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Mechanisms of the cyclic nucleotide cross-talk signaling network in cardiac L-type calcium channel regulation

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ABSTRACT

Regulation of L-type Calcium (Ca^{2+}) Channel (LCC) gating is critical to shaping the cardiac action potential (AP) and triggering the initiation of excitation-contraction (EC) coupling in cardiac myocytes. The cyclic nucleotide (cN) cross-talk signaling network, which encompasses the β-adrenergic and the Nitric Oxide (NO)/cGMP/Protein Kinase G (PKG) pathways and their interaction (cross-talk) through distinctively-regulated phosphodiesterase isoenzymes (PDEs), regulates LCC current via Protein Kinase A- (PKA) and PKG-mediated phosphorylation. Due to the tightly-coupled and intertwined biochemical reactions involved, it remains to be clarified how LCC gating is regulated by the signaling network from receptor to end target. In addition, the large number of EC coupling-related phosphorylation targets of PKA and PKG makes it difficult to quantify and isolate changes in L-type Ca^{2+} current (I_{CaL}) responses regulated by the signaling network. We have developed a multi-scale, biophysically-detailed computational model of LCC regulation by the cN signaling network that is supported by experimental data. LCCs are modeled with functionally distinct PKA- and PKG-phosphorylation dependent gating modes. The model exhibits experimentally observed single channel characteristics, as well as whole-cell LCC currents upon activation of the cross-talk signaling network. Simulations show 1) redistribution of LCC gating modes explains changes in whole-cell current under various stimulation scenarios of the cN cross-talk network; 2) NO regulation occurs via potentiation of a gating mode characterized by prolonged closed times; and 3) due to compensatory actions of cross-talk and antagonizing functions of PKA- and PKG-mediated phosphorylation of LCCs, the effects of individual inhibitions of PDEs 2, 3, and 4 on I_{CaL} are most pronounced at low levels of β-adrenergic stimulation. Simulations also delineate the contribution of the following two mechanisms to overall LCC regulation, which have otherwise been challenging to distinguish: 1) regulation of PKA and PKG activation via cN cross-talk (Mechanism 1); and 2) LCC interaction with activated PKA and PKG (Mechanism 2). These results provide insights into how cN signals transduced via the cN cross-talk signaling network are integrated via LCC regulation in the heart.

1. Introduction

The cardiac voltage-gated L-type calcium (Ca^{2+}) channel (LCC) initiates and coordinates a series of events that give rise to the cardiac myocyte action potential (AP) and mechanical contraction and relaxation within each heartbeat [1,2]. Activated upon membrane depolarization, LCCs allow Ca^{2+} influx across the sarcolemma [3] into nanostructures known as cardiac dyads, defined as regions where the sarcoplasmic reticulum (SR) membrane closely apposes (to within ~12 nm) the sarcolemma. Ca^{2+}-binding Ca^{2+} release channels, known as ryanodine receptors (RyRs), are localized to the junctional SR (JSR) membrane of the dyad. LCC openings increase dyad Ca^{2+} concentration ($[Ca^{2+}]$) and Ca^{2+} binding to RyRs. Upon Ca^{2+} binding, RyRs open to release Ca^{2+} from the JSR Ca^{2+} store in a process known as Ca^{2+}-induced Ca^{2+} release (CICR) [3,4]. The elevated $[Ca^{2+}]$ promotes Ca^{2+} binding to myofilaments, initiating contraction [5,6]. The process by which electrical excitation leads to mechanical contraction of the myocyte is referred to as the cardiac excitation-contraction (EC) coupling [3,7].

