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

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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

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Disclosures

None declared.

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reactions comprising cN cross-talk, the model characterizes the crosstalk response and reveals the underlying mechanisms of PDEs in this non-linear, tightly-coupled reaction system.

Keywords

Cyclic nucleotide cross-talk signaling network; Phosphodiesterases; β -adrenergic pathway; NO/cGMP/PKG pathway; cardiac myocytes; computational model

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 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 depends on the activities of PDEs 1, 2, 3, and 5 [32–34, 36]. Negative feedback on [cGMP] is accomplished by cAMP- and cGMP-dependent activation of PDE2 and cGMP-dependent activation of PDE5 [32, 33, 36, 40]. The presence of cAMP can potentially increase [cGMP] by inhibiting cGMP degrading activities of PDEs 1 and 3, while either inhibiting or potentiating [cGMP] by regulating PDE2 cGMP hydrolysis activity depending on [cAMP] [32, 36].

cAMP- and cGMP-mediated regulation of PDEs 1–5 has been studied primarily in protocols using purified protein extracts [34–36]. The interpretation of experiments investigating the roles of multiple PDEs by measuring [cAMP] and/or [cGMP] in response to application of selective PDE inhibitors can be confounded by compensatory network interactions between the remaining PDEs [39]. As a result, it is difficult to attain a systems level understanding of the signaling network that bridges the causal link between the characteristics of individual signaling proteins and the collective response of the entire network. To address this, we present a biophysically-detailed kinetic model of the cN cross-talk network (Fig. 1A) that includes mechanistic models of cN regulation of PDEs 1–5 (Fig. 1B–C). Three major novel predictions emerge from this model. First, simultaneous NO stimulation in the presence of sub-maximal β -adrenergic stimulation results in potentiation of whole-cell β -adrenergic response; reciprocally, β -adrenergic stimulation in the presence of sub-maximal NO stimulation results in increased [cGMP] and PKG activation. These increases, however, are small in magnitude when compared to direct activation of the NO/cGMP/PKG pathway by NO and the β -adrenergic pathway by isoproterenol (ISO). Second, PDE2 degrades increasing proportions of cAMP with increasing β -adrenergic stimulation; however, it degrades more cGMP under decreasing [NO]. Finally, the compensatory actions of PDE2 under selective PDE5 inhibition regulated the enhanced [cGMP] and PKG activation and maintain whole-cell β -adrenergic response similar to that prior to PDE5 inhibition.

2. Materials and Methods

In a previously developed model, we studied PDE regulation of cAMP and response of the β -adrenergic pathway by treating [cGMP] as a model parameter [39]. Here, we extend this model by incorporating a mechanistic description of cGMP production and degradation. Details of the model are presented in the Supplement. Symbols are defined in Suppl. Sect. I; Sect. II provides all model equations; Sect. III defines and gives nominal values for model parameters; Sect. IV lists state variable initial conditions. Synthesis of cGMP is modeled by incorporating a NO/sGC model by Batchelor et al. (Eqs. 15–20 Suppl. Sect. IIB) [41]. Models for PDEs 1–4 (Suppl. Sect. IIC) are those described previously in Zhao et al. [39], with PDEs 1–3 now regulating cGMP degradation as well as cAMP degradation (Fig. 1).

Based on the newly developed model of PKG activation by cGMP (Eqs. 40–43 Suppl. Sect. IIE; rationale Suppl. Sect. VB), the description of PDE5 degradation of cGMP is modified from that in Batchelor et al. [41] to explicitly include PDE5 activation upon phosphorylation by PKG [40, 42–46] (Eq. 32 Suppl. Sect. IIC; rationale Sect. VA). In order to replicate experimental protocols [1, 47–49], NO release from the NO donor SNAP is modeled according to existing literature [50–56] (Eqs. 12–14 Suppl. Sect. IIB; rationale Sect. VC). Experimental conditions for data depicted in figures are summarized in Suppl. Sect. VIII. Model signaling components are constrained by experimental data (Suppl. Sect. VIA–C) in conjunction with the experimentally determined ranges for model parameters (Suppl. Sect. III). The individual PDE component models are integrated within the rest of the network according to constraints in Suppl. Sect. VIB. The remaining model components and parameters are unchanged from those presented in Zhao et al. [39] and Batchelor et al. [41]. Model integration allowed for the construction of the entire cN signaling network which includes all biochemical actions outlined in Fig. 1. All figures contained in this paper, including those in the Supplement, are generated with the same set of parameters and state variable initial conditions.

