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Symposium: EC coupling and fatigue

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Chair: Gordon Lynch, Angela Dulhunty

Altered cellular Ca^{2+} handling in skeletal muscle fatigue

H. Westerblad, Department of Physiology & Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden.
(Introduced by G.D. Lamb)

Acute exercise results in impaired muscle function; that is, fatigue develops. The impaired muscle function in fatigue may be due to central (in the central nervous system) and/or peripheral (within the muscle) factors. My research group studies mechanisms of peripheral fatigue with a special interest in changes in sarcoplasmic reticulum (SR) Ca^{2+} handling. Experiments are performed on intact single muscle fibres obtained from mice and rats; all experiments are approved by the Stockholm North local ethics committee. Central fatigue is often assessed with the twitch interpolation technique, where electrical stimulation is superimposed on an ongoing voluntary contraction and the resulting change in force is measured. We have mimicked this stimulation pattern in single fibre experiments and observe a force increase that could be interpreted as central fatigue; the underlying principles will be discussed. SR Ca^{2+} release is decreased in later stages of fatigue. This decrease has been attributed to changes in energy metabolites but recent data indicate that it also depends on the phosphorylation status of proteins involved in the release mechanisms. Force recovery after fatiguing stimulation may be slow, especially at low stimulation frequencies. Recent data from our laboratory show that two different mechanisms can cause this prolonged force depression: decreased SR Ca^{2+} release and reduced myofibrillar Ca^{2+} sensitivity. The relative importance of these two mechanisms appears to depend on the production of different reactive oxygen species.

Importance of the t-system chloride conductance in muscle excitability and fatigue

G.D. Lamb, T.L. Dutka, R.M. Murphy and D.G. Stephenson, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

It is widely recognised that the chloride permeability of mammalian skeletal muscle fibres is important because if it is greatly reduced or absent muscles fibres readily display myotonia, an aberrant state in which the fibres generate their own action potentials (APs) and keep contracting without input from the central nervous system. The chloride conductance accounts for ~80% of the total resting conductance in mammalian muscle fibres, and there is some evidence that much of this resides in the transverse tubular (t-) system (Dulhunty, 1979). Currently, however, little is known about the importance, role(s) and molecular identity of t-system chloride conductance. This is a major issue because normal muscle function depends on excitation of the transverse-tubular (t-) system. It is frequently suggested the muscles readily fatigue with repeated activation owing to the build-up of K^+ in the t-system causing membrane depolarization and consequent failure of the action potentials and contraction, but the role of the t-system chloride conductance in this is not taken into consideration.

Here we present evidence that the chloride conductance in t-system is indeed high and that it plays a vital role in maintaining excitability during normal activity. It does this both by reducing the accumulation of potassium in the t-system and by opposing its depolarizing effects. This occurs because i) Cl^- ions carry part of the repolarizing current on each AP, reducing the extent of K^+ accumulation in the t-system, ii) the high relative Cl^- permeability of the t-system membrane strongly biases the membrane potential towards the Cl^- equilibrium potential, which is affected comparatively little by the ion concentration changes on each AP, and iii) K^+ accumulating in the t-system is likely driven back into the cytoplasm through the inward rectifier channels whenever the equilibrium potential for K^+ exceeds the membrane potential. The observed properties of the chloride conductance are consistent with it be meditated by ClC-1, which is putatively the dominant chloride channel in skeletal muscle, though frequently said to be only in the surface membrane and not in the t-system. We present Western blot analysis using antibodies to ClC-1 demonstrating that large amounts of ClC-1 channel proteins are indeed located inside muscle fibres.

Finally, if a fibre becomes depolarized after prolonged or vigorous activity, the t-system chloride conductance can be a hindrance to excitability because of its dampening effects on AP conduction. Intracellular acidification counters this effect by causing a partial reduction in the chloride conductance, thereby keeping the conductance in balance so that it maintains rather than antagonises t-system excitability (Pedersen *et al.*, 2004).

Dulhunty AF. (1979) *Journal Membrrane Biology*, **45:** 293-310.
Pedersen TH, Nielsen OB, Lamb GD & Stephenson DG (2004) *Science*, **305:** 1144-7.

Control of skeletal RyR channels by proteins in the SR lumen

N.A. Beard, L. Wei and A.F. Dulhunty, Muscle Research Group, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia.

