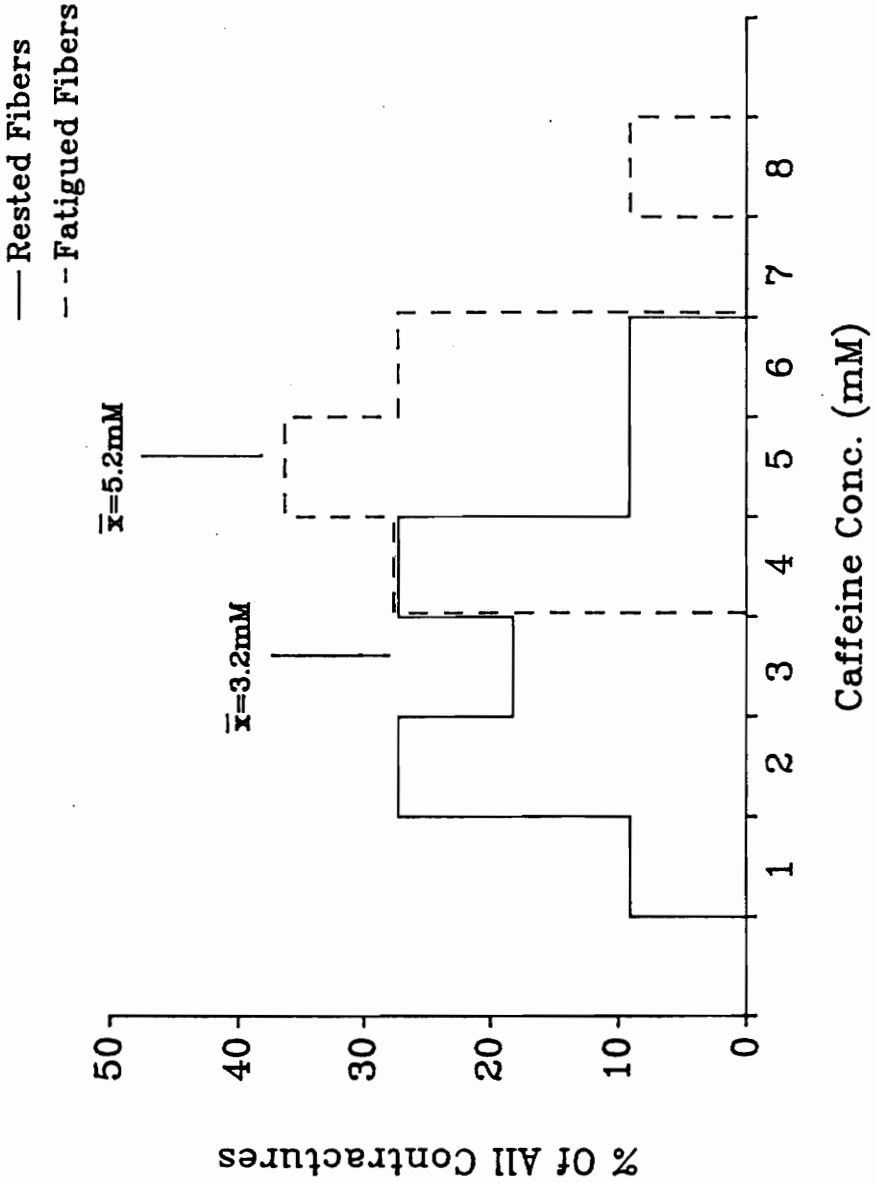

Figure 3. Percent of fibers contracting at each caffeine concentration between rested and fatigued fibers.

DISCUSSION

Fatigue Protocol

The purpose of the fatigue protocol was to depress force output for the duration of the fiber dissection and skinning period in order to assure a fatigued state during the measurements. Since $t_{1/2}$ for recovery was 66 min and all measurements were complete within 45 min; it is felt that the fibers were in a fatigued state during the measurement period. It is important to note that unlike in the case of post contractile depression (PCD), all muscles in this protocol exhibited a full uninterrupted increase in force during the recovery period. The fibers should be considered to be fatigued unless removal of the sarcolemma some how hastens recovery.

Contractile Properties

In order to depress tension development in this study a rigorous fatigue protocol was employed. Initial analysis of the maximal Ca^{2+} activated tension and rate of tension development was used to determine that the protocol had no effect on the ability of the fiber contractile apparatus to generate maximal tension. These results are in agreement with Lannergren and Westerblad (1988) who

found that caffeine elicited tensions in intact *Xenopus* skeletal muscle fibers were similar between rested and fatigued conditions. Our results, however, are somewhat contradictory to those reported by other authors using the skinned fiber preparation in which the bathing media was formulated to mimic that found in fatigued fibers (eg. low pH, ATP, PCr and elevated ADP) (Cooke and Pate. 1985, Nosek et al. 1987, Cooke et al. 1988). An explanation of these differences maybe that the composition of the "fatigue simulated" bathing media were such that the effects of certain metabolites could have had a stronger effect on tension generation than that found in intact fibers. Alternatively, it appears that the effects of the intracellular milieu are transient and that after returning to the rested composition, the maximal tension is restored. This would explain why no contractile disfunction was found in the present study since a bathing solution mimicking a rested state was used. Thus not including metabolites found in fatigued fibers which could affect force production. Nevertheless, fatigue does not appear to directly depress the contractile mechanism's ability to generate maximal force.

SR sensitivity

The major finding in the present study was that the absolute threshold concentration for caffeine contraction was significantly different between treatments regardless of loading condition. Specifically, the fatigued fibers tended to contract at a higher caffeine concentration after fatigue. This suggests that, following fatigue, the SR mechanism for Ca^{2+} release is less sensitive to caffeine. This notion is in agreement with resent findings that the rate of Ca^{2+} release evoked by submaximal silver ion concentration is depressed in fatigued isolated SR (G. Klug personal communication).

There are several other reasons why fatigued fibers could require increased concentrations of caffeine to cause contraction. Changes in the contractile apparatus's ability to generate force after fatigue could require a greater amount of Ca^{2+} and thus more caffeine induced Ca^{2+} release to cause contraction. In the present study, the maximal Ca^{2+} activated tension was similar in control and fatigue conditions. Allen et al.(1989) showed no difference in *vitro* force-pCa curves between rested and fatigued single fibers. Fitts et al. (1982) also showed that neither myofibrilar Ca^{2+} sensitivity or ATPase activity was reduced following intense exercise. Taken

together, these results suggest that the contractile apparatus from fatigued muscle does not appear to be directly affected by fatigue.

