Topical Review

Role of phosphate and calcium stores in muscle fatigue

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Intensive activity of muscles causes a decline in performance, known as fatigue, that is thought to be caused by the effects of metabolic changes on either the contractile machinery or the activation processes. The concentration of inorganic phosphate (P_i) in the myoplasm ($[P_i]_{myo}$) increases substantially during fatigue and affects both the myofibrillar proteins and the activation processes. It is known that a failure of sarcoplasmic reticulum (SR) Ca^{2+} release contributes to fatigue and in this review we consider how raised $[P_i]_{myo}$ contributes to this process. Initial evidence came from the observation that increasing $[P_i]_{myo}$ causes reduced SR Ca^{2+} release in both skinned and intact fibres. In fatigued muscles the store of releasable Ca^{2+} in the SR declines mirroring the decline in SR Ca^{2+} release. In muscle fibres with inoperative creatine kinase the rise of $[P_i]_{myo}$ is absent during fatigue and the failure of SR Ca^{2+} release is delayed. These results can all be explained if inorganic phosphate can move from the myoplasm into the SR during fatigue and cause precipitation of CaP_i within the SR. The relevance of this mechanism in different types of fatigue in humans is considered.

There is a decline in the performance of muscles when they are used at near their maximum capacity. The changes in performance include reduced force production, decreased velocity of shortening, and slowed relaxation; the combination of these factors can lead to profound reductions in performance particularly for rapidly repeated movements. This decline of performance, or fatigue, is particularly obvious and well recognized in athletes who use their muscles very close to their maximum capacity. What is less appreciated is that many patients suffer serious consequences from muscle fatigue and could benefit from strategies to reduce fatigue. Patients with a variety of diseases that affect muscle directly (e.g. muscular dystrophy) or indirectly (e.g. strokes, multiple sclerosis) have reduced muscle function. If the muscles involved are used for vital functions such as breathing or walking, then the remaining functional muscles are used much nearer to their maximal capacity. Consequently essential muscles fatigue rapidly with potentially serious outcomes. Elderly humans suffer a gradual loss of muscle mass and consequently muscles may fatigue during everyday activities causing loss of mobility and independence. Heart failure patients suffer from breathlessness on exertion but also experience excessive muscle fatigue and both of these factors contribute to their reduced exercise capacity (Wilson, 1996; Lunde *et al.* 2001*b*).

In the present article we explain the reasons for thinking that increased concentration of inorganic phosphate in the myoplasm ($[P_i]_{myo}$) has a central role in muscle fatigue including causing activation failure. We propose that inorganic phosphate (P_i), which increases substantially during fatigue, may enter the sarcoplasmic reticulum (SR), combine with Ca^{2+} and form an insoluble precipitate of calcium phosphate (CaP_i), leading to reduced SR Ca^{2+} release and a consequent decline in muscle performance as originally suggested by Fryer *et al.* (1995). This mechanism provides a simple explanation for the failure of SR Ca^{2+} release in fatigue and may lend itself to therapeutic interventions in the future.

Mechanisms of fatigue

In muscles contracting at high work loads, stores of energy within the muscle are consumed and products of these reactions accumulate. Thus muscles become acid because glycogen is broken down anaerobically to lactic acid; P_i accumulates because phosphocreatine (PCr) is broken down to creatine (Cr) and P_i. In addition there are, of course, numerous other metabolic changes in fatigue (for review see Fitts, 1994).

In principle, the cellular mechanisms which control force include: (i) the Ca^{2+} concentration surrounding the myofilaments, (ii) the sensitivity of the myofilaments to Ca^{2+}, and (iii) the force produced by the crossbridges characterized by the maximum Ca^{2+}-activated force. Thus, at a cellular level, fatigue is generally thought to be caused by the cumulative effects of the various metabolite changes acting through these three mechanisms (for

review see Westerblad *et al.* 1991; Allen *et al.* 1995). Historically by far the most popular hypothesis has been that intracellular acidosis associated with lactic acid accumulation is the major cause of fatigue acting through mechanisms (ii) and (iii). We have recently reviewed the evidence for this theory and outline why this theory is losing support, at least in mammals (Westerblad *et al.* 2001). As an alternative P_i has also been intensively investigated. Studies in intact muscles show that the resting $[P_i]_{myo}$ is 1–5 mM (Kushmerick *et al.* 1992) while during intense contraction it can rise to 30–40 mM (Cady *et al.* 1989) and the increased $[P_i]_{myo}$ may cause fatigue by acting through all three of the above mechanisms.

