Mechanisms of fatigue as studied in single muscle fibres

J. Lännergren, H. Westerblad & D.G. Allen

Department of Physiology II, Karolinska Institutet, 104 01 Stockholm, Sweden and Department of Physiology, University of Sydney, New South Wales, 2006, Australia.

Abstract Data from experiments on single, intact muscle fibres from Xenopus and mouse, aimed at elucidating cellular mechanisms of fatigue are summarized. During prolonged tetani there is evidence that conduction of action potentials down the t-tubuli may fail, leading to reduced Ca^{2+} release in the central part of the fibre. During repeated short tetani there is a uniform decline of Ca^{2+} across the fibre; in addition there is a reduction of force generating capacity of the crossbridges and diminished myofibrillar Ca^{2+} sensitivity. The possible relation of these alterations to changes in metabolite concentrations is discussed.

Muscle fatigue, here defined as a decline in force output during a period of activity, can be - and has been - studied in a number of different systems, from human athletes to minute myofibrillar preparations such as segments of 'skinned' muscle fibres. We have employed single, intact fibres for fatigue studies for the last 5-6 years. The advantage of this type of preparation is that adequate oxygenation is not a problem, the extracellular milieu can be controlled and changed rapidly if desired, and the action of drugs can be evaluated with high time resolution. Also, fibres of different types can be studied separately. It should be realized, however, that what we use is a *simplified model* of muscle fatigue and that in a whole muscle changes in the extracellular milieu are likely to contribute to fatigue development.

In the initial studies we used *amphibian* muscles because at that time this was the only kind which was amenable to single fibre dissection. We have used Xenopus muscle, because in this species fibres are relatively large and clearly differentiated into different types which can be identified and selected during the dissection. We chose to work on fibres from toe muscles (lumbricals) because these are short (1.5-1.8 mm) which facilitates electrical recordings. Lumbrical muscles contain fibres of amphibian types 1, 2, and 3, roughly corresponding to mammalian types IIb, IIa, and I, respectively. With time we developed a technique for dissecting single fibres from *mammalian* muscle as well (from mouse foot muscles) and results from experiments with such preparations will also be discussed.

Possible factors in fatigue

Signals for motor activity from the motor cortex are conveyed down the spinal cord to eventually activate motor neurons. Action potentials then travel out along motor nerve fibres, transmit across the neuromuscular junction and set up action potentials in the muscle fibres. Concomitant with the propagation along the surface membrane the action potential invades the t-tubules and triggers release of Ca^{2+} from adjacent regions of the sarcoplasmic reticulum (SR). This causes $[Ca^{2+}]_i$ to rise from a resting value of about 50 nM to 1-5 μM, which results in a conformational change in the troponin-tropomyosin complex occurs which then allows interaction of the mobile parts of myosin molecules (crossbridges) with actin, leading to force generation. Relaxation occurs when Ca^{2+} is pumped back from the myoplasm into the SR by an ATP-dependent Ca^{2+} pump in the wall of the SR.

It has been debated to what an extent the outflow of motor impulses from the CNS is reduced during fatiguing exercise. The concensus would appear to be that although such

reductions can and do occur, the major site of fatigue is the muscle itself (Bigland-Ritchie & Woods 1984). In the experiments we are concerned with here, the fibres are activated directly with transverse field stimulation, which means that neither neuromuscular transmission nor surface propagation of action potentials will be a limiting factor.

1. Changes in action potential configuration

We found, as have others, that there are marked changes in the shape of the action potential especially with continuous, high frequency stimulation: the upstroke is less steep, the amplitude is reduced and repolarization is slowed down. However, an action potential of this altered configuration is still capable of eliciting a full-size, or even augmented, twitch (Lännergren & Westerblad 1986) from which it appears that this change, *per se*, is not a cause of fatigue.

