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Review article

$Ca_V1.2$ signaling complexes in the heart

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ABSTRACT

L-type Ca^{2+} channels (LTCCs) are essential for generation of the electrical and mechanical properties of cardiac muscle. Furthermore, regulation of LTCC activity plays a central role in mediating the effects of sympathetic stimulation on the heart. The primary mechanism responsible for this regulation involves β-adrenergic receptor (βAR) stimulation of cAMP production and subsequent activation of protein kinase A (PKA). Although it is well established that PKA-dependent phosphorylation regulates LTCC function, there is still much we do not understand. However, it has recently become clear that the interaction of the various signaling proteins involved is not left to completely stochastic events due to random diffusion. The primary LTCC expressed in cardiac muscle, $Ca_V1.2$, forms a supramolecular signaling complex that includes the β_2AR, G proteins, adenylyl cyclases, phosphodiesterases, PKA, and protein phosphatases. In some cases, the protein interactions with $Ca_V1.2$ appear to be direct, in other cases they involve scaffolding proteins such as A kinase anchoring proteins and caveolin-3. Functional evidence also suggests that the targeting of these signaling proteins to specific membrane domains plays a critical role in maintaining the fidelity of receptor mediated LTCC regulation. This information helps explain the phenomenon of compartmentation, whereby different receptors, all linked to the production of a common diffusible second messenger, can vary in their ability to regulate LTCC activity. The purpose of this review is to examine our current understanding of the signaling complexes involved in cardiac LTCC regulation. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

Contents

Abbreviations: LTCC, L-type Ca^{2+} channel; βAR, β-adrenergic receptor; EPR, E type prostaglandin receptor; RyR2, type 2 ryanodine receptor; AC, adenylyl cyclase; PDE, phosphodiesterase; PP, protein phosphatase; AKAP, A kinase anchoring protein; PKA, protein kinase A; DCT, distal C terminus; SR, sarcoplasmic reticulum; GPCR, G protein coupled receptor; SERCA2a, type 2a sarcoplasmic reticulum Ca^{2+} ATPase; PLN, phospholamban; CAV-3, caveolin-3.

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1. Introduction

The proximity of the constituent components of a signaling pathway often plays a critical role in ensuring the speed, efficiency, and specificity of the functional responses they produce. This is especially true for the signaling mechanisms involved in regulating many different ion channels. Ion channels forming signaling complexes with kinase(s) and phosphatase(s) is a common theme [1]. While the localization of such signaling molecules is often achieved through direct protein-protein interactions, spatial organization can also be achieved indirectly via scaffolding proteins as well as the targeting of the relevant control elements to specific subcellular locations or lipid domains in the plasma membrane [1–3]. The spatial restriction of signaling also extends to aspects of those pathways that involve diffusible second messengers, such as cAMP. Accordingly, signaling complexes often include receptors and enzymes such as adenylyl cyclase that are responsible for second messenger production, as well as proteins such as phosphodiesterases (PDEs), which are involved in second messenger catabolism. The primary focus of this review will be on the signaling complexes important in maintaining the fidelity of L-type Ca^{2+} channel responses involving protein kinase A (PKA) in the heart.

2. Ca^{2+} channels in the heart

Ca^{2+} is a potent second messenger that controls a variety of cellular functions [4,5]. This is particularly true in the heart, where the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels plays an essential role in regulating action potential duration, triggering myocyte contraction, and controlling gene transcription [6]. Thus, they are an important target for regulating cellular function by a number of different signal transduction pathways, including those involving PKA.

3. Molecular structure of L-type Ca^{2+} channels

There are four types of LTCC, $Ca_V1.1$–1.4, each of which exists as a multimeric protein complex consisting of one of four different corresponding α_1 subunits ($\alpha_1$1.1–1.4) together with auxiliary β, $\alpha_2\delta$, and γ subunits [7] (Fig. 1). The α_1 subunit forms the ion-conducting pore and defines the specific type of Ca^{2+} channel. It consists of four homologous domains (I-IV) each containing six transmembrane segments (S1–S6) and a pore forming P-loop between segments 5 and 6. $Ca_V1.2$ is the predominant LTCC expressed in ventricular myocytes, while both $Ca_V1.2$ and $Ca_V1.3$ are expressed in atrial cells as well sinoatrial and atrioventricular node cells [8].

