Muscle Fatigue: The Role of Intracellular Calcium Stores

David G. Allen, Akram A. Kabbara, and Håkau Westerblad

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Abstract/Résumé

Force declines when muscles are used repeatedly and intensively and a variety of intracellular mechanisms appear to contribute to this muscle fatigue. Intracellular calcium release declines during fatigue and has been shown to contribute to the reduction in force. Three new approaches have helped to define the role of calcium stores to this decline in calcium release. Skinned fibre experiments show that when intracellular phosphate is increased the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR) declines. Intact fibre experiments show that the size of the calcium store declines during fatigue and recovers on rest. Intact muscles which lack the enzyme creatine kinase, do not exhibit the usual rise of phosphate during fatigue and, under these conditions, the decline of Ca^{2+} release is absent or delayed. These results can be explained by the "calcium phosphate precipitation" hypothesis. This proposes that if phosphate in the myoplasm rises, it enters the SR and binds to Ca^{2+} as Ca^{2+} phosphate. The resultant reduction in free Ca^{2+} within the SR contributes to the reduced Ca^{2+} release during fatigue.

D.G. Allen is with the Department of Physiology at the University of Sydney F13, NSW 2006, Australia; A.A. Kabbara is with the Department of Physiology at the University of Monash, Clayton, Victoria 3800, Australia; H. Westerblad is with the Department of Physiology and Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden.

La force diminue quand les muscles sont sollicités de façon intense et répétitive. Divers mécanismes intracellulaires contribuent à l'installation de la fatigue musculaire. La concentration intracellulaire de calcium diminue au cours de la fatigue et, selon des études, contribue à la diminution de la force. Trois récentes approches ont permis d'établir le rôle des réserves de calcium dans la réduction de la libération du calcium. Des études sur des fibres sans membrane rapportent que, avec l'augmentation de la concentration de phosphate intracellulaire, il y a une réduction de la quantité de Ca^{2+} libérée du réticulum sarcoplasmique. Des études sur des fibres intactes montrent que la quantité de calcium en réserve baisse avec la fatigue et s'accroît au cours de la récupération. Dans les fibres intactes, mais sans créatine kinase, il n'y a pas l'augmentation caractéristique de la concentration de phosphate au cours de la fatigue et, dans ces conditions, il y a soit un délai dans la libération du calcium ou encore pas de libération de clacium. Ces observations peuvent être expliquées par l'hypothèse de la « précipitation du phosphate de calcium ». Ainsi, si la concentration de phosphate dans le myoplasme augmente, le phosphate pénètre dans le réticulum sarcoplasmique et se lie au calcium pour former du phosphate de calcium, ce qui a pour effet de réduire la quantité de calcium libre dans le réticulum et, par conséquent, la libération de Ca^{2+} au cours de la fatigue.

Introduction

Muscles that are used repeatedly at near their maximum output exhibit a gradual decline of performance known as fatigue. Prominent features of fatigue are reduced force production, reduced shortening velocity and slower relaxation (for review see Allen et al., 1995; Fitts, 1994). It is accepted that the central nervous system, the motor nerves, the neuromuscular junction, and the muscle can all contribute to muscle fatigue (Gandevia et al., 1995). However under many circumstances the major component of fatigue arises in the muscle itself and can be conveniently studied in isolated muscles or single fibres (Allen et al., 1995). Force production requires consumption of energy and there are many studies of the metabolic changes which accompany muscle activity (for review see Spriet, 1998; Vollestad et al., 1988). In muscles that have a high aerobic capacity (e.g. heart muscle and slow muscle fibres) and can produce ATP faster than it is consumed, there are only minor changes in metabolite levels during activity and fatigue is largely absent. Conversely in fast muscle fibres aerobic capacity is limited and the rate of consumption of ATP can be much higher than the rate of resynthesis of ATP. When stimulated intensely these latter muscles show pronounced fatigue coupled to consumption of glycogen and phosphocreatine (PCr) and production of lactic acid, inorganic phosphate (P_i) and creatine. These observations have contributed to the widely held view that fatigue is closely related to the changes in metabolites which occur.

It is also well established that different patterns of activity in the muscle cause different mechanisms of fatigue to predominate. Important variables include the mechanical features of contraction (shortening, stretching, isometric, etc.), the duty cycle (fraction of time a muscle is excited), the pattern of activity, and the fraction of maximal output produced. Many experimental studies have focused on repeated, short, maximally-activated, isometric tetani for reasons of technical simplicity and this review is largely focused on this model of fatigue.