2. Failure of t-tubule propagation

It has often been suggested that propagation of action potentials down the t-tubules is a weak link in activation (e.g. Jones 1981). Some of our experiments on Xenopus fibres give support for this idea (Lännergren & Westerblad 1986). With continuous high frequency stimulation (70 Hz) force stays up for about 5 s and then declines rapidly. Three lines of evidence point towards t-tubule failure as a cause for the decline in this case: i) the early negative after-potential (EAP) becomes less evident. The EAP is usually taken as a sign of electrical activity in the t-tubules; ii) force recovery is very rapid, either at the end of stimulation, or also *during* stimulation if the stimulus frequency is suddenly reduced. The rapid recovery time is compatible with restitution of the ionic milieu in the t-tubules; iii) intracellular Ca^{2+} release, as monitored with fura-2 and imaging microscopy, changes from being homogeneous in the beginning, when the force is high, to showing a clear radial gradient with less Ca^{2+} in the core of the fibre when tension goes down (Westerblad et al. 1990)

It is worth noting that in our experiments failure of inward spread of action potentials can occur at a normal extracellular K^+ concentration. In a whole working muscle blood flow is occluded even at moderate forces and K^+ will accumulate in the extracellular space, reaching levels of 9-12 mM in mammalian muscle (Juel 1986; Medbø & Sejersted 1990). The increase in $[K^+]_o$ will accentuate K^+ accumulation in the t-tubule lumen due to repeated action potentials. It is thus possible that even at moderate impulse frequencies t-tubule failure may develop in whole muscle and contribute to fatigue development.

3. Decreased Ca^{2+} release

Before discussing the possible contribution of failing Ca^{2+} release to fatigue it may be appropriate to briefly summarise current ideas about EC-coupling (t-tubule-SR transmission). The t-tubules and SR membranes are separated by a narrow space (width 10-15 nm) bridged by structures known as 'foot proteins'. The t-tubule membrane contains a modified Ca^{2+} channel (the dihydropyridine receptor) which acts as a voltage sensor. Depolarization of the membrane changes the conformation of the dihydropyridine receptor and this change affects a large protein complex which is both the foot protein and a Ca^{2+} channel in the SR membrane. The final result of t-tubule depolarization is opening of SR Ca^{2+} channels which allows Ca^{2+} to diffuse from the very high concentration in the SR to the much lower concentration in the myoplasm, removal of the steric block by the troponin-tropomyosin complex and start of the crossbridge cycle.

Experiments performed nearly 30 years ago by Eberstein and Sandow (1963) suggested failing EC-coupling to be an important fatigue mechanism. They found that caffeine could restore twitch force in single frog fibres which had been fatigued by a long period of twitching. Caffeine acts directly on SR Ca^{2+} channels and facilitates Ca^{2+} release (Rousseau et al. 1988). We repeated this type of experiment both on Xenopus and mouse fibres, fatigued by repeated tetanic stimulation and found in both cases that caffeine caused a dramatic force

restoration (Westerblad & Lännergren 1986; Lännergren & Westerblad 1991). The result showed that in fatigue, the contractile machinery is still capable of substantial force production if sufficient Ca^{2+} release can be produced and suggested that failing Ca^{2+} release is an important mechanism of fatigue. Direct evidence for this view came from later experiments in which $[Ca^{2+}]_i$ was measured with aequorin (Allen et al. 1989) or with fura-2 in Xenopus fibres (Lee et al. 1991), later also in mouse fibres (Westerblad & Allen 1991). The change in amplitude of the Ca^{2+} transient during fatiguing, intermittent tetanic stimulation shows essentially the same pattern in Xenopus and mouse fibres. During the initial, fairly rapid period of tension decline to about 80% of the original the transient *increases,* it then slowly decreases while tension is relatively well maintained and then finally decreases markedly in parallel with a final, rapid tension decline (reviewed in Westerblad et al. 1991; see also Fig. 1). The importance of the reduction in Ca^{2+} release for the fall in tension is further substantiated by a concomitant increase in force and $[Ca^{2+}]_i$ when caffeine is applied (Westerblad & Allen 1991).

An important observation in studies of Ca^{2+} release in *Xenopus* fibres (Westerblad et al. 1990) was that with intermittent tetanic stimulation there was a *uniform* decline in $[Ca^{2+}]_i$ within fibres when force started to fall markedly, which indicates that t-tubule transmission does *not* fail in this fatigue model.

