Journal of Molecular and Cellular Cardiology 91 (2016) 215–227

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Roles of phosphodiesterases in the regulation of the cardiac cyclic nucleotide cross-talk signaling network

Claire Y. Zhao, Joseph L. Greenstein, Raimond L. Winslow *

Department of Biomedical Engineering and the Institute for Computational Medicine, The Johns Hopkins University School of Medicine and Whiting School of Engineering, 3400 N Charles Street, Baltimore, MD 21218, USA

ARTICLE INFO

Article history:
Received 25 October 2015
Received in revised form 12 December 2015
Accepted 4 January 2016
Available online 7 January 2016

Keywords:
Cyclic nucleotide cross-talk signaling network
Phosphodiesterases
β-adrenergic pathway
NO/cGMP/PKG pathway
Cardiac myocytes
Computational model

ABSTRACT

The balanced signaling between the two cyclic nucleotides (cNs) cAMP and cGMP plays a critical role in regulating cardiac contractility. Their degradation is controlled by distinctly regulated phosphodiesterase isoenzymes (PDEs), which in turn are also regulated by these cNs. As a result, PDEs facilitate communication between the β-adrenergic and Nitric Oxide (NO)/cGMP/Protein Kinase G (PKG) signaling pathways, which regulate the synthesis of cAMP and cGMP respectively. The phenomena in which the cAMP and cGMP pathways influence the dynamics of each other are collectively referred to as cN cross-talk. However, the cross-talk response and the individual roles of each PDE isoenzyme in shaping this response remain to be fully characterized. We have developed a computational model of the cN cross-talk network that mechanistically integrates the β-adrenergic and NO/cGMP/PKG pathways via regulation of PDEs by both cNs. The individual model components and the integrated network model replicate experimentally observed activation–response relationships and temporal dynamics. The model predicts that, due to compensatory interactions between PDEs, NO stimulation in the presence of sub-maximal β-adrenergic stimulation results in an increase in cytosolic cAMP accumulation and corresponding increases in PKA-I and PKA-II activation; however, the potentiation is small in magnitude compared to that of NO activation of the NO/cGMP/PKG pathway. In a reciprocal manner, β-adrenergic stimulation in the presence of sub-maximal NO stimulation results in modest cGMP elevation and corresponding increase in PKG activation. In addition, we demonstrate that PDE2 hydrolyzes increasing amounts of cAMP with increasing levels of β-adrenergic stimulation, and hydrolyzes increasing amounts of cGMP with decreasing levels of NO stimulation. Finally, we show that PDE2 compensates for inhibition of PDE5 both in terms of cGMP and cAMP dynamics, leading to cGMP elevation and increased PKG activation, while maintaining whole-cell β-adrenergic responses similar to that prior to PDE5 inhibition. By defining and quantifying reactions comprising cN cross-talk, the model characterizes the cross-talk response and reveals the underlying mechanisms of PDEs in this non-linear, tightly-coupled reaction system.

1. Introduction

The two cyclic nucleotides (cNs), cyclic adenosine-3′, 5′-monophosphate (cAMP) and cyclic guanosine-3′, 5′-monophosphate (cGMP), are intricately-regulated second messengers. Phosphodiesterase isoenzymes (PDEs) rely on the feedback of both cAMP and cGMP signals to modulate the rate at which they degrade these two cNs [1–3]. The integration of these feedback signals provides the means for communication between the β-adrenergic and Nitric Oxide (NO)/cGMP/Protein Kinase G (PKG) signaling pathways, which regulate the synthesis of cAMP and cGMP respectively. The phenomenon whereby one cN signal influences the dynamics of the other is commonly referred to as cN cross-talk [4–6]. It is known that stimulation of the β-adrenergic and NO/cGMP/PKG pathways exert opposing physiological responses, with the former enhancing cardiac inotropy and lusitropy [7,8] and the latter attenuating cardiac contractility [9–13] and antagonizing β-adrenergic tone [4–6,14–21]. Furthermore, many signaling components in the network are perturbed in cardiac hypertrophy and heart failure (HF), potentially contributing to disease remodeling [22–26]. As a result, it is important to understand the regulatory mechanisms that maintain balance between the cN signals in both health and disease.