Early ideas about the mechanism of fatigue were dominated by the observation that lactic acid can accumulate in muscles contracting anaerobically and by the strong correlation between the appearance of fatigue and the accumulation of lactic acid (Hill and Kupalov, 1929). One possible mechanistic role of lactic acid emerged when Eberstein and Sandow (1963) showed that fatigue can be partially reversed by agents which increase activation, notably high K^+ and caffeine, suggesting that the processes of activation could be affected by lactic acid or other metabolic changes. Currently it seems unlikely that intracellular lactic acid has a major effect on activation because if unfatigued fibres are made acidotic they show an increase, rather than a decrease, in the amplitude of the tetanic $[Ca^{2+}]_i$ (Westerblad and Allen, 1993). Furthurmore, studies of the effects of acidosis on Ca^{2+} release in the skinned fibre with intact T-tubular/sarcoplasmic reticulum (SR) coupling show that substantial degrees of acidosis appear to have little effect on the amount of Ca^{2+} released (Lamb et al., 1992). Another important mechanism whereby lactic acid might reduce force production arose from the demonstration by Fabiato (1978) that acidosis reduces the force production by the contractile proteins. However recent experiments show that at body temperature, as opposed to room temperature, the effect of acidosis on the contractile proteins is greatly reduced (Pate et al., 1995; Westerblad et al., 1997).

In order to determine the cellular mechanisms of fatigue in muscle in a more quantitative way it is convenient to use the concept that force production in muscle depends on (a) the level of $[Ca^{2+}]_i$ achieved in the myoplasm, (b) the Ca^{2+} sensitivity of the myofibrillar proteins, and (c) the force produced when the crossbridges are maximally activated. Experiments in intact fibres in which force and intracellular calcium concentration ($[Ca^{2+}]_i$) can be measured simultaneously in a single cell allow us to dissect out the contributions of these factors (for review see Allen et al., 1995; Westerblad et al., 1991). The consensus from experiments of this sort is that changes in maximum force and Ca^{2+} sensitivity occur during fatigue and are probably principally caused by accumulating metabolites such as phosphate (P_i) and, to a lesser extent, by the acidosis caused by lactic acid accumulation.

Changes in Ca^{2+} Handling During Fatigue

The changes in Ca^{2+} handling during fatigue caused by repeated short tetani are complex but seem to be reasonably similar in a variety of preparations (for review see Allen et al., 1995). During the first 5-20 tetani the amplitude of the tetanic rise in $[Ca^{2+}]_i$ increases by 10-40%. Subsequently the tetanic $[Ca^{2+}]_i$ gradually declines and often shows a more pronounced decline over the final part of fatigue. Both these changes can be observed in Figure 2 and Figure 3A. Typically when the force is reduced to 50% the tetanic $[Ca^{2+}]_i$ is also reduced to about 50% of the control level. The resting $[Ca^{2+}]_i$ gradually increases throughout fatigue often reaching 2 or 3 times the control level by the time force is reduced to 50%. It has also been demonstrated that the timecourse of the decline of $[Ca^{2+}]_i$ at the end of each tetanus is substantially slowed during fatigue. Not only is it clear that tetanic $[Ca^{2+}]_i$ falls in the latter phase of fatigue but it has been shown that if the tetanic $[Ca^{2+}]_i$ in fatigued muscles is increased by caffeine or drugs which block the SR Ca pump, then the reduction in tetanic $[Ca^{2+}]_i$ and force can both be partially reversed (Lännergren and Westerblad, 1991; Westerblad and Allen, 1991, 1994).

