AuPS/ASB Meeting - Adelaide 2010

Free communications: Calcium signalling in muscle

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Chair: Nicole Beard

SPontaneous Oscillatory Contraction (SPOC): Quantifying contractile performance in isolated human cardiomyocytes under partial activation

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Under conditions of partial activation, striated muscle fibres exhibit repetitive, cyclic auto-oscillation between rapid-lengthening (relaxation) and slow-shortening (contraction) phases. This phenomenon is termed SPontaneous Oscillatory Contraction (SPOC), and represents a third state of muscle that exists intermediate to contraction and relaxation. The cardiac SPOC period and shortening velocity have been correlated with heart rate in various animals. Thus, SPOC is likely to reflect the physiology of the heart as it functioned in life. Small bundles of skinned, immobilised human cardiomyocytes suspended by adhesive tape were exposed to precise ionic conditions to induce SPOC, and recorded at high spatial and temporal resolution using live cell microscopy. Quantitative analysis allows us to draw conclusions about how the SPOC parameters, including total SPOC period and rates of shortening and lengthening, change with age in non-failing human heart samples, from 3 weeks to 65 years. Further, we look at SPOC as a technique for demonstrating and quantifying a functional defect in cardiomyectomy samples from patients diagnosed with hypertrophic cardiomyopathy (HCM), where a causal genetic mutation has been identified. SPOC might be applied in future as a tool to assist with the diagnosis and risk stratification of HCM patients.

The effects of membrane potential and cytoplasmic calcium concentration on calcium extrusion across the tubular system in mammalian skeletal muscle

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The regulation of cytoplasmic Ca^{2+} is essential in the maintenance of skeletal muscle function and survival. This is achieved by the combined activities of ion transporters such as SERCA, NCX, PMCA, found embedded within the sarcoplasmic reticulum (SR) and plasma membranes. Upon Ca^{2+} release from the SR, $[Ca^{2+}]_{cyto}$ can globally reach ~2μM and it is during such an event that highlights Ca^{2+} extrusion is an essential regulatory mechanism, as this is ~20 times the resting $[Ca^{2+}]_{cyto}$. We have shown that during cell wide Ca^{2+} release events, there is an initial increase in t-system (extracellular) Ca^{2+}, followed by a net decline due to the activation of a store-operated calcium entry (SOCE) current. Although the SOCE current has recently generated significant levels of interest, information on the rates of t-system Ca^{2+} uptake (cytoplasmic Ca^{2+} extrusion) remains limited. Therefore, we aimed to characterise the effects of membrane potential and $[Ca^{2+}]_{cyto}$ on the rate of cytoplasmic Ca^{2+} extrusion in mammalian skeletal muscle.

Wistar rats were killed by asphyxiation in accordance to the guidelines set by the Animal Ethics Committee of the University of Queensland. *Extensor digitorum longus* and *soleus* muscles were rapidly excised, pinned out and fully immersed in paraffin oil. Small bundles of intact fibres were isolated and exposed to a Na^{+}-based physiological solution containing the fluorescent dye, fluo-5N salt. Single fibres were then isolated and mechanically skinned (resulting in the trapping the dye in the t-system) and transferred to a chamber containing a K^{+} or Na^{+} -based internal solution to set the membrane potential. A ‘release solution’ with low Mg^{2+}, 5mM BAPTA and 5mM caffeine was used to chronically deplete sarcoplasmic reticulum Ca^{2+} stores and activate SOCE. T-system fluo-5N fluorescence was imaged on an Olympus FV1000 confocal microscope in either xy or xyt mode in polarized and depolarized fibres with known cytoplasmic Ca^{2+} concentrations, at rest or during SR Ca^{2+} release. The net change in t-system fluo-5N signal was used as an indicator of Ca^{2+} movements across the t-system.