A change in the amount of Ca^{2+} stored in the SR could alter the amount of Ca^{2+} release in response to a caffeine stimulus. It has been reported by several authors that fatigued SR has a decreased ability to resequester Ca^{2+} (Sembrowich and Gollnick. 1977, Fitts et al. 1982 and Byrd et al. 1989). Although there is some question as to maximal loading, it is thought that a leaky SR or a dysfunctional Ca^{2+} ATPase could contribute.

The Ca^{2+} available at the contractile mechanism is the sum of the Ca^{2+} released and that which is resequestered. If the amount of Ca^{2+} that was not resequestered was left to accumulate during caffeine stimulation, the fibers should have contracted at a lower caffeine concentration which was not the case.

In the present study fairly long loading periods (2, 4 minutes) were employed which should have insured full loading. Also, no difference in the maximal caffeine contracture force between rested and fatigued muscle in either loading period were found. This suggests that the fibers were probably fully loaded in both cases. Thus it appears that differences in caffeine threshold are due to

differences in SR sensitivity to caffeine induced Ca^{2+} release.

Reasons for the change in SR sensitivity during fatigue are not readily apparent. One possibility is a decreased affinity of the caffeine binding site on the SR Ca^{2+} release channel. This would require an increased concentration of caffeine to cause a contracture. It has also been reported (Byrd et al. 1990, Luckin et al. 1990) that in fatigued SR, the affinity of ATP to the ATPase protien is depressed. Another possibility is action of Ca^{2+} activated proteases affecting the release channel. It has been reported that elevations in myoplasmic Ca^{2+} that occur in the fatigue process activate proteases that could affect the SR membrane (Bird et al. 1980). In either case, any disruption in the normal function of the SR Ca^{2+} release channel could result in a reduction of Ca^{2+} release by the SR and contribute to the fatigue process.

While caffeine is clearly a nonphysiological stimulus for Ca^{2+} release, it acts directly on the SR Ca^{2+} release mechanism (Weber and Herz. 1968). The interpretation of the results in this study should not focus on the use of caffeine as an agent but on the decreased sensitivity of the SR Ca^{2+} release channel to this agent.

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REFERENCES

Allen, D.G., Lee, J.A., & Westerblad, H. (1989). Intracellular calcium and tension during fatigue in isolated single muscle fibers from Xenopus Laevis. Journal of Physiology, 415, 433-458.

Bird, J.W., Carter, J.H., Triemer, R.M., (1980). Protienases in cardiac and skeletal muscle. Federation Proceedings, 39, 20-25.

Byrd, S.K., McCutcheon, L.J., Hodgson, D.R., & Gollnick, P.D., (1990). Altered sarcoplasmic reticulum function after high-intensity exercise. American Journal of Physiology, 67(5), 2072-2077.

Byrd, S.K., Bode, A.K., Klug, G.A., (1989) Effects of exercise of varying duration on sarcoplasmic reticulum function. Journal of Applied Physiology, 66(3), 1383-1389.

Cooke, R., & Pate, E., (1985). The effect of ADP and phosphate on the contraction of muscle fibers. Biophysical Journal. 48:789-798.

Cooke, R., & Pate, E., (1990). The inhibition of muscle contraction by the products of ATP hydrolysis. Biochemistry of Exercise VII. Human Kinetics Publishers. 21, 59-72.

Endo, M., (1977). Calcium release from the sarcoplasmic reticulum. Physiology Reviews, 57, 71-108.

Endo, M., & Iino, M., (1980). Specific perforation of muscle cell membranes with preserved SR functions by saponin treatment. Journal of Muscle Research and Cell Motility, 1, 89-100.

Fabiato, F., & Fabiato, F.,(1978). Effects of pH on the myofiliments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. Journal of Physiology, 276, 233-255.

Fabiato, A., (1988). Computer programs for calculating total and specific free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods in Enzymology, 157, 378-417.

Fitts, R.H., Courtright, J.B., Kim, D.H., & Witzmann, F.A., (1982). Muscle fatigue with prolonged exercise: contractile and biochemical alterations. American Journal of Physiology, 242, c65-c73.

Godt, R.E., & Nosek, T.M., (1989). Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. Journal of Physiology, 412, 155-180.

Lannergren, J., & Westerblad, H., (1989). Fatigue mechanisms in single Xenopus muscle fibers of different types. Muscle Energetics,Alan R. Liss, Inc, 99-107.

Lee, J.A., Westerblad, H., Allen, D.G., (1990). Changes in tetanic and resting $[Ca^{2+}]i$ during fatigue and recovery of single muscle fibers fron Xenopus Laevis. Journal of Physiology, 433, 307-326

Sembrowich, W.L., & Gollnick, P.D., (1977). Calcium uptake by heart and skeletal muscle sarcoplasmic reticulum from exercised rats (abstract). Medicine and Science in Sport and Exercise. 91, 64.

Weber, A., & Herz, R., (1968). The relationship between caffeine contracture of intact skeletal muscle and the effect of caffeine on reticulum. Journal of General Physiology, 52, 750-759.

CHAPTER IV

RESULTS AND DISCUSSION

SUMMARY

Several studies have shown that the loss in tension during fatigue can be virtually reversed by exposure of the muscle to agents which evoke Ca^{2+} release from the SR. These results suggest that an adequate amount of calcium remains in the SR to cause maximal crossbridge activation and that sensitivity of the contractile apparatus does not appear to be markedly affected in fatigue. It has also been shown that during fatigue, the amount of Ca^{2+} released by the SR during stimulation is markedly reduced (Allen et al., 1989; Lee et al., 1990). There appears to be two hypothesis which might explain this lack of Ca^{2+} release from the SR during fatigue. First, there may be less stimulus to release Ca^{2+} from fatigued SR. Second, the fatigued SR may be less sensitive to stimuli which cause the release of Ca^{2+}.

Because the exact stimulus by which the depolarized TT initiates Ca^{2+} release from the SR is unknown, it is impossible to test for its depletion during fatigue. It is, however, possible to test for a reduced Ca^{2+} release sensitivity of the SR by using agents known to evoke Ca^{2+} release such as caffeine. Therefore the purpose of this

study was to determine whether the SR Ca^{2+} release mechanism of fatigued muscle is less sensitive to caffeine than is the SR of rested muscle.

Experiments were performed on skinned skeletal muscle fibers obtained from fatigued and control (rested) male grass frogs (Rana Pipiens). Ca^{2+} release from the SR was indirectly monitored by isometric tension development. The effect of the fatigue protocol on the functioning of the SR was determined by the sensitivity of the SR to increasing concentrations of caffeine. Following a loading period, the threshold caffeine concentration, the maximal caffeine generated tension, and the rate of the tension development were measured. The Ca^{2+} release kinetics of the SR were also examined with a maximal caffeine contracture after loading. All rate and tension values were expressed as a percent of the maximal Ca^{2+} activated tension to normalize the data between fibers. The size of the caffeine contracture served as a measure the amount of Ca^{2+} stored in the SR (Endo, 1977).

