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Role of α_{1C} Carboxyl Terminal in Cardiac Ca^{2+} Signaling

Sun-Hee Woo

College of Pharmacy, Chungnam National University, Daegeon

Local cytosolic rises of Ca^{2+} appears to be critical in the regulation of many cellular activities, including muscle contraction, neurotransmitter secretion, and cell death. Cardiac Ca^{2+} signaling similarly begins with discrete and localized rises of Ca^{2+} (Ca^{2+} sparks) triggered by Ca^{2+} current (I_{Ca}). The large local releases of Ca^{2+} in turn modulate L-type $Ca_v1.2$ (α_{1C}) Ca^{2+} channels, suggesting that discrete Ca^{2+} cross-signaling may occur in the micro-domains of α_{1C}/ryanodine receptors (RyRs). Structural support for this scheme comes from the findings that the molecular determinants of Ca^{2+}-dependent inactivation are located on the cytosolic C-terminal tail some 100 amino acid away from the inner pore of the Ca^{2+} channel, possibly in the vicinity of RyRs, ~10 nm away.

Ca^{2+} influx through L-type $Ca_v1.2$ (α_{1C}) Ca^{2+} channels is a critical step in the activation of cardiac ryanodine receptors (RyRs) and release of Ca^{2+} (CICR). A large body of evidence suggests that cardiac RyRs are activated primarily by I_{Ca}. This includes: 1) the similarity in bell-shaped voltage dependence of intracellular Ca^{2+} transients and I_{Ca}, 2) the influx of only Ca^{2+}, but not gating charge or Na^+, triggers Ca^{2+} release, and 3) photolysis of caged Ca^{2+} activates Ca^{2+} release independent of membrane potential. The released Ca^{2+}, in turn, is the dominant determinant of inactivation of the Ca^{2+} current (I_{Ca}), and termination of release. Although Ca^{2+} cross-signaling is mediated by high Ca^{2+} fluxes in the micro domains of α_{1C}/RyR complexes, I_{Ca} gated Ca^{2+} cross-signaling is surprisingly resistant to intracellular Ca^{2+} buffering, and has steeply voltage-dependent gain, inconsistent with a strict CICR mechanism. The steep voltage-dependence of gain of CICR at negative potentials where I_{Ca} is minimally activated, suggesting that when Ca^{2+} influx through the channel is compromised other Ca^{2+} modulatory mechanism maybe activated. The impetus for this study came from identification of two Ca^{2+} sensing domains, LA (1571-1599) and K (1617-1636), on α_{1C} carboxyl-(C-) terminal tail that appear to be involved in the Ca^{2+}-dependent inactivation of the channel and may interact with RyRs. LA-peptide is composed of highly selective N-terminal Ca^{2+} sensor and the adjacent Ca^{2+}-independent tethering site for calmodulin. The latter region

is deleted for LM1-peptide. K-peptide contains IQ-like calmodulin binding region. To explore possible regulatory role of C terminal tail of α_{1C} in modulating Ca^{2+} signaling, we chose to study the effects of these peptides, by introducing them into atrial myocytes and focusing mostly on the central release sites where RyRs are free of association with Ca^{2+} channels and accessible to peptides mimicking Ca^{2+}-sensing domains of the α_{1C} channel. We used rapid (240 Hz) two-dimensional confocal Ca^{2+} imaging under voltage clamp to measure Ca^{2+} sparks, which represent gatings of RyRs. The frequency of spontaneously activating central sparks increased by ~4 fold on dialyzing LA-, but not K-peptide into myocytes voltage-clamped at –80 mV. The rate but not the magnitude of caffeine (10 mM)-triggered central Ca^{2+} release was significantly accelerated by LA- but not K-peptide. Individual Ca^{2+} spark size and flux were larger in LA-, but not in K-peptide dialyzed myocytes. Although LA-peptide did not change the amplitude or inactivation kinetics of I_{Ca}, LA-peptide did strongly enhance the central Ca^{2+} transients triggered by I_{Ca} at –30 (small I_{Ca}) but not at +20 mV (large I_{Ca}). In contrast, K-peptide had no effects on either I_{Ca} or local Ca^{2+} transients. LA-peptide with deleted calmodulin-binding region (LM1-peptide) had no significant effects on the central spark frequency but suppressed spontaneous spark frequency in the periphery. Our results indicate that calmodulin-binding LA motif of the α_{1C} C-terminal tail may sensitize the RyRs, thereby increasing their open probability and providing for both the voltage-dependence of CICR and the higher frequency of spark occurrence in the periphery of atrial myocytes where the native α_{1C}/RyR complexes are intact.

In cardiac hypertrophy it is now accepted that Ca^{2+} signaling efficacy is significantly down regulated. Cardiac remodeling under hypertrophic conditions includes poor development of dyadic junctions and less efficacy of CICR gain, thereby causing impaired contractility. Our data support that the α_{1C} C-terminal tail motif may improve the hypertrophy-induced impaired CICR gain and contractility by sensitizing and activating non-junctional RyRs that are more abundant in the hypertrophied heart.