3. Results

3.1 Validation of individual PDE models

The PDE models were validated against experimental data describing their interaction with cAMP and cGMP (Fig. 2), after being constrained by a separate set of experiments (Suppl. Sect. VIA). These model validation data (Fig. 2, dotted symbols) have not been used in the model fitting process. Data in Fig. 2A, B, and D (dotted symbols) are at different [cAMP] and [cGMP] than those used to constrain the respective PDE models (Suppl. Fig. S3A – C) from the same experimental studies. As shown in Fig. 2A, the PDE1 model reproduces cAMP hydrolysis rates at constant [cGMP] (6.25 μ M) while varying [cAMP] as reported by Yan et al. [57]. In addition, it replicates cGMP hydrolysis rates at constant [cAMP] (6.25 μ M) while varying [cGMP] as reported by Yan et al. [57] (Fig. 2B). As shown in Fig. 2C, the PDE2 model replicates the dome-shaped cAMP hydrolysis curve as a function of [cGMP] reported by Prigent et al. [58], as well as cAMP regulation of PDE2 cGMP hydrolysis with 10 μ M [cGMP] (Fig. 2D) observed by Russell et al. [59]. When only one cN is present in the reaction system, the PDE2 EC₅₀s (half-maximal activation concentrations) for both cAMP and cGMP hydrolysis also agree with experimental averages [28, 34] (Suppl. Fig. S7). As shown in Fig. 2E and F respectively, PDE3 cAMP and cGMP hydrolysis are suppressed by the other cN, in agreement with experimental data of He et al. [60].

3.2 Validation of the integrated signaling network model

The behavior of the full signaling network model (Fig. 1) is validated by comparison with experimental data obtained from rat cardiac ventricular myocytes (Fig. 3 dotted symbols), which have not been used in model development. The model qualitatively replicates whole-cell [cAMP] responses to ISO with (blue) and without (black) inhibition of PDEs by IBMX (Fig. 3A) as measured by Vila-Petroff et al. [47] (blue dots) and Kuznetsov et al. [62] (black dots). In addition, the model approximates the time course of cytosolic [cAMP] transients in response to acute β -adrenergic stimulation (Fig. 3B) as reported by Vila-Petroff et al. [63]

(filled black dots) and Zaccolo et al. [48] (hollow blue dots) and to specific PDE inhibitors according to Rochais et al. [64] (Suppl. Fig. S8).

The model replicates experimentally measured [cGMP] under basal, non-stimulated conditions (Fig. 3C, first two columns) [40, 49] as measured by Castro et al., 2006 [49] (black dots), both before (gray) and after (white) inhibition of all PDEs by IBMX. The basal [cGMP] of ~ 10 nM (first column) also agrees with data of Götz et al. [65]. In addition, simulated [cGMP] under NO stimulation from SNAP follows data of Castro et al., 2010 [40] (blue dots), before (gray) and after (white) IBMX application (Fig. 3C, last two columns). Furthermore, the temporal dynamics of [cGMP] accumulation and degradation approximates data from Castro et al. [49] with SNAP and subsequent IBMX application (Fig. 3D). Finally, as shown in Fig. 3E, [cGMP] time course under specific PDE inhibitors for PDE2 and PDE5 agrees with data of Castro et al. [49]. Details on simulations and data processing for Fig. 3C–E are provided in Suppl. Sect. VIIIA. The agreement of the model with data from a wide range of experiments (Fig. 2 and Fig. 3; Suppl. Figs. S3–8) indicates the assumptions underlying model mechanisms are reasonable.

3.3 β -adrenergic pathway and NO/cGMP/PKG pathway responses are potentiated upon NO and ISO stimulation respectively via cN cross-talk

Cross-talk between the cN pathways refers to the signaling mechanisms by which β -adrenergic stimulation leads to PKG activation and/or NO elevation leads to PKA activation, i.e. the phenomenon whereby one pathway's input signal influences the response of another pathway. The responses of the pathways to their respective stimuli are referred to as the direct responses, which consist of changes in β -adrenergic responses upon ISO stimulation and changes in NO/cGMP/PKG pathway responses upon NO stimulation. In Fig. 4, we compare the cross-talk responses (right-hand axes, gray color scheme) to the direct responses of each pathway (left-hand axes, black color scheme). As shown in Fig. 4A–C (black lines), under 1 nM ISO, increasing [NO] via an increase in [SNAP] leads to substantial activation of the NO/cGMP/PKG pathway, with large increases in [cGMP] (Fig. 4A) and PKG activation (Fig. 4B and C). Comparatively, increases in the β -adrenergic cross-talk responses measured in terms of [cAMP] and activation of PKA-I and PKA-II are smaller in magnitude (Fig. 4A–C respectively, gray lines). In order to fully characterize the cross-talk responses, percent increases in β -adrenergic pathway activations are calculated under varying [ISO] with low, medium, and high [SNAP] of 5 nM, 50 nM, and 500 nM (Suppl. Fig. S9A–C). It can be seen that the NO cross-talk effects on the β -adrenergic pathway increases with [NO], reaching up to ~ 30% in potentiation of [cAMP] and activation of PKA-I and PKA-II. On the other hand, this cross-talk response diminishes with increasing [ISO], reaching negligible values for [ISO] above 100 nM (Suppl. Fig. S9A–C). Under the same simulation protocol, the percent increases in [cGMP] and PKG activation (Suppl. Fig. S10A and B) are comparable in magnitude as those for the β -adrenergic pathway (Suppl. Fig. S9A–C).

