Effects of membrane cholesterol depletion on excitation-contraction coupling in mammalian skeletal muscle

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Cholesterol in the lipid bilayer of plasma membranes is present in relatively large proportions compared to the internal membranes of eukaryotic cells. Its presence affects the biophysical properties of the membrane-embedded proteins. An excellent membrane signaling system to study the effects of membrane cholesterol is excitation-contraction coupling (EC coupling). This involves transduction of electrical signals throughout the sarcolemma and its invagination into the cell, the tubular (t-) system and the transduction of this signal to internal sarcoplasmic reticulum (SR) membrane for initiation of Ca^{2+} release. Therefore for functional EC coupling in skeletal muscle, Cl^-, K^+, Na^+ and the voltage sensor must work at the level of surface membrane/t-system and Ca^{2+} release channels and Ca^{2+} pumps must work at the SR, two membranes with significantly different cholesterol contents. We aimed to assess the role of membrane cholesterol in these membranes by assessing the functionality of EC coupling after manipulating membrane cholesterol with methyl-β-cyclodextrin (MβCD) and saponin, agents that deplete membrane cholesterol and bind with membrane cholesterol and aggregate to form pores, respectively (Launikonis & Stephenson, 2001).

The Animal Ethics Committee at The University of Queensland approved the use of animals in this study. 2-3 mo old C57 mice and 2-3 mo old Wistar rats were killed by asphyxiation and the interossei and *extensor digitorum longus* (EDL) muscles were removed, respectively. Interossei fibres were transferred to a chamber with Na^+-based physiological solution (with or without Cl^-) and either impaled with a glass microelectrode to measure resting membrane potential (Vm) with a GeneClamp 500 amplifier, or enzymatically dissociated and loaded with fura-2AM for imaging cytoplasmic Ca^{2+} on a Nikon wide-field fluorescence microscope equipped with a camera during field stimulation at 10 Hz with a pulse strength of ~30 V/cm for 0.5 ms. Intact EDL fibre bundles were exposed to a Na^+-based physiological solution containing fluo-4 salt or fluorescein dextran. Fibres were mechanically skinned, trapping the dye in the t-system, and transferred to a chamber containing a K^+-based internal solution. In some experiments, skinned fibres were bathed in an internal solution with 20 μM fluo-4 and release of SR Ca^{2+} was evoked by exposure to 30 mM caffeine. Fluo-4 or fluorescein dextran fluorescence signals were imaged on an Olympus FV1000 confocal microscope.

Treatment of skinned fibres with 10 mM MβCD for up to 10 min did not result in significant loss of fluorescence signal from the t-system (n = 5). Treatment of skinned fibres with 1 mg/ml saponin caused a rapid decline in fluorescence from the t-system due to creating cholesterol-dependent pores in the t-system (n = 3). The saponin-induced loss of t-system trapped dye was progressively reduced after 2 and 10 min pretreatment of the fibre with 10 mM MβCD (n = 4 each), indicating significant cholesterol is removed from the t-system by MβCD, without affecting t-system membrane integrity. Exposure of 10 mM MβCD to isolated intact fibres caused the formation of pores at the myotendonus junction but not elsewhere in the preparation, as assessed by the loss of cytoplasmic fura-2. This may be a consequence of membrane weakening following collagenase treatment or a non-fluid nature of this section of membrane in these fibres. Assaying SR Ca^{2+} content with caffeine showed that 2 min treatment of the skinned fibre with 10 mM MβCD for 2 min reduced SR Ca^{2+} loading ability by about 60 ± 6 % (n = 10). In a normal physiological solution, resting Vm initially hyperpolarized and then slowly depolarized in the presence of 30 mM MβCD over an hour (n = 3). In a Cl^--free physiological solution, 30 mM MβCD caused a slow depolarization of the resting Vm over an hour (n=3). Resting Vm remained relatively stable for more than an hour in the absence of MβCD (n = 6). Electrical stimulation of isolated intact fibres loaded with fura-2 induced partially fused Ca^{2+} transients that initially were potentiated by 2 or 3 mM MβCD and then declined within 1.5 and 4 min, respectively. These results suggest that depleting membrane cholesterol affects the function of t-system Cl^- and K^+ channels. An effect of membrane cholesterol manipulation on the t-system Na^+ channel and voltage-sensor cannot be excluded.

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