Submicroscopic cAMP/PKA compartmentalization: Ion flux at the cardiomyocyte plasmalemma

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In the heart cyclic AMP-dependent Protein Kinase A (PKA) mediates the catecholaminergic control over the force and frequency of cardiac contraction via phosphorylation of proteins that are involved in excitation-contraction coupling (ECC). As part of the ECC machinery, catecholamine dependent activation of PKA leads to phosphorylation of the L-type Ca^{2+} channels (LTCC) resulting in increased systolic Ca^{2+}. PKA also phosphorylates phospholemman (PLM), a regulator of the cardiac Na^{+}/K^{+} ATPase (NKA), which leads to decreased systolic Ca^{2+} content. Thus, PKA activation appears to mediate opposing effects on intracellular Ca^{2+} levels and how cAMP/PKA signaling is coordinated at these two sites remains unclear.

Aims:

The aim of this study was to investigate cAMP/PKA signaling at the A Kinase Anchoring Protein 79(AKAP79)/150 which binds PKA that phosphorylates LTCC and at the PLM/NKA complex and determine whether the two sites are under distinct cAMP pools which could explain the resulting opposing effects on Ca^{2+} ion flux.

Methods:

Selectively targeted FRET-based sensors for cAMP (CUTie) and PKA-dependent phosphorylation (AKAR4) were used for the detection of cAMP amplitudes and PKA activity respectively at PLM/NKA and AKAP79/LTCC nanodomains at the plasmalemma. This allowed for the direct comparison of cAMP levels, PKA and phosphatase activity in real time with high spatial and temporal resolution at the two sites. Experiments were conducted in rat ventricular myocytes.

Results and Conclusions:

A heterogeneous cAMP response was observed at the two sites on β-AR stimulation with Isoproterenol (Iso), with cAMP increase at PLM/NKA being significantly lower than that at AKAP79/LTCC. We demonstrated that this differential local regulation of cAMP response is due to PDEs which degrade cAMP and contain its increase at PLM/NKA. We found that PDE2 and PDE8 play a major role in selectively shielding the PLM/NKA complex from cAMP generated on activation of the β-adrenergic receptor. However, despite the difference in the amplitude of the cAMP response to catecholamines at the AKAP79/LTCC and PLM/NKA complexes, we found that the PKA-dependent phosphorylation of local targets is similar at the two sites and this apparent discrepancy is explained by a more robust phosphatase activity at AKAP79 compared to PLM. Our findings show profound differences in local handling of cAMP levels and phosphatase activity

at these two plasmalemma sites and reveal that adrenergic regulation of Ca^{2+} flux across the plasmalemma relies on submicroscopic compartmentalization of cAMP/PKA signals.

Importantly, our data points to the functional relevance of targeting PDE2, and PDE8 and phosphatases as a way to manipulate NKA activity in the treatment of heart disease. Our findings will most likely open new avenues in drug research in the area of heart failure.

References:

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