Role of ClC-1 Cl^- ion channels for skeletal muscle function in health and disease

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Skeletal muscle activation requires that muscle fibres respond appropriately to neuronal commands. This entails that every action potential that arrives at a motor nerve terminal is transmitted to a corresponding muscle fibre action potential. Once excited at the neuromuscular junction the action potential must propagate along the sarcolemma membrane of the fibre while exciting action potentials in the t-tubular network systems that it encounters along its propagation path. Failure to excite or propagate action potentials will compromise contractile activity and introduce task failure (fatigue). In contrast, in hyper-excitable muscle one nerve action potential can give rise to more than one muscle fibre action potential, and this underlies the delayed muscle relaxation and loss of coordination that is observed in patients with myotonia congenita.

ClC-1 Cl^- ion channels are muscle specific ion channels that carry inhibitory ionic current that counteracts action potential excitation and propagation. While the role of ClC-1 for myotonia is well known from myotonia congenita patients, the normal physiological role of ClC-1 has been less clear. Over the last decade the understanding of the physiological role of ClC-1 for determining muscle excitability in active muscle has however improved substantially. Contributing to this are studies performed with different approaches and in several different laboratories.

Using expression systems it has been shown that adenosine nucleotides bind to ClC-1 ion channels and effectively reduces the number of active channels at membrane potentials that correspond to the membrane potential in resting muscle fibres (Bennetts *et al.*, 2005). While ATP, ADP and AMP all appear to have this inhibiting effect on ClC-1 channels, a corresponding inhibition of IMP does not exist. Also, the inhibition of adenosine nucleotides is enhanced at acidic pH (Bennetts *et al.*, 2007), and it can be completely lost with oxidation (Zhang *et al.*, 2008). These studies suggest that ClC-1 could be functioning as a metabolic and redox sensor in muscle fibres .

Studies in both skinned muscle fibres and isolated muscle from rodents and human strongly support that ClC-1 function is regulated by metabolic and redox state in active muscle (Pedersen *et al.*, 2004; Pedersen *et al.*,2016). Thus, it has been demonstrated in slow- and fast-twitch muscle from mouse, rats and human that onset of repeated action potential firing induces a ClC-1 channel inhibition *via* acidification and protein kinase C activation. This initial inhibition contributes to maintaining muscle excitability when fibres fire action potentials repeatedly. With prolonged activity in fast-twitch muscle, this state of partial ClC-1 inhibition and well maintained excitability is interrupted by large and fully reversible ClC-1 activation (Pedersen *et al.*, 2009a,b). This ClC-1 activation is closely associated with depression of action potential amplitude and reduced Ca^{2+} release from the sarcoplasmic reticulum. As such the ClC-1 activation could be an important contributing factor to fatigue in working muscle by reducing muscle excitability and lowering the action potential amplitude. The ClC-1 activation during activity can be greatly accelerated in fast-twitch muscle fibres and also made to develop in slow-twitch muscle when the metabolic state of the fibres is depressed through mild glycolytic inhibition or exposure to oxidizing agents.

Taken together it has become clear that regulation of ClC-1 Cl^- ion channels plays a central role for determining the excitability and function of working skeletal muscle. There is compelling experimental evidence that ClC-1 is regulated by both metabolic state and redox state. Such regulation of ClC-1 implement ClC-1 activation in disorders characterized by metabolic and/or oxidative stress but such a role of ClC-1 in disease has not yet been explored.

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