Analyses of variance adjusted for repeated measures determined that while the tensions and rates of the maximal caffeine , Ca^{2+} and threshold caffeine contractures were similar, the threshold for caffeine contraction was significantly different. These results suggest that fatigue

elicits a decreased sensitivity in the SR Ca^{2+} release mechanism.

RESULTS AND DISCUSSION

The purpose of the fatigue protocol was to depress force output for the duration of the fiber dissection and skinning period in order to assure a fatigued state during the measurements. Data showed that the protocol caused tension reduction to 1.8 $\pm$ 0.2 % of initial tension (P_0). The half-time of recovery ($t_{1/2}$) was 66.0$\pm$4.7 min and recovery was complete within 3.0 hr of fatiguing stimulation. Since $t_{1/2}$ for recovery was 66 min and all measurements were complete within 45 min; it is felt that the fibers were in a fatigued state during the measurement period. It is important to note that unlike in the case of post contractile depression (PCD) all muscles in this protocol exhibited a full uninterrupted increase in force during the recovery period. The fibers should be considered to be fatigued unless removal of the sarcolemma hastened recovery.

In order to depress tension development in this study a rigorous fatigue protocol was employed. Initial analysis on the maximal Ca^{2+} activated tension and rate of tension

development was used to determine that the protocol had no effect on the ability of the fibers contractile apparatus to generate maximal tension. Figure 1a (Appendix B) shows a typical tracing of maximal Ca^{2+} activated contractures between control and fatigue conditions. Results show no significant difference between the maximal Ca^{2+} activated tension measurements between the control and fatigue treatments (Table 1, Appendix B).

These results are in agreement with Lannergren and Westerblad (1988) who found that caffeine elicited tensions in intact *Xenopus* skeletal muscle fibers were similar between rested and fatigued conditions. Our results, however, are somewhat contradictory to those reported by other authors using the skinned fiber preparation in which the bathing media was formulated to mimic that found in fatigued fibers (eg. low pH, ATP, PCr and elevated ADP) (Cooke and Pate, 1985; Nosek et al., 1987; Cooke et al., 1988). An explanation of these differences maybe that the composition of the "fatigue simulated" bathing medias were such that the effects of certain metabolites could have had a stronger effect on tension generation than that found in intact fibers. It appears that the effects of the intracellular milieu are transient and that after returning to the rested composition, the tension is restored. This

could be the explanation of no contractile disfunction in the present study since a bathing solution mimicking a rested state was used thus not including metabolites found in fatigued fibers which could effect force production. Nevertheless, fatigue does not appear to directly depress the contractile mechanism's ability to generate maximal force.

Following the second loading period a 25mM caffeine contracture was evoked to determine if the maximal tension and rate of tension generation was different between the 2 and 4 minute loading periods in either loading period. Representative maximal caffeine contractures between the control and fatigue experiments are shown in figure 1b (Appendix B). Statistical analysis revealed no differences in the maximal caffeine activated tension and rate of tension rise between the control and fatigue treatments in either the 2 or 4 minute loading periods (Table 2, Appendix B).

In order to investigate whether the SR sensitivity to caffeine was altered during fatigue, maximal tension and rate of tension development was measured at the caffeine concentration required for contraction. Rate and tension measurements at the threshold concentration were not significantly different between control and fatigue in

either loading condition (Table 3, Appendix B). The major finding in the present study was that the absolute threshold concentration for caffeine contraction was significantly different between treatments regardless of loading condition (Table 3, Appendix B). Specifically, the fatigued fibers tended to contract at a higher caffeine concentration after fatigue. This suggests that, following fatigue, the SR mechanism for Ca^{2+} release is less sensitive to caffeine. This notion is in agreement with resent findings that the rate of Ca^{2+} release evoked by submaximal silver ion conc. is depressed in fatigued isolated SR (G. Klug personal communication).

There are several other reasons why fatigued fibers could require increased concentrations of caffeine to cause contraction. Changes in the contractile apparatus's ability to generate force after fatigue could require a greater amount of Ca^{2+} and thus more caffeine induced Ca^{2+} release to cause contraction. In the present study, the maximal Ca^{2+} activated tension was similar in control and fatigue conditions. Allen et al.(1989) showed no difference in *vitro* force-pCa curves between rested and fatigued single fibers. Fitts et al. (1982) also showed that neither myofibrilar Ca^{2+} sensitivity or ATPase activity was reduced following intense exercise. Taken together, these results suggest that

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The Ca^{2+} available at the contractile mechanism is the sum of the Ca^{2+} released and that which is resequestered. If the amount of Ca^{2+} that was not resequestered was left to accumulate during caffeine stimulation, the fibers should have contracted at a lower caffeine concentration which was not the case.

In the present study fairly long loading periods (2, 4 minutes) were employed which should have insured full loading. Also, no difference in the maximal caffeine contracture force between rested and fatigued muscle in either loading period were found. This suggests that the fibers were probably fully loaded in both cases. Thus it appears that differences in caffeine threshold are due to differences in SR sensitivity to caffeine induced Ca^{2+}

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While caffeine is clearly a nonphysiological stimulus for Ca^{2+} release, it acts directly on the SR Ca^{2+} release mechanism (Weber and Herz, 1968). The interpretation of the results in this study should not focus on the use of caffeine as an agent but on the decreased sensitivity of the SR Ca^{2+} release channel to this agent.

SUGGESTIONS FOR FUTURE RESEARCH

The findings of this study contribute to the scientific body of knowledge in the area of SR function in skeletal muscle fatigue. While the results of this study do show a decrease in SR sensitivity to caffeine during fatigue, it will be important to establish the same response with other agents (eg. silver ions) which also evoke Ca^{2+} release from the SR. Because these agents work directly on the Ca^{2+} channel, future studies should also focus on characterizing the SR caffeine binding site and the channel itself to see if any functional or physically alteration has occured following fatigue, thus possibly explaining the decreased effect of caffeine on the SR. Finally, to characterize this change in SR sensitivity, it is important to investigate the SR following other intensities of contraction and degrees of fatigue. This will allow further generalization to other less depressed fatigue states.

REFERENCES

Adrian,R.H.,& Peachy,L.D. (1973). Reconstruction of the action potential of frog sartorius muscle. Journal of Physiology, 235, 103-131.

Allen, D.G., Lee, J.A., & Westerblad, H. (1989). Intracellular calcium and tension during fatigue in isolated single muscle fibers from Xenopus Laevis. Journal of Physiology, 415, 433-458.

Bianchi, C.P., & Narayan, S., (1982). Muscle fatigue and the role of the transverse tubules. Science, 215, 295 -296.

