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SHORT COMMUNICATION

Oscillation of cAMP and Ca^{2+} in cardiac myocytes: a systems biology approach

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Abstract Cyclic adenosine monophosphate (cAMP) and Ca^{2+} levels may oscillate in harmony within excitable cells; a mathematical oscillation loop model, the Cooper model, of these oscillations was developed two decades ago. However, in that model all adenylyl cyclase (AC) isoforms were assumed to be inhibited by Ca^{2+}, and it is now known that the heart expresses multiple AC isoforms, among which the type 5/6 isoforms are Ca^{2+}-inhibitable whereas the other five (AC2, 3, 4, 7, and 9) are not. We used a computational systems biology approach with CellDesigner simulation software to develop a comprehensive graphical map and oscillation loop model for cAMP and Ca^{2+}. This model indicated that Ca^{2+}-mediated inhibition of AC is essential to create oscillations of Ca^{2+} and cAMP, and the oscillations were not altered by incorporation of phosphodiesterase-mediated cAMP hydrolysis or PKA-mediated inhibition of AC into the model. More importantly, they were created but faded out immediately in the co-presence of Ca^{2+}-noninhibitable AC isoforms. Because the subcellular locations of AC isoforms are different, spontaneous cAMP and Ca^{2+} oscillations may occur within microdomains containing only Ca^{2+}-inhibitable isoforms in cardiac myocytes, which might be necessary for fine tuning of excitation–contraction coupling.

Keywords Adenylyl cyclase · Subtype · Computational analysis · Oscillation · Cyclic AMP · Calcium

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Introduction

β-Adrenergic receptor (β-AR) signaling is of crucial importance in regulating normal cardiac function, and abnormality of β-AR signaling contributes to the development of heart failure via altered cyclic AMP (cAMP) and calcium (Ca^{2+}) signaling [1–3]. Cardiac excitation–contraction coupling (E–C coupling) is the process that links electrical excitation of cardiac myocytes to contraction of heart muscle. Ca^{2+} is essential for cardiac electrical activity and is a direct activator of myofilaments, causing both contraction and relaxation [4–6]. Dysregulation of cAMP and the subsequent Ca^{2+} oscillation are fundamental causes of both contractile and diastolic dysfunction, and arrhythmia among heart failure patients [1, 3, 5, 7–10].

Adenylyl cyclase (AC) is a membrane-bound enzyme that catalyzes the conversion of ATP to cAMP [1, 11]. cAMP, an intracellular second messenger, activates protein kinase A (PKA), leading to phosphorylation of multiple molecules involved in cardiac contraction, including the L-type Ca^{2+}-channel [1]. Phosphorylation of the L-type Ca^{2+}-channel is known to increase the influx of Ca^{2+}, resulting in increased intracellular levels of Ca^{2+}. In the 1990s, AC isoforms directly inhibited by Ca^{2+} were identified, i.e., types 5 and 6 AC isoforms (AC5 and AC6) [11–14]. Because these isoforms are dominantly expressed in the heart, it was proposed that cAMP levels may oscillate in harmony with Ca^{2+} levels; an increase in cAMP, as generated by AC5/6, phosphorylates L-type Ca^{2+} channels, and induces influx of Ca^{2+} into the cytosol [11]. An increase of cytosolic Ca^{2+} inhibits AC5/6 and reduces phosphorylation of the L-type Ca^{2+} channel. Thus, the activity of AC5/6 and the L-type Ca^{2+} channel may work synergistically to generate an oscillation loop of cAMP and Ca^{2+} in cardiac myocytes [11].

It is now well known that the heart expresses not only AC5 and AC6, but also many other AC isoforms (AC2, 3, 4, 5, 6, 7, and 9) [2]. Although these isoforms are all expressed in the heart, recent studies have indicated they may have different subcellular locations [15, 16]. AC5 is a major cardiac isoform in adults, and AC6 is a fetal or neonatal cardiac AC isoform [1, 17–19]. AC5 and AC6 share most, if not all, of their biochemical properties, and are inhibited not only by Ca^{2+}, but also by Gi and PKA [2, 11, 13, 20–22]. In contrast, the other AC isoforms (AC2, 3, 4, 7, and 9), which are ubiquitously expressed throughout the body, are not inhibited by Ca^{2+}, Gi, or PKA [13], and their involvement in cAMP and Ca^{2+} oscillations is poorly understood.

We therefore examined the involvement of AC isoforms in cAMP and Ca^{2+} oscillations [1, 12]. Because it is difficult to examine these issues by means of traditional in vitro or in vivo biochemical approaches, we used a computational systems biology approach with CellDesigner software, a recently developed, structure diagram editor for drawing gene-regulatory and biochemical networks [23–25].

Materials and methods

CellDesigner version 4.2 (http://www.celldesigner.org/) enables users to describe molecular interactions by using well-defined and consistent graphical notions and to create a comprehensive model incorporating positive feedforward or negative feedback loops among AC, cAMP, Ca^{2+}, phosphodiesterase (PDE), and PKA within the β-AR signaling pathway [23–25].