In addition, the β -adrenergic response is substantially activated by increasing [ISO] under 5 nM [SNAP], with large increases in [cAMP], PKA-I activation, and PKA-II activation (Fig. 4D–F respectively, black lines). Under the same simulation protocol, the cross-talk response

in the NO/cGMP/PKG pathway is also potentiated by ISO, although the increase in [cGMP] (Fig. 4D, gray line) and PKG activation (Fig. 4E and F, gray lines) are relatively small in magnitude compared to that of the direct pathway response (black lines). In Suppl. Fig. S9D and E, the cross-talk response is characterized by calculating percent increases in NO/cGMP/PKG pathway activation under low, medium, and high [ISO] (1 nM, 10 nM, and 1 μ M [ISO] respectively) across various [SNAP]. The potentiation in [cGMP] and PKG activation increases with increasing [ISO], reaching maximal percent increases of ~ 60% and ~ 90% for [cGMP] and PKG activation respectively. The cross-talk response decreases with increasing [SNAP] until reaching a nadir for [SNAP] above 1 μ M (Suppl. Fig. S9D and E). In addition, percent increases in [cAMP] and PKA-I and PKA-II activation (Suppl. Fig. S10C–E) are an order of a magnitude larger than those for the NO/cGMP/PKG pathway (Suppl. Fig. S9D and E).

3.4 Mechanisms underlying cross-talk responses

Results shown in Fig. 5 attempt to tease apart the interactions between PDEs (black, red, blue, green, and cyan for PDEs 1–5 respectively) that give rise to the observed cross-talk responses characterized in Fig. 4 and Suppl. Figs. S9 and S10. At both low (1 nM; Fig. 5A) and high (1 μ M; Fig. 5B) [ISO], similar mechanisms account for the observed increase of [cAMP] with increasing [NO] (or [SNAP]). Increasing [NO] increases [cGMP], and cGMP competes with cAMP for the catalytic site of PDE3. This results in a progressive decrease in PDE3 cAMP hydrolysis rate (blue), leading to a net increase in [cAMP]. The observed increases in PDE2 (red) and PDE4 (green) cAMP hydrolysis rates due to feedback activation by increased [cAMP], and additional allosteric activation of PDE2 by cGMP are not sufficient to compensate for the steep decrease in PDE3 rate, especially at higher [SNAP] (e.g. 50 nM and 500 nM). These changes in PDE rates result in a net decrease in cAMP hydrolysis rates across all PDEs (gray) and larger cAMP accumulation at higher [SNAP]. In Fig. 5B, the same [SNAP] under high [ISO] conditions (e.g. 1 μ M) produces much smaller changes in PDE rates. This occurs because [cAMP] is elevated in response to strong β -adrenergic stimulation, and cAMP then binds to a larger proportion of PDEs, making them less available to interact with cGMP. As such, increases in [cGMP] result in much less changes in PDE activities under high [ISO] than that under low [ISO]. The much smaller magnitude of decrease in PDE3 rates observed at high [ISO] is further compensated by increases in PDE2 and PDE4 rates, resulting in a smaller net rate increase across all PDEs (Fig. 5B). Consequently, the impact of NO stimulation on the β -adrenergic pathway becomes less significant under stronger β -adrenergic stimulation.

With regard to cGMP hydrolysis, as shown in Fig. 5C and D, increases in PDE2 (red) and PDE5 (cyan) rates over a range of [ISO] cannot fully counteract the decreases in PDE3 (blue) and PDE1 (black) cGMP hydrolysis rates, leading to a net decrease in cGMP hydrolysis rate across all PDEs (gray) and therefore the observed [cGMP] accumulation (Fig. 4D) and increased PKG activation (Fig. 4E). The decreased PDE1 (black) and PDE3 (blue) cGMP hydrolysis rates are due to increased competition of cAMP, arising from β -adrenergic stimulation, over cGMP for binding the catalytic domains of these PDEs. Increases in PDE2 and PDE5 cGMP rates are due to feedback signaling from increased [cGMP], with some additional increase in PDE2 cGMP rate arising from allosteric

activation by increased [cAMP]. A comparison of Fig. 5D to C, in which an increased [cGMP] lessens the availability of PDEs to interact with cAMP, the impact of ISO stimulation on the NO/cGMP/PKG pathway becomes less significant under stronger NO stimulation (Fig. 4D and E).

3.5 Role of PDE2 in cN cross-talk network

Using the model, the roles of individual PDEs can be probed in the complete cross-talk network. In particular, because PDE2 exhibits multiple modes of interaction with cAMP and cGMP (Fig. 1B and C), it is challenging to experimentally quantify its actions in the cross-talk network. PDE2 cAMP hydrolysis rates can be allosterically activated by cGMP binding to its GAF-B regulatory domain; however, high [cGMP] can also suppress PDE2 cAMP hydrolysis rate as it competes with cAMP for its catalytic domain (Fig. 1B). Similarly, lower [cAMP] can increase PDE2 cGMP hydrolysis rate by occupying GAF-B regulatory domain, while higher [cAMP] can suppress its rate by out-competing cGMP in occupying the catalytic domain (Fig. 1C).

