In coupled regions, TF50 occurred within 18 ms, 17 ms and 12 ms, respectively. Whole-cell voltage clamp was subsequently used to elicit Ca\(^{2+}\) sparks. In isolated pig ventricular myocytes consecutive vertical confocal images were taken after labeling with wheat germ agglutinin-Alexa594. After image processing, the 3-D TT geometry was assessed using Euclidean distance mapping. ATP (PO\(_{4}\)) at intermediate [Ca\(^{2+}\)] was recorded in a confocal line scan (0.5 micron pixel-1, 649 Hz). The temporal mid-point of the local Ca\(^{2+}\) transient upstroke (T\(_{50}\)) was used to assess latency of release. Correlation of the T\(_{50}\) for each site with distance to TT in 3-D yielded a linear relationship for distance between 0.5 and 3 \(\mu\)m from T-tubules. This allowed the use of T\(_{50}\) to map subcellular Ca\(^{2+}\) release regions as coupled (< 0.5 \(\mu\)m) and uncoupled (> 2 \(\mu\)m). In coupled regions, T\(_{50}\) occurred within 18 ms, 17 ms and 12 ms, respectively for 0.5, 1 and 2 Hz. This resulted in 56% (0.5 Hz), 57% (1 Hz) and 49% (2 Hz) of coupled regions. Spark frequency increased with stimulation frequency in coupled release sites but significantly less so in uncoupled ones. In the presence of the F-actin disrupter Cytochalasin D, this increase in spark frequency was abolished and there was no difference between coupled and uncoupled sites. Therefore, the regional discrimination of RyR re-veals a preferential activation of coupled RyRs with frequency, which is dependent on a cytoskeletal interaction.

Control of Diastolic Activity of the RyR2 Channel by Luminal Calcium and ATP
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The mechanism of activation of the cardiac ryanodine receptor (RyR2) by luminal Ca\(^{2+}\) in the presence of ATP was examined in planar lipid bilayers. The dose response of the RyR2 channel to ATP was characterized at a range of cytosolic (100-400 nM) and luminal (0.01 - 53 mM) Ca\(^{2+}\) concentrations. Luminal Ca\(^{2+}\) markedly increased the maximal open probability in the presence of ATP (P\(_{0}\)max) and markedly decreased EC\(_{50}\) for ATP (EC\(_{50}\)ATP). Cytosolic Ca\(^{2+}\), without substantially activating the RyR2 channel in the absence of ATP, greatly amplified the effects of 1 mM [Ca\(^{2+}\)]\(_L\) on P\(_{0}\)max and EC\(_{50}\)ATP. An allosteric model of RyR2 interaction with ATP showed that the increase in RyR2 open probability by luminal Ca\(^{2+}\) in the presence of ATP is induced by a decrease of the ATP dissociation constant (K\(_{\text{ATP}}\)) at low [Ca\(^{2+}\)]\(_L\), by an increase in the positive allosteric effect of ATP on channel opening (decrease of f\(_{\text{ATP}}\)) at intermediate [Ca\(^{2+}\)]\(_L\), and by an increase in the stability of the ATP-free RyR2 open state (decrease of K\(_{\text{ATP}}\)) at the highest [Ca\(^{2+}\)]\(_L\). Increasing [Ca\(^{2+}\)]\(_L\) did not affect K\(_{\text{ATP}}\) but led to a parallel decrease of K\(_{\text{ATP}}\) and f\(_{\text{ATP}}\). These results suggest that the allosteric effect of ATP might be mediated by the energy of the ATP-TT open state, i.e., indirectly by the effect of the occupancy of the channel by Ca\(^{2+}\) at the cytosolic and luminal sites on the stability of the open state. The increase of RyR2 open probability at diastolic levels of cytosolic Ca\(^{2+}\) by elevated luminal Ca\(^{2+}\) may play a role in the calcium over-load induced Ca\(^{2+}\) release.

Supported by APVV-0441-09, APVV-0721-10, VEGA 2/0118/09 and VEGA 2/0197/11.

References

Ryanodine Receptor Recruitment and Construction of Calcium Release Sites in Cardiac Myocytes

Local character of calcium release in cardiac myocytes implies independent recruitment of calcium release units by triggering stimuli. Calcium release from individual CRUs also displays quantal character, interpreted either as recruitment of small cohorts of independent RyRs (1, 2), or as recruitment of small cohorts of RYR clusters with coupled RYRs gating (3). We tested both interpretations on published experimental data using a model of virtual calcium release units (vCRUs) consisting of 1-10 clusters that were constructed in accordance with the experimentally observed cluster size distribution (4). If RyR gating was independent, vCRUs consisting of 3 or more clusters provided a good agreement between the model and experimental data (1). If RyR gating was coupled, the calcium release flux of vCRUs did not display quantal structure that would correspond to in situ observations. The model of RyR gating (2) combined with the model of independent RyRs in vCRUs (2) matched the experimentally observed calcium dependence of calcium spark frequency (5) under all conditions. However, the Mg\(^{2+}\)-binding parameters of RyRs were in accordance with the single-channel observations (6) only for vCRUs composed of 3 or more clusters. In conclusion, these results favor independent over fully coupled RyR gating in situ, and predict the presence of at least 40 RyRs per cardiac release unit.

Supported by grants APVV-0441-09, APVV-0721-10, VEGA 2/0190/10, 2/0203/11, and 2/0197/11.

References

Exocytosis & Endocytosis

Properties of the Weibel-Palade Body Fusion Pore
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Weibel-Palade bodies (WPBs) are regulated secretory organelles found in endothelial cells (ECs). The major WPB constituent is the haemostatic