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Interaction between phosphodiesterases in the regulation of the cardiac β -adrenergic pathway

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Abstract

In cardiac myocytes, the second messenger cAMP is synthesized within the β -adrenergic signaling pathway upon sympathetic activation. It activates Protein Kinase A (PKA) mediated phosphorylation of multiple target proteins that are functionally critical to cardiac contractility. The dynamics of cAMP are also controlled indirectly by cGMP-mediated regulation of phosphodiesterase isoenzymes (PDEs). The nature of the interactions between cGMP and the PDEs, as well as between PDE isoforms, and how these ultimately transduce the cGMP signal to regulate cAMP remains unclear. To better understand this, we have developed mechanistically detailed models of PDEs 1–4, the primary cAMP-hydrolyzing PDEs in cardiac myocytes, and integrated them into a model of the β -adrenergic signaling pathway. The PDE models are based on experimental studies performed on purified PDEs which have demonstrated that cAMP and cGMP bind competitively to the cyclic nucleotide (cN)-binding domains of PDEs 1, 2, and 3, while PDE4 regulation occurs via PKA-mediated phosphorylation. Individual PDE models reproduce experimentally measured cAMP hydrolysis rates with dose-dependent cGMP regulation. The fully integrated model replicates experimentally observed whole-cell cAMP activation-response relationships and temporal dynamics upon varying degrees of β -adrenergic stimulation in cardiac myocytes. Simulations reveal that as a result of network interactions, reduction in the level of one PDE is partially compensated for by increased activation of others. PDE2 and PDE4 exert the strongest compensatory roles among all PDEs. In addition, PDE2 competes with other PDEs to bind and hydrolyze cAMP and is a strong regulator of PDE interactions. Finally, an increasing level of cGMP gradually out-competes cAMP for the catalytic sites of PDEs 1, 2, and 3, suppresses their cAMP hydrolysis rates, and results in amplified cAMP signaling. These results provide insights into how PDEs transduce cGMP signals to regulate cAMP and how PDE interactions affect cardiac β -adrenergic response.

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Disclosures

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yjmcc.2015.09.011>.

Keywords

Phosphodiesterase; Cyclic nucleotides; β -adrenergic pathway; Signaling networks; Cardiac myocytes; Computational model

1. Introduction

Cyclic nucleotide (cN) phosphodiesterase isoenzymes (PDEs) regulate intracellular levels of second messengers, cyclic adenosine-3', 5'-monophosphate (cAMP) and cyclic guanosine-3', 5'-monophosphate (cGMP), by controlling their degradation. They are ubiquitous in mammalian cells and are critical to the regulation of numerous physiological processes, such as cell signal transduction, proliferation and differentiation, apoptosis, and metabolism [1–3]. In the cardiovascular system, through controlling the degradation of cAMP, distinct PDE isoenzymes regulate contractility and relaxation, cell growth/survival, and cardiac structural remodeling [4–6]. Ablation of specific PDE activities through pharmacological inhibition or gene depletion is observed to promote cardiac apoptosis [7], accelerates development of heart failure (HF) [8] and increases likelihood of cardiac arrhythmias [8,9]. The importance of delicate regulation by distinct PDE families is also reflected by their isoform-specific alternations in cardiac diseases [8,10–13]. As examples, PDE2 upregulation in the failing heart is observed to attenuate β -adrenergic signaling [12], decreased PDE3 activity promotes cardiac myocyte apoptosis [10], and PDE4 downregulation is associated with arrhythmias in cardiac hypertrophy and HF [8]. Drugs that restore specific PDE activities [14], such as PDE3 activity in ischemic and dilated cardiomyopathies [15] and PDE1 and PDE4 activities in cardiac ischemia [16] have a cardio-protective effect. However, it has been challenging to obtain a quantitative understanding of the contribution of different PDEs in modulating intracellular signaling [1,17–21], in part because the participation of multiple PDEs in the common task of cAMP degradation creates complex interactions between them. Accordingly, in this work, we develop and apply quantitative, experimentally-based models of the PDE network to better understand these complex interactions.