Fig. 6A shows predicted PDE2 cAMP hydrolysis rates as a percentage of the total cAMP hydrolysis rates of all PDEs; similarly, Fig. 6B shows predicted PDE2 cGMP hydrolysis rates as a percentage of the total cGMP hydrolysis rates of all PDEs. The proportion of cAMP degraded by PDE2 increases with increasing [ISO] (Fig. 6A). This is due to increased substrate availability from increased cAMP synthesis produced by increased β -adrenergic stimulation. The proportion of cAMP degraded by PDE2 also increased with increased [NO] from SNAP due to allosteric activation of PDE2 and compensation for the competitive inhibition of PDE1 and PDE3 cGMP activities by cGMP. As shown in Fig. 6B, PDE2 is simultaneously responsible for hydrolyzing up to ~ 60% of cGMP. Increasing [ISO] potentiated the proportion of cGMP hydrolyzed by PDE2 across all [SNAP] primarily because PDE2 compensated for the decrease in PDE1 and PDE3 rates arising from competitive inhibition by elevated [cAMP]. On the other hand, increased [cGMP] from increased [SNAP] increased PDE5 cGMP hydrolysis rate such that the proportion of cGMP hydrolyzed by PDE2 is lower at higher [SNAP].

3.6 NO/cGMP/PKG activity potentiated by PDE5 inhibition is partially compensated by PDE2

The effect of PDE5 inhibition has been investigated extensively because of its therapeutic promise in treating heart diseases such as cardiac hypertrophy and heart failure [38, 66–68]. The model makes predictions about the impact of PDE5 inhibition on changes in PDE interactions under varying extent of β -adrenergic and NO stimulation (Fig. 7). Figure 7 shows effects of strong PDE5 inhibition (90%) on all PDEs (Fig. 7A), and varying levels of PDE5 inhibition on PDE2 cGMP hydrolysis rates (Fig. 7B), [cGMP] (Fig. 7C), and PKG activation (Fig. 7D) as [ISO] is varied in the presence of medium level of [SNAP] (50 nM). In order to study the effect of PDE5 inhibition under varying [ISO], percent increase in response is given relative to values prior to PDE5 inhibition at the selected [SNAP] without ISO. As shown in Fig. 7A, upon PDE5 inhibition, the compensatory increase in PDE2 cGMP rate is the greatest among the cGMP hydrolyzing PDEs regardless of [ISO], with magnitudes that also scale with the extent of PDE5 inhibition (Fig. 7B). The cGMP rate

increases in PDEs 1–3 are due to increased cGMP-occupancy of their catalytic domains (Suppl. Fig. S12A). Reciprocally, compensatory increase in PDE5 cGMP rates is also the greatest upon PDE2 inhibition (Suppl. Fig. S11). As a result, among all PDEs, PDE2 and PDE5 compensate most strongly for a reduction in activity of the other, a behavior we refer to as strong coupling. In addition, as shown in Fig. 7C and D, PDE5 inhibition leads to sizable increases in [cGMP] and consequently PKG activation.

3.7 Whole-cell [cAMP] is stabilized under PDE5 inhibition via PDE2 activation

Fig. 8 shows the impact of PDE5 inhibition on β -adrenergic response. As shown in Fig. 8A, PDE5 inhibition indirectly leads to a decrease in PDE3 cAMP hydrolysis rates. There is also a smaller magnitude of PDE1 cAMP hydrolysis rate decrease, which is less significant due to the smaller overall PDE1 cAMP degradation capacity compared to that of PDE3. Changes in PDE rates are observed because increased [cGMP] from PDE5 inhibition (Fig. 7A) competes with cAMP for the catalytic domains of PDEs 1 and 3 (Suppl. Fig. S12B) and suppresses their cAMP hydrolysis rate. On the other hand, a large increase in PDE2 cAMP hydrolysis rate results from allosteric activation by cGMP and feedback regulation by the elevated [cAMP] (Suppl. Fig. S12B). The resulting elevation in [cAMP] is therefore almost completely compensated for by PDE2 (Fig. 8B). The increase in PDE2 (Fig. 8C) and decrease in PDE3 (Fig. 8D) rates vary depending on the extent of PDE5 inhibition, preventing changes in β -adrenergic response under PDE5 inhibition regardless of [ISO] (Fig. 8B).

