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CHARACTERIZATION OF THE INTERACTIONS OF CALMODULIN WITH THE CARDIAC RYANODINE RECEPTOR.

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Abstract

Contraction of heart muscle is initiated by release of calcium from the sarcoplasmic reticulum through the ryanodine receptor/Ca^{2+} release channel (RyR) in response to an action potential. RyR is a homotetramer consisting of ~5,000 amino acids per subunit. Computer modeling of RyR cDNA suggested the presence of consensus binding sites for three calmodulins (CaM) at regions spanning amino acids 2775-2807, 2877-2898, and 2998-3016, one ATP at 2619-2652, and one Ca^{2+}/CaM kinase II phosphorylation site at serine 2809. Thus, the region on the channel defined by residues 2600-3100 was hypothesized to be important for channel regulation by intracellular ligands. CaM is a small, ubiquitous Ca^{2+}-sensing protein that transduces changes in intracellular Ca^{2+} levels via conformational changes which effect target protein functions. CaM targets the RyR in muscle and regulates its Ca^{2+} release function. The biochemical basis for CaM regulation of the RyR attracts much interest due to the fundamental role RyR plays in muscle contraction.

The number and location of CaM binding sites on the cardiac RyR was examined experimentally by photoaffinity labeling of RyR with a radiolabeled and chemically modified CaM. Cross-linking of CaM to RyR followed by proteolysis and peptide mapping with anti-RyR antibodies localized the CaM binding site to a proteolytic fragment encompassing residues 1,800-2,800 of RyR protein. While the location of the CaM binding site is adjacent to the proposed regulatory domain, results suggest that only one, not three, CaM bound RyR. Dose response photoaffinity labeling of RyR indicated that CaM binds to RyR with a K_D of 84 nM and 290 nM under active and resting muscle Ca^{2+} conditions, respectively. The results of this study suggest that the cardiac RyR contains only one CaM binding site within the proposed regulatory domain. Given that CaM concentration in heart muscle is in the micromolar range, the CaM binding site on RyR is most likely in the occupied state throughout Ca^{2+} cycling in a beating heart. Therefore, CaM may serve as RyR built-in Ca^{2+} sensor, which permits the channel to respond to fluctuating Ca^{2+} levels in a living muscle cell.

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