Investigations on mechanism (i) started with the classic work of Eberstein & Sandow (1963). They fatigued intact muscles with repeated tetani until force was greatly reduced and then increased the level of activation by increasing extracellular K^+ or application of caffeine. Both these manoeuvres increased force substantially in the fatigued muscle suggesting that a reversible failure of activation was an important contributor to fatigue. A recent example of this approach is shown in Fig. 1*A* which illustrates how caffeine can reverse much of the decline of force in a fatigued muscle. The rise in tetanic $[Ca^{2+}]_{myo}$, which activates the contractile proteins (Fig. 1*B*(i)), initially increases (Fig. 1*B*(ii)), but then declines during fatigue (Fig. 1*B*(iii)). Agents such as caffeine, which increase the opening of the SR Ca^{2+} release channels (ryanodine receptors), can increase the amplitude of tetanic $[Ca^{2+}]_{myo}$ (Fig. 1*B*(iv)) and thus overcome much of fatigue. Thus the partial failure of SR Ca^{2+} release is accepted to be one of the causes of muscle fatigue (Allen *et al.* 1995; Williams & Klug, 1995; Favero, 1999).

The main focus of the present review is the role of P_i in the failure of SR Ca^{2+} release during fatigue. It is already established that increasing $[P_i]_{myo}$ reduces crossbridge force and Ca^{2+} sensitivity of the myofilaments (Cooke & Pate, 1985; Millar & Homsher, 1990) and probably contributes to the early fall in force (within 1 min) shown in Fig. 1*A*. Inorganic phosphate also increases the open probability of the SR Ca^{2+} release channels (Fruen *et al.* 1994) and is a probable cause of the early increase in tetanic $[Ca^{2+}]_{myo}$ illustrated in Fig. 1*B*(ii). Inorganic phosphate also contributes to a slowing of the SR Ca^{2+} pump (Dawson *et al.* 1980*a*; Duke & Steele, 2000). These roles of increasing $[P_i]_{myo}$ in early fatigue are each supported by the finding that when creatine kinase is inoperative (see later section) the early fall of force, the early rise of tetanic $[Ca^{2+}]_{myo}$ and the early slowing of the decline of $[Ca^{2+}]_{myo}$ during relaxation are all abolished (Dahlstedt *et al.* 2001).

Dawson *et al.* (1978) initially suggested that slowing of the SR Ca^{2+} pump could explain the reduced force in

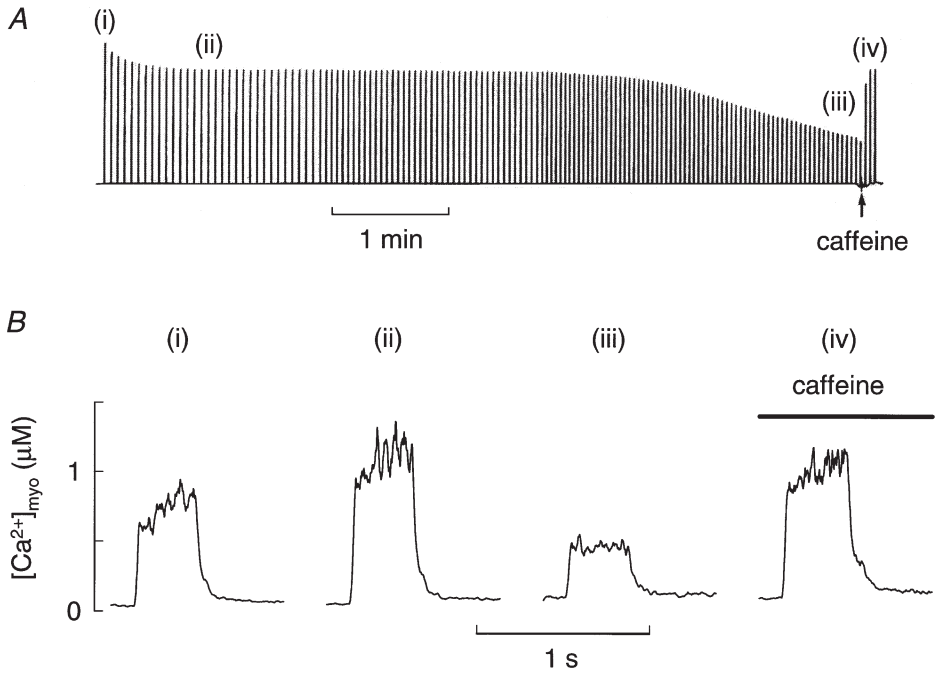

Figure 1. Muscle fatigue is partly caused by failure of SR Ca^{2+} release

A, force production from a mouse single fibre stimulated to give repeated brief tetani at gradually reducing intervals until force had declined to ~40 % of control. At that time caffeine (10 mM) was applied, which reversed much of the decline of force. *B*, $[Ca^{2+}]_{myo}$ records of selected tetani from experiments similar to *A*; (i) is the first tetanus, (ii) is at the end of the early decline of force, (iii) is a fatigued tetanus just before the addition of caffeine, and (iv) is in the presence of caffeine. *A* is taken from Lännergren & Westerblad (1991). *B* is modified from data in Westerblad & Allen (1991).

