Time resolved depression of isometric force by Mavacamten in single myofibrils from rabbit psoas and human cardiac muscle

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Mavacamtem (MYK-641, Axon Medchem BV) is a promising small molecule designed to act as allosteric inhibitor of sarcomeric myosins and presently used in preclinical/clinical trials for HCM treatment (Anderson et al., 2018)). Studies of the effects of ligands on the force generation mechanism in intact or skinned striated muscle fibres are complicated by diffusional barriers. These limitations can be overcome by the use of single myofibrils submitted to perturbations of the contractile environment by sudden solution changes (Tesi et al., 2000). Here, single myofibrils or thin bundles of myofibrils from rabbit fast skeletal muscle (psoas) and human donor ventricle (frozen biopsies) have been used to study the effects of μmolar Mavacamtem on maximal isometric force. Both myofibril types were mounted in relaxing solution (pCa 9; [Pi] ~ 200 μM, 15 °C,) and then fully activated (pCa 3.5) by fast alternation of perfusing fluxes. Myofibrils were then suddenly moved to and from a second flux of activating solution containing selected concentrations of Mavacamtem ("jumps"; solution change ~ 10 ms). Relaxation of force was achieved by returning myofibrils to the relaxing solution. When submitted to Mavacamten jumps, both myofibril types responded with a rapid, relaxation-like force drop. The effect was fully reversible but with significantly slower kinetics than that of force development. Dose–response curves confirmed a higher sensitivity of cardiac muscle (IC50 ~ 0.5 μM) compared to fast skeletal (IC50 ~ 5 μM), as previously reported for pCa50-activated ATPase of the same myofibrillar systems (Kawas et al., 2017). Interestingly, Mavacamtem also decreased the rate of force development, in agreement with the reported inhibition of Pi release rate but also with a possible effect on the regulation state mediated by the availability of strongly actin binding heads. SilicoFCM grant agreement n. 777204.

Session II: Muscle: close-up

Locating titin on the muscle thick filament

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The complex sequence of titin reflects features of the structure and function of the vertebrate striated muscle sarcomere. The C-terminal ~ 200 immunoglobulin (Ig) and fibronectin (Fn) domains have been associated with the thick filament from the tip to the centre, however the precise axial register of titin on the thick filament has not been determined. To do this we have characterised several titin antibodies and determined their positions on the thick filament using super resolution microscopies, STORM and STED. These data plotted together with published information show that the antibody positions are linearly related to domain number in the crossbridge region of the A-band, where the data is in excellent agreement with the regression line ($R^2 = 0.999$). The slope of the line is 4 nm per domain, the expected span of an Ig or Fn domain. This is also in agreement with the assumption that 11 titin super repeats (CSR) of 11 domains (Ig-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn) are related to the disposition of the accessory protein, myosin binding protein C (MyBP-C), on 9 stripes at ~ 43 nm intervals in each half of the thick filament. Using the known position of MyBP-C stripes establishes that the furthest MyBP-C position from the M-band coincides with the end of the second CSR (domains 8–10). The 9 MyBP-C stripes are therefore related to the ends of CSRs 2–10. This is consistent with a recent publication in which the first two CSRs were deleted with the loss of only one MyBP-C stripe. Proximal to the MyBP-C positions are two stripes of unknown origin, one of which is located at the edge of the bare zone. We find the last Fn domain close to this position, refining the start of the titin bare zone sequence. Furthermore, titin kinase neighbours this Fn domain, suggesting that it may contribute to the stripe 1 density.

Localisation of individual nebulin molecules in sarcomeres of Nebulin-Dendra2-KI mice

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Nebulin spans the length of the thin filament, with its C-terminus located in the z-disc and its N-terminus near the thin filament pointed-end. Genetic mutations in nebulin, as well as in proteins that bind to nebulin cause myopathy. The pathophysiological mechanisms are incompletely understood, in part because of a lack of tools to precisely localize nebulin on the thin filament. We localized nebulin N-termini using a mouse with photoconvertable Dendra2 inserted at the nebulin N-terminus (Dendra2-KI).

Individual nebulin molecules were visualized by photoactivated localization microscopy (PALM). In PALM, Dendra2 switches between fluorescent states so that in every snapshot, only a small, optically resolvable fraction of Dendra2 is detected. Single FDB fibers of Dendra2-KI mice were isolated and imaged with PALM in TIRF mode. The Dendra2 blinks were background subtracted and Gaussian fitted with a reconstruction resolution of 10 nm. Dendra2 blinks were fitted with a precision of 10–15 nm. Individual nebulin N-termini were normally distributed within the sarcomere, with a width at half-maximum of ~ 120 nm. The average position of the nebulin N-terminus molecules was 1.1 um from the Z-disk. Based on 908,078 localizations, nearly 40% of the theoretical number of nebulin molecules in the sarcomere was detected, assuming a lattice spacing of 35 nm and a hexagonal distribution of thin filaments with 2 nebulin molecules per thin filament. Leiomodin3 (Lmod3) is a thin filament associated molecule, and mutations in LMOD3 cause myopathy. Pilot experiments in Lmod3-KO x nebulin-Dendra2-KI mice (Lmod3-KO Dendra2-KI) show less nebulin molecules, with a wider distribution of nebulin N-termini, and with the average position closer to the z-disc. Thus, the nebulin-Dendra2 mouse model is a useful tool to localize individual nebulin molecules in the muscle's sarcomere.

Super-resolution 3D mapping of site-specific phosphorylation signatures of ryanodine receptors in the healthy and failing heart

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The primary intracellular calcium release mechanism in cardiac muscle is dependent on the tightly clustered ryanodine receptors (RyRs), found in compact signalling domains called couplons. Classical super-resolution microscopy methods (e.g. dSTORM, PALM) have previously been used to visualise RyR clusters, however