Excitation contraction (EC) coupling is the mechanism linking depolarization of the surface membrane with Ca^{2+} release from the sarcoplasmic reticulum (SR) and is the process which initiates skeletal and cardiac muscle contraction. Ca^{2+} release during EC coupling is triggered by a transverse-tubule action potential. The SR Ca^{2+} release channel the ryanodine receptor (RyR) - has recruited a surface membrane Ca^{2+} channel (the dihydropyridine receptor) as a voltage sensor to detect the action potential, and Ca^{2+} binding protein calsequestrin (CSQ) to sense the environment inside the SR. Both the skeletal (RyR1) and cardiac (RyR2) isoforms of the RyR form the hub of giant macromolecular complexes with transmembrane, luminal and cytoplasmic proteins. The activity of the channels is set by the integrated effects of associated proteins, ligands, covalent modification by redox reactions and phosphorylation. Luminal interactions, such as that with CSQ, are essential for communicating the $[Ca^{2+}]$ in the store to the RyR, but are not well understood. We have identified several key luminal mechanisms which control RyR1 activity and communication of store load to the RyR. CSQ regulates the RyR1 via two distinct processes, physically coupling either directly to the RyR or to the channel via anchoring proteins triadin and junctin. CSQ can inhibit native RyR1s (via triadin and junctin) under physiological Ca^{2+} conditions (1 mM), but it directly activates the purified RyR1. Additionally, we have found that CSQ regulation of RyR1 channel activity allows CSQ to communicate the SR store Ca^{2+} load to the RyR in a phosphorylation-dependent manner. Phosphorylation of CSQ is important not only for RyR1 regulation, but it also influences the Ca^{2+} binding capacity of CSQ (which in turn, influences the size of the SR Ca^{2+} store). We have defined junctin as the key anchoring protein required in mediating the inhibition imposed by CSQ on the RyR1. In addition, we have shown that CSQ is able to regulate the way in which RyR1 responds to changes in luminal Ca^{2+} concentration. CSQ is now defined as a luminal Ca^{2+} sensor for RyR1. These different mechanisms of CSQ regulation of the RyR1 allow for a complex regulation system that is sensitive to changes in the cellular environment. The formation of a luminal protein complex between triadin, junctin, CSQ and the RyR1 allows enhancement of Ca^{2+} release from the SR when the store is fully loaded but protects the SR from Ca^{2+} depletion when the Ca^{2+} load falls. The results of current research highlight the complex nature of luminal regulation of the RyR1 and the importance of luminal SR proteins in maintaining store load and in controlling processes leading to muscle contraction.

Molecular recognition of the disordered dihydropyridine receptor II-III loop by a conserved domain in the type 1 ryanodine receptor

Y. Cui, H-S. Tae, N.C. Norris, Y. Karunasekara, P. Pouliquin, P.G. Board, A.F. Dulhunty and M.G. Casarotto , John Curtin School of Medical Research, Australian National University, ACT 0200, Australia.

Conformational coupling between the dihydropyridine receptor (DHPR) and the skeletal ryanodine receptor (RyR1) is essential for excitation-contraction (EC) coupling in skeletal muscle. The II-III loop of the DHPR α_{1s} subunit is central to this coupling process, but neither its structure nor its mode of binding to RyR1 are known. We have investigated the NMR-derived structure of the α_{1s} II-III loop and its binding to a region of the skeletal RyR1. We find that the II-III loop is highly flexible, with a strong N-terminal helix followed by several nascent helical/turn elements and unstructured segments, but it possesses no stable tertiary fold. The II-III loop thus belongs to a burgeoning class of functionally important, intrinsically unstructured, proteins. We have mapped the area of II-III loop interaction with RyR1 as a SPRY domain (1085-1208) and have identified which regions of II-III loop that are directly involved in binding by NMR methods. The principle site of interaction is through the N-terminal helix (A region, 671-690) but the B region (691-723) is also found to participate in binding. Confirmation that the A region of the II-III loop is indeed involved in SPRY2 binding is demonstrated through a series of mutations that clearly implicate a stretch of basic residues (R^{681}-K^{685}) as an important structural determinant. Evidence was found suggesting that there are weaker interactions between the C region of the loop and SPRY2. We propose that the flexible nature of the II-III loop is required for segments of the loop to associate and disassociate with RyR1 when the surface membrane potential changes, so that the loop may act as a conformational switch in EC coupling.