SA1

Calcium sparks in frog and dedifferentiating mammalian skeletal muscle fibres

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Activation of a skeletal muscle fibre is initiated by electrical depolarization of its transverse tubules (TTs), which penetrate the fibre at each sarcomere. Membrane voltage sensors within the TT dihydropyridine receptor (DHPR) molecules in the TT membrane respond to TT depolarization, and trigger sarcoplasmic reticulum (SR) Ca^{2+} release via the abutting ryanodine receptor (RyR) Ca^{2+} release channels in the adjacent SR membrane. In *frog* muscle, the 'macroscopic' Ca^{2+} release caused by fibre depolarization has been shown by us and others to be composed of huge numbers of discrete local Ca^{2+} release events, the Ca^{2+} sparks, which blend together to form the macroscopic Ca^{2+} transient. Ca^{2+} sparks also occur spontaneously in resting frog fibres, but at much lower frequencies. We have used ultra high speed confocal line scan imaging of fluo-4 fluorescence in frog fibres slightly depolarized by exposure to elevated K^+ Ringer solution in an attempt to characterize the gating properties of the few channels that underlie each voltage-activated Ca^{2+} spark, and to determine whether the group of channels always gates in unison, or may sometimes gate independently during a spark, as indicated by a change in the rate of rise of fluorescence prior to the final exponential decay of spark fluorescence. In *mouse* muscle, Ca^{2+} sparks similar to those in frog muscle are extremely infrequent in resting adult muscle, but are present in embryonic muscle and may appear under abnormal or pathological conditions, possibly due to DHPR-RyR uncoupling. We have found that Ca^{2+} sparks reappear during dedifferentiation of adult mouse flexor digitorum brevis muscle fibres in culture, and that the sparks that appear are blocked by the plasma membrane (PM) and TT L-type Ca^{2+} channel blocker nifedipine, as well as by the non-specific PM and TT Ca^{2+} channel blocker Co^{2+}. These agents were previously shown to block Ca^{2+} sparks in embryonic muscle. Thus the Ca^{2+} sparks that appear during fibre dedifferentiation may represent a reversal of the embryionic muscle development/maturation process. Adult mammalian fibres express predominantly only the RyR1 isoform, which is present in an alternating checkerboard pattern of RyRs that are coupled and not coupled to DHPRs. Frog fibres express similar amounts of both the homologs of RyR1 (in an alternating coupled pattern) and of RyR3 (all uncoupled), as does embryionic mammalian muscle. Arrays of uncoupled RyRs may be needed for production of frog-like Ca^{2+} sparks in either frog or mammalian muscle.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA2

Regulation of calcium signalling from within the cellular store. New methods and surprises

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The functional cycle of striated muscles requires a fast contraction followed by full relaxation, which demand in turn a fast activation of Ca^{2+} release channels followed by their rapid closing. At the cell-wide level, this is manifested by a rapidly increasing Ca^{2+} release flux in response to cell membrane depolarization, followed by its spontaneous decay even if the depolarizing stimulus is maintained. At the local level of Ca^{2+} sparks, the changes in flux correspond to synchronized opening and closing of channel clusters. The termination of flux in Ca^{2+} sparks thus represents at the local level the physiologically relevant process of termination of Ca^{2+} release. While a growing body of evidence points at depletion of SR-lumenal Ca^{2+} as an agent of Ca^{2+} spark termination in the heart, the mechanism of termination remains unknown in skeletal muscle. We will report on advances in methods to study the Ca^{2+} store of skeletal muscle and the effects of its depletion, using the more developed studies in the cardiac field as a term of comparison. Global estimates of depletion upon release elicited by an action potential range between 5 and 20% in skeletal muscle, while in cardiac muscle they reach easily 40%. Estimates of local depletion after a spark are of less than 7% in skeletal muscle, but close to 50% in the heart. Frequency and morphology of sparks in skeletal muscle depend weakly on SR $[Ca^{2+}]$, while in cardiac ventricular muscle an increase in SR $[Ca^{2+}]$ is believed to strongly promote Ca^{2+} release and its decrease to terminate Ca^{2+} release. In sum, there is no evidence of a role of depletion in termination of Ca^{2+} release and sparks in skeletal muscle, in striking contrast with the wealth of support for this role in the heart. In spite of this missing control, sparks are briefer and more stereotyped in skeletal than cardiac muscle, suggesting the presence of an alternative mechanism. We will present preliminary data exploring two possibilities: (1) the inactivation of channels by released Ca^{2+} and (2) a gating role of the intra-SR protein calsequestrin, suggested - among other indications - by the surprising time course of intra-SR Ca^{2+} depletion during a Ca^{2+} spark.

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SA3

The effect of a myocardial infarction on the electrical activity of the epicardial surface of isolated rabbit hearts

