Journal of Molecular and Cellular Cardiology 52 (2012) 376–387

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

# Different subcellular populations of L-type $Ca^{2+}$ channels exhibit unique regulation and functional roles in cardiomyocytes

Jabe M. Best, Timothy J. Kamp *

*Cellular and Molecular Arrhythmia Research Program, Department of Medicine, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, Madison, WI 53792, USA*

ARTICLE INFO

*Article history:*
Received 14 April 2011
Received in revised form 11 July 2011
Accepted 17 August 2011
Available online 23 August 2011

*Keywords:*
Cardiomyocyte
Calcium channel
Subcellular localization
Microdomain
Calcium signaling

ABSTRACT

Influx of $Ca^{2+}$ through L-type $Ca^{2+}$ channels (LTCCs) contributes to numerous cellular processes in cardiomyocytes including excitation–contraction (EC) coupling, membrane excitability, and transcriptional regulation. Distinct subpopulations of LTCCs have been identified in cardiac myocytes, including those at dyadic junctions and within different plasma membrane microdomains such as lipid rafts and caveolae. These subpopulations of LTCCs exhibit regionally distinct functional properties and regulation, affording precise spatiotemporal modulation of L-type $Ca^{2+}$ current ($I_{Ca,L}$). Different subcellular LTCC populations demonstrate variable rates of $Ca^{2+}$-dependent inactivation and sometimes coupled gating of neighboring channels, which can lead to focal, persistent $I_{Ca,L}$. In addition, the assembly of spatially defined macromolecular signaling complexes permits compartmentalized regulation of $I_{Ca,L}$ by a variety of neurohormonal pathways. For example, β-adrenergic receptor subtypes signal to different LTCC subpopulations, with $\beta_2$-adrenergic activation leading to enhanced $I_{Ca,L}$ through caveolar LTCCs and $\beta_1$-adrenergic stimulation modulating LTCCs outside of caveolae. Disruptions in the normal subcellular targeting of LTCCs and associated signaling proteins may contribute to the pathophysiology of a variety of cardiac diseases including heart failure and certain arrhythmias. Further identifying the characteristic functional properties and array of regulatory molecules associated with specific LTCC subpopulations will provide a mechanistic framework to understand how LTCCs contribute to diverse cellular processes in normal and diseased myocardium. This article is part of a Special Issue entitled "Local Signaling in Myocytes".



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*Abbreviations:* LTCC, L-type $Ca^{2+}$ channel; EC, excitation–contraction; $I_{Ca,L}$, L-type $Ca^{2+}$ current; SR, sarcoplasmic reticulum; T-tubules, transverse tubules; CICR, $Ca^{2+}$-induced $Ca^{2+}$ release; $[Ca^{2+}]_i$, free intracellular $Ca^{2+}$ concentration; PKA, protein kinase A; Cav, caveolin; PTRF, Polymerase I and Transcript Release Factor; C-terminus, carboxyl terminus; CDI, $Ca^{2+}$-dependent inactivation; CaM, calmodulin; CREB, cAMP-responsive element-binding protein; TIRF, total internal reflection fluorescence; AKAP, A-kinase anchoring protein; PKCα, protein kinase C alpha; CaMK, $Ca^{2+}$-CaM-dependent kinase; PP2B, protein phosphatase 2B; NFAT, nuclear factor of activated T cells; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment protein receptor; ANP, atrial natriuretic peptide; PP2A, protein phosphatase 2A; FRET, fluorescence resonance energy transfer; GPI, glycosylphosphatidyl inositol; TS, Timothy syndrome; LQTS, congenital long QT syndrome.

* Corresponding author at: Box 3248, Clinical Science Center, 600 Highland Avenue, Madison, WI 53792-3248, USA. Fax: +1 608 263 0405.
*E-mail address:* tjk@medicine.wisc.edu (T.J. Kamp).

0022-2828/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2011.08.014

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## 1. Introduction

In the heart, voltage-dependent L-type $Ca^{2+}$ channels (LTCCs) are essential to numerous cellular processes including excitability, excitation–contraction (EC) coupling, hormone secretion, and regulation of gene expression. Participation in such diverse functions demands that the influx of $Ca^{2+}$ through L-type channels (L-type $Ca^{2+}$ current, $I_{Ca,L}$) is tightly controlled and compartmentalized within the cardiac myocyte. It has long been recognized that discrete clusters of LTCCs exist along the sarcolemma, and studies in recent years have greatly extended our understanding of how specific subcellular localization impacts channel function and regulation by a variety of neurohormonal and second messenger pathways [1–6].