The focus of the present short review is the cause of this decline in tetanic $[Ca^{2+}]_i$ during fatigue. Many hypotheses have been explored but there is no consensus view at present (Favero, 1999; Westerblad et al., 1998). Currently the best developed hypothesis is that either the rise in $[Mg^{2+}]_i$ or the fall in $[ATP]_i$ or both inhibit the opening of the SR Ca^{2+} release channels in fatigued muscles. It is known that a rise in $[Mg^{2+}]_i$ or a fall in $[ATP]_i$ causes reduced Ca^{2+} release in skinned fibre preparations with intact SR/T-tubular connections (Owen et al., 1996). During the final stages of fatigue there can be net consumption of ATP and since this is in the form MgATP the result is that ATP falls and Mg^{2+} rises (Nagessar et al., 1992; Westerblad and Allen, 1992). Westerblad and Allen (1992) measured the changes in $[Mg^{2+}]_i$ during fatigue and concluded that the changes in $[Mg^{2+}]_i$ were not great enough to explain the reduction in SR Ca^{2+} release. However, a recent study by Blazev and Lamb (1999) indicates that the combination of fall in $[ATP]_i$ and rise in $[Mg^{2+}]_i$ has an enhanced inhibitory effect on SR Ca^{2+} release suggesting that together they may make a substantial contribution during fatigue. On the other hand, muscles which have a high initial glycogen content may show little change in $[ATP]_i$ or $[Mg^{2+}]_i$ at the end of a single fatigue run (Kabbara et al., 2000). Thus the contribution of this mechanism in fatigue remains uncertain.

Effects of P_i on the SR Ca^{2+} Handling

Early experiments on isolated SR established the principle that organic acids, such as oxalic acid, can enter the SR in their uncharged form and can substantially increase the Ca^{2+} storage capacity of the SR by binding with Ca^{2+} (Hasselbach, 1964). Fryer et al. (1995) extended these ideas to phosphate using the skinned fibre preparation with intact SR. They showed that when the pool of Ca^{2+} and P_i available for SR uptake was unlimited, the caffeine-induced Ca^{2+} release was increased in the presence of additional phosphate. This was probably because the free Ca^{2+} in the SR ($[Ca^{2+}]_{SR}$) was unchanged but was buffered by a large pool of CaP_i. However under the more realistic conditions of a limited pool of Ca^{2+} around the SR, the Ca^{2+} release was reduced when P_i was increased, presumably because the binding of Ca^{2+} to P_i reduced the $[Ca^{2+}]_{SR}$. The proposal of Fryer et al. is based on the fact that under control conditions the $[P_i]$ concentration in the myoplasm is low (1-5 mM) whereas it rises to high levels during muscle fatigue (20-40 mM) (Cady et al., 1989). In addition it is assumed that P_i can cross the SR membrane and equilibrate with the myoplasm but at slow rate. Under resting conditions, if the $[P_i]$ in the SR is 3 mM and the $[Ca^{2+}]_{SR}$ is 1 mM, then the product (3 mM^2) is below the solubility product for precipitation of calcium phosphate (6 mM^2; Fryer et al. 1995). Under these circumstances ($[Ca^{2+}]_{SR} \sim 1$ mM) much of the total SR calcium will be bound to calsequestrin but because this reaction has rapid forward and reverse rate constants (personal communication; Dr C. Hidalgo) calsequestrin will act as a rapid buffer for SR Ca^{2+} and help minimise the fall which would otherwise tend to occur when the SR Ca^{2+} release channels open. In contrast during fatigue the myoplasmic $[P_i]$ rises to high levels and $[P_i]_{SR}$ will also rise towards this level. Once the product of $[Ca^{2+}]_{SR}$ and $[P_i]_{SR}$ exceeds 6 mM^2 then calcium phosphate (CaP_i) will start to precipitate and will continue to do so until the above product falls below 6 mM^2. This will tend to reduce $[Ca^{2+}]_{SR}$ and there will also be a reduction in Ca^{2+} bound to calsequestrin which will reduce the buffering of $[Ca^{2+}]_{SR}$.

For both these reasons, the efflux of Ca^{2+} through the SR Ca^{2+} release channels will be reduced. Clearly, critical factors in such a model are the rate of precipitation of CaP_i and the rate of re-solution of the precipitate when the $[Ca^{2+}]_{SR}$ X $[P_i]_{SR}$ product falls below 6 mM^2. In simple aequous solutions precipitation is rapid while rentry of CaP_i into solution has a half-time of 10 s (Fryer et al., 1995) but in the complex environment of the SR these may well be quite different.