4. Reduced Ca^{2+} sensitivity of the contractile proteins

There are three principal mechanisms for reduced tension output in fatigue: i) reduced Ca^{2+} release; ii) reduced Ca^{2+} sensitivity of the contractile machinery; iii) reduced maximum force production by crossbridges. The first mechanism has just been discussed. As for the second possibility, experiments on skinned fibres have shown that metabolic changes which are likely to occur in fatigue, such as an increase in H^+ and P_i concentration, displace the curve relating force to $[Ca^{2+}]$ to the right, i.e. decrease the myofibrillar Ca^{2+} sensitivity (Godt & Nosek 1989). Recent experiments on mouse fibres by Westerblad and Allen (1991) show that at the end of a fatigue run, when force is down to about 35% of the original, the force-pCa curve is shifted by about 0.3 pCa units. Also in intact Xenopus fibres evidence for a diminished Ca^{2+} sensitivity was found (Lee et al. 1991).

5. Decreased force production by crossbridges

Several studies on 'skinned' fibre preparations have shown that the maximum force generation is depressed by metabolic changes which are likely to occur in fatigue. Thus, Cooke et al. (1988) and Godt & Nosek (1989) have demonstrated that a fall in pH from 7.0 to 6.5 together with an increase in P_i to 15 mM depresses maximum force by about 50%. Further, intracellular acidification of rested intact fibres by exposure to high CO_2 reduces maximum tension by 15-20% (e.g. Edman & Mattiazi, 1981).

Before discussing how the observed changes in tetanic force, Ca^{2+} release and Ca^{2+} sensitivity might relate to changes in metabolite concentrations a brief summary of such changes will be given, together with reference to some new data on metabolite concentrations in fatigued single Xenopus fibres.

Metabolite changes during fatigue

There is a wealth of data on metabolic changes during fatigue. The values obtained depend on various factors such as the preparation, type of fatiguing stimulation, aerobic/anaerobic conditions and so forth. Typical changes are a decrease in PCr from 35 to 2.4 mM, a decrease in ATP from 6 to 4.6 mM, an increase in P_i from 5 to 25 mM and a decrease in pH from 7.0 to 6.5 (reviewed by Vøllestad & Sejerstedt 1988; see also Godt & Nosek 1989; Westerblad et al. 1991). A recent study by Nagesser et al. (1992) is of particular relevance in the present context. They stimulated single Xenopus fibres (types 1 and 3) with intermittent tetani with a pattern similar to ours and measured, at various stages of fatigue, several metabolites of

interest. Their findings are plotted in schematic form in Fig. 1. It can be seen that at 0.7-0-8 P_o, when tetanic force starts to fall rapidly, PCr is fully depleted, ATP starts to decrease and IMP starts to rise. At 0.3-0.4 P_o, the end-point of our fatigue runs, ATP is decreased by about 40%, P_i would have risen by about 30 mM, IMP has increased to ~2 mM and lactate to ~40 mM (type 1 fibres).

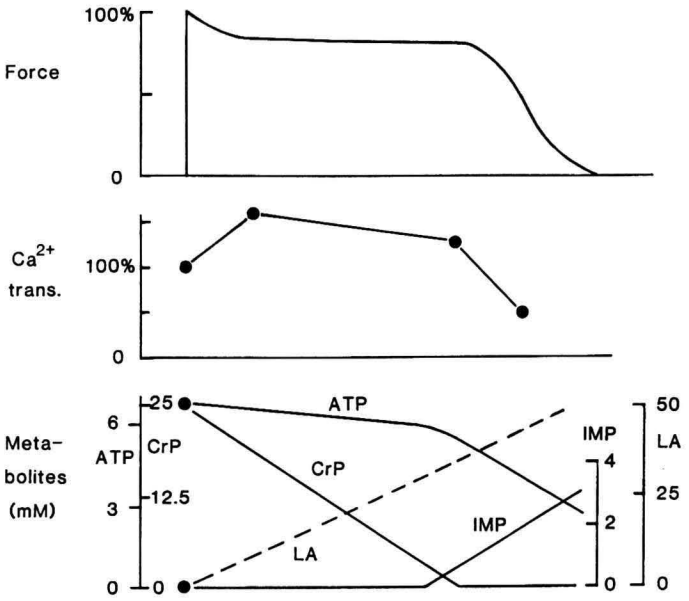

Fig. 1. Schematic representation of changes occurring in fatigue. Top panel: force production in repeated 70 Hz tetani; middle panel: amplitude of Ca^{2+} transients; bottom panel: metabolite concentrations. Metabolite values refer to Xenopus type 1 fibres. Data compiled from Westerblad & Allen (1991) and from Nagesser et al. (1992).