As shown in Fig. 1, synthesis of cAMP (dark red oval) is governed by the β -adrenergic pathway (red shaded background) in response to elevated catecholamines (e.g. norepinephrine and epinephrine) [22–24]. These ligands bind to and activate β -adrenergic receptors (β -ARs), which via a G-protein (Gs) mediated process activate adenylyl cyclase (AC), the enzyme which catalyzes cAMP synthesis [25,26]. PDEs 1–4 (orange symbols) are primarily responsible for the degradation of cAMP (yellow shaded background) in cardiac myocytes [1,5,6,17,19, 27–29]. The cAMP-hydrolyzing activities of PDEs 1, 2, and 3 are in turn modulated by their interaction with cGMP (dark blue oval) [3,6,30]. The net cAMP signal controls the level of Protein Kinase A (PKA) activation (isoforms PKAI and PKAII [31]), and hence, its phosphorylation of downstream targets to regulate contraction and relaxation of cardiac myocytes [6,26].

cAMP- and cGMP-mediated regulation of the individual PDE isoenzymes has been studied primarily in protocols using purified protein extracts of individual PDEs [1,6,27]. We

therefore lack a systems level understanding of their network interactions. In addition, existing computational models of the β -adrenergic pathway [31–36] have not incorporated isoform-specific reaction mechanisms of PDE isoenzymes or their cGMP-mediated regulation. Therefore, the ways in which cGMP influences β -adrenergic response through PDE activities, and the specific roles of each individual PDE isoenzyme in the regulation of β -adrenergic responses remains un-quantified [17–20,27]. Here, we present a biophysically-detailed kinetic model of the β -adrenergic pathway with integrated PDE isoenzyme activity as shown in Fig. 1. Models of PDEs 1–4 are developed and incorporated into an existing model of the β -adrenergic pathway [31]. This new model both reconstructs and predicts experimental data describing cAMP hydrolysis activities and regulation by cGMP.

The model is used to investigate the regulation of PDEs and β -adrenergic response by changes in cGMP concentration ([cGMP]). Three major findings are presented here. First, simulations reveal the nature of network interactions between distinct isoforms of PDEs, and indicate that activities of PDE2 and PDE4 adjust to compensate for reduced activity of other PDEs including themselves, a behavior which we refer to as “strong coupling”. Second, it is demonstrated that PDE2 exerts control over PDE interactions and cytosolic cAMP signals, which is masked in cAMP readouts under PDE2 inhibition by compensatory actions of other PDEs. Finally, with increasing [cGMP], cGMP out-competes cAMP for binding to the catalytic sites of PDEs 1–3, thereby suppressing cAMP hydrolysis rates of these PDEs, leading to a net accumulation of cytosolic cAMP. This contradicts the hypothesis that cAMP concentration ([cAMP]) can be suppressed through cGMP interactions with the PDEs [5,37,38], at least on the global whole-cell/cytosolic level.

2. Materials and methods

The model presented here expands upon the single, lumped PDE cAMP degradation component of the Saucerman et al. β -adrenergic pathway model [31] to include the activities of multiple PDEs and their cAMP- and cGMP-mediated regulation, while the remainder of the signaling pathway remains the same as that of the original model [31]. Model constraints, equations, and parameters are listed in Supplement Sections 2, 4, and 5, respectively.

2.1. Model formulation of cN-mediated regulation of PDE activities

Panels A–D in Fig. 2 show state diagrams of cN-mediated regulation of cAMP degradation activities for PDEs 1–4 respectively. Each of the PDE isoenzymes are modeled as dimers of two identical subunits [3, 18,31,39,40]. For simplicity, each panel of Fig. 2 shows only one of the two subunits. All mammalian PDE subunits are made up of a catalytic and a regulatory domain [3,17,18], denoted by the letters “C” and “R” respectively. The catalytic domain (oval) contains a conserved active site (semi-circular socket) that can bind either cAMP or cGMP [3,17,18]. The regulatory domains differ markedly among PDEs [3] and consequently are denoted by symbols of different shapes in Fig. 2. Among PDEs 1–4, only PDE2 contains a cN-binding site in its regulatory domain, namely the GAF-B regulatory domain [3,30,41].

As shown in Fig. 2A, both cNs compete for binding to the catalytic domain of PDE1. In PDE2 (Fig. 2B), both cNs compete for binding to both the regulatory and catalytic domains.

Allosteric activation of PDE2 enzymatic activity is induced by binding of either cN to the GAFB regulatory domain (vertical transitions). This regulates the binding affinity of cNs for the catalytic domain (horizontal transitions). The PDE3 model (Fig. 2C) also incorporates competitive binding of both cNs to the catalytic domain. Finally, as shown in Fig. 2D, PKA-mediated phosphorylation of PDE4 induces a conformational change (vertical transitions), allowing the PKA-phosphorylated form to hydrolyze cAMP with faster maximal rate than does the non-phosphorylated form (horizontal transitions).