Bigland-Ritchie, B., Furbush, F., & Woods, J.J.,(1986). Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. Journal of Applied Physiology, 61, 421-429.

Bigland-Ritchie, B.,& Thomas, C., (1987). Impulse propagation and muscle activation during prolonged human muscle contractions. Journal of Physiology, 391, 91P.

Bigland-Ritchie, B., & Woods, J.J., (1984). Changes in muscle contractile properties and neural control during human muscle fatigue. Muscle and Nerve, 7, 691-699.

Bird, J.W., Carter, J.H., Triemer, R.M., (1980). Protienases in cardiac and skeletal muscle. Federation Proceedings, 39, 20-25.

Brandt, P.W., Cox R.N., Kawai, M., Robinson, T., (1982) Regulation of tension in skinned muscle fibers. Journal of General Physiology, 79, 997-1016.

Byrd, S.K., McCutcheon, L.J., Hodgson, D.R., & Gollnick, P.D., (1990). Altered sarcoplasmic reticulum function after high- intensity exercise. American Journal of Physiology, 67(5), 2072-2077.

Byrd, S.K., Bode, A.K., Klug, G.A., (1989) Effects of exercise of varying duration on sarcoplasmic reticulum function. Journal of Applied Physiology, 66(3), 1383-1389.

Constantin, L.L., (1971). Biphasic potassium contractures in frog muscle fibers. Journal of General Physiology, 58,117-130.

Cooke, R., & Pate, E., (1985). The effect of ADP and phosphate on the contraction of muscle fibers. Biophysical Journal. 48:789-798.

Cooke, R., & Pate, E., (1990). The inhibition of muscle contraction by the products of ATP hydrolysis. Biochemistry of Exercise VII. Human Kinetics Publishers. 21, 59-72.

Deleze, J., Lazrak, A., Rakotonirinia, V.S., (1986) Isolation of the t-tubule action potential in the frog skeletal muscle fiber. Journal of Physiology, 371, 149p.

Donaldson, S.K.B., Hermansen, L., Bolles, L., (1978). Differential direct effects of H^+ and Ca++ -activated force of skinned fibers from the soleus, cardiac, and adductor magnus muscles of rabbits. Plugers Archives, 376, 55-65.

Edwards, R.H.T., (1986). Interaction of chemical with electromechanical factors in human skeletal muscle fatigue. Acta Physiologica Scandinavia, 128, 149-155.

Endo, M., (1977). Calcium release from the sarcoplasmic reticulum. Physiology Reviews, 57, 71-108.

Endo, M., & Iino, M., (1980). Specific perforation of muscle cell membranes with preserved SR functions by saponin treatment. Journal of Muscle Research and Cell Motility, 1, 89-100.

Fabiato, F., & Fabiato, F.,(1978). Effects of pH on the myofiliments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. Journal of Physiology, 276, 233-255.

Fabiato, A., (1988). Computer programs for calculating total and specific free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods in Enzymology, 157, 378-417.

Fink, R., Luttgau, H.C., (1976). An evaluation of the membrane constants and the potassium conductance in metabolically fatigued fibers. Journal of Physiology, 263, 215-238.

Fitts, R.H., Courtright, J.B., Kim, D.H., & Witzmann, F.A., (1982). Muscle fatigue with prolonged exercise: contractile and biochemical alterations. American Journal of Physiology, 242, c65-c73.

Fitts, R.H., & Hollozy, J.O., (1976). Lactate and contractile force in frog muscle during development of fatigue and recovery. American Journal of Physiology, 231(2), 430-433.

Godt, R.E., & Nosek, T.M., (1989). Changes of intracellular milieu with fatigue or hypoxia depress conrtaction of skinned rabbit skeletal and cardiac muscle. Journal of Physiology, 412, 155-180.

Grabowski, W., Lobsiger, E.A., Luttgau, H., (1972). The effect of repetitive stimulation at low frequencies upon the electrical and mechanical activity of single muscle fibers. Pflugers Archive, 334, 222-239.

Green, H.J., (1990). Manifestations and sites of neuromuscular fatigue. Biochemistry of Exercise VII, Human Kinetics Publishers, 21, 13-35.

Ikai, M,. & Stenhaus, A.H., (1961). Some factors modifying the expression of human strength. Journal of Applied Physiology, 16, 157-163.

Jones, D.A., (1979). Change in excitation threshold as a cause of muscular fatigue. Journal of Physiology, 295, 90p-91p.

Lannergren, J., & Westerblad, H., (1989). Fatigue mechanisms in single Xenopus muscle fibers of different types. Muscle Energetics, Alan R. Liss, Inc, 99-107.

Lee, J.A., Westerblad, H., Allen, D.G., (1990). Changes in tetanic and resting $[Ca^{2+}]i$ during fatigue and recovery of single muscle fibers fron Xenopus Laevis. Journal of Physiology, 433, 307-326

MacLaren, D.P.M., Gibson, H., Parry-Billings, M., (1989). A review of metabolic and physiologic factors in fatigue. Exercise and Sport Science Reviews, 17, 29-65.

Mainwood, G.W. & Renaud, J.M., (1984). The effect of acid-base balance on fatigue of skeletal muscle. Canadian Journal of Physiology and Pharmacology, 63, 403-416.

Mainwood, G.W., Renaud, J.M., Mason, M.J., (1986). The pH dependence of the contractile response of fatigued skeletal muscle. Canadian Journal of Physiology and Pharmacology, 65, 648-658.

Mandel, F., Kranias, E.G., DeGende, A.C., Sumida. M., and Schwartz, A., (1982) The effect of pH on the transient -state kinetics of Ca++ - Mg^{2+} - ATPase of cardiac sarcoplasmic reticulum. A comparison with skeletal sarcoplasmic reticulum. Circ Res, 50, 310-317.

Merton, P.A., (1954). Voluntary strength and fatigue. Journal of Physiology, 123, 553-564.

Nassar-Gentina, V., Passonneau, J.V., Rapoport, S.I, (1981) Fatigue and metabolism of frog muscle fibers during stimulation and in responce to caffeine. American Journal of Physiology, 241: c160-c166.

Nassar-Gentina, V., Passonneau, J.V., Vergara, J.L., Rapoport, S.I, (1978) Metabolic correlates of fatigue and of recovery from fatigue in single frog muscle fibers. American Journal of Physiology, 72, 593-606.

Nosek, T.M., Fender, K.Y., Godt, R.E., (1987) It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. Science. 236, 191-193.

Salviati, G., & Volpe, P., (1988). Ca^{2+} release from sarcoplasmic reticulum of skinned fast and slow-twitch muscle fibers. American Journal of Physiology, 254, c459 -c465.

