Calcium-dependent proteolysis of junctophilin-1 and junctophilin-2 in skeletal and cardiac muscle

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Excessive increases in intracellular [Ca^{2+}] in skeletal muscle fibres can cause prolonged muscle weakness by disrupting normal communication between the dihydropyridine receptors (DHPRs) in the transverse tubular (T-) system and the Ca^{2+} release channels (RyRs) in the sarcoplasmic reticulum (SR), but the exact basis of this effect is unknown. Our previous work suggested a possible role of Ca^{2+}-dependent proteolysis in this uncoupling process (Lamb *et al.*, 1995; Murphy *et al.*, 2006; Verburg *et al.*, 2009), but found no proteolysis of the DHPRs, RyRs or triadin. Junctophilin1 (JP1) (~90kDa) stabilizes close apposition of the T-system and SR membranes in adult skeletal muscle fibres; its C-terminal end is embedded in the SR and its N-terminal associates with the T-system membrane (Takeshima *et al.*, 2000). Junctophilin-2 (JP2) has an analogous role in cardiac cells. This study examined whether JP1 and JP2 undergo Ca^{2+}-dependent proteolysis, and whether this occurs in conjunction with the disruption of EC-coupling in Ca^{2+}-treated skinned fibres and in other intact muscle situations.

Extensor digitorum longus (EDL), *tibialis anterior* and diaphragm muscles were dissected from rats and mice killed by isoflurane overdose, and whole heart obtained from adult rats anaesthetized with sodium pentobarbitone (60mg/kg i.p.) and injected with sodium heparin (200IU) *via* the femoral vein. Depolarization-induced force responses were recorded in mechanically-skinned fibres of rat EDL muscle as previously described (Lamb *et al.*, 1995). Hearts were retrogradely perfused with oxygenated bicarbonate buffer in non-recirculating Langendorff mode at a constant pressure equivalent to 73mmHg. Left ventricular pressure measurements were performed using a fluid-filled balloon connected to a pressure transducer and recorded on a data acquisition system. Hearts were subjected to 20 min global ischemia (37˚C) and 60 min reperfusion. Control hearts were perfused aerobically throughout. Western blotting of proteins was performed as in Murphy *et al*. (2011).

Exposure of skeletal muscle homogenates to precisely set [Ca^{2+}] revealed that JP1 undergoes Ca^{2+}-dependent proteolysis over the physiological [Ca^{2+}] range in tandem with autolytic activation of endogenous μ-calpain. JP1 cleavage occurs close to the C-terminal, yielding a ~75kDa diffusible fragment and a fixed ~15kDa fragment. Depolarization-induced force responses in rat skinned fibres were abolished following 1min exposure to 40μM Ca^{2+}, with accompanying loss of full-length JP1. Supra-physiological stimulation of rat skeletal muscle *in vitro*, by repeated tetanic stimulation in 30mM caffeine, also produced marked proteolysis of JP1 (and not RyR1). In dystrophic *mdx* mice, there was marked proteolysis of JP1 in limb muscles at 4 and not at 10 weeks of age, and in diaphragm at >6 months of age. JP2 also underwent Ca^{2+}-dependent proteolysis and JP2 levels were reduced following cardiac ischaemia-reperfusion. It is concluded that junctophilin proteolysis may contribute to skeletal muscle weakness and cardiac dysfunction in a range of circumstances.

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