Experiments in intact fibres micro-injected with substantial amounts of Na^+ P_i showed that after a few min the Ca^{2+} release and force declined substantially (Figure 1). In addition the expected reductions of maximum Ca^{2+} activated force and of Ca^{2+} sensitivity were absent (Westerblad and Allen, 1996). These results could all be explained if much of the injected P_i left the myoplasm and entered the SR. There the P_i could precipitate with Ca^{2+} while the $[Ca^{2+}]_{SR}$ and $[P_i]_{SR}$ product was > 6 mM^2. If these CaP_i complexes were only slowly reversible when $[Ca^{2+}]_{SR}$ was reduced, then the effect would be to reduce the $[Ca^{2+}]_{SR}$ and hence to reduce Ca^{2+} release on stimulation. The results of Fryer et al. (1995) and the results of P_i injections are both consistent with the idea that P_i may enter the SR, bind or precipitate with Ca^{2+} and reduce SR Ca^{2+} release, but they do not establish whether this phenomenon contributes to muscle fatigue.

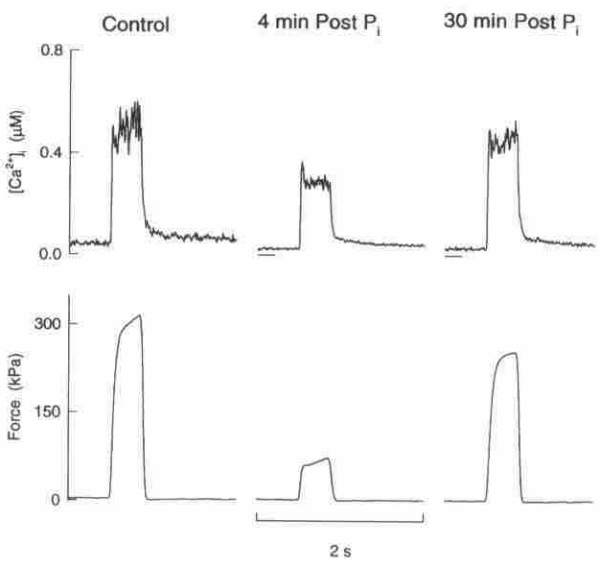

Figure 1. Phosphate injection into a muscle fibre causes reduced SR Ca^{2+} release. Each panel shows $[Ca^{2+}]_i$ measured with indo-1 (above) and force (below) from a single mouse skeletal muscle fibre. Left hand panel shows a brief 100 Hz tetanus. The fibre was then micro-injected with a concentrated solution of Na^+ phosphate resulting in a final concentration of 20 mM P_i if distributed throughout the myoplasm. The middle panels shows the reduction of tetanic $[Ca^{2+}]$ and force 4 min after the injection. The right hand panel shows that the muscle had substantially recovered after 30 min. From Westerblad and Allen (1996).

Subsequent experiments from Fryer's group have sought to establish the mechanism and timecourse with which P_i enters and leaves the SR. Two main mechanisms for transport of P_i across the SR membrane have been considered. Early work suggested the presence of a P_i transporter which was ATP-dependent and blocked by phenylphosphonic acid (Stefanova et al., 1991). Recent work has suggested a P_i permeable channel which may also be ATP-dependent (Posterino and Fryer, 1998). This possibility is supported by the discovery that the small chloride channel of the SR has significant P_i permeability (Kourie et al., 1996; Laver and Dulhunty, 1999). Thus these results suggest that P_i may enter and leave the SR by a P_i permeable channel. Posterino and Fryer (1998) showed that the influx of P_i into SR was related to myoplasmic $[P_i]$ over the range 10 to 50 mM. Of particular interest was the observation that the rate of influx of P_i into the SR increased as ATP was reduced to zero; conversely the rate of efflux of P_i appeared to be unaffected by ATP. The efflux of P_i from the SR had a half time of 35s when myoplasmic P_i was reduced to zero and was inhibited by elevated P_i .

P_i has also been shown to increase the opening of isolated SR Ca^{2+} release channels of skeletal muscle (Fruen et al., 1994). The significance of this observation in vivo is not clear but it could underlie the increase in Ca^{2+} release, which normally occurs over the first 10 or 20 tetani in a fatigue run. Duke and Steele (2000) have also observed Ca^{2+} efflux from skinned muscle with intact SR when P_i was elevated but this efflux was unaffected by ryanodine but eliminated by cyclopriazonic acid, an inhibitor of the SR Ca^{2+} pump. These authors concluded that elevated P_i is leading to SR pump reversal which would also tend to deplete the SR of Ca^{2+}.