Sembrowich, W.L., & Gollnick, P.D., (1977). Calcium uptake by heart and skeletal muscle sarcoplasmic reticulum from exercised rats (abstract). Medicine and Science in Sport and Exercise.91, 64.

Vollestad, N.K., & Sejersted, O.M., (1988). Biochemical Correlates of Fatigue: A brief review. European Journal of Applied Physiology, 57, 336-347.

Weber, A., & Herz, R., (1968). The relationship between caffeine contracture of intact skeletal muscle and the effect of caffeine on reticulum. Journal of General Physiology, 52, 750-759.

Williams J.H., & Ward C.W. (1991) Reduced pH depresses Ca++ induced Ca++ release from skeletal muscle sarcoplasmic reticulum.Medicine and Science in Sport and Exercise. 23, 4; S263.

APPENDIX A

DETAILED METHODOLOGY

Experimental Solutions

For whole muscle preparations a normal Ringer's solution (NR) containing (mM): NaCl (115), KCl (2.5), Ca_2Cl (1.8), Na_2HPO_4 (0.85) NaH_2PO_4 (2.15) and 0.1 mg/ml D-tubocurarine was used.

All skinned fiber solutions were made with deionized water and contained 20mM imidazole as a pH buffer, with the final pH adjusted to 7.0 with KOH. Sufficient potassium methanosulfate was also added to all solutions to maintain an ionic strength of 180 mM. The standard relaxing solution (G10) also contained (mM), $MgSO_4$ (4.0), Na_2ATP (4.0), ethylene glycol-bis(B-aminoethyl ether)-N,N,N',N',-tetra acetic acid (EGTA, 10). An additional relaxing solution was prepared (G2) as above except that the EGTA concentration was reduced to 2mM. Caffeine solutions of 0-10 mM (1 mM increments) and 25 mM were made by adding appropriate amounts caffeine stock (100mM) to the G2 solution. The loading and activating solutions were prepared as G10 except that $CaSO_4$ was added to obtain pCa 6.3 and 4.5 (-log free[Ca^{2+}]), respectively. The amount of $CaSO_4$ added to obtain each pCa solution was calculated using apparent stability constants adjusted for pH 7.0 and 20°C. (Fabiato, 1988). All solutions were prepared in Nalgene containers

which had been thouroughly rinsed first with a EDTA solution then rinsed with distilled/deionized water.

Isolated Muscle/Skinned Fiber Preparations

Experiments were performed using skeletal muscle of male grass frogs Rana Pipiens obtained from Carolina Biological Supply and stored under refrigeration prior to use. After a cold-induced torpor, frogs were killed by decapitation and paired semitendinosus muscles were dissected free (tendon to tendon). Paired muscles were randomized for either control or fatigue treatments. In both cases muscles were stored in aereated NR until use.

For the fatigue treatment, muscles were tied at each tendon with surgical suture(5-0). The muscle was then suspended horizontally at optimal resting length the muscle chamber with one end fixed to a stationary post and the other to a calibrated Harvard isometric tension transducer. The bath was then filled NR solution (20°C) and bubbled with room air. Fatigue was induced in the muscle by electrical field stimulation provided by a Grass S48 stimulator coupled to a SIU5 stimulis isolation unit. Pulses were delivered by platinum wire electrodes positioned on oppisite ends of the muscle. A 5 minute protocol employing supramaximal 100msec,

100hz square wave pulses delivered at 2 per second was used to induce fatigue.

In both cases the muscle was placed into G10 solution and fiber bundles (1-5 fibers) dissected free. Fatigue muscle were dissected and used immediatly following the fatigue treatment.

Muscle bundles were then chemically skinned for 20 minutes in G10 (20°C) containing saponin(20 ug/ml). The 20 minute skinning protocol allows for permeablization of the sarcolemma without damage to the SR (Endo and Iino, 1980).

Mechanical Measurements

Following the 20 minute skinning period, fiber bundles were placed in G10 and dissected to single fibers. Segments of skinned fiber(2-5mm) were then suspended horizontally between a pair of jewelers forceps one fixed to a micromanipulator and one fixed to a calibrated GRASS FT-03 isometric tension transducer. Force output was amplified by a low level DC amplifier (Grass P122, 0-30Hz)and displayed for permanent record on a flat bed strip chart recorder (BD8, Kipp & Zoen). Fiber length was adjusted to 130% of slack length.

Solutions were poured into 400ul wells milled in a plexiglass block so that a convex bubble of solution

remained above the surface. Fibers were positioned just above the surface of the block and were introduced to different solutions by sliding the block until the fiber was submerged in the solution. Fatigued fibers were only used if measurements were completed within 45 minutes of the fatigue protocol, thus assuring a fatigued state.

Measurement Protocol

The SR of the skinned fiber was first depleted of Ca^{2+} by exposure to 25mM caffeine. The SR was then loaded for 2 or 4 minutes with the loading solution. Following loading, the fibers are exposed to G10 to remove any free Ca^{2+}. Fibers were then exposed to increasing caffeine concentrations until a concentration was reached which caused tension development was reached. This concentration was termed the threshold concentration. 25 mM Caffeine was then applied and the fiber was again loaded for either 2 or 4 minutes. Following this loading period a 25 mM caffeine contracture was evoked. Each experiment was bracketed by a max Ca^{2+} contracture(pCa4.5) to adjust for fiber rundown. All measuremnets were made at pH 7.0 and $20^{0}C$.

Data Reduction

Following the experiment, the smallest and largest

fiber diameter was measured by calibrated micrometer (Reichert-Jung) to determine cross-sectional area (CSA). Peak tension values and the rates of tension increase were then normalized for CSA.

All rate and tension values were also expressed as a percent of the maximal Ca^{2+} activated tension to normalize the data between fibers. The size of the Ca^{2+} contracture served as a measure the amount of Ca^{2+} stored in the SR (Endo, 1977). Similar tensions suggested that the amount of Ca^{2+} released from the SR was similar in both conditions. The rate measurements were calculated to elucidate any differences in the rate of Ca^{2+} release from the SR (Salvati & Volpe, 1988). Data between the 2 and 4 minute loading periods were compared to assure maximal loading of the SR.

Statistical Analysis

Preliminary studies suggested that while the caffeine sensitivity of muscle fibers between muscles is somewhat variable, the sensitivity of muscles taken from a single frog was not. Thus, analyses of variance adjusted for repeated measures was used to examine difference in the parameters measured between rested and fatigued muscles. Significance was established at the $p<.05$ level.