4. Discussion

4.1 Multi-scale modeling of cN signaling reveals cross-talk responses and mechanisms

By measuring responses downstream of the signaling network, independent activation of the β -adrenergic pathway by β -agonists and the NO/cGMP/PKG pathway by NO are observed to result in antagonistic actions, with the former strengthening and the latter attenuating cardiac contractility [7–13]. Balanced signaling between cAMP and cGMP is crucial in maintaining normal cardiac function, especially when prominent remodeling of the cN signaling network is also observed in cardiac hypertrophy and heart failure, potentially contributing to altered and/or untargeted cN signals, aberrant phosphorylation of target proteins, and cardiac dysfunction [22–26]. However, relatively little is known about the ways in which different PDE isoforms regulate the cN cross-talk that enables communication between the β -adrenergic and NO/cGMP/PKG pathways. Several factors make it difficult to understand these interactions: a) PDEs degrade the same cAMP and cGMP signals that regulate their activities (Fig. 1A); b) multiple PDE isoenzymes function synergistically in the common task of cAMP and cGMP degradation (Fig. 1A); c) a variety of positive and negative regulatory relationships exist between PDEs, cGMP, and cAMP (Fig. 1B and C). As a result, when the activity of one PDE is altered, the activities of other PDEs will also change to partially compensate for the change in cN signals, activating a subset or all of the many reaction mechanisms identified in Fig. 1B–C on a scenario-dependent basis. By precisely defining biochemical reactions comprising the cN cross-talk network (Fig. 1), models are useful tools for revealing the mechanisms of PDE interaction underlying the communication between the β -adrenergic and NO/cGMP/PKG pathways.

The model presented here enables analysis of the roles of each PDE in the context of the full signaling network. Model analyses demonstrate that: a) at sub-maximal levels of β -adrenergic stimulation, simultaneous NO stimulation decreases PDE3 cAMP hydrolysis rate, which overcomes increases in PDE2 and PDE4 rates, resulting in a modest increase of whole-cell [cAMP] and the observed NO-mediated potentiation of Protein Kinase A (PKA) activation; b) at sub-maximal levels of NO stimulation, the decreased PDE3 cGMP hydrolysis rates produced by simultaneous β -adrenergic stimulation overcomes increases in the rates of PDE2 and PDE5, resulting in a net increase of [cGMP] and PKG activation (Figs. 4–5); c) PDE2 exerts more control of cytosolic [cAMP] and [cGMP] under increasing β -adrenergic stimulation and decreasing NO stimulation (Fig. 6); d) due to PDE2 compensation, the anti-adrenergic effect of PDE5 inhibition is mediated via increased PKG activation (Fig. 7) while changes in whole-cell β -adrenergic response are kept small (Fig. 8). These results reflect that redundancy of the PDEs in the cN signaling network contributes a degree of robustness to perturbations. This compensatory coupling between PDEs may be an important mechanism in controlling cN dynamics in the heart [17, 22, 69], including disease settings where alterations in isoenzyme-specific PDE expression and/or activity have been implicated [17, 22, 69–79].

4.2 PKG is likely to suppress downstream target activity as cross-talk responses are stimulatory

Understanding the effects of NO on biological tissues remains challenging in part because NO, a highly reactive gas, is difficult to manipulate and has many reaction pathways and targets [12, 13, 80–85]. Prior experimental results have found that NO decreases the cardiac functional response to β -adrenergic stimulation [13, 80, 86–88]. For instance, in cardiac ventricular myocytes, pre-stimulated whole-cell LCC current via β -adrenergic pathway stimulation is diminished by increased [NO] [89] and inotropic responses to β -adrenergic stimulation are enhanced in eNOS knockout (eNOS^{−/−}) mice [90]. On the other hand, the exact mechanisms underlying these findings remain unclear [86, 88, 91], in part because vast majority of these experimental readouts have not been actual cAMP and cGMP signals but have been inferred by their effects on other targets following interventions aimed to alter their concentrations.

To refine our understanding, we precisely defined the signal transduction mechanisms elicited by NO in the cN signaling network in terms of a model. Simulations show that, NO produced modest increases of [cAMP] and PKA activation at levels of [ISO] that do not saturate β -adrenergic response (gray lines in Fig. 4A–C and Suppl. Fig. S9A–C). This potentiation under sub-maximal β -adrenergic stimulation results from increased PDE2 and PDE4 and decreased PDE3 cAMP hydrolysis rates (Fig. 5A). Under strong β -adrenergic tone, the PDEs are more fully saturated with cAMP and thus become less available for cGMP binding (Fig. 5B); thereby, the β -adrenergic pathway becomes less responsive to NO stimulation (Suppl. Fig. S9A–C). Our model therefore suggests that, in cardiac myocytes, NO-mediated inhibition of β -adrenergic response is not likely to arise from cGMP-mediated stimulation of PDE cAMP hydrolysis activities. On the global whole-cell/cytosolic level, the proposed mechanism is contrary to previous conclusions that NO suppresses [cAMP] via cN cross-talk based on observations of suppressed β -adrenergic responses of downstream

targets upon NO stimulation [4, 81, 91]. The model predicts that despite NO-mediated stimulation of [cAMP] and PKA activation, NO more strongly activates the NO/cGMP/PKG pathway (Fig. 4A–C, black lines) and the inhibitory effects of NO on the β -adrenergic responses are likely due to the direct actions of PKG on its molecular targets. This direct response is likely to be strong enough to overcome the additional cross-talk activation, as well as attenuating the ISO-stimulated activation of β -adrenergic pathway. This mechanism is supported by experimental observations that PKG phosphorylates LCCs on α_1 - and β -subunits [92, 93] and that activation of PKG attenuates the β -adrenergic potentiation of LCC single channel open probability [94–97] and whole-cell current [89, 98]. Overall, the model suggests that NO activation of PKG dominates over its positive effects on cAMP/PKA. As a result, the model-predicted NO potentiation of β -adrenergic pathway activity is consistent with experimentally observed NO-mediated anti-adrenergic effects on target actives [13, 80, 86–88]. These predictions of the model could be tested experimentally.

