PROMISCUOUS ATTRACTION OF LIGANDS WITHIN THE ATP BINDING SITE OF RYR2 PROMOTES DIVERSE GATING BEHAVIOUR

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The cardiac ryanodine receptor, RyR2, is a ligand-gated ion channel that is triggered to open by rises in cytosolic [Ca^{2+}]. The changes in gating caused by Ca^{2+} binding to RyR2, however, depend heavily on the coincident interaction of RyR2 with other regulators. ATP is an important constitutive cellular regulator of RyR2 and can fully open the channel in the presence of activating cytosolic Ca^{2+}. Since other physiologically relevant adenine nucleosides and ATP fragments can also activate RyR2, we have used published cryo-EM structures of both RyR2 (Peng et al. (2016) Science 354, 5324) and ATP-bound RyR1 (des Georges (2016) Cell 167, 145-157) to investigate the likely relationships between ligand binding in the RyR2-ATP site and the subsequent modulation of channel gating.

RyR2 open probability and gating behaviour was monitored using [^{3}H]ryanodine binding and single-channel recording of RyR2 after incorporation of sheep cardiac SR into artificial membranes under voltage-clamp. Ligand docking into the RyR2-ATP site was performed using AutoDock 4.2.3.

We found that most fragments of ATP could activate RyR2 but exhibited reduced affinity or efficacy. Even triphosphate (PPPi) alone could activate RyR2 demonstrating that the adenine moiety is not essential for binding, however, irreversible inactivation was observed in 53% of single-channel recordings (n=15). Combinations of complementary fragments of ATP (Pi+ADP or PPi+AMP or PPPi+adenosine) could not reproduce the effects of ATP, however, prior treatment with adenosine protected against PPPi-induced inactivation. Therefore, while the RyR2-ATP binding site can accommodate a wide variety of ligands, including PPPi, the most effective ligands have at least three phosphate groups that are guided into place by an adenine nucleoside thus preventing destabilising interactions that lead to channel inactivation.

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