APPENDIX B

TABLES AND FIGURES

Table 1. Repeated Measures ANOVA Results for Maximal Calcium Contractures

2 Minute loading period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	190.22	351.09	95.52	233.07
2	218.01	210.83	119.49	77.87
3	333.54	243.74	118.12	151.35
4	153.90	92.87	78.10	87.33
5	124.25	93.75	52.96	48.84
6	206.77	127.30	90.97	67.57
7	158.22	169.34	55.15	81.50
x	190.68	184.13	87.18	106.79
S.E.M.	25.76	35.21	10.19	24.24
F value	.17		.79	
Signif.	N.S.		N.S.	

4 Minute Loading Period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	299.27	340.64	165.89	93.85
2	157.29	220.29	125.11	135.91
3	167.70	231.73	85.27	139.98
4	103.13	133.08	53.44	77.23
5	190.21	-	61.03	-
x	183.52	231.46	98.14	111.74
S.E.M.	32.28	42.54	21.04	15.52
F value	34.92		.02	
Signif.	N.S.		N.S.	

* Means are significantly different $p<.05$
-All data expressed as a percent of the max. Ca^{2+} contrature.

Table 2. Repeated Measures ANOVA Results of 25mM Caffeine Activated Contractures

2 Minute loading period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	92.42	77.80	66.65	100.69
2	64.11	92.15	143.50	95.97
3	106.02	105.12	167.90	106.12
4	86.78	91.30	130.94	113.26
5	80.64	90.00	194.25	238.13
6	81.36	88.90	189.07	149.31
x	85.22	90.87	148.21	133.91
S.E.M.	5.68	3.56	19.07	22.24
F value	.98		.62	
Signif.	N.S.		N.S.	

4 Minute Loading Period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	107.14	75.27	158.80	90.36
2	69.23	83.33	122.48	38.14
3	83.32	62.51	181.23	87.56
4	76.87	87.51	112.40	242.89
5	119.99	87.49	130.87	111.93
x	91.31	79.22	141.15	114.17
S.E.M.	9.57	4.73	12.64	34.37
F value	2.07		.42	
Signif.	N.S.		N.S.	

* Means are significantly different $p<.05$
-All data expressed as a percent of the max. Ca^{2+} contracture.

Table 3. Repeated Measures ANOVA Results of Caffeine Threshold Tension and Rate

2 Minute loading period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	15.70	26.46	5.55	23.92
2	64.00	73.68	77.31	51.11
3	23.13	62.50	12.95	44.48
4	23.45	51.61	22.38	7.90
5	21.81	24.23	28.77	24.14
6	33.03	33.33	10.87	30.24
x	30.19	45.30	26.30	30.30
S.E.M.	7.13	8.33	5.68	3.56
F value	5.7		.18	
Signif.	N.S.		N.S.	

4 Minute Loading Period

	Tension		Rate	
	Control	Fatigue	Control	Fatigue
1	70.82	36.55	87.94	73.42
2	62.50	37.50	19.19	48.52
3	50.00	62.51	199.93	85.23
4	28.56	19.99	12.08	18.28
5	45.45	41.66	7.32	35.97
x	51.46	39.34	65.29	52.28
S.E.M.	7.27	6.81	36.71	12.18
F value	2.07		.23	
Signif.	N.S.		N.S.	

* Means are significantly different $p<.05$
-All data expressed as a percent of the max. Ca^{2+} contracture.

Table 4. Repeated Measures ANOVA
Results of Caffeine Threshold

	2 min Load		4 min Load	
	Control	Fatigue	Control	Fatigue
1	4.0	8.0	4.0	4.0
2	6.0	6.0	3.0	5.0
3	2.5	6.0	3.0	5.0
4	4.0	5.0	2.0	4.0
5	5.0	6.0	2.0	4.0
6	1.0	5.0	-	-
x	3.75	6.0	2.8	4.4
S.E.M.	0.73	0.45	0.37	0.24
F value	9.55		16	
Signif.	<.05		<.05	

Table 5. Raw Data Table for 2 Minute Loading Period

		Frog #					
		1	2	3	4	5	6
CafIR	C	49.78	77.58	36.37	59.38	15.24	50.00
CafIR	F	91.47	29.32	61.48	-	-	48.33
CafIT	C	50.96	47.25	43.43	54.46	24.29	50.00
CafIT	F	74.07	36.28	44.23	-	-	33.69
CafTR	C	5.55	77.31	12.95	22.38	28.77	10.87
CafTR	F	23.92	51.11	44.48	7.90	24.14	30.24
CafTT	C	15.70	64.00	23.13	23.45	21.81	33.03
CafTT	F	26.46	73.68	62.50	51.61	24.23	33.33
CafIIR	C	66.65	143.50	167.90	130.94	194.25	189.07
CafIIR	F	100.69	95.97	106.12	113.26	238.13	149.31
CafIIT	C	92.42	64.11	106.02	86.78	80.64	81.36
CafIIT	F	77.80	92.15	105.12	91.30	90.00	88.90
CafTh	C	4	6	2.5	4	5	1
CafTh	F	8	6	6	5	6	5

C = control
F = fatigue

-All rate and tension data is expressed as a percent of the max. Ca^{2+} activated contracture.

Table 6. Raw Data Table for 4 Minute Loading Period

		Frog #				
		1	2	3	4	5
CafIR	C	33.01	170.73	84.38	63.42	114.99
CafIR	F	83.33	76.47	100.00	71.87	-
CafIT	C	54.28	93.02	82.86	54.54	55.76
CafIT	F	77.68	73.91	74.90	52.17	-
CafTR	C	87.94	19.19	199.93	12.08	7.32
CafTR	F	73.42	48.52	85.23	18.28	35.97
CafTT	C	70.82	62.50	50.00	28.56	45.45
CafTT	F	36.55	37.50	62.51	19.99	41.66
CafIIR	C	158.80	122.48	181.23	112.40	130.87
CafIIR	F	90.36	38.14	87.56	242.89	111.93
CafIIT	C	107.14	69.23	83.32	76.87	119.99
CafIIT	F	75.27	83.33	62.51	87.51	87.49
CafTh	C	4	3	3	2	2
CafTh	F	4	5	5	4	4

C = control
F = fatigue

-All rate and tension data is expressed as a percent of the max. Ca^{2+} activated contracture.