fatigue. This could come about if the Ca^{2+}, which is not returned to the SR, were sequestered in some organelle, e.g. mitochondria, or removed from the cell. However, the amount of Ca^{2+} sequestered by mitochondria during fatigue seems to be relatively small (Gonzalez-Serratos *et al.* 1978; Länngergren *et al.* 2001) and the rate of removal of Ca^{2+} to the extracellular space is also very slow (Balnave & Allen, 1998). Furthermore, studies in which the SR pump is slowed by pharmacological means show that the amplitude of the tetanic $[Ca^{2+}]_{myo}$ becomes *larger* during pump inhibition presumably because the activity of the pump usually opposes the rise in tetanic $[Ca^{2+}]_{myo}$ caused by SR Ca^{2+} release (Westerblad & Allen, 1994). Thus current evidence shows that slowing of the SR Ca^{2+} pump, while important to relaxation, is not the primary cause of reduced SR Ca^{2+} release.

Precipitation of calcium phosphate

None of the established effects of P_i described above appear capable of explaining the substantial changes in activation observed during fatigue. The first suggestion that increased $[P_i]_{myo}$ might have novel effects through precipitation of CaP_i came from an important study by Fryer *et al.* (1995) . They used a skinned fibre preparation with intact SR and tested the effects of increased $[P_i]_{myo}$ on the SR Ca^{2+} content, which was estimated from the force produced by caffeine. As background information it has long been known that the Ca^{2+} uptake of isolated SR vesicles can be greatly increased by agents such as oxalate or P_i (Hasselbach, 1964). These substances enter the SR, bind to Ca^{2+}, and eventually precipitate with Ca^{2+}. Consequently such substances buffer the Ca^{2+} within the SR and greatly increase the Ca^{2+} uptake capacity of the SR. Fryer *et al.* confirmed such effects of elevated $[P_i]_{myo}$ when the solution around the preparation was large. More importantly, they also showed that when the volume of solution around the fibre was restricted, elevation of $[P_i]_{myo}$ reduced the subsequent caffeine-induced contraction (Fig. 2*A*). They proposed that P_i is capable of entering and leaving the SR leading to the events shown schematically in Fig. 2*B*. It is known that the Ca^{2+} concentration in the SR ($[Ca^{2+}]_{SR}$) is of the order of 1 mM (Somlyo *et al.* 1981) with the total Ca^{2+} being much higher because much of the Ca^{2+} is bound to calsequestrin (CS), a Ca^{2+}-binding protein