Possible relation between metabolite changes and force reducing mechanisms

It has long been recognized that there is a close correlation between metabolic capacity and the resistance to fatigue (eg. Kugelberg & Lindegren 1979). A very clear demonstration of this at the single fibre level was recently given by van der Laarse et al. (1991) where the time to fatigue (0.75 P_o) was related to the energy balance of Xenopus fibres of types 1-3, measured as SDH activity/myofibrillar ATPase activity, which gave a correlation coefficient of 0.93. The point of discussion is now how metabolite changes might have a bearing on the three cellular fatigue mechanisms referred to above and how they might develop with time.

i) Reduced force generation of cross-bridges. This change develops early as indicated by the finding that when force has fallen to about 0.8 P_o (after 10-20 tetani), caffeine application does not give any force enhancement. The most likely candidates for force depression, P_i and H^+, have already been discussed above. It should be pointed out here that isolated mouse fibres show little pH change in fatigue produced by repeated tetani (Westerblad & Allen 1992) so for these fibres P_i would be the dominant factor.

ii) Reduced Ca^{2+} sensitivity. Force-pCa curves have mainly been measured towards the end of fatiguing stimulation, i.e. when force is down to 0.3-0.4 P_o, and compared with resting conditions so it is not possible to state in detail how sensitivity changes with time. Judging from skinned fibre results the metabolic changes which are most likely to be responsible are inceases in P_i and H^+, similar to the case of maximum force depression.

iii) Reduced Ca^{2+} release. In our experimental model there was evidence for failing t-tubule function during a prolonged tetanus at high frequency, but *not* with repeated tetanic stimulation. In principle, a homogeneous depression of Ca^{2+} release might be due to either a decreased Ca^{2+} content of the SR or inhibition of the release mechanism. The finding that caffeine can release substantial amounts of Ca^{2+} in fatigued fibres is difficult to reconcile with SR depletion. Further, electron probe microanalysis of the ionic content of the SR of fatigued fibres failed to show a decrease in Ca^{2+} concentration (Gonzalez-Serratos et al. 1978).

Considering the second possibility, i.e. failure of Ca^{2+} release, Fig. 1 suggests that the failure might be connected with metabolic changes. One suggestive point is that the marked fall in the amplitude of the Ca^{2+} transient coincides with the time when ATP starts to decline. ATP is known to be required for the opening of SR Ca^{2+} channels (Smith et al. 1985), but it appears that also ADP and AMP can fulfil this role, at least for channels incorporated into artificial membranes. However, it has recently been demonstrated that phosphorylation of the voltage-sensitive DHP-channels in the t-tubules increases their readiness to open in response to depolarization (Mundina-Weilenmann et al. 1991). It is conceivable that phosphorylation also of the SR Ca^{2+}-channels is important for their opening probability, thus giving the phosphorylation potential ([ATP]/[ADP][P_i] ratio) in the vicinity of the channels a possible functional role.

A second feature of the metabolic changes depicted in Fig. 1 is that decreased Ca^{2+} release coincides with a rise in IMP concentration. The effect of IMP on channel opening appears not to have been investigated but it cannot be excluded that it has an inhibitory effect.