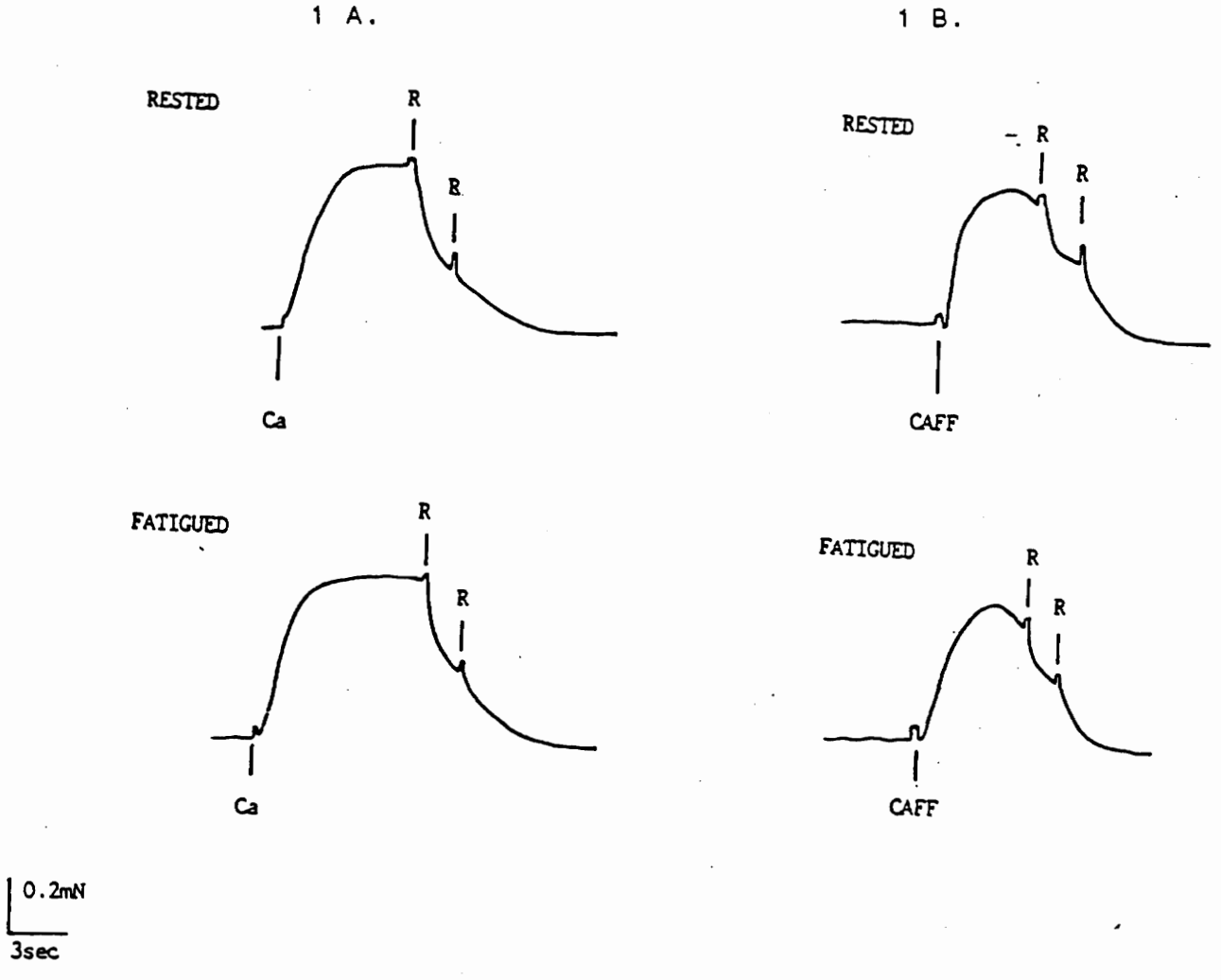


Figure 1. A) Representitive Maximal (pCa4.5) Calcium Contracture.
B) Representitive Maximal (25mM) Caffeine Contractures.

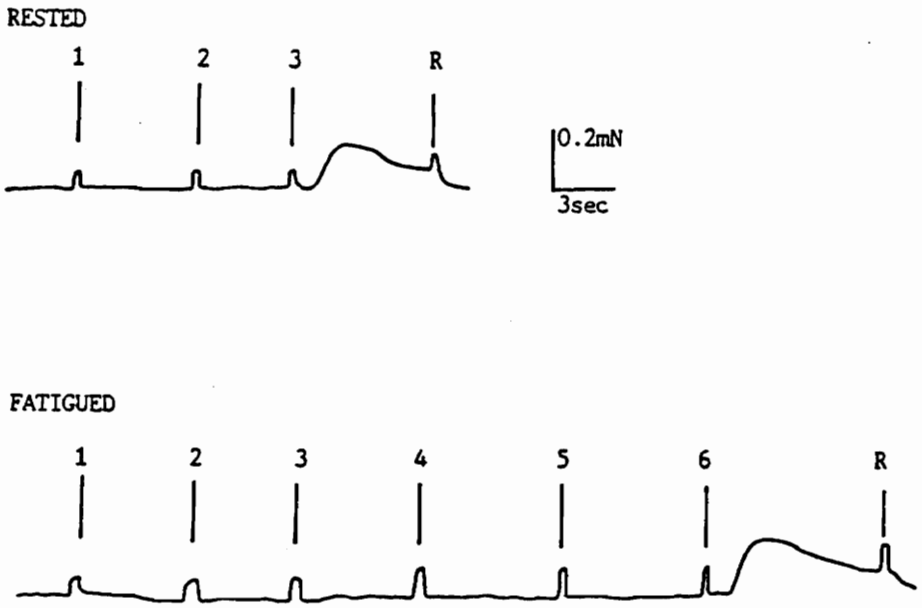


Figure 2. Representitive tracings of fibers at the threshold for caffeine contraction.