Reciprocally, the model shows that β -adrenergic stimulation can result in increased [cGMP] and subsequent PKG activation (gray lines in Fig. 4D–E), especially under lower levels of NO stimulation (Suppl. Fig. S9D and E). Increased [cAMP] from β -adrenergic stimulation out-competes cGMP for catalytic domains of PDE1 and PDE3 suppressing their cGMP hydrolysis rates, which are not fully counteracted by the compensatory increase in PDE2 and PDE5 rates, leading to a net decrease in cGMP hydrolysis rate across all PDEs. In a manner similar to the diminished cross-talk response of the β -adrenergic stimulation under high [ISO] (Suppl. Fig. S9A–C), the impact of ISO stimulation on the NO/cGMP/PKG pathway becomes less significant under strong NO stimulation (Suppl. Fig. S9D and E), because the resulting higher [cGMP] reduces the availability of PDEs to interact with cAMP resulting in smaller changes in PDE rates. At present, experimental studies are limited with respect to the effects of β -adrenergic stimulation on the NO/cGMP/PKG pathway. This may be the case because simultaneous ISO stimulation with NO produced much larger effects on [cAMP] and PKA activation (Suppl. Fig. S10C – E) than on [cGMP] and PKG activation (Suppl. Fig. S9D and E). On the other hand, Sumii and Sperelakis showed that ISO stimulated whole-cell LCC current is inhibited by PKG [99]. In addition, it has long been established that β -adrenergic stimulation of LCC occurs via PKA-phosphorylation of the channel [100]. Interpreting these data through the model, we expect that β -adrenergic activation of PKA is likely to dominate over its potentiation of the NO/cGMP/PKG pathway. This is supported by our simulation result that direct effect of ISO on β -adrenergic pathway activation (black) is much more pronounced than its cross-talk effect (gray) on the NO/cGMP/PKG pathway (Fig. 4D–E).

4.3 PDE2 serves as a nexus in cAMP-cGMP cross-talk

PDE2 exhibits complex interactions with both cNs and serves as a nexus for cAMP-cGMP crosstalk. Increased PDE2 expression in HF is observed to blunt β -adrenergic signaling with PDE2 inhibition partially restoring β -adrenergic responsiveness in diseased cardiac myocytes [75]. Adenoviral PDE2 overexpression in healthy cardiac myocytes also markedly decreased [cAMP] upon β -adrenergic stimulation with effects reversed by PDE2 inhibition [75]. However, PDE2 EC50s are at least tenfold higher than the cellular-average [cAMP] and [cGMP], and approximately tenfold higher than that of other PDEs [4, 27, 34, 101],

properties which are replicated by the model (Suppl. Fig. S7). By itself, these high EC50s suggest that PDE2 would play a minor role in regulating cytosolic cAMP and cGMP in cardiac myocytes [32, 33, 102, 103]. When interpreted through a quantitative model which accounts for the complex interactions among PDEs, the role of PDE2 in cN hydrolysis manifested in experiments is exposed.

PDE2 hydrolyzes a larger proportion of cAMP than what may be inferred from the minute changes in [cAMP] observed upon PDE2 inhibition [32, 33, 102, 103]. Via negative feedback by cAMP, the hydrolysis rates of the remaining PDEs increase to compensate for the loss of PDE2 activity. Therefore the negligible net change in [cAMP] under PDE2 inhibition is not a true reflection of the proportion of cAMP hydrolyzed by PDE2 [39]. By direct measurement of its hydrolysis activity, simulations reveal that despite having an EC50 at least tenfold higher than the other PDEs, PDE2 is responsible for hydrolyzing a sizable proportion of cAMP in the cross-talk (Fig. 6A). Simulation also reveals that PDE2 hydrolyzes larger proportions of cGMP than it does cAMP. It exerts more control of cGMP with increasing [ISO] but lower [NO]. In addition, because of its high EC50s, PDE2 can be further activated at elevated [cAMP] and [cGMP] when other PDE isoenzymes have already reached their maximum hydrolysis capacity. As a result, PDE2 may exert a greater functional role than previously concluded based on experiments which showed only small changes of cN signals upon PDE2 inhibition [32, 33, 102, 103].