Reactions consisting of binding of cNs to PDEs, PDE conformational changes, and cN degradation via breakage of the 3'-cyclic phosphate bond are assumed to reach equilibrium rapidly with respect to the time-scale of other reactions in the signaling network. This assumption is supported by experiments demonstrating near instantaneous decay of cN signals upon withdrawal of PDE 1 – 4 inhibitors [19,42,43] and upon increased PDE activation [44]. Parameters “ K ” denote binding affinities, whereas parameters “ k ” denote rate constants. The final degraded products 5'-AMP and 5'-GMP are inactive in cN signaling pathways [3]. Equations and the experimental basis for each PDE model in Fig. 2 are given in Sections 4B and 6 of the Supplement. PDE parameters are set within the ranges identified in the cited literature where available, as tabulated in Supplement Section 5B.

2.2. Model formulation and analysis of cGMP regulation of the β -adrenergic pathway

All four PDE models (Fig. 2) are integrated into the β -adrenergic model of Saucerman et al. [31] by replacing its lumped PDE cAMP degradation component. The relative cAMP hydrolysis rates of PDEs 1–4 are constrained using data from Rochais et al. [45] and Verde et al. [46] (Supplement Fig. S4). All figures, including those in the Supplement, are produced with the same set of parameters and initial conditions (Supplement Section 5). For model analysis, we also defined control, low, medium, and high concentrations of input isoproterenol ([ISO]) and [cGMP] (Supplement Section 3A).

3. Results

3.1. Model validation

Having constrained the individual models for PDEs 1–4 to reproduce experimental data [31–32] (Supplement Section 2A), we tested the ability of each model to reconstruct experimental data not included in the fitting process. The PDE1 model reproduces cAMP hydrolysis rates in the presence of 6.25 μ M cGMP as reported by Yan et al. [47] (Fig. 3A). At 1 μ M cAMP, the PDE2 cAMP hydrolysis rate (Fig. 3B) is potentiated by cGMP in a concentration-dependent manner. The PDE2 cAMP hydrolysis rate is approximately twice that of basal rate under maximal stimulation at ~ 3 μ M cGMP. With further increases of [cGMP], PDE2 potentiation diminishes, with suppression occurring at and above ~ 30 μ M cGMP. This dome-shaped relationship agrees with in vitro assay data for PDE2 [48–50]. As shown in Supplement Fig. S2, the sigmoidal shape of the PDE2 cAMP hydrolysis curve measured in the presence of cAMP alone predicts experimental results [51–53], and the simulated EC50 concentration (36 μ M) agrees with average values from experiments [6,54,55]. As shown in Fig. 3C, the cGMP-dependent rate of cAMP hydrolysis by PDE3 agrees with experiment [56]. Behavior of the integrated signaling network model is validated against cN

measurements from Wild-Type (WT) adult rat ventricular myocytes (Figs. 3, D and E). In Fig. 3D, the dose–response of whole-cell [cAMP] as a function of [ISO] (at basal [cGMP]) both with (blue) and without (black) the non-specific PDE inhibitor IBMX agree qualitatively with experimental data [57,58]. In addition, the model approximates the time course of cytosolic cAMP transients in response to acute β -adrenergic stimulation (Fig. 3E) [57,59] as well as to specific PDE inhibitors [45] (Supplement Fig. S5). The agreement of the model with a wide range of experiments indicates the assumptions underlying model mechanisms are reasonable.

3.2. cAMP dynamics arises from tight interactions between PDEs

Multiple PDE isoenzymes hydrolyze cAMP simultaneously, making regulation of cAMP degradation a tightly coupled and intertwined reaction system. Fig. 4 shows results of simulations designed to tease apart the relationships between individual PDEs. It depicts the response of the β -adrenergic pathway when PDE2 (Fig. 4A–C) or PDE4 (Fig. 4D–F) is inhibited during and after β -adrenergic stimulation. Inhibition of PDE2 produces little change in both whole-cell [cAMP] (Fig. 4A) and PKA activation (Fig. 4B) before or after ISO application. Fig. 4C shows that an increase of the PDE4 cAMP hydrolysis rate (green line) compensates for the ablation of PDE2 activity (red line). Inhibition of PDE4 (Fig. 4D) produces a large increase in whole-cell [cAMP] which is greater than that which occurs in response to 10 nM ISO stimulation alone, resulting in further activation of PKAI and PKAII (Fig. 4E, black and gray lines respectively). Fig. 4F shows that the increase in PDE2 hydrolysis activity (red line) upon PDE4 inhibition, together with smaller increases from PDE1 (black line) and PDE3 (blue line), provide partial compensation for the loss of PDE4 activity (green line) to limit the overall rise in whole-cell [cAMP]. Fig. 4 demonstrates that cAMP dynamics arise from tight interactions between the PDEs.