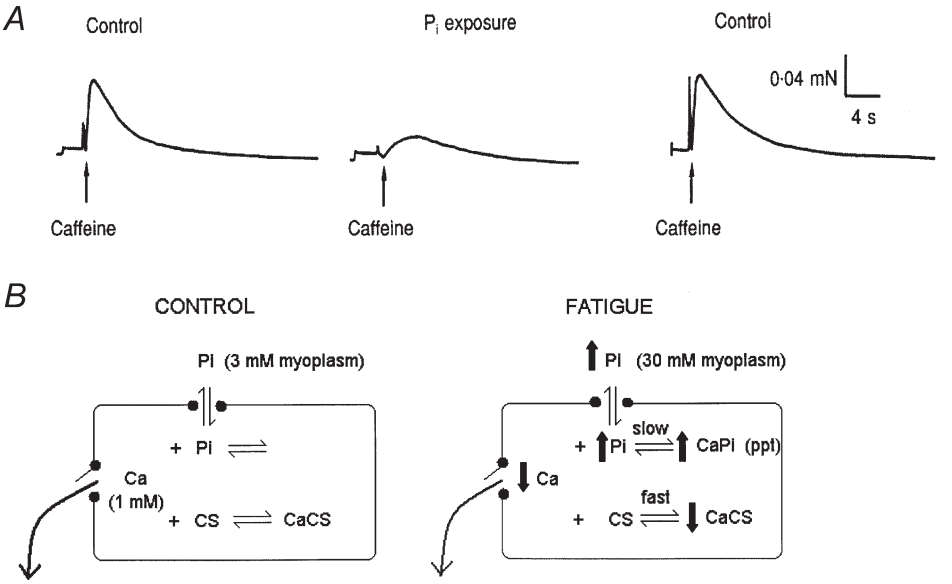

Figure 2. Increased $[P_i]_{myo}$ reduces SR Ca^{2+} release

A, force records from skinned fibres with intact SR. Caffeine was used to release SR Ca^{2+} producing the contractures shown; thus the size of the contracture is an indication of the Ca^{2+} available for release in the SR. In the middle record, the muscle was exposed to 50 mM P_i for 20 s, the P_i was then washed off and caffeine applied. Adapted from Fryer *et al.* (1995) with permission. *B*, schematic diagram of Ca^{2+} and P_i movements across the SR membrane and binding sites within the SR. Under control conditions $[P_i]_{myo} = [P_i]_{SR} = 3$ mM and $[Ca^{2+}]_{SR} = 1$ mM. Thus $[Ca^{2+}]_{SR} \times [P_i]_{SR} = 3$ mM^2 and because this is below the solubility product of CaP_i (which is 6 mM^2) none of this product is present. Ca^{2+} in the SR, however, binds rapidly and reversibly to calsequestrin (CS) so that there is a large pool of CaCS which buffers $[Ca^{2+}]_{SR}$. When the SR Ca^{2+} release channel opens, a large flux of Ca^{2+} into the myoplasm occurs because $[Ca^{2+}]_{SR}$ is high and $[Ca^{2+}]_{SR}$ is maintained high by the buffering of CaCS. In fatigue $[P_i]_{myo}$ is 30 mM and P_i enters the SR via anion channels. Once $[P_i]_{SR}$ exceeds 6 mM, the product of $[Ca^{2+}]_{SR} \times [P_i]_{SR}$ exceeds the solubility product of CaP_i and precipitation of CaP_i starts to occur slowly in the SR. As a consequence $[Ca^{2+}]_{SR}$ and CaCS fall and when the SR Ca^{2+} release channels are open the flux is smaller both because $[Ca^{2+}]_{SR}$ is lower and the buffering of $[Ca^{2+}]_{SR}$ by CaCS is reduced. Dissociation of CaP_i is assumed to be too slow to contribute to Ca^{2+} release. Heavy arrows indicate changes of key concentrations during fatigue.

with high capacity and low affinity (MacLennan & Holland, 1975). Ca^{2+} is thought to bind to, and dissociate from, calsequestrin relatively quickly so that it acts as a rapid buffer for Ca^{2+}. The solubility product of CaP_i is 6 mM^2 (Fryer *et al.* 1995) so that with a $[Ca^{2+}]_{SR}$ of 1 mM, if $[P_i]_{SR}$ exceeds 6 mM then CaP_i will start to precipitate. The net effect is that $[Ca^{2+}]_{SR}$ declines as an increasing fraction of the total SR Ca^{2+} becomes precipitated as CaP_i. This mechanism can explain the reduced contraction caused by elevated $[P_i]_{myo}$ (Fryer *et al.* 1995).

A second indication that P_i has effects other than directly on the myofilaments came from a study in which P_i was directly injected into muscle cells (Westerblad & Allen, 1996). We were expecting to see reduced force and Ca^{2+} sensitivity due to the direct effects of P_i on the myofilaments but, to our surprise, these effects were hardly apparent and instead there was a drastic reduction in SR Ca^{2+} release which caused a fall in force. Since the expected effects of P_i on myofilaments were largely absent, we reasoned that most of the injected P_i had entered the SR, precipitated as CaP_i, and consequently reduced SR Ca^{2+} release.

Role of calcium stores in fatigue

The studies cited above show that precipitation of CaP_i can occur but it still has to be established that this process takes place in the more complex intracellular environment of fatigue. An important contribution to this debate arises from a landmark study by Gonzalez-Serratos *et al.* (1978). They fatigued single frog muscle fibres with repeated tetani and measured ionic concentrations by electron probe microanalysis. The electron microprobe could be focused down onto individual terminal cisternae of the SR and the total Ca^{2+} and phosphorus were measured. The important finding was that both total Ca^{2+} and phosphorus (which would be largely P_i) increased significantly in the fatigued muscle. The authors concluded that the Ca^{2+} store was not depleted during fatigue and hence the decline in tetanic $[Ca^{2+}]_{myo}$ which occurs during fatigue has usually been interpreted as a failure of opening of the SR Ca^{2+} release channels.