The complex interactions comprising cN cross-talk, and the participation of multiple PDEs in the common task of cN degradation make understanding the nature of these regulatory mechanisms challenging [1,27–29]. As shown in Fig. 1A, the synthesis of cAMP and cGMP is regulated by the β-adrenergic pathway (red background) and the NO/cGMP/PKG pathway (blue background) in response to external and/or internal stimuli [11,30,31]. Synthesis of cAMP is catalyzed by adenylyl cyclase (AC) via a stimulatory G-protein (Gs) mediated process that occurs

* Corresponding author.
E-mail addresses: czhao10@jhu.edu (C.Y. Zhao), jgreenst@jhu.edu (J.L. Greenstein), rwinslow@jhu.edu (R.L. Winslow).

http://dx.doi.org/10.1016/j.yjmcc.2016.01.004
0022-2828/

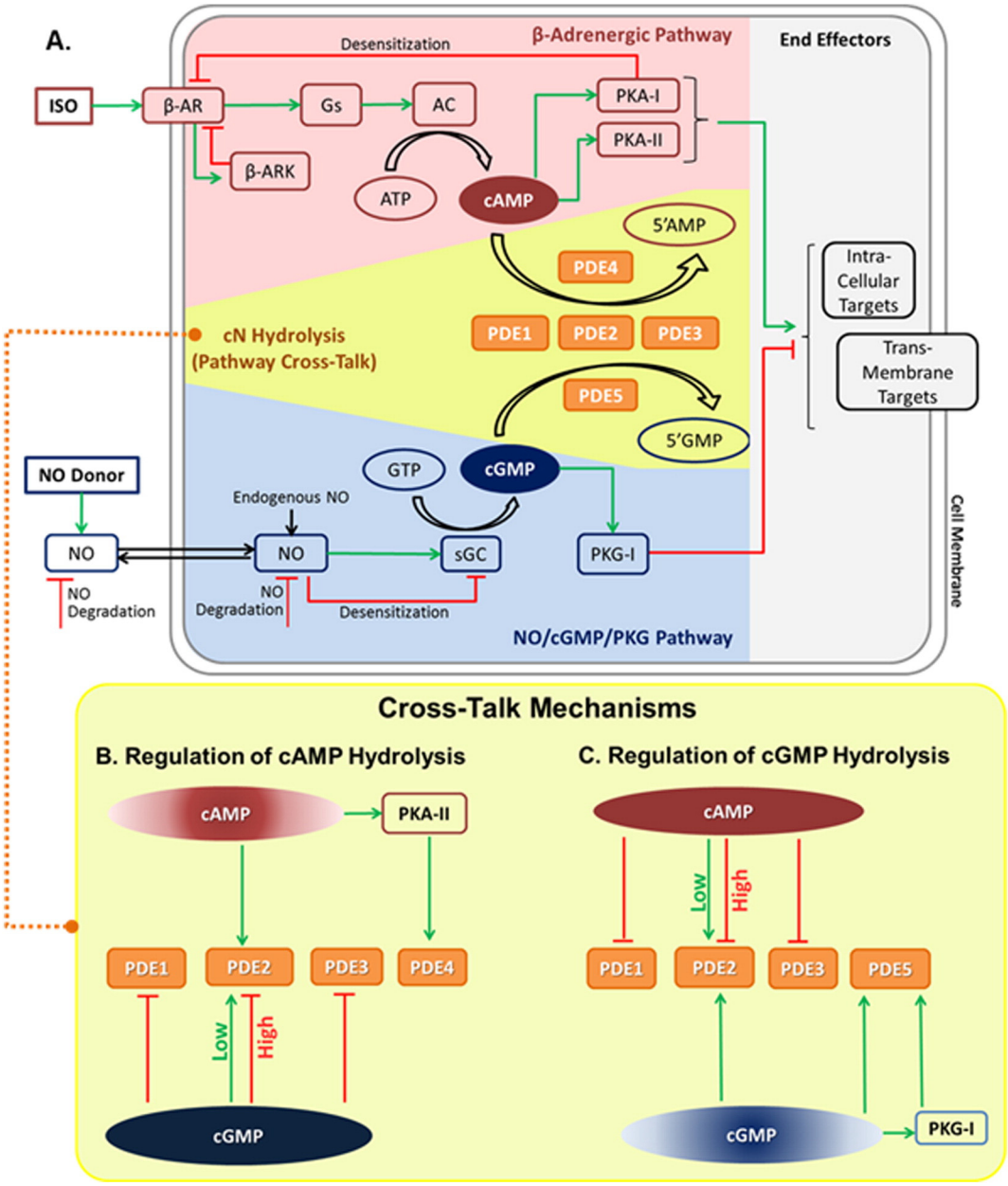

Fig. 1. Cyclic nucleotide cross-talk signaling network. (A) The cN cross-talk signaling network model is composed of the β-adrenergic pathway (red background), the NO/cGMP/PKG signaling pathway (blue background), and cross-talk between them (yellow background). Cross-talk is mediated by PDEs 1–5. In the regulation of cAMP- (B) and cGMP- (C) hydrolysis, cNs exert positive (green arrows) or negative (red arrows) regulation of PDE activities. In particular, PDE2 hydrolysis rate of either cN is stimulated (green arrow) by low concentrations of the other cN but is suppressed (red arrow) if the concentrations are sufficiently high. To avoid crowding the figure, the hydrolysis reactions of cNs are omitted in (B) and (C), which would have been drawn as red arrows originating from each PDE to cAMP in (B) and cGMP in (C). Instead, hydrolysis of cAMP and cGMP are respectively represented by ovals of faded red in (B) and faded blue in (C).