A number of important LTCC subpopulations have been identified in cardiomyocytes that associate with unique macromolecular signaling complexes and scaffolding proteins, which enables spatiotemporal modulation of $I_{Ca,L}$. These include channels that are localized to dyadic junctions as well as extradyadic channels that reside in biochemically distinct regions of surface membrane known as membrane microdomains. Plasma membrane microdomains, including lipid rafts and caveolae, exhibit unique lipid composition and protein components and coordinate numerous cellular functions including various signal transduction pathways and protein recycling [7–9]. Numerous signaling molecules have been localized to caveolae including components of the $\beta_2$-adrenergic receptor/adenylyl cyclase/protein kinase A (PKA) cascade [5,6]. This review will highlight the evolving understanding of distinct subcellular populations of LTCCs in cardiomyocytes and their differing regulation and contributions to $Ca^{2+}$ signaling in the heart.

## 2. LTCCs in the heart

### 2.1. Molecular composition of cardiac LTCCs

LTCCs are multimeric complexes consisting of a pore forming $\alpha_1$ subunit and auxiliary β, $\alpha_2\delta$, and γ subunits [10]. The $\alpha_1$ subunit serves as the main functional component of the channel complex and consists of four homologous domains (I–IV) each containing six transmembrane segments (S1–S6). $Ca_v1.2$ ($\alpha_{1C}$, encoded by the *CACNA1C* gene) is the predominant $\alpha_1$ subunit in ventricular myocardium, whereas both $Ca_v1.2$ and $Ca_v1.3$ ($\alpha_{1D}$, encoded by *CACNA1D*) are expressed in atrial tissue as well as nodal cells, where $I_{Ca,L}$ contributes to automaticity [11–15]. Extensive alternative splicing of $Ca_v1.2$ has been reported, and these splice variants play unique roles in cardiovascular physiology, pharmacology, and disease [16,17]. One important example is alternative splicing of $Ca_v1.2$ within transmembrane segment IS6, which impacts sensitivity to the dihydropyridine class of LTCC blockers. Differential expression of these splice variants lead to higher or lower sensitivity in smooth and cardiac muscle, respectively [18,19].

$Ca^{2+}$ channel auxiliary subunits further add to the functional diversity of LTCCs. The cytosolic β subunits promote trafficking of the channel complex to the plasma membrane and modulate gating properties of the channel [20–22]. The β subunits are encoded by four distinct genes (*CACNB1–4*), each of which undergoes alternative splicing to generate a total of 18 or more unique β subunit isoforms in human myocardium [23]. The $\alpha_2\delta$ subunits arise from a common precursor protein that is post-translationally cleaved and relinked via a disulfide bridge. The extracellular $\alpha_2$ peptide is heavily glycosylated and the δ peptide contains a single transmembrane domain [24]. Of the four $\alpha_2\delta$ subunits (encoded by *CACNA2D1–4*), $\alpha_2\delta$-1–3 are expressed in atrial tissue whereas $\alpha_2\delta$-1 and $\alpha_2\delta$-2 are present in ventricular myocardium [25,26]. The $\alpha_2\delta$ subunits modify both channel gating properties and surface membrane expression of the L-type channel complex [20,27]. $Ca^{2+}$ channel γ subunits, of which eight exist (encoded by *CACNG1–8*), were originally demonstrated to associate with voltage-dependent $Ca^{2+}$ channels in skeletal muscle and brain [28,29]. However, recent evidence suggests several γ subunits including γ4, γ6, γ7, and γ8 are present in cardiac muscle and associate with the cardiac $Ca_v1.2$ channel complex, altering both activation and inactivation properties of the channel [30].

### 2.2. Distinct LTCC subpopulations in cardiac myocytes

#### 2.2.1. Dyads

A critical subpopulation of LTCCs is that which participates in EC coupling. A number of studies applying immunoconfocal and electron microscopy techniques have demonstrated that a subset of LTCCs form dyadic complexes with $Ca^{2+}$-release channels (ryanodine receptors) on apposing junctional sarcoplasmic reticulum (SR) [1,2,31,32]. Upon membrane depolarization, activation of these LTCCs leads to an influx of $Ca^{2+}$ into the dyadic cleft space, which triggers the opening of ryanodine receptors and subsequent release of SR $Ca^{2+}$ stores. This $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) mechanism underlies the rise in free intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) that activates myofilament proteins leading to muscle contraction [33].

Studies have estimated that approximately 75% of LTCCs reside at dyad junctions in cardiac myocytes [34]. In mammalian ventricular cardiomyocytes, dyadic couplings occur predominantly within the transverse (T)-tubule network, which represents a complex system