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Auto-regulation of Cardiac L-type Calcium Channels

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Abstract

L-type Ca channels (LTCC) are major contributors to electrical and contractile function of the heart. LTCC regulate action potential duration, allow Ca entry into cardiac myocytes for contraction, and regulate growth-related signaling in the heart. In cardiac development and in mature heart disease, LTCC are regulated at levels of acute function, and transcription. In addition, LTCC are clinically relevant therapeutic targets for anti-hypertensive medications. In this review we discuss LTCC homeostasis whereby cardiac myocytes maintain LTCC expression via a novel transcriptionally regulated pathway that includes a segment of the LTCC that moves between surface membrane and nucleus.

Introduction

Calcium ions (Ca^{2+} ; abbreviated here as Ca) serve a broad range of function in the heart over a wide range of time scales. Beat to beat rise and fall of intracellular Ca in cardiomyocytes governs contraction and relaxation at typical frequencies of once per second. In addition, Ca regulates much longer-term signaling such as that involved in cardiac hypertrophy. A common Ca-signaling element in cardiac myocytes is L-type Ca-channels (LTCC), which are a major pathway for Ca-entry into cells. There are several excellent reviews and monographs on Ca-regulation of beat-to-beat function (Bers 2008), and Ca regulation of long-term signaling (Molkentin 2006). The goal of this review is to highlight new information linking LTCC function and gene expression, including auto-regulation of LTCC expression.

Compensatory Changes of LTCC

Cells must compensate changes in Ca-handling protein expression and/or function to maintain Ca homeostasis. LTCC are the major sarcolemmal influx pathway for Ca, and Na-Ca-exchangers (NCX) are the major efflux pathway. To maintain Ca homeostasis the amount of Ca that enters the cytosol via LTCC is extruded in the same amount via NCX (Eisner et al. 2000). NCX-deletion induces Ca-overload and lethal cardiac arrhythmia (Ebert et al. 2005). Surprisingly however, cardiac specific NCX-knock-out mice are viable, with near normal cardiac function (Henderson et al. 2004). Apparently, Ca homeostasis is maintained by a compensatory down-regulation of $\text{Ca}_v1.2$ and LTCC current ($\text{I}_{\text{Ca,L}}$) (Pott et al. 2005). Conversely, genetic long-term blockade of LTCC attenuates $\text{I}_{\text{Ca,L}}$ (Crump et al. 2007), and causes a compensatory down-regulation of NCX (Lester et al., 2008).

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Pharmacological blockade of LTCC is a clinically relevant situation by which $I_{Ca,L}$ may be altered. There are also precedents for physiological perturbations that alter $I_{Ca,L}$. LTCC channel levels are plastic, they change in response to Ca (Davidoff et al. 1997), angiotensin II (Gassanov et al. 2006), cardiac denervation in heart failure (Yatani et al. 2006), or prolonged exposure to catecholamines (Maki et al. 1996). The most consistent finding for myocytes isolated from hypertrophied and failing hearts is a prolongation of the action potential (AP). *A priori*, an increase of $I_{Ca,L}$ density can explain a longer AP, but there is little evidence for this, with the exception of studies of mildly hypertrophied hearts (Kleiman and Houser 1988). There is, however, a trend for Ca channel current density to decline with the progression of hypertrophy (Mukherjee and Spinale 1998). Moderate hypertrophic hearts show no significant change in current density. By contrast severe and failing hearts showed significant reduction of Ca channel density (reviewed by Mukherjee and Spinale 1998). Reduced Ca channel density accompanying heart failure can be rescued in humans with a left ventricular assist device (Chen et al. 2002). Reduced LTCC density is counter to AP prolongation; however, in heart failure there is a marked decrease of K currents, and changes in K channel density are in fact the most consistent finding in the study of ionic remodeling in heart failure progression. The more controversial findings examining Ca channel regulation may underscore the critical and central role of LTCC in excitation and excitation-contraction coupling in the heart. We posit that even a small alteration of Ca channel density or function would be expected to have profound effects on heart function. Finally, atrial fibrillation-induced electrical remodeling includes shortening of AP duration and reversal of its physiologic rate adaptation. Changes in the time course of repolarization of the AP are associated with changes in mRNA expression of $Ca_v1.2$ and the $K_v1.5$ channel (van der Velden et al. 2000), and this is reduced by LTCC blockade by verapamil (Tieleman et al. 1997). In summary, during pathogenesis there is a coordinated ionic remodeling that occurs in the myocardium. In addition, pharmacological LTCC blockade *in vivo* causes a compensatory up-regulation of $Ca_v1.2$ and $I_{Ca,L}$ remodeling (Schroder et al. 2007, Chiappe De Cingolani et al. 1994).