The CellDesigner notation used in this paper is briefly illustrated for a simple reaction scheme in Supplemental Fig. 1. Protein A is transformed to protein B and protein C promotes this transition (Supplemental Fig. 1a). Supplemental Fig. 1b shows the notation for degradation (upper) or production (lower) of protein A; their balance determines the concentration of protein A in cells under physiological conditions.

In this study, most of the variables were the same as in Cooper's original model [12]; in future work, it would be desirable to optimize the variables used in the oscillation loop model to match physiological conditions.

The formulas and values used to generate the oscillation models shown in the figures can be found in the online Supplemental methods and Supplemental Tables 1–3, available on http://www.link.springer.com/journal/12576.

Results

Cooper's model mimicked by CellDesigner

We first mimicked Cooper's original model by using CellDesigner to create a graphical comprehensive map (Fig. 1a) and oscillation loop model of cAMP and Ca^{2+} (Fig. 1b). We also incorporated the activity of PKA and AC. For AC, we used AC5/6 because they are the major cardiac isoforms and are directly inhibited by Ca^{2+} [2, 13]. We obtained stable and spontaneous oscillation curves for cAMP and Ca^{2+}, as demonstrated in the original model [12]. The activity of AC and PKA also oscillated (Fig. 1b).

The formulas and values used to generate this oscillation model are shown in online Supplemental methods and Supplemental Table 1.

Incorporation of the PDE-mediated cAMP hydrolysis

Intracellular cAMP concentration is determined by the balance between its production via AC and its hydrolysis

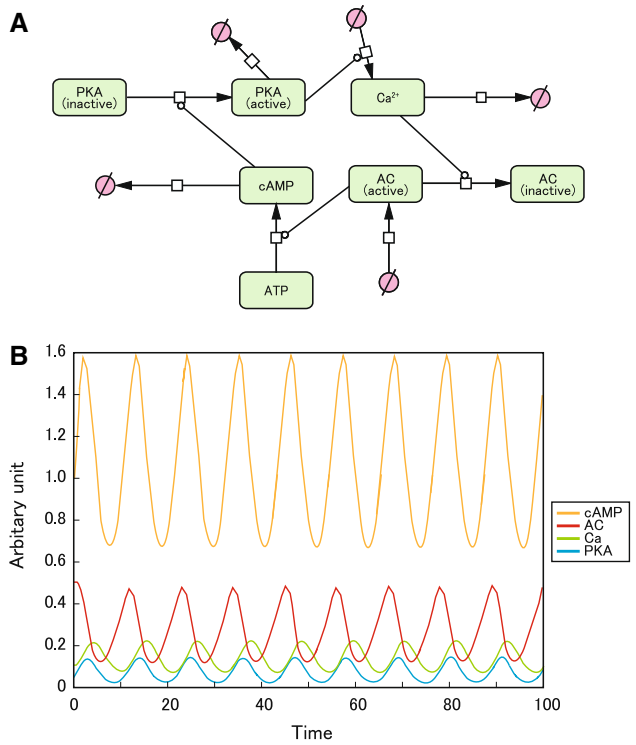

Fig. 1 Cooper's model mimicked by CellDesigner. **a** Graphical notations used in CellDesigner to depict Cooper's model. **b** Computational oscillation loop of β-AR signaling molecules. cAMP, Ca^{2+}, AC, and PKA formed a stable and persistent negative feedback loop. The intracellular concentration of cAMP at baseline (0 min) was taken as 1 (arbitrary units)