Very recently Duke and Steele (2001) have shown in skinned fibres that when creatine phosphate was reduced to zero, the precipitation of CaP_i caused by elevated P_i was eliminated. They suggest this is because the increase in ADP when the creatine kinase reaction is prevented causes a leakage of Ca^{2+} from the SR so that the Ca X P_i product in the SR no longer exceeds the solubility product. However during fatigue the level of creatine phosphate will decline slowly and the P_i will rise slowly so that precipitation of CaP_i in the SR may well occur before the creatine phosphate falls so low that precipitation ceases. Further complications could arise from the dependence of P_i movements across the SR channel on $[ATP]_i$ (Posterino and Fryer, 1998) and the changes in pH_i associated with fatigue might also influence the situation.

Another difficulty for the CaP_i precipitation hypothesis is the observation that resting $[Ca^{2+}]_i$ generally rises during fatigue. If precipitation of CaP_i were the only factor affecting Ca^{2+} handling we would expect that resting $[Ca^{2+}]_i$ to fall as the leak declined in proportion to the $[Ca^{2+}]_{SR}$. A possible explanation for this observation is that the SR pump is inhibited independently by one of the metabolic changes which occurs. This issue has been considered in more detail elsewhere (Kabbara and Allen, 1999a; Westerblad and Allen, 1996).

Measurements of Calcium Stores

Important developments by the Somlyo's group led to the application of the electron microprobe to skeletal muscle (Gonzalez-Serratos et al., 1978). For this approach muscle fibres need to be rapidly frozen, dehydrated and cut into ultra-thin

slices; the concentration of total ions per unit volume of dry tissue can then be measured using an electron microprobe. Gonzalez-Serratos et al. (1978) measured the total calcium in the SR both under control conditions and in muscles fibres which were profoundly fatigued. Surprisingly, in the fatigued muscles the total SR calcium was increased by 55% (36 mmol/kg dry weight) and phosphorus also showed a smaller percentage rise of 8% though the absolute increase was similar to calcium (34 mmol/kg dry weight). The main conclusion at the time was that total SR calcium was not reduced and therefore the failure of activation during fatigue described by Eberstein and Sandow (1963) did not appear to be caused by depletion of the total SR calcium. For the same reason the reduced Ca^{2+} release observed during fatigue has generally been ascribed to reduced permeability of the SR Ca^{2+} release channels or failure of coupling between the voltage sensors and the Ca^{2+} release channels.

These methods, while a technical tour-de-force, have only limited time resolution and have not been exploited by other groups. We therefore sought other methods of measuring the stored calcium. In skinned fibres and in intact cardiac muscle rapid application of caffeine or 4-chloro-*m*-cresol (4-CmC) has proved a simple and effective method to estimate the SR Ca^{2+} content (Callewaert et al., 1989; Lamb and Stephenson, 1990). These agents open SR Ca^{2+} release channels; the ensuing rise of $[Ca^{2+}]_i$ in the myoplasm can be measured either by the activation of the contractile proteins or by a Ca^{2+} indicator introduced into the myoplasm. In mammalian skeletal muscle the SR release channels are relatively insensitive to caffeine and caffeine contractures are small and unreliable. In amphibian skeletal muscle it is possible to induce a maximal contracture with 5-10 mM caffeine but fibres are often damaged by this procedure so that it cannot be used repetitively in one fibre. We found that provided the application of caffeine was brief (< 30 s) no damage was apparent and that if a calcium indicator was included in myoplasm then the large rise in $[Ca^{2+}]_i$ which resulted represented the amount of releasable Ca^{2+} in the SR (Kabbara and Allen, 1999b). An important consideration in analysing such records of $[Ca^{2+}]_i$ is whether the Ca^{2+} in the SR is completely released in such a caffeine application. There are several issues here. First, Ca^{2+} is undoubtedly bound to binding sites within the SR including calsequestrin. Ca^{2+} will only be released from these sites if the effective dissociation rate is fast compared to the period of opening of the release channels. For calsequestrin, the association and dissociation rates have recently been measured and appear to have time constants of < 6 ms for physiological conditions and 25 °C (Dr. C. Hidalgo, personal communication, 2000). Such rapid rates would not exert any restriction on Ca^{2+} release from the SR during caffeine application of 10-20 s. But other binding sites, such as P_i, may be much slower. A second issue is the fraction of SR Ca^{2+} released by caffeine. Although Ca^{2+} is obviously released at a rapid rate by caffeine presumably it is also taken up at a substantial rate by the SR. Thus the fraction of Ca^{2+} released from the SR will depend on the ratio of (release rate)/(reuptake rate) and will only approach completion when release rate >> reuptake rate. This can be tested by the use of drugs which inhibit the SR reuptake; if the peak Ca^{2+} achieved in caffeine is unaffected by the presence of an SR Ca^{2+} blocker this suggests that the release rate is so much greater than the reuptake rate that slowing the reuptake rate does not affect the fraction of SR Ca released. This was shown to be the case for both caffeine and 4-CmC in the toad muscle (Kabbara and Allen, 1999b).