The distal C terminus (DCT) of the $\alpha_1$1.2 subunit can undergo proteolytic cleavage by the Ca^{2+}-activated protease calpain resulting in a long and a short form [9,10]. The DCT can remain associated with the truncated $\alpha_1$1.2 subunit after cleavage via a non-covalent interaction [9,11,12]. Expression of truncated $\alpha_1$1.2 alone produces currents that are significantly greater than those produced by the full length subunit [9,11–14], suggesting that the DCT has an autoinhibitory effect. Consistent with this idea, coexpression of the DCT as an independent polypeptide together with the $\alpha_1$1.2 short form results in a decrease in the current produced by the truncated subunit alone [12]. It has been reported that the majority of $\alpha_1$1.2 in heart may exist in the cleaved state [10,15], however, see Dai et al. 2009 for evidence that 50% or more may exist in its uncleaved, full length form [1]. The C terminal fragment of $\alpha_1$1.2 has also been reported to act as a Ca^{2+} channel associated transcription factor (CCAT) regulating a wide range of genes affecting neuronal signaling and excitability [16]. Whether or not it serves such a function in the heart has yet to be determined.

There are four different β subunit genes (β_1–β_4). These can undergo alternative splicing, resulting in numerous isoforms [17]. In the cytosol, the β subunit promotes surface expression of the channel complex. Once at the membrane, the β subunit is found at the cytosolic face of the channel, where it affects voltage-dependent activation and inactivation [18–20]. The $\alpha_2\delta$ subunits are generated from a single gene product, which is proteolytically cleaved but rejoined by disulfide linkage. The α_2 subunit is extracellular, while the δ subunit consists of a single transmembrane segment with short intracellular and extracellular segments [21]. Like the β subunit, the $\alpha_2\delta$ subunits promote surface expression of the channel complex in addition to affecting channel gating [18,22,23]. In cardiac muscle, the $Ca_V1.2$ complex also includes a γ subunit, which can affect both activation and inactivation [24].

4. Regulation of $Ca_V1.2$

The regulation of $Ca_V1.2$ has been extensively studied because of its central role in contributing to the electrical and mechanical properties of the heart. Influx of Ca^{2+} through LTCCs is responsible for maintaining

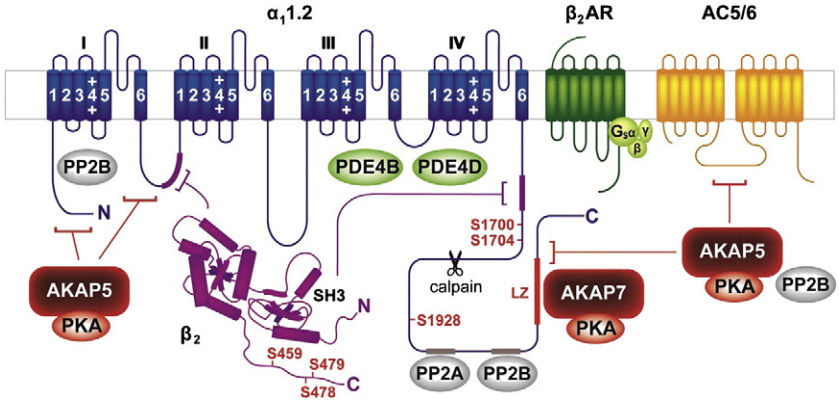

Fig. 1. The $Ca_V1.2$ signaling complex includes the $\alpha_1$1.2 subunit, β_2 subunit, A kinase anchoring proteins 5 and 7 (AKAP5 and AKAP7), protein kinase A (PKA), protein phosphatases 2A and 2B (PP2A and PP2B), the β_2-adrenergic receptor (β_2AR), adenylyl cyclase 5/6 (AC5/6), stimulatory G protein (G_s), and phosphodiesterases 4B and 4D (PDE4B and PDE4D). PKA phosphorylation sites are indicated in red; sites of interaction with binding partners are indicated by corresponding color-coded segments or brackets. Site cleaved by calpain indicated by scissors. See text for details.