4.4 Anti-adrenergic effect of PDE5 inhibition is facilitated by PDE2

Selective inhibition of PDE5 has been intensively investigated because of its potential cardio-protective effects in various cardiac pathologies, such as heart failure, cardiac hypertrophy, and ventricular arrhythmias [38, 66–68]. Model simulations provide insights into the effects of PDE5 inhibition on changes in PDE interactions under differing conditions of β -adrenergic and NO stimulation (Fig. 7 and Fig. 8). The model demonstrates that PDE5 inhibition is partially compensated for by an increase in PDE2 cGMP rate (Fig. 7A); therefore, [cGMP] and PKG activation are prevented from further elevation above the observed respective maxima (Fig. 7C and D). Modeling results further show that PDE2 strongly compensates for the reduced activity of PDE5 and, similarly, that PDE5 strongly compensates for PDE2 inhibition, (Fig. 7 and Suppl. Fig. S11). In addition, an increase in PDE2 cAMP rates prevented an increase in β -adrenergic activation under PDE5 inhibition, especially at high [ISO] (Fig. 8), which could have resulted from decline in PDE3 cAMP rates due to competitive inhibition by the elevated [cGMP] (Fig. 7). As a result, the anti-adrenergic effect of PDE5 inhibition is likely mediated by PKG phosphorylation of downstream targets, instead of suppressing cAMP via pathway cross-talk. Such anti-adrenergic effect is further facilitated by the prevention of sizable increases in β -adrenergic activation (Fig. 8). This is a novel insight regarding the role of PDE2 in HF. This compensation will be further enhanced through inhibition of PDE5 in HF and cardiac hypertrophy, as both PDE2 and PDE5 are up-regulated [75, 77–79].

4.5 Model rationale, limitations, and future work

Our current study focuses on investigating PDE interactions that give rise to cross-talk behavior of the cN signaling network (Fig. 1). Recent advances have revealed that many

signaling components in the network form compartments and/or multi-protein signaling complexes (“signalosomes”) [1, 2, 25, 104–107]. With advancements in methods for spatiotemporally-resolved recording of cAMP [1, 2, 25, 104–107] and cGMP [49, 108, 109], extending the model to include these additional mechanisms is an important next step to understand diversification of cN signals in subcellular micro-domains and communication between these cellular compartments. Under conditions exhibiting high [cGMP], cAMP-cGMP positive cross-talk is likely to be more substantial than that shown in Fig. 4 [39]. In addition, NO-derived cGMP and natriuretic peptide (NP)-derived cGMP reside in distinct subcellular compartment [49, 110], exerting differential regulation of β -adrenergic responses [4, 81, 91, 108, 111]. Functional redistribution of PDEs between these two cGMP compartments has been observed in diseased cardiac myocyte [108–110]. These discoveries together with more in-depth analysis of the cN cross-talk signaling network provide new opportunities for model expansion in future research. Changes in cN signals can be magnified many folds in the responses of downstream targets. For instance, LCCs, critical to cardiac excitation-contraction coupling [112, 113], respond sensitively to β -adrenergic stimulation [8, 114]. Important next steps will be extensions of the signaling model to study its regulation of transmembrane channels and calcium cycling proteins, and eventually for the model to be incorporated into integrative models of the ventricular myocyte.

5. Conclusions

We developed a computational model of the cN cross-talk signaling network (Fig. 1) that mechanistically integrates the β -adrenergic and the NO/cGMP/PKG pathways (Fig. 2 and Fig. 3) to investigate the roles of distinctively-regulated PDE isoenzymes in regulating cAMP and cGMP signal dynamics. Using the model, we deciphered the underlying mechanisms of three model observations a) via cN cross-talk, β -adrenergic pathway and NO/cGMP/PKG pathway responses are potentiated upon stimulation by NO and β -agonist respectively (Fig. 4 and Fig. 5), but this cross-talk response is dominated by the direct response; b) PDE2 exerts increasing control of cytosolic [cAMP] under higher β -adrenergic stimulation, but exerts increasing control of [cGMP] at decreasing levels of NO stimulation (Fig. 6); c) due to compensatory action by PDE2, PDE5 inhibition enhanced [cGMP] and PKG activation but not whole-cell β -adrenergic response (Fig. 7 and Fig. 8).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Model analyzes roles of phosphodiesterases in cyclic nucleotide signaling network.
- Whole-cell [cAMP] and PKA activation are potentiated by NO stimulation.
- NO/cGMP/PKG pathway response is potentiated by β -adrenergic stimulation.
- PDE2 regulation of cytosolic [cAMP] and [cGMP] depends on PDE interaction.
- PDE2 compensation regulates anti-adrenergic effect of PDE5 inhibition.

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).

Fig. 2. Experimental validation of PDE models

Experimental data predicted by the PDE models. Dotted symbols are experimental data (obtained from purified PDEs); lines are simulation results. **(A)** PDE1 cAMP hydrolysis rate at 6.25 μM [cGMP] (normalized to the maximum rate) versus data of Yan et al. [57]. **(B)** Normalized PDE1 cGMP hydrolysis rate at 6.25 μM [cAMP] versus data of Yan et al. [57]. **(C)** Effects of [cGMP] on PDE2 cAMP hydrolysis rates (1 μM [cAMP]) compared to data of Prigent et al. [58]. Reported rates are normalized against rate without cGMP. **(D)** PDE2 cGMP hydrolysis rates (10 μM [cGMP]) as a function of [cAMP] compared to data of Russell et al. [59], normalized to that without cAMP. **(E)** PDE3 cAMP hydrolysis (0.2 μM [cAMP]) as a function of [cGMP] versus data of He et al. [60], normalized to that without cGMP. **(F)** Normalized PDE3 cGMP hydrolysis (0.2 μM [cGMP]) versus data of He et al. [61].