In order to characterize interaction between the PDEs, we simulated inhibition of a specific PDE isoenzyme with simultaneous application of 10 nM ISO and varying [cGMP] and recorded PDE rate changes from that without PDE inhibition (Figs. 5 and S9). Under 10 nM ISO and the indicated [cGMP], Fig. 5 shows percent increases in the activities of each PDEs upon PDE4 inhibition, relative to their respective hydrolysis rates before inhibition at basal [cGMP] of 10 nM and 10 nM ISO. At 10 nM cGMP, the percent rate increases of Fig. 5A correspond approximately to that at the termination of concomitant β -agonist stimulation and PDE4 inhibition in Fig. 4F above. As shown in Fig. 5A, the compensatory increase in PDE2 rate (red line) is the greatest among the PDEs across all levels of [cGMP], with magnitudes that scale with varying degrees of PDE4 inhibition (Fig. 5B). Conversely, increases in PDE4 rates respond similarly to varying levels of PDE2 inhibition (Supplement Fig. S9), despite the small total PDE rate increases. As shown in Supplement Fig. S10A–D, PDE3 inhibition is also partially compensated for by primarily an increase in PDE4 activity and a smaller magnitude of increase in PDE2 activity. PDE4 rate is also increased in response to inhibition of PDE1 (Supplement Fig. S10E). Consequently, PDE2 and PDE4 show the strongest coupling among the PDEs in cAMP hydrolysis because either one can partially compensate for reduced activity of the other, and for reduced PDE3 activity.

These model results show that the interpretation of experiments investigating the roles of multiple PDEs by measuring [cAMP] in response to application of selective blockers can be confounded by network interactions between the different PDEs. For instance, many experiments show no or negligible changes in whole-cell [cAMP] before and after PDE2 inhibition [12,45,61–67]. Figs. 4 and 5 demonstrate that multiple PDE isoenzymes work together to lessen changes to the cAMP signal when some of the PDE mechanisms are altered. Much greater increases in [cAMP] than that in Fig. 4D would result without PDE2 compensation (Supplement Fig. S11B), although increases in PDE2 rates (Fig. 4F) cannot fully prevent [cAMP] increases due to PDE4 inhibition (Fig. 4D). Reciprocally, PDE4 can also partially compensate for the rises in [cAMP] due to PDE2 inhibition (Supplement Fig. S11A). As a result, the aforementioned experimental approach [12,45,61–67] underestimates cAMP degradation by the inhibited PDE, because it does not account for compensatory actions of the remaining PDEs.

3.3. cGMP regulates PDE2 cAMP hydrolysis in a biphasic manner

The cAMP binding affinity is significantly lower for PDE2 (Supplement Fig. S2) than those for the other PDEs [5,6,17,68], making it more resistant to activation by cNs [6,54]. Furthermore, the fact that cytosolic [cAMP] does not rise appreciably upon PDE2 inhibition (Fig. 4A) can lead to the inference that PDE2 plays little role in cAMP regulation in the cytosol of cardiac myocytes [28,29,63,64]. To examine this, model PDE2 hydrolysis rate is shown as a percentage of the total hydrolysis rate by all PDEs (i.e. PDEs 1–4) at each [ISO] and indicated [cGMP] (Fig. 6A).

Simulations shown in Fig. 6A demonstrate that PDE2 is responsible for a non-negligible proportion of cAMP degradation (up to ~22%) in the cytosol of cardiac myocytes, which increases with increasing levels of ISO stimulation. Under control [cGMP] (10 nM), PDE2 is responsible for hydrolyzing ~5% of cytosolic cAMP under basal conditions (i.e. no ISO), and up to ~10% at higher [ISO]. Addition of cGMP changes the behavior of PDE2 in the pathway. At higher [cGMP] of 1 μ M and 5 μ M, the proportion of cytosolic cAMP hydrolyzed by PDE2 increases to ~15% respectively with no ISO and up to ~22% at high [ISO]. Under these elevated [cGMP] (e.g. 1 μ M and 5 μ M), PDE2 surpasses PDE1 and PDE3 to become the second most potent regulator after PDE4. However, at 50 μ M cGMP, the activity of PDE2 is suppressed close to that at basal [cGMP].