Our previous work constructed a mechanistic model of the cyclic nucleotide (cN) cross-talk signaling network (Fig. 1A) [8,9], composed of the β-adrenergic signaling pathway (red color scheme), the nitric oxide (NO)/cGMP/PKG signaling pathway (blue color scheme), and cross-talk between them (yellow color scheme) as facilitated by five distinct phosphodiesterases (PDEs) (orange boxes) [11–13]. Stimulation of the β-adrenergic and NO/cGMP/PKG pathways exert opposing physiological responses, with the former enhancing cardiac inotropy

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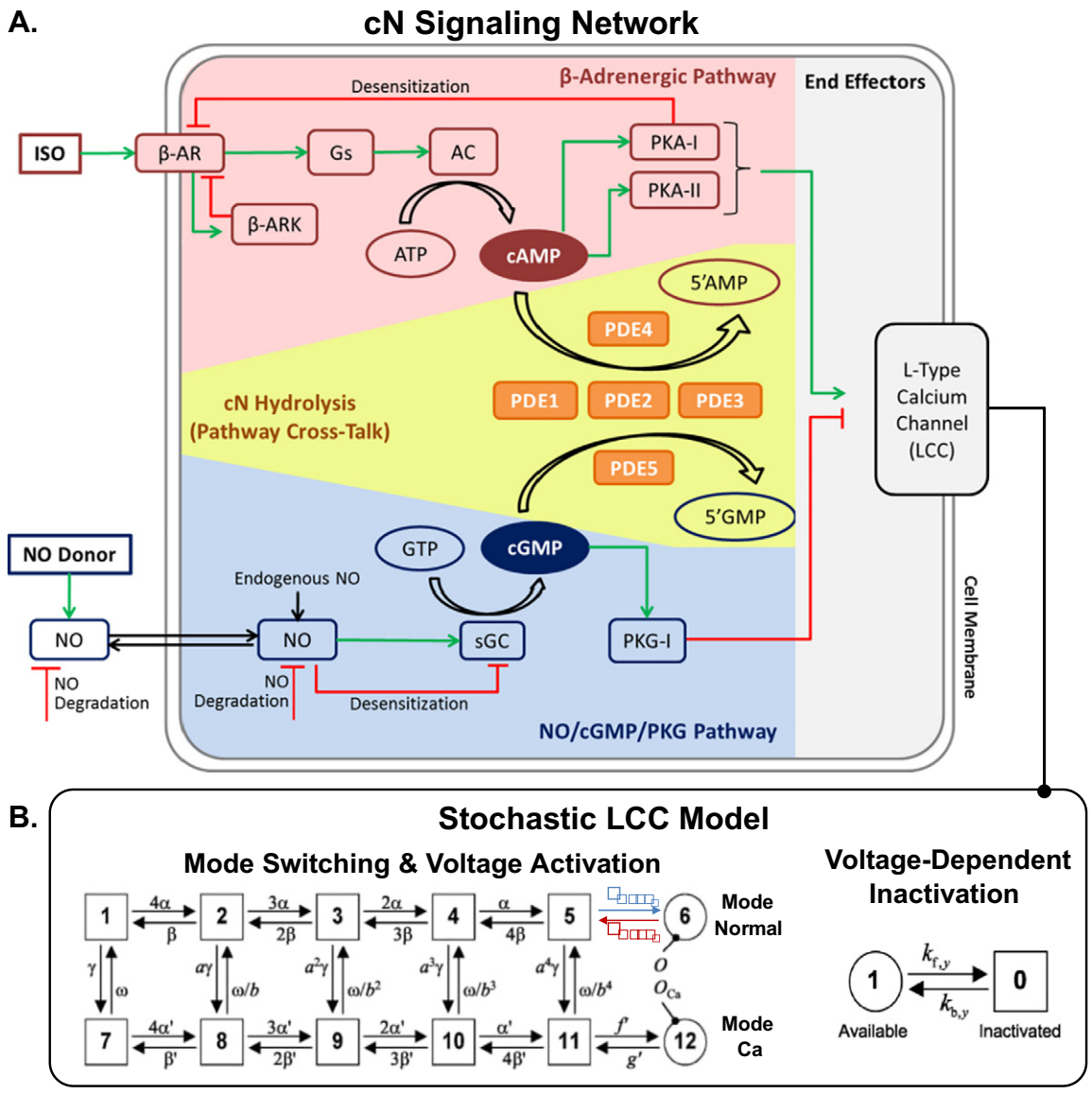

