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Modification of cardiac RYR2 gating by a peptide from the central domain of the RYR2

Research Article

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Abstract: The effect of a domain peptide DP_{CPVTc} from the central region of the RYR2 on ryanodine receptors from rat heart has been examined in planar lipid bilayers. At a zero holding potential and at 8 mmol L^{-1} luminal Ca^{2+} concentration, DP_{CPVTc} induced concentration-dependent activation of the ryanodine receptor that led up to 20-fold increase of P_0 at saturating DP_{CPVTc} concentrations. DP_{CPVTc} prolonged RyR2 openings and increased RyR2 opening frequency. At all peptide concentrations the channels displayed large variability in open probability, open time and frequency of openings. With increasing peptide concentration, the fraction of high open probability records increased together with their open time. The closed times of neither low- nor high-open probability records depended on peptide concentration. The concentration dependence of all gating parameters had EC_{50} of 20 μmol L^{-1} and a Hill slope of 2. Comparison of the effects of DP_{CPVTc} with the effects of ATP and cytosolic Ca^{2+} suggests that activation does not involve luminal feed-through and is not caused by modulation of the cytosolic activation A-site. The data suggest that although "domain unzipping" by DP_{CPVTc} occurs in both modes of RyR activity, it affects RyR gating only when the channel resides in the H-mode of activity.

Keywords: *Ryanodine receptor • Planar lipid bilayer • Domain peptide • Allosteric activation*

1. Introduction

Cardiac ryanodine receptors (RyR2 channels) are intracellular ion channels in the membrane of the sarcoplasmic reticulum (SR) that provide a pathway for release of calcium ions from the SR into the cytosol. They are made up of four identical monomers, each consisting of approximately 5,000 amino acids. The large N-terminal cytoplasmic part of the RyR2 comprised of the N-terminal 80-90% of amino acids contains a multitude of regulatory ligand-binding and phosphorylation sites [1]. The trans-membrane region, comprised of the remaining C-terminal 10-20% of amino acids, is responsible for the channel function of the protein [2].

Mutations of RyR2 channels are responsible for the genetic diseases CVPT (catecholaminergic polymorphic ventricular tachycardia) and ARVD2 (arrhythmogenic right ventricular dysplasia) that lead to life-threatening arrhythmias, and have been found in cases of idiopathic ventricular fibrillation, syncope of unknown origin and sudden cardiac death associated with drowning (http://www.fsm.it/cardmoc/). These mutations alter the activity of the RyR2 channel and may result in premature calcium release in the absence of the action potential [1,3]. Similar mutations in the skeletal muscle isoform of the RyR lead to malignant hyperthermia and central core disease [4]. The clustering of mutations into several regions of high occurrence led to the hypothesis that domains containing disease-causing mutations are involved in inter-domain interactions within the ryanodine receptor that keep the channel closed, and that these mutations result in defective inter-domain interaction ("domain unzipping") that leads to increased resting open probability [5]. Inter-domain interaction was proposed to be competitively inhibited by peptides from the interacting domains [1,5]. Indeed, it has been shown that in agreement with this hypothesis the domain peptide from the central domain, DPc10 ($RyR2^{2460-2495}$),

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