when catecholamines (e.g. norepinephrine and epinephrine) bind to and activate β-adrenergic receptors (β-ARs) [23]. Synthesis of cGMP is catalyzed by soluble guanylate cyclase (sGC) in response to an increase in NO concentration ([NO]) [9,13]. In cardiac myocytes, the degradation of cAMP and cGMP are primarily catalyzed by PDEs 1–5, each with its own unique biochemical characteristics and regulatory mechanisms [1,4,27,32–36] (yellow background). PDEs 1, 2, and 3 are capable of hydrolyzing both cNs, while PDE4 and PDE5 are specific to cAMP and cGMP respectively. The coordinated regulation of PDEs 1, 2, and 3 activities by both cNs thus functionally bridge together the β-adrenergic and NO/cGMP/PKG pathways. The net cN signals regulate downstream targets (gray background) via phosphorylation of these targets by Protein Kinase A (PKA) (isoforms PKA-I and PKA-II) and/or PKG (Fig. 1A). As examples, these targets include L-type Calcium (Ca^{2+}) channels (LCCs), phospholamban (PLB), and Troponin I (TnI) for both PKA [8] and PKG [9,37,38].

The cross-talk between β-adrenergic and NO/cGMP/PKG pathways consists of a variety of cN-mediated reactions that regulate PDE activities (Fig. 1A and B). As shown in Fig. 1B, cAMP degradation is regulated by PDEs 1–4 in cardiac myocytes [1,4,27,32–36]. As a form of negative feedback, cAMP can stimulate its own degradation through activation of PDEs 2 and 4 (green arrows) [39]. The presence of cGMP can potentially increase cAMP concentration ([cAMP]) by inhibiting cAMP hydrolysis rates of PDEs 1 and 3 (red arrows) [39]. Depending on its concentration ([cGMP]), cGMP can either inhibit or potentiate [cAMP] by regulating PDE2 cAMP hydrolysis activity (alternating red/green arrows) [39]. As shown in Fig. 1C, cGMP dynamics depend on the activities of PDEs 1, 2, 3, and 5 [32–34,36]. Negative feedback on [cGMP] is