This technique of assessing SR Ca^{2+} content was then applied during fatigue (Kabbara and Allen, 1999a). Figure 2 illustrates the $[Ca^{2+}]_i$ recorded before, during and after a fatigue run. The initial tracing shows a single tetanus followed immediately by a 4-CmC application whose amplitude is roughly twice that of a maximal tetanus (similar results were obtained with caffeine). After a 30 min rest regular tetanic stimulation was started and continued until the force had fallen to 50% (force record not shown). Note the usual early rise in the amplitude of the stimulation-induced tetanic $[Ca^{2+}]_i$; subsequently as the muscle fatigued the tetanic $[Ca^{2+}]_i$ declined to about half the control value. Finally when the force had fallen to 50% of the control level stimulation was stopped and 4-CmC immediately applied. It is apparent that the peak $[Ca^{2+}]_i$ in response to 4-CmC, assumed to represent the Ca^{2+} available for rapid release in the SR, has approximately halved over this period. The muscle was then rested for 20 min, which allows force to largely recover, and then the store Ca^{2+} was again tested with 4-CmC and shown to have recovered completely. Such experiments do not distinguish whether the Ca^{2+} in the SR has been lost to the cell, transferred to another intracellular compartment or become bound to a site from which dissociation is too slow for it to be released even in 20-30 s 4-CmC exposure. While we cannot entirely resolve this question, we do show that the SR releasable Ca^{2+} recovers in the absence of extracellular Ca^{2+}, suggesting that the Ca^{2+} is not lost to the cell (Kabbara and Allen, 1999a). However, the store does not recover if cyanide is present during the recovery which prevents most of the metabolic recovery.

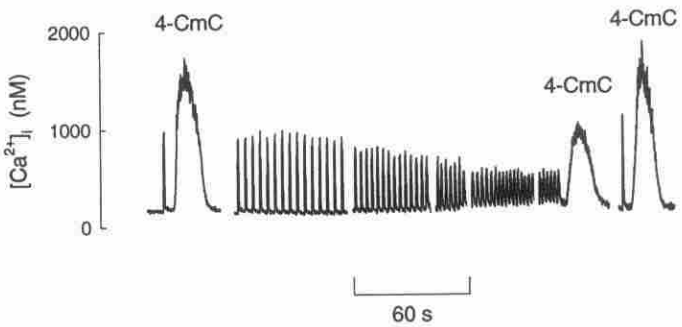

Figure 2. SR Ca^{2+} stores during muscle fatigue. $[Ca^{2+}]_i$ measurements from a single cane toad muscle fibre during stimulation to fatigue. The rapidly releasable Ca^{2+} in the SR was assessed by brief applications of 4-chloro-m-cresol which opens SR Ca^{2+} release channels and allows the rapidly releasable Ca^{2+} to diffuse into the myoplasm. Each 4-chloro-*m*-cresol (4-CmC) application is preceded by a stimulated tetanus. The first application of 4-CmC was before fatigue. There was then a 20 min break in the record before fatiguing stimulation started. Note the characteristic rise then fall in the peak tetanic $[Ca^{2+}]_i$ (note breaks in record) during repeated tetanic stimulation. When force had fallen to 50%, stimulation was stopped and 4-CmC applied and shows a reduction in the rapidly releasable SR Ca^{2+}. The fibres was then rested for 20 min and 4-CmC reapplied and shows that the rapidly releasable SR Ca^{2+} has returned to control levels. From Kabbara and Allen (1999b).