As shown in Fig. 6B, this cGMP-dependent biphasic response in PDE2 cAMP hydrolysis (Fig. 6A) arises from the fact that the proportion of PDE2 with cAMP occupying its catalytic domain (Fig. 6B, solid black line) is in the range of ~15–20% at lower [cGMP] (e.g. 1 μ M and 5 μ M), but is suppressed to much lower levels at [cGMP] above ~10 μ M. At these high [cGMP], cGMP successfully competes with cAMP to bind the PDE2 catalytic binding site and cGMP-bound PDE2 (solid gray line) dominates. Although cGMP stimulates PDE2 hydrolytic activity upon binding to the GAF-B regulatory domain (Fig. 2B), high [cGMP] can still suppress cAMP hydrolysis by preventing cAMP-binding at the catalytic site.

3.4. Activation of the β -adrenergic pathway is potentiated by cGMP

Understanding the way in which cGMP regulates β -adrenergic function is challenging due to the complex behavior of the individual PDEs (Figs. 2 and 6) and their interactions (Fig. 4). To better understand this regulation, activation of the β -adrenergic pathway at different [cGMP] was simulated. Increasing [cGMP] increases the amplitude of whole-cell cAMP transients as well as PKAI and PKAII activation (Supplement Fig. S6 A – C). This occurs as result of a net decrease in PDE cAMP hydrolysis rates (Supplement Fig. S6D). Fig. 7A–C shows percent increases in steady state, cellular-average [cAMP], PKAI and PKAII activation in response to elevated [cGMP] of 1 μ M, 5 μ M, and 50 μ M, relative to that measured at basal [cGMP] (10 nM). At each level of ISO stimulation, an increase in [cGMP] leads to an increase in cellular-average [cAMP] (Fig. 7A) as well as increased PKAI and PKAII activation (Fig. 7B and C).

Fig. 7D shows increases in steady state cAMP hydrolysis rates for PDEs 1–4 under elevated [cGMP] from their respective rates under control cGMP (10 nM). There are two distinct modes of PDE interactions underlying the observed increases of whole-cell cAMP with increasing [cGMP]. First, at 1 μ M and 5 μ M cGMP, the hydrolysis rates of PDE2 and PDE4 increase (red and green bars respectively); however, the hydrolysis rate of PDE3 (blue) is reduced sufficiently to produce a net reduction in cAMP hydrolysis (gray). Second, at 50 μ M cGMP, the hydrolysis rate of PDE2 is decreased to less than the control rate as are those of PDEs 1 and 3. This is due to the fact that at high levels, cGMP overtakes cAMP in its competition for the catalytic sites of PDEs 1, 2, and 3, suppressing their cAMP hydrolysis rate (e.g. Fig. 6B). The increase in PDE4 rates (green) is no longer sufficient to compensate, resulting in a larger net reduction of cAMP hydrolysis rate (gray).

4. Discussion

4.1. Integrative modeling of cN signaling reveals interactions between PDEs

Therapeutic agents have been developed on the basis of their ability to potently and selectively inhibit specific PDE isoenzymes for the treatment of various diseases [17,69–73]. However, we know relatively little about the mechanisms and contribution of the various PDEs in modulating intracellular signaling [1,17–21]. As shown in Fig. 1, PDEs 1–4 function synergistically to regulate the strength of β -adrenergic signaling in cardiac myocytes [1,5,6,17,19,26–29]. Several factors make it difficult to understand these interactions: 1) PDEs degrade the same cAMP signal that regulates their activities; 2) PDE activities are regulated by cGMP (Fig. 2); 3) Multiple PDE isoenzymes work in concert to hydrolyze cAMP (Fig. 1). When the activity of one PDE isoenzyme is altered, activities of others change due to the presence of cAMP-induced negative feedback (Fig. 4). For these reasons, biophysically-detailed computational models are useful for studying interactions between different types of PDEs and cGMP, and their role in the regulation of the β -adrenergic pathway.

A wealth of past research on cGMP regulation of PDEs and the β -adrenergic pathway (e.g. Fig. 3; Supplement Figs. S1–S6, Tables S9–S12, Section 5) has allowed us to develop a functionally integrated mechanistic model of cGMP regulation of the β -adrenergic pathway

(Figs. 1 and 2). Recent live-cell imaging studies, such as those by Nikolaev et al. [44], Herget et al. [43], and Mehel et al. [12], have further advanced our understanding of PDE family regulation. Using the model reported here, we are able to perform in-depth analyses of and tease out the complex interactions underlying these experimental observations. There are three major findings from this work. First, model analyses demonstrate compensatory regulation of PDEs, with PDE2 and PDE4 being the major factors in this compensatory regulation. In particular, the increase in PDE2 rate is the most sensitive of all PDEs to PDE4 inhibition (Figs. 4 and 5). Reciprocally, upon PDE2 inhibition, the increase in PDE4 rate is the greatest among the PDEs (Supplement Fig. S9). Second, PDE2 regulates cAMP dynamics in a cGMP-dependent manner (Fig. 6). Third, cGMP potentiates whole-cell [cAMP] and PKA activation in a concentration-dependent manner (Fig. 7).