The LTCC complex: focus on $Ca_v1.2$ C-terminus

The LTCC α -subunit ($Ca_v1.2$) is the predominant voltage-gated Ca channel in mammalian myocardium. Functional $Ca_v1.2$ is localized to t-tubules, and it is also present in the sarcolemma (SL). Functional $Ca_v1.2$ exists as a heteromultimer consisting of the α_1 subunit ($Ca_v1.2$), a cytosolic β -subunit ($Ca_v\beta_2$), and covalent-linked α_2 - δ subunits. Additionally, several other proteins associate with $Ca_v1.2$ (reviewed by Dai et al. 2009) that are crucial for $I_{Ca,L}$ include calmodulin. Calmodulin is pre-bound to the proximal $Ca_v1.2$ -C-terminal on the IQ-motif, where this complex serves as a sensor for local Ca (Liang et al. 2003).

The carboxyl-terminal domain contains two regions of interest distal to the IQ motif, including a proteolytic cleavage site, and a PKA substrate site (De Jongh et al. 1996, Hulme et al. 2006a). $Ca_v1.2$ may be proteolytically modified while on the surface membrane or during synthesis; in heart, a 240 and a ~190kD $Ca_v1.2$ are both present (De Jongh et al. 1996). The $Ca_v1.2$ -C-terminus (CCt) is cleaved and re-associates with the Ca channel complex (Hulme et al. 2006b). Moreover, re-association via AKAP scaffold protein appears to be a pre-condition for $I_{Ca,L}$ to respond to PKA-mediated stimulation (Hulme et al. 2003). Although it is not known whether this cleavage is a regulated process, calpain, which is Ca-activated can catalyze $Ca_v1.2$ -C-terminal cleavage (De Jongh et al. 1994). It is equally possible that alternative splicing of the $Ca_v1.2$ gene may independently generate CCt. This is an important area for further study.

The demonstration that CCt exists independently of the main pore-forming channel subunit warrants re-evaluation of a study that was intended to interrogate the contribution of the $Ca_v1.2$ -C-terminus to channel gating. Anchoring of the $Ca_v1.2$ -C-terminus to the plasma

membrane caused an inhibition of its state-dependent mobility, inhibition of channel inactivation, and inhibition of CREB-dependent transcription. Release of the tail restored these functions, suggesting a direct role for voltage-gated mobility of the CCt in Ca signaling (Kobrinisky et al. 2003). Taken together, these studies provided us the impetus to examine whether CCt are mobile in heart cells (Schroder et al. 2009).

LTCC Promoters

Ca_v1.2 is encoded by the *CACNA1C* gene which is located on chromosome 6 in mouse and 12 in human. Human *cacna1c* contains 7 predicted alternative promoters with variations in the mRNA including 5' and 3' truncations (AceView Database). Ca_v1.2 expression is regulated by β adrenergic stimulation (Fan et al. 2000), α adrenergic stimulation (Fan et al. 2000), androgens (Golden et al. 2002), and Ca entry through the channel (Davidoff et al. 1997, Schroder et al. 2009).

Initial work on rat *CACNA1C* promoter demonstrated an approximately 2KB 5' flanking region as a member of the TATA-less class of core promoters (Liu et al. 2000). Binding sites for transcription factors such as NKX2.5, Mef2c, AP-1, a cAMP response element (cre), and hormone binding sites were identified, along with a minimal promoter sequence (Liu et al. 2000).

Research revealing alternatively spliced first exons in the *cacna1c* gene led to the discovery of two promoters in human and rat with tissue specific expression and distinct 5' flanking sequences (Pang et al. 2003, Saada et al. 2005). The majority of the Ca_v1.2 in human cardiac tissue expresses a longer first exon (Dai et al. 2002).

Recent work in HL-1 cells showed increased expression of Ca_v1.2 mRNA, protein and I_{Ca,L} in response to Angiotensin II treatment (ANG II) (Tsai et al. 2007). This work utilized a rat *cacna1c* promoter reporter to show that ANG-II enhanced transcriptional activity. A combination of mutation and truncation analysis of the promoter suggested that the upstream cAMP response element was the ANG II interaction site.

Autoregulation of LTCC

Given the finding that sustained LTCC blockade up-regulated Ca_v1.2 protein (Crump et al. 2006, Schroder et al. 2007), we tested the hypothesis that LTCC complex sensed a reduction in Ca-influx, and responded by signaling to the nucleus to up-regulate *cacna1c*. Cardiac myocytes were transfected with a plasmid encoding an eGFP-CCt fusion protein, and we tracked CCt to the nucleus in live cells (Schroder et al. 2009). Chromatin immunoprecipitation demonstrated that endogenous CCt interacts with the Ca_v1.2 promoter at NKX2.5/MEF-, C/EBP-, and CRM1-containing sites, but not at a cre-element (Schroder et al. 2009). *Cacna1c* promoter reporter assays revealed that CCt inhibits Ca_v1.2 transcription (Schroder et al. 2009). Thus, CCt is a transcription regulator in cardiac myocytes, consistent with a similar role in neurons (Gomez-Ospina et al. 2006).