These results show the rapidly releasable Ca^{2+} in the SR undergoes a reversible decline during fatigue which is similar in magnitude to the decline of the tetanic $[Ca^{2+}]_i$. This is consistent with the CaP_i precipitation previously observed in skinned fibres or intact fibres injected with P_i. In particular the failure of recovery of the store Ca^{2+} when cyanide was present, which is known to prevent the fall of P_i which normally occurs during recovery (Dawson et al., 1980a), is additional evidence that P_i may be involved in this effect. Interestingly our results are not in conflict with those of Gonzalez-Serratos et al. (1978) who showed that the total SR calcium (ionised + non-ionised) was increased while we show that the rapidly releasable Ca^{2+} is decreased. If much of the SR Ca^{2+} is bound to P_i which is not able to rapidly release the Ca^{2+} under the present conditions then these two results are compatible.

Recently we have explored another aspect of store Ca^{2+} which is of particular relevance to human sporting achievement. It is well known that exercise that continues at a high level for more than one hour is likely to be accompanied by glycogen depletion when the muscles become exhausted (Bergström et al., 1967). However current evidence is the associated muscle fatigue cannot be explained by accumulation of metabolites and that excitation-contraction failure occurs (Green, 1990; Chin and Allen, 1997). We have explored this issue by fatiguing cane toad muscle fibres repeatedly in the absence of glucose. As expected, glycogen levels gradually decreased and the rate of decline of both force and tetanic $[Ca^{2+}]_i$ during each fatigue run became more rapid. As noted above when fibres had a large store of glycogen the decline of tetanic $[Ca^{2+}]_i$ was mirrored by a decline in the rapidly releasable Ca^{2+} store. However, when the glycogen was partially consumed the Ca^{2+} store no longer became depleted during a fatigue run despite the fact that the tetanic $[Ca^{2+}]_i$ fell more rapidly than usual (Kabbara et al., 2000). These results suggest that there are several mechanisms contributing to the decline of SR Ca^{2+} release during fatigue. One is the decline of Ca^{2+} stores, contingent on a rise in P_i, and probably only occurring when the P_i has been elevated for sufficiently long to enter the SR and cause Ca^{2+} binding and/or precipitation. The second mechanism, which is prominent in glycogen depleted preparations, appears to cause a decline of SR Ca^{2+} release by an independent mechanism. The nature of this mechanism is unclear but it may well be the in vivo analogue of the decline in SR Ca^{2+} release observed in skinned fibres which also correlates with glycogen levels and occurs in the presence of normal levels of creatine phosphate and ATP (Stephenson et al., 1999). These observations suggest that glycogen or perhaps breakdown products have some specialised role in the maintainence of excitation-contraction coupling. It is known that glycogen is closely associated with the SR/T-tubular junction (Fridén et al., 1989) and there are suggestions in the literature that glycolytic intermediates are necessary for EC coupling (Han et al., 1992).

Fatigue in the Absence of Creatine Kinase

Recently mice deficient in both myofibrillar and mitochondrial creatine kinase ($CK^{-/-}$ mice) were developed (Steeghs et al., 1997). These mice provide a good model to study the effect of metabolic changes on SR Ca^{2+} release during fatigue, since the rapid CK energy buffering cannot operate and muscle fibres will fatigue without PCr breakdown or P_i accumulation. If, on the one hand, the decline of

$[Ca^{2+}]_i$ in fatigue is due to a direct inhibition of the SR Ca^{2+} release channels by reduced $[ATP]_i$ and/or increased $[Mg^{2+}]_i$, then $CK^{-/-}$ muscles would be expected to fatigue more rapidly than their wild-type littermates. The opposite would be expected if, on the other hand, CaP_i precipitation is an important mechanism of the reduced SR Ca^{2+} release in fatigue.

Recent experiments on single muscle fibres from $CK^{-/-}$ mice show that both the above mechanisms may be operating. With a very intense stimulation pattern (67% duty cycle), $CK^{-/-}$ fibres display a transient decline of both tetanic $[Ca^{2+}]_i$ and force, which was not observed in wild-type fibres (Dahlstedt et al., 2000). With a less intense stimulation pattern similar to that used in studies described above (350 ms tetani every 2.5 s; duty cycle 14%), $CK^{-/-}$ fibres displayed a transient decline of tetanic $[Ca^{2+}]_i$ in the second fatiguing tetanus. These findings are consistent with a direct inhibition of SR Ca^{2+} release by reduced $[ATP]_i$ and/or increased $[Mg^{2+}]_i$. However, after the transient decline in the second tetanus, $CK^{-/-}$ were markedly more fatigue resistant than wild-type fibres during the less intense fatiguing stimulation (Figure 3). For instance, after 100 fatiguing tetani neither tetanic $[Ca^{2+}]_i$ nor force was significantly reduced in $CK^{-/-}$ fibres, whereas tetanic force fell to 30% of the control and stimulation stopped after about 80 tetani in wild type fibres. Since $CK^{-/-}$ muscles fatigue without any significant increase of P_i, there will be no drive for P_i to enter SR and consequently CaP_i precipitation would not occur. Thus, the increased fatigue resistance of $CK^{-/-}$ fibres fits with the concept that CaP_i precipitation in the SR is an important mechanism of the decline in SR Ca^{2+} release in fatigue. However, this conclusion is not unequivocal. Like many genetically modified mice, $CK^{-/-}$ mice show major adaptations which develop as a consequence of the absent gene. For instance, $CK^{-/-}$ muscles show higher mitochondrial content and oxidative enzyme activities as compared to wild-type muscles (Steeghs et al., 1997). Therefore the increased fatigue resistance in $CK^{-/-}$ fibres could simply be due to an increased oxidative capacity. Further studies are required to distinguish between these two possibilities.