4.2. Tight coupling between PDE2 and PDE4 drives cAMP dynamics

The redundancy of the PDE network, with multiple isoenzymes performing the same hydrolytic function (Fig. 1) contributes a degree of robustness to perturbations. Specifically, a decrease of activity of a particular PDE isoenzyme is partially compensated for by the remaining PDE isoenzymes, with PDE2 and PDE4 exhibiting the strongest interdependence among the PDEs (Figs. 4, 5, and S9). Such coupling between PDE isoenzymes may be an important mechanism in stabilizing cAMP dynamics in the heart [11,13,74], including disease settings where alterations in isoenzyme-specific PDE expression and/or activity have been implicated [8,10–13,21,74–76]. Modeling results show that the ability of PDE2 to compensate for reduced activity of PDE4 is particularly strong. Partial PDE2 compensation for reduced PDE4 activity is consistent with experimental data showing that PDE4 inactivation produces only small effects, if any, on basal blood pressure, heart rate, and inotropy [77]. It is clearly established that PDE4 expression is reduced in HF [8,77,78]. Our modeling predicts that under this condition PDE2 may partially compensate for the loss of cAMP hydrolytic activity of PDE4. This is a novel insight regarding the role of PDE2 in HF. This compensation will be further enhanced through upregulation of PDE2 that has been observed in HF [12]. Improved understanding of the modes of interactions between PDEs may therefore provide insights on disease-induced changes in signal transduction and the efficacy of drug actions.

4.3. PDE2 integrates cAMP and cGMP signals in regulation of the β -adrenergic pathway

The presence of PDE2 in cardiac myocytes and its degradation of cAMP have been demonstrated in many studies using a variety of experimental techniques, such as chromatography [79,80], electrophoresis [48], electrophysiological measurements [45,46], and live-cell imaging [62,81]. Increased PDE2 expression in HF is observed to blunt β -adrenergic signaling with PDE2 inhibition partially restoring β -adrenergic responsiveness in diseased cardiac myocytes [12]. In addition, adenoviral PDE2 overexpression in healthy cardiac myocytes markedly decreased [cAMP] upon β -adrenergic stimulation with effects reversed by PDE2 inhibition [12]. As highlighted by our PDE2 model (Fig. 2B), PDE2 exhibits complex interactions with both cNs, and serves as a nexus through which cGMP influences cAMP dynamics. Our model, therefore, helps tease out the complex interactions and interpret experimental observations.

When interpreted through a quantitative model of the complex interactions involved, the role of PDE2 in cAMP hydrolysis manifested in experiments is exposed. Experiments demonstrate that PDE2 EC50 is at least tenfold higher than the cellular-average [cAMP] and approximately tenfold higher than that of other PDEs [5,6,17,68], therefore appearing to play a minor role in regulating cytosolic cAMP in cardiac myocytes [28,29,63,64]. Simulations reveal that despite these biochemical properties, PDE2 does hydrolyze appreciable amounts of cytosolic cAMP (up to ~22%), especially under increased β -adrenergic stimulation (Fig. 6A); however, its role is partially masked in the small increases in [cAMP] upon PDE2 inhibition due to the compensatory actions of the remaining PDEs (Fig. 4A – C).

Because of its high EC50, PDE2 can be further activated at elevated [cAMP] when other PDE isoenzymes have already reached their maximum hydrolysis capacity. Furthermore, cGMP increases the proportion of cAMP hydrolyzed by PDE2, until very high levels suppress its activity (Fig. 6A). This cGMP-mediated dome-shaped biphasic behavior of PDE2 cAMP hydrolysis rate arises from the competitive binding of cNs to its regulatory and catalytic domains (Figs. 6B and 2B). In addition, as the changes in PDE rates (Fig. 5) reflect the dome-shaped hydrolysis curves of PDE2 (Fig. 6), it can be seen that cGMP regulation of PDE2 influences the activities of other PDEs, and therefore shapes synergistic interactions between them. Our simulations show the aforementioned PDE2 behaviors exist without having to hypothesize the existence of a specialized nano-compartment with elevated cN levels or limited expression of other PDEs [61]. However, our work does not rule out the existence of such micro-domains. In such cases, PDE2 will exert an even greater control over cAMP dynamics than that shown in Figs. 5 and 6, as it would be further activated by the higher [cAMP], and/or be the only PDE isoenzyme appreciably degrading cAMP.