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The pattern of electrical activation of the ventricle of the heart determines the efficiency of ventricular function and the elec-

trical stability of the heart. The pattern of epicardial activation was examined in rabbit hearts with large transmural apical infarcts. The hearts, isolated from adult male New Zealand rabbits under terminal pentobarbitone anaesthesia, were Langendorff-perfused with Tyrode solution. Hearts were isolated 8 weeks after coronary artery ligation performed under halothane anaesthesia. Membrane voltage from the epicardial surface of the left ventricle (LV) including the infarct was monitored using the voltage-sensitive dye RH237. Optical action potentials were detected from the epicardial surface of the infarct; the signal amplitude was ~20% of those in the non-infarcted zone (NZ). Epicardial activation mapping of the mid-region of the LV free wall, including the infarct, showed that during right atrial (RA) or LV endocardial pacing, the activation sequence was not significantly different between infarcted and sham-operated groups. Direct stimulation of the epicardium in the NZ revealed an area of slow conduction velocity (CV ~5 cm s^{-1}, ~10% of normal values) at the margin of the infarct zone (IZ). Within the IZ, CV was ~50% of normal. Epicardial action potential rise time was longer in the IZ (23.4±2.1 vs. 42.4±1.2 ms, $p<0.05$) and repolarization interval was shorter (88.9±3.7 vs. 73.0±4.4 ms, $p < 0.05$). Premature stimuli applied to NZ during RA pacing produced regions of very slow conduction (2-3 cm s^{-1}) at the margins of the IZ. Chemical ablation of the endocardium of the LV free wall (including the infarct) dramatically altered the epicardial activation pattern on RA pacing, but the pattern during epicardial stimulation was not significantly altered. In conclusion, the presence of a scar did not disrupt the ventricular epicardial activation pattern during RA or endocardial pacing. Regions of slow conduction were seen in the margin of the IZ during epicardial pacing or with epicardial extra stimuli. Optical action potentials were recordable from the epicardial surface of the transmural infarct scar. These signals were not altered by endocardial ablation. Therefore remnant groups of myocytes in the ventricular wall may support electrical activity even in a transmural scar.

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SA4

Novel regulators of RyR Ca^{2+} release channels: insight into molecular changes in genetically linked myopathies

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There are many mutations in the ryanodine receptor (RyR) Ca^{2+} release channel that are implicated in skeletal muscle disorders and cardiac arrhythmias. More than 80 mutations in RyR1 have been identified and linked to malignant hyperthermia, central core disease or multi-minicore disease, while more than 40 mutations in RyR2 lead to ventricular arrhythmias and sudden cardiac death. These RyR mutations cause diverse changes in RyR activity which either excessively activate or block the channel in a manner that disrupts Ca^{2+} signalling. There are two regions of RyR1 that are variably spiced and developmentally regulated (ASI and ASII). In myotonic dystrophy (DM), the less active juvenile isoform of the skeletal RyR, ASI(-), is preferentially expressed in adults and may contribute to functional changes in the dystrophic muscle. Finally, mutations in an important regulator of the RyR, the Ca^{2+} binding protein calsequestrin (CSQ), have been linked to a disruption of Ca^{2+} homeostasis in cardiac myocytes that results in arrhythmias. We discuss evidence supporting the hypothesis that mutations in each of these situations alter protein-protein interactions within the RyR complex or between the RyR and its associated proteins. The disruption of these protein-protein interactions can lead either to excess Ca^{2+} release or reduced Ca^{2+} release and abnormal Ca^{2+} homeostasis. Much of the evidence for disruption of protein-protein interactions has been provided by the actions of a group of novel RyR regulators, domain peptides with sequences that correspond to sequences within the RyR and which compete with the endogenous residues for their interaction sites.

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SA5

Tubular system excitability: an essential component of excitation-contraction coupling in fast-twitch fibres of vertebrate skeletal muscle

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The tubular (t-) system is the main interface between the myoplasm and the extracellular environment and is responsible for the rapid inward spread of excitation from the sarcolemma to the inner parts of the skeletal muscle fibre as well as for signal transfer to the sarcoplasmic reticulum to release Ca^{2+} that, in turn, activates the contractile apparatus. In this presentation I explore the insights provided by the mechanically skinned muscle fibre preparation to the better understanding of the importance of the t-system excitability in determining the force response under physiologically relevant conditions. In the mechanically skinned muscle fibre, the t-system seals off after it is physically separated from the sarcolemma and its excitability can be investigated by electrical stimulation under controlled conditions. Parameters that can be assessed include the threshold for action potential generation, specific electrical resistance and time constant of the tubular wall, quantity of charge transferred during an action potential, refractory period, length constant and velocity of excitation propagation. Results obtained with mechanically skinned fibres from fast-twitch muscles show that decreased t-system excitability does not necessarily translate into reduced force output, but for any particular set of physiologically relevant conditions there is a level below which a further decrease in t-system excitability markedly decreases the force output. There are several built-in mechanisms linked to the metabolic/energetic state of the muscle fibre which prevent complete action potential failure in the t-system, thus allowing the muscle to respond to nerve stimulation, even if the response becomes markedly attenuated.

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SA6

Compartmentalized cAMP-PKA signalling regulates cardiac excitation-contraction coupling

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The sympathetic control over excitation-contraction coupling (ECC) is mediated by the cAMP-PKA signalling pathway. It is becoming increasingly evident that the compartmentalization of the pathway components within specific regions of the cell is pivotal to the specific signalling of the cAMP/PKA transduction system (Tasken & Aandahl, 2004). Very little is known, however, on the intracellular dynamics of cAMP and on how such a freely diffusible second messenger can transduce localized signals and mediate specific responses. By using a FRET-based real-time imaging approach (Zaccolo et al. 2000), we are studying the spatio-temporal dynamics of cAMP in isolated intact living rat cardiac myocytes. We were able to directly visualize microdomains of high cAMP in neonatal myocytes stimulated with catecholamine (Zaccolo & Pozzan, 2002). We found that PDEs have a key role in shaping such intracellular steep gradients. In particular, PDE4 (Mongillo et al. 2004)and PDE2 (Mongillo et al. 2006), rather than PDE3, are responsible for modulating the amplitude and duration of the cAMP response to beta-agonists and the consequent effect on the strength of myocyte contraction. Consistent with their distinct function, we found that PDE3 and PDE2/PDE4 localize to different subcellular compartments.

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