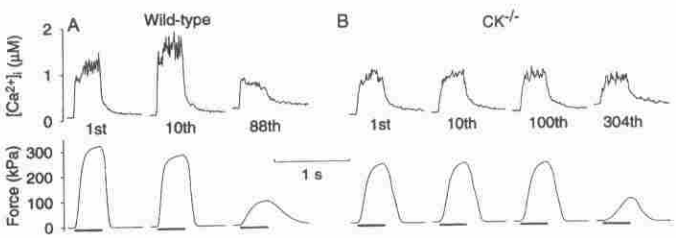

Figure 3. Creatine kinase knockout muscles show reduced fatigue. Each panel shows $[Ca^{2+}]_i$ (above) and force (below) from mouse single fibres during fatigue caused by repeated tetani. The number of the tetanus is shown beneath the $[Ca^{2+}]_i$ record. Panel A shows representative records from a normal mouse; note the increased tetanic $[Ca^{2+}]_i$ on the 10th tetanus and the reduced tetanic $[Ca^{2+}]_i$ at the end of fatigue (88th tetanus). Panel B shows results from a mouse fibre with no creatine kinase. Independent experiments showed that the initial P_i was higher but there was no rise in P_i during fatigue. Note the absence of the early rise in $[Ca^{2+}]_i$ and the absence of the subsequent decline in $[Ca^{2+}]_i$ during fatigue and the fact that fatigue was much slower in this muscle. From Dahlstedt et al. (2000).

Another interesting aspect of the $CK^{-/-}$ fibres was that they did not show the early increase in tetanic $[Ca^{2+}]_i$ associated with reduced tetanic force (compare Figure 3A and B). This suggests that these changes in early fatigue are due to increased myoplasmic P_i. A P_i-induced reduction of the number of cross-bridges in strong, force-generating states can explain the reduced tetanic force normally observed in early fatigue (Millar and Homsher, 1990). One possible mechanism of the increased tetanic $[Ca^{2+}]_i$ is that P_i stimulates the SR Ca^{2+} release channels (Fruen et al., 1994). An alternative mechanism is that the increased P_i inhibits the SR Ca^{2+} pumps (Dawson et al., 1980b; Duke and Steele, 2000), which at least in the short term might lead to increased tetanic $[Ca^{2+}]_i$ (Westerblad and Allen, 1994). Yet another possibility is that the increased P_i, by reducing the number of strong cross-bridges interacting with the thin filament (Guth and Potter, 1987), caused reduced myoplasmic Ca^{2+} buffering and therefore an increased tetanic $[Ca^{2+}]_i$.

Conclusions

The evidence is now strong that a decline in the rapidly releasable Ca^{2+} in the SR can contribute to the reduced Ca^{2+} release occurring during fatigue. The reduction in rapidly releasable Ca^{2+} is probably a consequence of Ca^{2+} within the SR binding or precipitating with P_i. However, it also appears that other mechanisms contribute to the decline of SR Ca^{2+} release during fatigue. If $[ATP]_i$ falls and $[Mg^{2+}]_i$ rises then these changes will be expected to contribute to the decline of SR Ca^{2+} release. There is also increasing evidence that glycogen is an essential requirement for normal excitation-contraction coupling and that when glycogen levels decline this also inhibits SR Ca^{2+} release. It is probable that these factors contribute to variable extents in different types of muscle and different models of fatigue.

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