4.4. Whole-cell cAMP is potentiated by cGMP

The model demonstrates that cGMP potentiates cellular-average cAMP signals, resulting in increased PKA activation due to a net reduction of hydrolysis rates across all PDEs (Fig. 7A–C). The cGMP-mediated dome-shaped biphasic behavior of PDE2 cAMP hydrolysis rate contributes two modes of PDE interaction underlying the observed cAMP potentiation in the cellular average sense (Fig. 7D). Upon sensing lower [cGMP] (e.g. 1–5 μ M, Fig. 7D), the increases in PDE2 and PDE4 rates serve to compensate for the suppressed PDE3 rate. At higher [cGMP] (e.g. 50 μ M, Fig. 7D), PDE2 rate is suppressed and contributes to, instead of compensating for, cGMP-mediated cAMP potentiation. In the latter case, despite PDE4's large contribution to cAMP degradation, the strength of PDE4-mediated feedback driven by the elevated [cAMP] is not sufficient to completely compensate for the suppression of other PDE activities (Fig. 7D). Model results therefore suggest that, in mammalian cardiac myocytes, reduction of β -adrenergic response, as measured by whole-cell [cAMP] and PKA activation, upon stimulation of the Nitric Oxide (NO)/cGMP/Protein Kinase G (PKG) pathway is not likely to be mediated by cGMP regulation of the PDEs. In fact the model predicts that this regulation potentiates [cAMP] and PKA activation. This is contrary to what has been previously hypothesized based on observations of responses of downstream targets of the β -adrenergic pathway [5,37,38], at least on the global whole-cell/cytosolic level. Instead, we speculate that the inhibitory activities may result from pathways downstream of

the PDEs, such as PKG-mediated phosphorylation of downstream targets. This prediction of the model could be tested experimentally.

4.5. Model rationale, limitations, and future work

In order to study synergistic interactions between multiple PDE families in regulating whole-cell cAMP dynamics, we focused on studying the signal transduction mechanisms, particularly with regard to the PDEs, and sought to quantify the biochemistry of the signal components (Fig. 2) and cGMP regulation of cAMP dynamics (Fig. 1). Recent advances in spatiotemporally-resolved recording of cAMP signals [2, 19,82–86] now make it possible to measure the compartmentalized activities of different PDEs. For instance, in the SERCA/PLB/AKAP signalosome, PDE3A1 phosphorylation by PKA is recently shown to promote its targeting to the signalosome, where it may modulate [cAMP] in a highly localized manner [87]. Our model lacks such compartmentalization, and extending the model to allow its use in studying the diversification of cN signals in subcellular micro-domains is an important next step. The current model does not incorporate the bidirectional functional relationships between cAMP and cGMP, which (respectively) potentiate and attenuate cardiac contractility [26,88,89]. To advance our investigation of the cAMP–cGMP cross-talk signaling network, we are incorporating more mechanistic models of cGMP dynamics, guided by recent advancements in cGMP recording [60,81]. Further extensions to the model must ultimately include regulation of transmembrane channels and calcium cycling proteins, and incorporation of these mechanisms into integrative models of the ventricular myocyte.

4.6. Multi-scale modeling bridges causal link between individual signaling protein characteristics and collective pathway response

This multi-scale model mechanistically integrates interactions between the cNs within the domains of each PDE isoenzymes to the emergent network responses of the β -adrenergic pathway. By enabling system-level analysis, the model provides insight regarding the ways in which each distinct PDE regulates cAMP signals, and the ways in which cAMP signals feedback to influence the behavior of each PDE. In particular, the synergistic, compensatory behaviors of PDE2 and PDE4 would not have been revealed without this network-level analysis. Further studies of the signaling pathway will reveal how isoform specific changes in PDE expression in hypertrophy and HF [11,16,90, 91] contribute to disease-related remodeling. Understanding information processing through signaling pathways is critical to deciphering the mechanisms behind observed physiological responses in health and disease.

5. Conclusions

We have developed a computational model of the β -adrenergic pathway to investigate the roles and interactions of distinctively-regulated PDEs 1–4, in regulating cAMP signal dynamics in the cardiac myocyte. Using the model, we have shown that 1) PDE2 and PDE4 exhibit the strongest coupling among the PDEs; 2) PDE2 and its regulation by cGMP regulate cAMP dynamics; and 3) The presence of cGMP potentiates whole-cell [cAMP], which subsequently increases PKA activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1. PDEs 1–4 (orange symbols) hydrolyze cAMP (yellow shaded background) generated by the β -adrenergic signaling pathway (red shaded background). cGMP, in turn, regulates the hydrolytic activity of PDEs 1, 2, and 3. Schematic for the β -adrenergic pathway is adapted from Saucerman et al. [31].