Another approach to this problem is to try to measure the size of the SR Ca^{2+} store in intact muscle. This has frequently been done in cardiac myocytes in which caffeine causes rapid SR Ca^{2+} release. We have recently applied this approach to single cane toad fibres, as illustrated in Fig. 3 (Kabbara & Allen, 1999). Note that application of a drug which increases the opening probability of SR Ca^{2+} release channels, causes a large rise in $[Ca^{2+}]_{myo}$ which is typically twice the size of a maximally activated tetanus. We call this the rapidly releasable SR Ca^{2+} content to indicate that Ca^{2+} bound to slowly releasable sites within the SR would not be detected by this approach. During fatigue it is clear from Fig. 3 that the rapidly releasable SR Ca^{2+} falls substantially; it also recovers after a rest period as does the tetanic $[Ca^{2+}]_{myo}$. In order to try to identify the whereabouts of the missing SR Ca^{2+} two types of experiments were performed. First, recovery from fatigue was performed in the absence of extracellular Ca^{2+}; if the missing Ca^{2+} had been pumped out of the cell this would presumably have prevented recovery of SR Ca^{2+} release (Balnave & Allen, 1998). However, SR Ca^{2+} stores recovered completely in the absence of extracellular Ca^{2+} suggesting that the missing Ca^{2+} remained within the fibre. Second, recovery was performed in the presence of cyanide to delay the

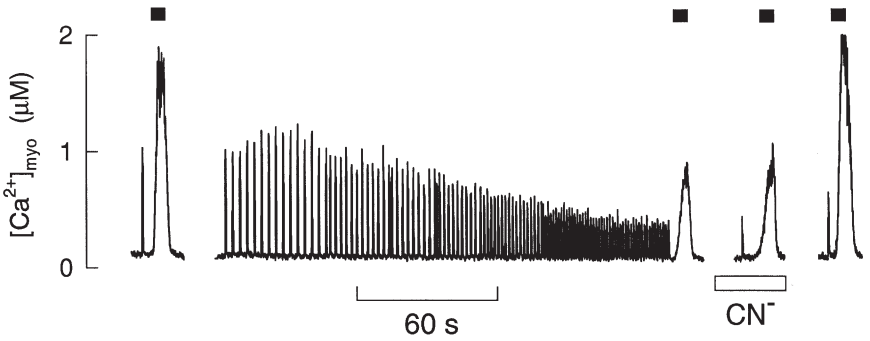

Figure 3. SR Ca^{2+} stores decline during fatigue and recovery is prevented by inhibition of oxidative metabolism

$[Ca^{2+}]_{myo}$ recorded from a single cane toad muscle fibre. The first record shows a single short tetanus followed by ~10 s application of 4-chloro-*m*-cresol (4-CmC, indicated by bar above record). This drug opens SR Ca^{2+} release channels and the large rise in $[Ca^{2+}]_{myo}$ represents the amount of rapidly releasable SR Ca^{2+}. Similar results are obtained with caffeine. The fibre was then rested for 20 min and then fatigued with repeated brief tetani until the tetanic force (not shown) was reduced to 40 %. 4-CmC was then reapplied and the amount of rapidly releasable SR Ca^{2+} was reduced compared to control. The fibre was then allowed to rest for 20 min in the presence of 2 mM cyanide, which inhibits oxidative metabolism, and a tetanus and 4-CmC application repeated. Neither tetanic $[Ca^{2+}]_{myo}$, the rapidly releasable SR Ca^{2+} store, nor the force (not shown) recovered. The fibre was then rested for 20 min in cyanide-free solution and partial recovery of all three parameters occurred. Adapted from Kabbara & Allen (1999).

recovery of metabolic changes associated with fatigue (Fig. 3). It is known, for instance, that the elevated $[P_i]_{myo}$ recovers very slowly in the absence of oxidative metabolism (Dawson *et al.* 1980*b*). Cyanide prevented the recovery of the SR Ca^{2+} release, showing that oxidative metabolism is required for store recovery. These experiments show that the rapidly releasable SR Ca^{2+} declines during fatigue and are compatible with the hypothesis that the missing Ca^{2+} is within the SR as a precipitate of CaP_i. Note that there is no conflict between these results, which show reduced rapidly releasable SR Ca^{2+} ($[Ca^{2+}]_{SR}$ + [CaCS], see Fig. 2*B*), and the earlier results of Gonzalez-Serratos *et al.* (1978) which showed increased total SR Ca^{2+}, which would include $[Ca^{2+}]_{SR}$ + $[CaP_i]$ + [CaCS].