LTCC blockade inhibits cellular hypertrophy (Sucharov et al. 2006). To test the hypothesis that the cellular hypertrophic response contributes to CCt signaling, we treated cardiac myocytes with 10% serum and compared them to serum withdrawal for 48 hours. Serum withdrawal increased Ca_v1.2 promoter activity, mRNA, protein, and I_{Ca,L}. Concomitantly, serum withdrawal caused a re-distribution of CCt with less nuclear localization consistent with reduction of transcriptional inhibition and subsequent increase of Ca_v1.2 transcription (Schroder et al. 2009). Ca_v1.2 promoter reporter assays revealed complexities of CCt's contribution to Ca_v1.2 regulation in the presence of hypertrophic signaling. Deletion of the 5' segments of the Ca_v1.2 promoter, including cre- and NKX2.5-containing elements, reduces

promoter activity (Saada et al. 2005, Schroder et al. 2009); however, these 5' elements require serum. In the absence of serum, 5' deletion has no significant effect (Schroder et al. 2009). Moreover, CCt inhibition is similar between serum and serum-free conditions for the full-length promoter, but is enhanced for the truncated promoter in serum compared to serum-free conditions. This raises the interesting new hypothesis that CCt governs positive effects on Cav1.2 via interaction with transcription factors bound to cre-containing elements.

Having established an effect of growth factors on CCt, we next tested the reciprocal. Namely, does CCt alter hypertrophic signaling? We found that CCt represses growth factor-induced ANF promoter activity. In addition, CCt over-expression blocked the serum-induced hypertrophy of cardiac myocytes *in vitro* (Schroder et al. 2009). Thus, CCt is influenced by growth factor signaling, and CCt is a mediator of hypertrophic signaling.

Perspectives

We propose a novel mechanism for long-term regulation of Ca-homeostasis. This model incorporates recent work by others and key findings from our ongoing studies. **Step #1** (Figure 1) depicts cardiac myocyte Cav1.2 configuration. The LTCC-C-terminus (CCt) is cleaved at position 1821 (Hulme et al. 2006b); CAC1C_rabbit numbering). Several interacting proteins are omitted for clarity. These include, Cavβ2, α2δ, CaM, CaMKIIδc, AKAP15, & Rem. The red & grey 'P' represent substrate sites for CaMKIIδc and PKA, respectively. We show them for orientation, and we are not implying that phosphorylation is required or regulates CCt re-association. Note that CCt may be auto-inhibitory for ICaL. **Step #2** represents pharmacological or Rem blockade of ICaL (or in the less extreme, simply reduced Ca-entry). **Step #3** depicts our finding that LTCC blockade remodels ventricular myocytes, and our new findings that CCt interacts with the *CACNA1C* promoter, and CCt reduces *CACNA1C* promoter activity (Schroder et al. 2009). The end result of a tonic LTCC blockade is an increase of Cav1.2 protein. Our new data now suggest that a plausible mechanism for tonic drug-induced increase of protein expression is reduction of net CCt nuclear accumulation, thus relieving CCt net repression of Cav1.2 transcription. It will be important to test the hypothesis that ICaL, or local Ca may modulate CCt translocation. **Step #4** represents the Ca-activated proteolytic cleavage of the full-length Cav1.2 protein. The net result, then of prolonged LTCC blockade would be an increase of LTCC. Our functional studies also point to a commensurate change in NCX (Lester et al. 2008), the main SL extrusion mechanism, to balance changes in LTCC-mediated SL-entry.

Many studies have linked Cav1.2 activity to the transcriptional regulation of other genes. Termed excitation transcriptional coupling this group of genes includes but is not limited to c-fos in hypoxia (Premkumar et al. 2000), Rho A/ROK, myocardin and SRF pathways in smooth muscle (Wamhoff et al. 2006), and in cardiac memory (Plotnikov et al. 2003). LTCC have multi-modal functions – they serve as sensors for Ca, mediate excitability, contractility, and potentially integrate acute function with long-term transcriptional signaling. It will be important to unravel the intricate feedback systems between LTCC function, CCt, and downstream effectors – including the LTCC itself.

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Figure 1.

A cell/molecular model of LTCC auto-regulation depicts the dynamic homeostatic regulation of LTCC and Ca via CCT. '1' indicates mature channel complex with auto-inhibitory CCT interaction. '1β' represents β-adrenergic stimulated myocytes. '2' corresponds to reduced Ca-influx, for example, stemming from therapeutic LTCC block. '3' shows that nuclear CCT causes Ca_v1.2 transcriptional repression. '4' relates synthesis and processing of mature LTCC complex.