Purification And Biochemical Characterization Of The Cardiac SR Calcium Release Channel

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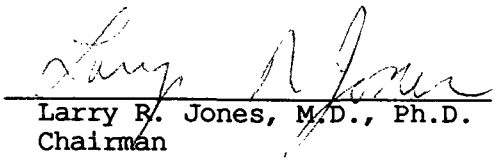

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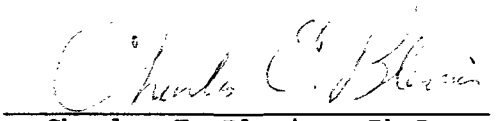

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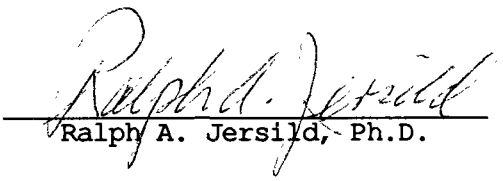

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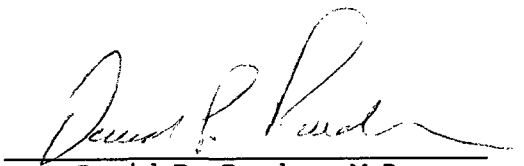

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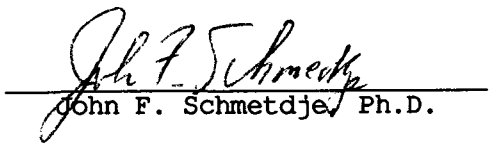

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ABSTRACT

Purification and Biochemical Characterization of the Cardiac SR Calcium Release Channel

Dominic C. Cefali

High molecular weight proteins (HMW) that demonstrate large conductance calcium channel activity were purified to homogeneity from cardiac junctional sarcoplasmic reticulum vesicles. These high molecular weight proteins were solubilized from junctional sarcoplasmic reticulum in either zwitterionic (Z-14 or CHAPS) or anionic (SDS) detergents and purified by sucrose density gradient centrifugation and/or size exclusion chromatography. The purified proteins possessed an apparent M_r = 400-350,000. CHAPS purified HMW proteins bound [^{3}H]ryanodine with a K_D of a 4.6 nM and a B_{max} of 140-280 pmoles/mg protein. Reconstitution of the detergent-purified high molecular weight proteins into artificial lipid membranes demonstrated that they contained divalent cation channel activity of which two subtypes were discovered. With 1 μM cis $CaCl_2$ and 50 mM trans $Ba(OH)_2$, large conductance channels had a slope conductance of 96 pS and a reversal potential of 42 mV. Small conductance channels had a slope conductance of 5.5 pS. After reconstitution, SDS detergent purified channels were found to contain only the 5.5 pS conductance channel. Chelation of cis calcium from 1 μM to 1 nM with EGTA reduced the percent open time of the large conductance channel from 7% to 0.1%. ATP (1 mM) in the cis chamber increased percent channel open time from 6% to 52%. Depending on concentration, ryanodine increased (10 nM) and

decreased (100 μM) percent open time of the 96 pS channel, without altering unitary channel conductance. Large conductance calcium release channel activity seen in purified HMW protein preparations was similar to channel activity observed in native canine cardiac junctional sarcoplasmic reticulum vesicles. These data suggest that the HMW proteins, the ryanodine receptor, and the calcium release channel are the same proteins. All contain allosteric regulatory sites for calcium, ATP, and ryanodine.

Calpain II, an endogenous cytosolic protease, selectively degraded the HMW proteins in cardiac and skeletal muscle JSR vesicles. Proteolysis of the HMW proteins resulted in cleavage of an extravesicular projection of the junctional "feet" from the vesicular membrane surface. Digestion by calpain converted the intact 450-400 KD protein to an ~150 KD limit peptide through a series of higher molecular weight intermediates. The maximal number of binding sites as well as the affinity of the receptor for [^{3}H]ryanodine were unchanged by calpain. $^{45}Ca^{++}$ efflux measurements established that the functional properties of the calcium release channel were maintained following calpain II digestion. The channel activating character of Ca and nucleotides remained, as did Mg block of release. Calcium release channels recorded from undegraded cardiac and skeletal JSR membrane preparations had slope conductances of 85 and 110 pS, respectively, in 1 μM cis $CaCl_2$ and 50 mM trans Ba $(OH)_2$. Although degradation did not change the unitary conductance of the channel, it did result in an increased probability of finding the channel in an open state (36 to > 90%). Following degradation, regulation of channel opening remained Ca-dependent. Percent channel open time was decreased in the presence of

high (300 μM) concentrations of ryanodine. These data suggest that proteolysis of the HMW ryanodine receptor by calpain selectively impairs inactivation of the JSR calcium release channel by cleaving a required cytoplasmic domain, a domain not required for Ca-dependent channel activation or [^{3}H]ryanodine binding.

TABLE OF CONTENTS