Fig. 3. Experimental validation of cross-talk signaling network model

Model predicts experimental data from rat cardiac ventricular myocytes. Dotted symbols are experimental data; lines are simulation results. **(A)** Dose-response relationship of [cAMP] in response to ISO, with (blue) and without (black) the PDE inhibitor IBMX (100 μM), versus data of Vila-Petroff et al. [47] (blue dots) and Kuznetsov et al. [62] (black dots). **(B)** Simulated [cAMP] time course in response to 10 nM [ISO] compared to data of Vila-Petroff et al. [63] (filled black dots) and Zaccolo et al. [48] (hollow blue dots). **(C)** Simulated [cGMP] with (gray bar) and without 100 μM [IBMX] (white bar) in the absence and presence of SNAP (100 μM) compared to data of Castro et al., 2006 [49] (black dots) and 2010 [40] (blue dots). **(D)** Simulated [cGMP] time course under 100 μM [SNAP] perfusion followed by 100 μM [IBMX] application compared to data of Castro et al. [49]. **(E)** Simulated [cGMP] time course in presence of SNAP (100 μM) with intermittent application of the specific PDE inhibitors EHNA (PDE2 inhibitor, 10 μM) and Sildenafil (Sil, PDE5 inhibitor, 0.1 μM), and the non-specific PDE inhibitor (100 μM [IBMX]) compared to data of Castro et al. [49]. (C)–(D) Details on simulations and data processing are provided in Suppl. Sect. VIIIA.

Fig. 4. Cross-talk responses as compared to direct pathway responses

Direct and cross-talk responses are shown in black and gray respectively. (A)–(C) NO-elicited direct response in the NO/cGMP/PKG pathway (black) is compared to its cross-talk response in the β -adrenergic pathway (gray) at 1 nM [ISO] and with varying [SNAP]. (D)–(F) ISO-elicited direct response of the β -adrenergic pathway (black) is compared to its cross-talk response in the NO/cGMP/PKG pathway (gray) at 5 nM [SNAP] and with varying [ISO]. (A) [cGMP] versus [cAMP]. (B) PKG versus PKA-I activation as a percentage of total [PKG] and [PKA-I] respectively. (C) PKG versus PKA-II activation as a percentage of total [PKG] and [PKA-II] respectively. (D) [cAMP] versus [cGMP]. (E) PKA-I versus PKG activation as a percentage of total [PKA-I] and [PKG] respectively. (F) PKA-II versus PKG activation as a percentage of total [PKA-II] and [PKG] respectively.

Fig. 5. PDE interactions underlying cross-talk response

Shadings for PDEs 1–5 are respectively black, red, blue, green, and cyan, with gray indicating net increases across all PDEs. (A) and (B) Percent increases in PDE cAMP hydrolysis rates, under low (1 nM) and high (1 μM) [ISO] respectively. The percent increases are calculated against PDE2 cAMP hydrolysis rates without SNAP but with the same indicated [ISO]. (C) and (D) Percent increases in PDE cGMP hydrolysis rates under low (5 nM) and high (500 nM) [SNAP] and indicated [ISO], normalized to PDE2 cGMP hydrolysis rates before ISO application but under the same indicated [SNAP].

Fig. 6. Role of PDE2 in cross-talk signaling network

(A) Percent cAMP hydrolyzed by PDE2 relative to total cAMP hydrolyzed at each [ISO], without SNAP and with simultaneous applications of 5 nM, 50 nM, and 500 nM [SNAP]. (B) Percent cGMP hydrolyzed by PDE2 against total cGMP hydrolyzed at each [SNAP], without ISO and with simultaneous applications of 10 nM, 100 nM, and 1 μM [ISO].

Fig. 7. NO/cGMP/PKG pathway responses under PDE5 inhibition

All simulation data shown are percent increases upon PDE5 inhibition relative to their respective values prior to inhibition under a medium level of [SNAP] (50 nM) without ISO stimulation. Varying degrees of PDE5 inhibition (20%, 50%, and 90%) is simulated with simultaneous SNAP application under the indicated [ISO] for 30 min. **(A)** Percent increases in cGMP hydrolysis rates of PDEs 1–3 and 5 upon 90% PDE5 inhibition. **(B)–(D)** respectively, percent increases in PDE2 cGMP rates, [cGMP], and PKG activation upon 20%, 50%, and 90% PDE5 inhibition.

Fig. 8. β -adrenergic pathway activity under PDE5 inhibition

All simulation data shown are percent increases upon PDE5 inhibition relative to their respective values prior to inhibition under the same $[SNAP]$ (50 nM) without ISO stimulation (same normalization as in Fig. 7). (A) Percent increases in cAMP hydrolysis rates of PDEs 1–4 upon 90% PDE5 inhibition. (B)–(D) respectively, percent increases in $[cAMP]$, PDE2 cAMP rates, and PDE3 cAMP rates upon 20%, 50%, and 90% PDE5 inhibition.