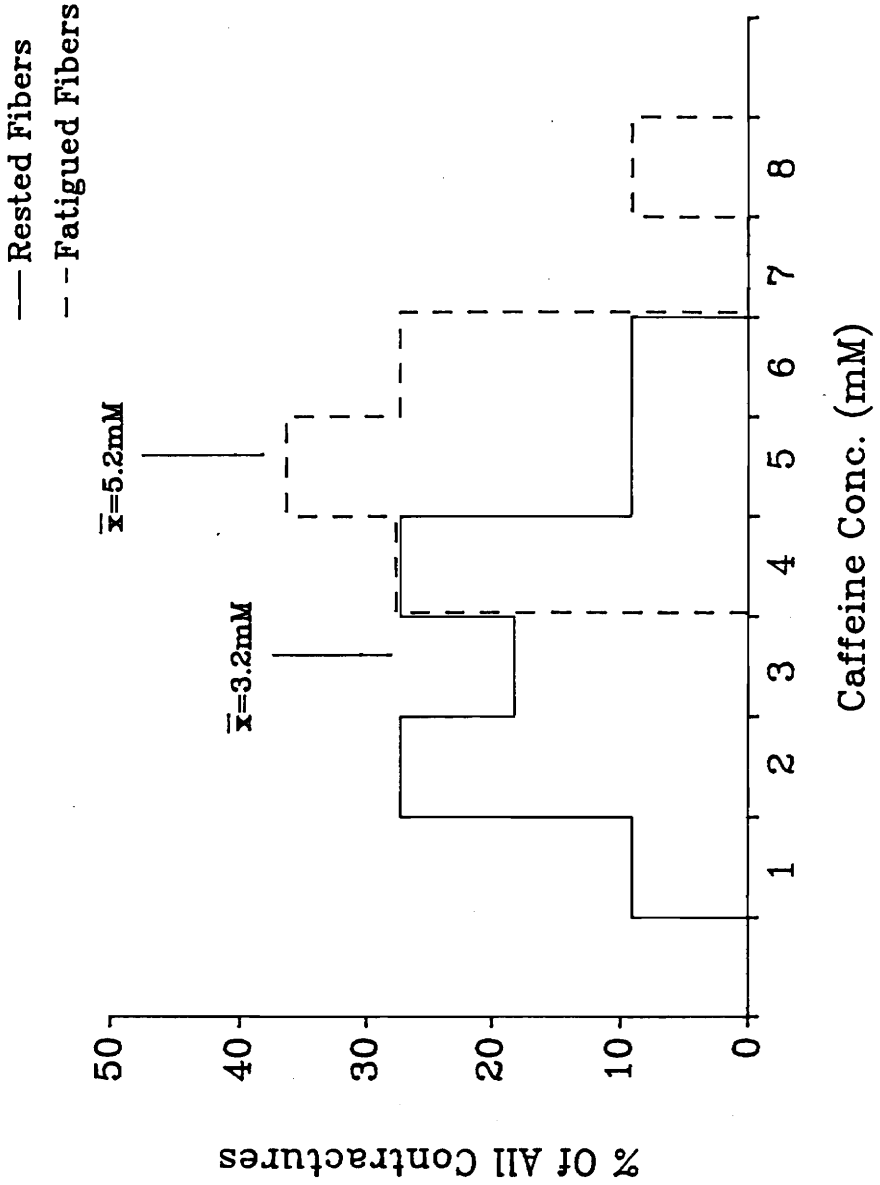
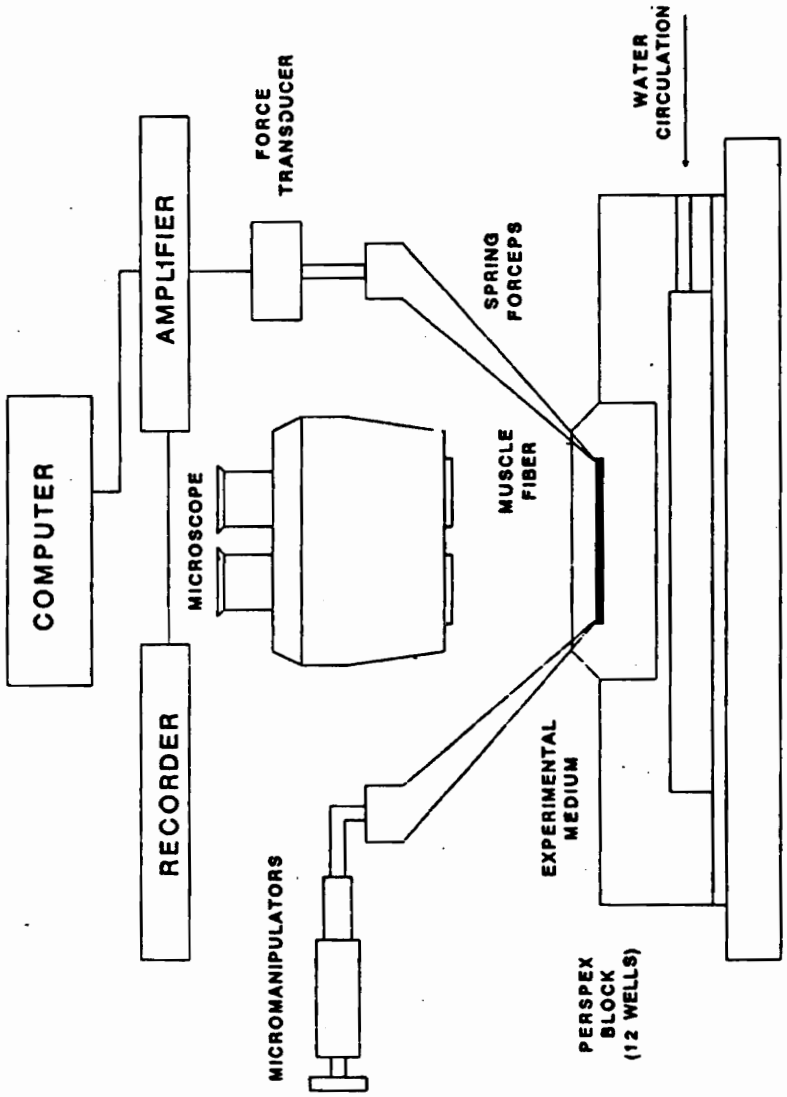


Figure 3. Percent of fibers contracting at each caffeine concentration between rested and fatigued fibers.



Equipment Set-up

VITA

Christopher William Ward was born in Miami, Florida on November 28, 1967. Chris's life was forever changed on December 20, 1967 when he was adopted by two loving parents William and Virginia Ward. The next year his parents adopted LoriAnn, undoubtedly the best sister in the world.

Chris's father worked in government Foriegn Service which allowed the family to travel and live in several countries throughout his childhood. The experiences of living abroad and the guidence of his parents gave Chris the appreciation of other cultures, of a good education, of the freedom afforded to us in America and of the benifits of haveing two wonderful parents.

Upon returning to the United States in 1979, Chris's family settled in Manassas, Virginia where they still reside to this day. Chris attended Stonewall Jackson H.S. and while pursuing his achedemics also participated in various varsity sports. In his high school senior year Chris meet Michelle Maire a very special person who he dated for several years to follow. Although no longer together, the years with Michelle were very special and will never be forgotten.

Graduation from H.S. in 1985 found Chris attending Virginia Tech to pursue a degree in engineering. The decision to change into the field of exercise physiology was one of great difficulty for Chris. The support of his

parents allowed him to do this with confidence resulting in a love for the field of physiology and the desire for further education. The following year chris re-enrolled at Virginia Tech as a Masters student.

Several things highlight his masters degree experience at Tech. The course work and friendships with the faculty and students at Tech were great and will never be forgotten. The guidence and friendship of Dr, Jay Williams was instrumental in developing a appreciation for the field of physiology and the desire to continue with further education. To Dr William's, I can't give enough thanks. Finally is the friendship and love I have found with Jodi Kramer. In one short year I realized how strong a love can grow. Jodi is the most special person in the world to me and I hope we will continue to forever grow closer.

Upon graduation with a masters degree from Virginia Tech in 1991, Chris will attend the University of Florida to obtain a PhD in Exercise Physiology.