One weakness of the above approach is the possibility that some change in the SR Ca^{2+} release channels or their sensitivity to caffeine (Duke & Steele, 1998) is the cause of the reduced SR Ca^{2+} release in fatigue. Furthermore, we have no information about the time course of the changes in stored Ca^{2+}. Both these weaknesses can be resolved by direct measurement of $[Ca^{2+}]_{SR}$. This has been achieved in a number of tissues (Golovina & Blaustein, 1997; Shmigol *et al.* 2001), and we have recently shown that it is possible in skeletal muscle (Kabbara & Allen, 2001). The essence of the method is to use a low affinity Ca^{2+} indicator loaded in its membrane-permeant form. Assuming there is esterase present in the SR, some indicator should be localized within the SR site and the indicator in the myoplasm will give little response to $[Ca^{2+}]_{myo}$ because of the low Ca^{2+} sensitivity of the indicator. These studies showed that $[Ca^{2+}]_{SR}$ declined throughout a period of fatiguing stimulation and recovered afterwards. These experiments do not allow unequivocal identification of the mechanism involved but they do support the previous studies in showing a reduction and recovery of $[Ca^{2+}]_{SR}$ which presumably contribute to the changes in SR Ca^{2+} release.

Inhibition of creatine kinase prevents the rise of P_i

A prerequisite for precipitation of CaP_i in the SR to occur is that $[P_i]_{myo}$ increases. The P_i accumulating in the myoplasm during fatigue mainly stems from PCr breakdown. Creatine kinase (CK) holds the following reaction near equilibrium:

$$PCr + ADP \rightleftharpoons Cr + ATP.$$

Thus the net reaction when ATP is rapidly consumed is as follows:

$$PCr \rightarrow Cr + P_i.$$

Consequently when the CK reaction is inhibited, fatigue occurs without any major increase in $[P_i]_{myo}$ (Dahlstedt *et al.* 2000) and then precipitation of CaP_i within the SR should be avoided. On these grounds it might be hypothesized that tetanic $[Ca^{2+}]_{myo}$ will remain unchanged during fatiguing stimulation in muscle fibres with inoperative CK. This hypothesis has recently been tested both with genetically engineered mice, which have no detectable CK in their skeletal muscles ($CK^{-/-}$) (Dahlstedt *et al.* 2000), and with pharmacological inhibition of the CK reaction using 2,4-dinitro-1-fluorobenzene (DNFB) (Dahlstedt & Westerblad, 2001). The basic results of these two studies were similar: there was a markedly slower decline of tetanic $[Ca^{2+}]_{myo}$ during fatigue after CK inhibition (Fig. 4). Both these models have limitations: $CK^{-/-}$ muscle displays adaptations with, for instance, an increased mitochondrial content (Steeghs *et al.* 1997) and DNFB has non-specific inhibitory effects on muscle function (for discussion see Dahlstedt & Westerblad, 2001). Since the limitations of the two models are quite disparate and they both cause a slower decline of tetanic $[Ca^{2+}]_{myo}$ during fatigue, it appears safe to conclude that the preserved tetanic $[Ca^{2+}]_{myo}$ in fatigue is due to inhibition of CK. Thus, the results from muscle fibres with inoperative CK strengthen the proposal that CaP_i precipitation is an important mechanism underlying the failure of SR Ca^{2+} release in fatigue.

P_i appears to enter the SR via ATP-sensitive anion channels

The results of an early study looking at P_i transport over the SR membrane indicate that P_i enters the SR via an ATP-dependent P_i transporter (Stefanova *et al.* 1991; Fryer *et al.* 1997). However, the results of a subsequent study suggest that P_i entry into the SR is a passive process, which was *inhibited* in the presence of ATP (Posterino & Fryer, 1998). It has now become clear that the SR membrane contains small conductance chloride channels, which may conduct P_i (Ahern & Laver, 1998; Laver *et al.* 2001). Interestingly the open probability of these channels increases at low $[ATP]_{myo}$ confirming the above study on intact SR (Posterino & Fryer, 1998). This dependence on $[ATP]_{myo}$ can explain one apparent weakness of the hypothesis that raised $[P_i]_{myo}$ causes CaP_i precipitation in the SR: $[P_i]_{myo}$ increases relatively early during fatiguing stimulation while the decline of tetanic $[Ca^{2+}]_{myo}$ generally occurs quite late. Moreover, in mouse fast-twitch fibres the decline of tetanic $[Ca^{2+}]_{myo}$ temporally correlates with an increase in $[Mg^{2+}]_{myo}$, which presumably stems from a net breakdown of ATP (Westerblad & Allen, 1992), and it is not obvious why CaP_i precipitation in the SR should show a temporal correlation with ATP breakdown. The ATP dependence of the presumed SR P_i channels can explain both why P_i enters the SR with a delay and why there is a temporal correlation between declining $[ATP]_{myo}$ and declining tetanic $[Ca^{2+}]_{myo}$.