A consequence of the fall in ATP is that cytoplasmic [Mg^{2+}] will rise since ATP forms a complex with Mg^{2+} with a higher binding constant than for other nucleotides. Westerblad & Allen (1992) have shown that free $[Mg^{2+}]_i$ does indeed rise during the final phase of a fatigue run. This is of particular interest since it has been demonstrated by Lamb and Stephenson (1991) that an increase of [Mg^{2+}] from its normal resting value of 1 mM to 3 mM significantly inhibits Ca^{2+} release from semi-intact fibre preparations. A direct test of the Mg^{2+} inhibition hypothesis by injection of Mg^{2+} into intact mouse fibres showed that the inhibition was not large enough to explain the tension reduction in fatigue. However, the combination of increasecd Mg^{2+} and low ATP might be more effective in inhibiting Ca^{2+} release.

Yet another connection between a fall in ATP and reduced Ca^{2+} release might be provided by ATP-sensitive K^+ channels which exist in skeletal muscle (Spruce et al 1987). These channels are opened at low $[ATP]_i$, augmenting K^+ efflux (Castle & Haylett 1987) and changing the shape of the action potential (Sauviat et al 1991) which could lead to impaired activation of the Ca^{2+} release channels by the t-tubule voltage sensors.

Previous experiments on isolated Ca^{2+} channels in artificial membranes indicated that their opening probability is greatly reduced by low pH (Ma et al. 1988). However, using mechanically skinned fibres, where channels remain in a more natural environment, Lamb et al. (1992) have shown that acidosis in all likelihood has very little effect on Ca^{2+} release under physiological conditions. Thus, a fall in pH_i, as observed especially in amphibian type 1 fibres (but not in mouse fibres, see above) does not depress force via interference with Ca^{2+} release but more likely through an effect at the crossbridge level.

Relevance of single fibre studies

The results summarized here are all derived from experiments on isolated fibres. A question which naturally arises is how representative they are for fatigue in vivo. One factor which differs is that in the single fibre experiments the preparation is continuously superfused with fresh solution so that the extracellular environment remains constant. In whole muscles, on the other hand, blood flow is impeded already during moderate contractions, which means that substances such as lactic acid and K^+ ions will accumulate in the extracellular space. These

changes in extracellular composition may aggravate some of the fatigue mechanisms described. For instance, changes in pH_i are likely to be more pronounced than we have observed and ionic changes in the t-tubule lumen may occur more readily, increasing the risk of failure of inward spread of action potentials. On the other hand, in single fibre experiments the extracellular milieu can be modified in a controlled way, allowing a systematic analysis of the contribution of extracellular changes to the development of muscle fatigue.

References

Allen DG, Lee JA & Westerblad H (1989). Intracellular calcium and tension in isolated single muscle fibres from Xenopus. *Journal of Physiology* **415**, 433-458.

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

Castle NA & Haylett DG (1987). Effect of channel blockers on potassium efflux from metaboilically exhausted frog skeletal muscle. *Journal of Physiology* **383**, 31-43.

Cooke R, Franks K, Luciani GB & Pate E (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ion and phosphate. *Journal of Physiology* **345**, 77-97.

Eberstein A & Sandow A (1963). Fatigue mechanisms in muscle fibres. In: *The Effect of Use and Disuse on Neuromuscular Functions*. Elsevier. Amsterdam. pp 515-526

Edman KAP & Mattiazzi AR (1981). Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibres. *Journal of Muscle Research and Cell Motility* **2**, 321-334.

Godt RE & Nosek TM (1989). Changes of intracellular milieu with fatigue or hypoxia depress contraction of striated rabbit skeletal and cardiac muscle. *Journal of Physiology* **412**, 155-180.

Gonzalez-Serratos H, Somlyo AV, McClellan G, Shuman H, Borrero LM & Somlyo AP (1978). Composition of vacuoles and sarcoplasmic reticulum in fatigued muscle: electron probe analysis. *Proceedings of the National Academy of Science (USA)* **75**, 1329-1333.

Juel C (1986). Potassium and sodium shifts during in vitro isometric contraction, and the time course of the ion-gradient recovery. *Pflügers Archiv* **406**, 458-463

Jones DA (1981). Muscle fatigue due to changes beyond the neuromuscular junction. In: Human Muscle Fatigue: Physiological Mechanisms (Ciba Foundation Symposium 82) Eds, Porter R and Whelan J. Pitman Medical. London. pp 178-196

Kugelberg E & Lindegren B (1979). Transmission and contractile fatigue of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. *Journal of Physiology* **288**, 285-300.