Fig. 2.

PDE monomer subunits are shown with catalytic domains (ovals, denoted by “C”) and regulatory domains (varied shapes, denoted by “R”) for PDEs 1–4 in (A)–(D) respectively. Active sites within catalytic domains that bind cAMP and/or cGMP are represented by semi-circular sockets; the GAF-B regulatory domain of PDE2 is represented by open rectangular socket. Reversible and irreversible reactions are denoted by double-headed and single-headed arrows respectively. (A)–(C) Competitive binding of cAMP and cGMP to PDEs 1–3 respectively. (D) cAMP hydrolysis by PDE4 is regulated by PKA-mediated phosphorylation.

Fig. 3.

Experimental data predicted by the model. (A) PDE1 cAMP hydrolysis rate with 6.25 μM cGMP (normalized to maximum rate) versus data of Yan et al. [47]. (B) PDE2 cAMP hydrolysis rates (1 μM cAMP) as a function of [cGMP] versus data of Prigent et al. [48]. Hydrolysis rates are normalized against cAMP hydrolysis rate at 1 μM cAMP without cGMP. (C) PDE3 cAMP hydrolysis rates as a function of [cGMP] versus data of He et al. [56] at 0.2 μM cAMP. Rates are normalized to cAMP hydrolysis rates at 0.2 μM cAMP without cGMP. (A)–(C) shows data (dots) from experiments performed using purified

PDEs. (D) ISO dose–response relationship of [cAMP], both with (blue) and without (black) 100 μ M PDE inhibitor IBMX versus data of Vila-Petroff et al. [57] and Kuznetsov et al. [58]. (E) Time-varying [cAMP] in response to 10 nM ISO versus data of Vila-Petroff et al. [57] (filled black dots) and Zaccolo et al. [59] (hollow blue dots). (D) and (E) Simulations are performed with basal [cGMP] of 10 nM from Götz et al. [60].

Fig. 4.

Responses of the β -adrenergic pathway to 10 nM ISO and subsequent inhibition of PDE2 in (A–C) or PDE4 in (D–F). Shown are whole-cell [cAMP] in (A, D), activation of PKAI (black) and PKAII (gray) in (B, E), and hydrolysis rates of PDEs 1–4 (black, red, blue, and green lines respectively) in (C, F). All simulations are performed with basal [cGMP] of 10 nM from Götz et al. [60].

Fig. 5.

Percent increases in PDE rates upon PDE4 inhibition relative to their respective rates prior to inhibition under 10 nM ISO and basal [cGMP] (10 nM). Varying degrees of PDE4 inhibition is simulated with simultaneous ISO stimulation under the indicated [cGMP] for 30 min. (A) Percent increases in the hydrolysis rates of PDEs 1–4 upon 90% PDE4 inhibition are shown with black, red, blue, green lines respectively. (B) Percent increases in PDE2 cAMP hydrolysis rate upon 20%, 50%, and 90% PDE4 inhibition.

Fig. 6.

PDE2 cAMP hydrolysis rates under cGMP regulation. (A) Proportion of total cAMP hydrolysis attributed to PDE2 as a function of ISO stimulation and concomitant applications of cGMP at 10 nM (control), 1 μM , 5 μM , and 50 μM . (B) Percentages of PDE2 with cAMP (solid black) and cGMP (solid gray) occupying its catalytic domain under 1 μM ISO as a function of [cGMP]. Percentages of PDE2 with unoccupied free catalytic domains are shown in dashed gray line. The dotted vertical lines correspond to elevated [cGMP] simulated in (A) (i.e. 1 μM , 5 μM , and 50 μM cGMP).

Fig. 7. cGMP regulation of β -adrenergic response. (A)–(C) Percent increases of steady state cellular-average [cAMP] and PKAI and PKAII activations are shown respectively under various [cGMP] and [ISO]. Percent increases are shown relative to that measured at basal [cGMP] of 10 nM. (D) Increases in individual PDE hydrolysis rates ($\mu\text{M}/\text{min}$) with 10 nM ISO and indicated [cGMP] from their respective rates at basal cGMP (10 nM). The shadings for PDEs 1–4 are respectively black, red, blue, and green. The net change in PDE hydrolysis rate is shaded gray.