Relevance to exercise in humans

The evidence is now reasonably strong that precipitation of CaP_i in the SR can contribute to the decline of SR Ca^{2+} release in muscles and is therefore one important cause of fatigue. In what kinds of exercise is this mechanism likely to be important? Clearly a number of processes are involved and these place some limit on the time course

over which such a mechanism could develop. The first process is breakdown of PCr which is the main source of P_i. This can occur quickly (~1 min) if the muscle is maximally and continuously activated (Kushmerick & Meyer, 1985; Cady *et al.* 1989). The second process is that the P_i has to move from the myoplasm to the SR presumably by the P_i permeable channel in the SR membrane noted above. To calculate the time course of entry of P_i into the SR requires knowledge of the number of channels and their P_i permeability. An upper limit can be obtained by assuming the channels exhibit their maximum permeability and that $[P_i]_{myo} = 20$ mM and these assumptions suggest an initial flux of 0.7 mM s^{-1} (Laver *et al.* 2001). Assuming further that $[P_i]_{SR} = 2$ mM, it would only take 6 s for the $[P_i]_{SR}$ to exceed 6 mM. Fryer *et al.* (1997) measured P_i flux as 0.05 mM s^{-1} which would give a much longer equilibration time though this time would be shorter if $[P_i]_{SR}$ is higher than 2 mM. Thirdly, in many systems it is common for precipitation to fail to occur immediately the solubility product threshold is exceeded; a nucleus for precipitation is required and the solubility product may be exceeded transiently before precipitation occurs. Since these processes are in series it would seem unlikely that precipitation of CaP_i would occur in fatiguing activities shorter than at 1–2 min. During recovery all these processes have to be reversed and this may be one factor why P_i recovery often precedes force recovery by some minutes (Cady *et al.* 1989).

In fatigue of a very long duration (> 1 h), muscle activation is generally quite low and the final decline of performance correlates well with glycogen depletion (Bergström *et al.* 1967). In this kind of exercise $[P_i]_{myo}$ is only moderately raised (Vøllestad *et al.* 1988) and it is unclear whether CaP_i precipitation would occur. Furthermore, in muscles which have been glycogen depleted by repeated bursts of activity in the absence of glucose, fatigue occurs very rapidly but is still caused by a failure of SR Ca^{2+} release (Chin & Allen, 1997; Kabbara *et al.* 2000). Interestingly, such muscles do not show reduction in SR Ca^{2+} stores at the point of fatigue (Kabbara *et al.* 2000) possibly because the muscles fatigue so quickly that the series of events outlined above cannot occur. These results suggest that failure of SR Ca^{2+} release in glycogen-depleted muscles involves a different mechanism. For instance, studies on skinned fibres suggest that glycogen may, in addition to its energy storage role, have a structural role which is essential for normal excitation–contraction coupling (Stephenson *et al.* 1999).

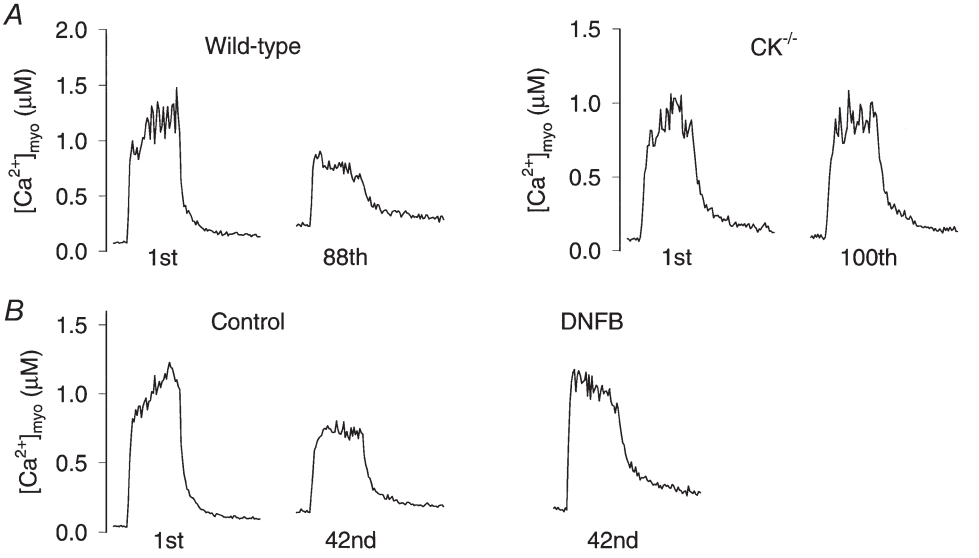

Figure 4. The decline of tetanic $[Ca^{2+}]_{myo}$ is delayed in muscle fibres which fatigue without an increase in $[P_i]_{myo}$