Lamb GD & Stephenson DG (1991). Effect of Mg^{2+} on the control of Ca^{2+} release in skeletal muscle fibres of the toad. *Journal of Physiology* **434**, 507-528.

Lamb GD, Recupero E & Stephenson (1992). Effect of myoplasmic pH on excitation-contraction coupling in skeletal muscle fibres of the toad. *Journal of Physiology* **448**, 211-224.

Lännergren J & Westerblad H (1986). Force and membrane potential during and after fatiguing, continuous high frequency stimulation of single Xenopus muscle fibres. *Acta Physiologica Scandinavica* **128**, 359-368.

Lännergren J & Westerblad H (1991). Force decline due to fatigue and intracellular acidification in isolated fibres from mouse skeletal muscle. *Journal of Physiology* **434**, 307-322.

Lee JA, Westerblad H & Allen DG (1991). Changes in tetanic and resting $[Ca^{2+}]_i$ during fatigue and recovery of single muscle fibres from Xenopus laevis. *Journal of Physiology* **415**, 433-458.

Ma J, Fill M, Knudson M, Campbell KP & Coronado R (1988). Ryanodine receptor of skeletal muscle is a gap junction-type channel. *Science* **242**, 99-102.

Medbø JI & Sejersted OM (1990). Plasma potassium changes with high intensity exercise. *Journal of Physiology* **421**, 105-122.

Mundina-Weilenmann C, Ma J, Rios E & Hosey MM (1991). Dihydropyridine sensitive skeletal muscle channels in polarized bilayers. 2. Effects of phosphorylation by a cAMP-dependent protein kinase. *Biophysical Journal* **60**, 902-909.

Nagesser AS, van der Laarse WJ & Elzinga G (1992). Metabolic changes with fatigue in different types of single muscle fibres of Xenopus laevis. *Journal of Physiology* **448**, 511-523.

Rousseau E, LaDine J, Lin Q-Y & Meissner G (1988). Activation of the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum by caffeine and related compounds. *Archives of Biochemistry and Biphysics* **267**, 75-86.

Sauviat M-P, Ecault E, Faivre J-F & Findlay I (1991). Activation of ATP-sensitive K channels by a K channel opener (SR 44866) and the effect upon electrical and mechanical activity of frog skeletal muscle. *Pflügers Archiv* **418**, 261-265.

Smith JS, Coronado R & Meissner G (1985). Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature* **316**, 446-449.

Spruce AE, Standen NB & Stanfield PR (1985). Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* **316**, 736-738.

van der Laarse WJ, Lännergren J & Diegenbach PC (1991). Resistance to fatigue of single muscle fibres from Xenopus related to succinate dehydrogenase and myofibrillar ATPase activities. *Experimental Physiology* **76**, 589-596.

Vøllestad NK & Sejersted OM (1988). Biochemical correlates of fatigue. *European Journal of Applied Physiology* **57**, 336-347.

Westerblad H & Lännergren J (1987). Tension restoration with caffeine in fatigued, single Xenopus muscle fibres of various types. *Acta Physiologica Scandinavica* **130**, 357-358.

Westerblad H, Lee JA, Lamb AG, Bolsover SR & Allen DG (1990). Spatial gradients of intracellular calcium in sketal muscle during fatigue. *Pflügers Archiv* **415**, 734-740.

Westerblad H & Allen DG (1991). Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *Journal of General Physiology* **98**, 615-635.

Westerblad H, Lee JA, Lännergren J & Allen DG (1991). Cellular mechanisms of fatigue in skeletal muscle. *American Journal of Physiology* **261**, C195-C209.

Westerblad H & Allen DG (1992) Changes of intracellular pH due to repetitive stimulation of single fibres from mouse skeletal muscle. *Journal of Physiology* **449**, 49-71.

Westerblad H & Allen DG (1992). Myoplasmic free Mg^{2+} concentration during repetitive stimulation of single muscle fibres from mouse skeletal muscle. *Journal of Physiology* (in press).