Fluo 5N trapped in the sealed t-system was calibrated (*in situ*) and in the presence of different concentrations of bovine serum albumin. Following low $[Ca^{2+}]_{t\text{-}system}$ (achieved by chronic activation of SOCE with ‘release solution’) the t-system could be reloaded with internal solutions containing 1 mM EGTA (either 100, 200 or 800 nM free Ca^{2+}). This rate of uptake was markedly greater in depolarized cells (Na+ based solutions) compared to polarized cells (K^{+} based solutions), most likely due to an increased driving force for Ca^{2+} to exit the cell. Interestingly, vacuoles were seen in some fibres. Vacuoles retained fluo 5N for > 20mins in the presence of a ‘release solution’ whereas a fluorescence signal from transverse tubules was rapidly lost. We have also measured for the first time, a SOCE flux and t-system uptake in slow-twitch fibres from the *soleus* muscle which are currently being explored.

The effect of suramin (a calmodulin antagonist) on caffeine-induced Ca^{2+}-release in mechanically skinned fast-twitch skeletal muscle fibres of the rat

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Calmodulin (CaM) is a ubiquitous, multifunctional calcium binding protein, which binds to the ryanodine receptor (RyR1) in skeletal muscle (Tripathy *et al.*, 1995). Ca^{2+}-free CaM binds to RYR1 and increases RYR1s affinity for calcium, while Ca^{2+}-bound CaM inhibits RYR1s affinity for calcium (Rodney *et al.*, 2000). The precise role of CaM in the regulation of calcium release in physiological preparations remains unknown. Suramin is a broad acting CaM antagonist known to displace bound CaM from RyRs (Sigalas *et al.*, 2009). In this study we investigated the effects of suramin on Ca^{2+} release from the sarcoplasmic reticulum (SR) in freshly mechanically skinned muscle fibres where the SR is intact under conditions where the suramin treatment did not affect the ability of the contractile apparatus to develop force.

All experiments conducted were approved by the La Trobe University Animal Ethics committee. Rats were killed by an overdose of isoflurane (4% volume: volume) and whole EDL muscles were removed and pinned out on a bed of Sylgard at resting length under paraffin oil. Single fibres were then isolated under a dissecting microscope, mechanically skinned, and mounted between a pair of forceps and a sensitive force transducer. After mounting to the force recording apparatus, fibres were bathed in a K-based solution that mimics the normal intracellular environment with respect to pH (7.10); monovalent ions (137 mM); Mg^{2+} (1 mM); divalent cations (60 mM); total ATP (8 mM); osmolality (295 mOsm/L); [Ca^{2+}] ~100 nM. The relative SR Ca-content and ease of Ca^{2+} moving through the RyR1s can be estimated from the force response following the transfer of the fibre to a SR-Ca^{2+}-depleting solution containing caffeine (30 mM) and low [Mg^{2+}] (50 μM) (Lamb & Stephenson, 1991). A similar area under the caffeine-induced force response is indicative of similar SR Ca-content and a faster rate of rise of the caffeine-induced force and/or a greater force peak (for same SR Ca-content) indicate a greater SR Ca^{2+}-efflux through the RyR1. Fibres were repeatedly bathed in a SR Ca-loading solution ([Ca^{2+}] ~300 nM) for increasing amounts of time (10-120 s) to reload the SR Ca to various levels and then the SR Ca^{2+} was fully released in the SR-Ca-depleting solution . Fibres were randomly divided into two groups (control and test) then exposed for 2 min to 100 μM suramin (test) or exposed to a like solution *sans* suramin (control). After the suramin/*sans* suramin treatment, the fibres were washed for 10 min and the SR Ca-loading/caffeine release protocol was repeated for both groups. Suramin treatment resulted in a marked reduction in the caffeine induced rate of rise (time between 20 and 80% of peak caffeine response decreased by 25.9 ± 7.1% (S.E.M.) of control ($p < 0.01$) at 30 s load without changing the area under the caffeine-induced force responses ($p > 0.1$). Suramin also increased the peak force of submaximal caffeine responses by 20.70 ± 5.99 % of maximum force for a 30 s load (n=4). Thus, the results show that treatment with 100 μM suramin for 30 s increases the SR Ca^{2+}-efflux through the RyR1, which is consistent with suramin removing the CaM from RyRs on the intact SR.

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