A, left: tetanic $[Ca^{2+}]_{myo}$ records obtained in a wild-type mouse muscle fibre at the start and the end (tetanic force reduced to 30 %; not shown) of fatigue caused by repeated tetani. This fibre was fatigued after 88 tetani and at that time tetanic $[Ca^{2+}]_{myo}$ was markedly reduced. *A*, right: records from a genetically engineered fibre lacking creatine kinase ($CK^{-/-}$). In this fibre 100 tetani had no significant effect on either tetanic $[Ca^{2+}]_{myo}$ or force (not shown). Adapted from Dahlstedt *et al.* (2000). *B*, the first two records show tetanic $[Ca^{2+}]_{myo}$ at the start and end of fatigue produced in a mouse muscle fibre under control conditions. In this fibre 42 tetani were required to reduce force to 30 %. The fibre was then allowed to recover for 90 min before 10 μM 2,4-dinitro-1-fluorobenzene (DNFB), which inhibits CK, was applied and the fibre fatigued again. This procedure produced some reduction in the first tetanic $[Ca^{2+}]_{myo}$ (not shown; for discussion of this point see Dahlstedt & Westerblad, 2001). Note that after DNFB exposure, tetanic $[Ca^{2+}]_{myo}$ was not reduced by 42 tetani. Adapted from Dahlstedt & Westerblad (2001).

Patients with heart failure experience rapid and disabling fatigue in their skeletal muscles and the mechanism of this fatigue has been the subject of considerable debate (Wilson, 1996; Lunde *et al.* 2001*b*). Obviously cardiac output, particularly during exercise, is reduced in heart failure patients so that blood supply to muscles will tend to be smaller. Consequently for a given level of exercise the metabolic changes are usually larger; for instance, in heart failure subjects the resting $[P_i]_{myo}$ in muscles is higher in some studies (Massie *et al.* 1987; van der Ent *et al.* 1998) and the increase in $[P_i]_{myo}$ on exercise is greater in most studies (Wilson *et al.* 1985; Arnolda *et al.* 1991). However, the changes are not large and it seems unlikely that the direct effects of P_i on the myofilaments are sufficient to explain the increased degree of fatigue. Therefore we suggest that raised $[P_i]_{myo}$ may contribute to the more rapid fatigue by causing precipitation of CaP_i in the SR. In addition there are many changes in the protein composition of skeletal muscles in heart failure (for review see Lunde *et al.* 2001*b*) and there have been attempts to discover whether isolated skeletal muscles taken from animals with heart failure fatigue more rapidly. Perreault *et al.* (1993) used bundles of fast muscle and showed that those from an animal with heart failure fatigued more rapidly and had a larger decline in tetanic $[Ca^{2+}]_{myo}$. This result suggested that an activation defect develops in skeletal muscle in animals with heart failure and that reduced blood flow is not the sole or major cause. However, more recent studies on single fibres from a similar model of heart failure did not show any major increase in fatiguability and only modest changes in $[Ca^{2+}]_{myo}$ (Lunde *et al.* 2001*a*). If changes in activation are modest in isolated single fibres this may be because the oxygen supply is more than adequate in this preparation and consequently aerobic metabolism can provide for much of the metabolic requirements. Conversely when muscles are *in situ* in heart failure subjects, the oxygen supply will be more limited so that anaerobic metabolic changes, such as a rise in $[P_i]_{myo}$, will occur earlier and may lead to activation changes by means of the CaP_i precipitation mechanism.

Conclusion

Failure of SR Ca^{2+} release has been shown to be a major contributor to muscle fatigue. Increasing evidence supports the hypothesis that precipitation of CaP_i in the SR contributes to the failure of SR Ca^{2+} release. We suggest that this mechanism may be important in high intensity activities which lead to fatigue in >1–2 min but other mechanisms are probably more important in lower intensity activities which cause fatigue in >1 h. CaP_i precipitation in the SR may contribute to the disabling fatigue common in many muscle disabilities and is especially likely to contribute in heart failure where $[P_i]_{myo}$ levels are higher.

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