Fig. 1. Model representation of LCC regulation by the cN cross-talk signaling network. (A) The cyclic nucleotide (cN) cross-talk signaling network model from Zhao et al. [8] is composed of the β-adrenergic and NO/cGMP/PKG pathways (red and blue color schemes respectively) as well as the PDEs that regulate cN degradation (yellow color scheme). The PDEs are in turn regulated by the cNs; therefore, they also facilitate cross-talk between the two pathways [8,9]. The entire signal network transduces stimuli (e.g. ISO and NO) to the activation of PKA (isoforms PKA-I and PKA-II) and PKG (isoform PKG-I), which then respectively deliver stimulatory (green arrow) and inhibitory (red arrow) control of LCC (grey color scheme). Model schematics adapted from Zhao et al. [8]. (B) Each individual LCC undergo voltage- and Ca^{2+}-dependent gating (left) and an independent process of voltage-dependent inactivation (VDI) (right), according to a model from Greenstein and Winslow [10]. PKA- and PKG-mediated phosphorylation of LCC promotes LCCs to open and close in distinct gating modes ($Mode_i$), characterized by distinct gating parameters f_{mode_i} (blue) and g_{mode_i} (red). Model schematics adapted from Greenstein and Winslow [10].

and lusitropy [3,14] and the latter attenuating cardiac contractility [15–19] and antagonizing β-adrenergic tone [11–13,20–27]. The importance of a delicate balance of cN signaling is reflected by isoform-specific alternations in PDEs in cardiac diseases [23,26,28–30]. As examples, PDE2 upregulation in the failing heart is observed to attenuate β-adrenergic signaling [26], decreased PDE3 activity promotes cardiac myocyte apoptosis [28], and PDE4 downregulation is associated with arrhythmias in cardiac hypertrophy and HF [29]. Drugs that target specific PDE activities have cardio-protective effects [31], such as restoration of PDE3 activity in ischemic and dilated cardiomyopathies [32], restoration of PDE1 and PDE4 activities in cardiac ischemia [33], and inhibition of PDE5 in heart failure, cardiac hypertrophy, and ventricular arrhythmias [34–37].

The cN signaling cross-talk network (Fig. 1A) exerts both stimulatory (green arrow) and inhibitory (red arrow) regulation of LCCs (Fig. 1B). These actions are mediated by the dynamics of the two cyclic nucleotides (cNs), cyclic adenosine-3′, 5′-monophosphate (cAMP) and cyclic guanosine-3′, 5′-monophosphate (cGMP), as well as the subsequent activation of protein kinase A (PKA) and protein kinase G (PKG) [8,9]. More specifically, PKA isoforms, PKA-I and PKA-II, and PKG isoform, PKG-I, are predominant in cardiac myocytes [8,9]. As shown in Fig. 1B, the random openings and closings of LCCs result from an interplay between the processes of voltage-dependent activation (left model, horizontal transitions), Ca^{2+} dependent inactivation (CDI; left model, vertical transitions), and voltage-dependent inactivation (VDI; right model) [10]. In addition, Fig. 1B (left model) represents one of a number of possible LCC gating modes ($Mode_i$) depending upon PKA- and PKG-mediated phosphorylation of the channel, in response to the cN cross-talk signaling network [3,35]. The values of transition rates, f_{mode_i} and g_{mode_i} (colored), depend on the gating mode ($Mode_i$). Explanation of gating mode transitions will follow in Methods below. In diseases such as cardiac hypertrophy and heart failure (HF) [30,38–41], the imbalances between β-adrenergic and NO/cGMP/PKG signaling and the resultant changes in L-type Ca^{2+} current (I_{CaL}) lead to alterations in EC