Effects of PKA and CaMKII phosphorylation on Ryanodine Receptor phosphorylation domain

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Contraction of cardiac myocytes requires the rapid influx of Ca2+ into the cytosol. Ryanodine Receptors (RyRs) form ion channels that release Ca2+ from the sarcoplasmic and endoplasmic reticula into the cytosol. These large (2.2 MDa) ion channels are targets for hundreds of disease causing mutations. In particular, mutations in the cardiac isoform (RyR2) are known to cause catecholaminergic polymorphic ventricular tachycardia (CPVT), which can result in sudden cardiac death. RyRs are targets of oxidative stress, and their hyperphosphorylation has been proposed to underlie heart failure and atrial fibrillation. As such, the cardiac isoform (RyR2) is a major drug target to treat arrhythmias and heart failure.

A tandem repeat domain of RyRs contains multiple phosphorylation sites for kinases PKA and CaMKII; notably at residues S2808 and S2814, but the mechanism via which phosphorylation alters channel activity is not understood. Docking into cryo-electron microscopy maps suggests a location in the turret region, implying that mutations and phosphorylation may affect the allosteric motions within this area. However, the cryo-EM density in this area is poorly resolved, so other methods are required to look at the precise impact of phosphorylation.

We recently determined the high-resolution crystal structure of PKA in complex with its target sequence within RyR2, and using NMR, we show the effects of phosphomimetics (S2808D, S2814D) on the structure and dynamics of RyR2 phosphorylation domain. Importantly, both S2808D and S2814D show different fingerprints on the spectra, suggesting that they may have different functional impacts on the channel. Finally, ITC and kinase assay show the preference of PKA for S2808, and that the affinity and activity of PKA is increased towards S2814D phosphomimetic, while the affinity and activity is drastically reduced for the S2808D phosphomimetics and R2806C CPVT mutant.