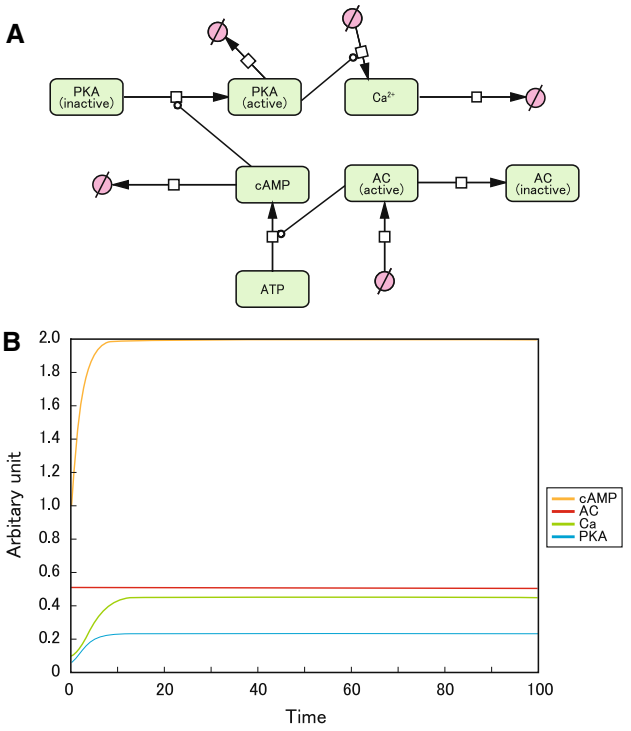

Fig. 2 Effects of Ca^{2+}-mediated inhibition of AC. **a** Graphical notation used in CellDesigner to depict β-AR signaling molecules AC, cAMP, Ca^{2+}, and PKA. Ca^{2+}-mediated inhibition of AC was deleted from Cooper's original model. **b** Computational oscillation loop of β-AR signaling molecules. cAMP, Ca^{2+}, AC, and PKA did not form a negative feedback loop. The intracellular concentration of cAMP at baseline (0 min) was taken as 1 (arbitrary units)

via PDE in the heart under physiological and pathological conditions [26]. Because PDE is activated by cAMP, we incorporated its activity into the model (Supplemental Fig. 2a). We found that oscillations of the molecules involved in the β-AR signaling pathway were maintained, even though their amplitudes were increased by approximately 1.6-fold, compared with those of Cooper's original model (Supplemental Fig. 2b). Thus, PDE-mediated cAMP hydrolysis did not appear to change the behavior of the oscillation, but exaggerated its amplitude.

The formulas and values used to generate this oscillation model are shown in online Supplemental methods and Supplemental Table 2.

Effect of Ca^{2+}-mediated inhibition of AC

The heart expresses multiple AC isoforms (AC2, 3, 4, 5, 6, 7, and 9) [2], of which AC5 and AC6 are directly inhibited by submicromolar Ca^{2+} [2, 13]. Thus, to examine the effect of Ca^{2+}-mediated inhibition of AC, we modeled the situation in which all AC isoforms in the heart are not Ca^{2+}-inhibitable in the heart (Fig. 2a). As shown in Fig. 2b, we found that no oscillation appeared. This result indicates that the presence of Ca^{2+}-inhibitable AC isoforms is essential for stable and spontaneous cAMP and Ca^{2+} oscillations to occur.

The formulas and values used to generate this oscillation model are shown in online supplemental methods and supplemental Table 1.

Incorporation of PKA-mediated inhibition of cardiac AC isoforms into the model

Recent studies have indicated that AC5 and AC6 are inhibited not only by Ca^{2+}, but also by PKA [13, 20–22]. Therefore, we next incorporated PKA-mediated inhibition into Cooper's model (Fig. 3a). Oscillations of cAMP and Ca^{2+}, as well as AC and PKA, were observed, but the amplitudes were reduced by approximately 13 %, compared with those in Cooper's original model (Fig. 3b). Thus, PKA-mediated inhibition of cardiac AC isoforms did not seem to change the behavior of the oscillation, but reduced its amplitude.

The formulas and values used to generate this oscillation model are shown in online Supplemental methods and Supplemental Table 1.

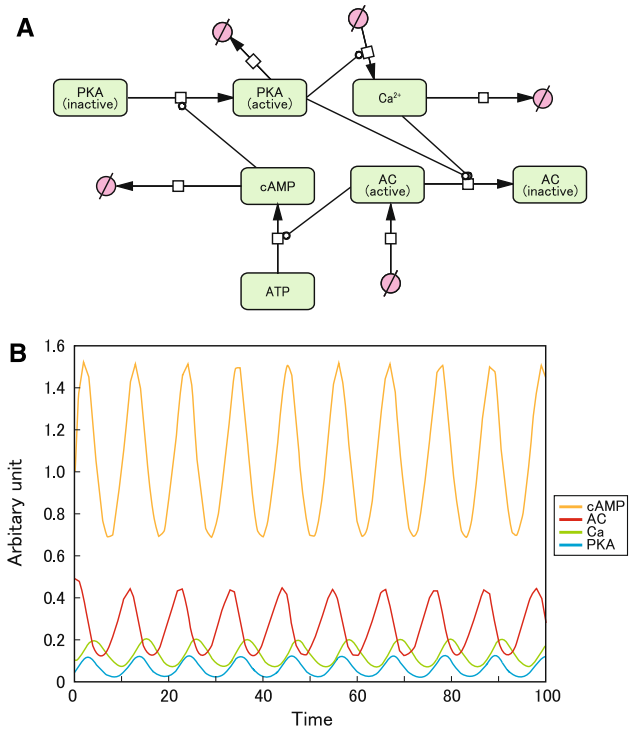

Fig. 3 Effects of PKA-mediated inhibition of AC. **a** Graphical notation used in CellDesigner to depict β-AR signaling molecules AC, cAMP, Ca^{2+}, and PKA. PKA-mediated inhibition of AC was incorporated into Cooper's original model. **b** Computational oscillation loop of β-AR signaling molecules. cAMP, Ca^{2+}, AC, and PKA formed a stable and spontaneous negative feedback loop. The intracellular concentration of cAMP at baseline (0 min) was taken as 1 (arbitrary units)

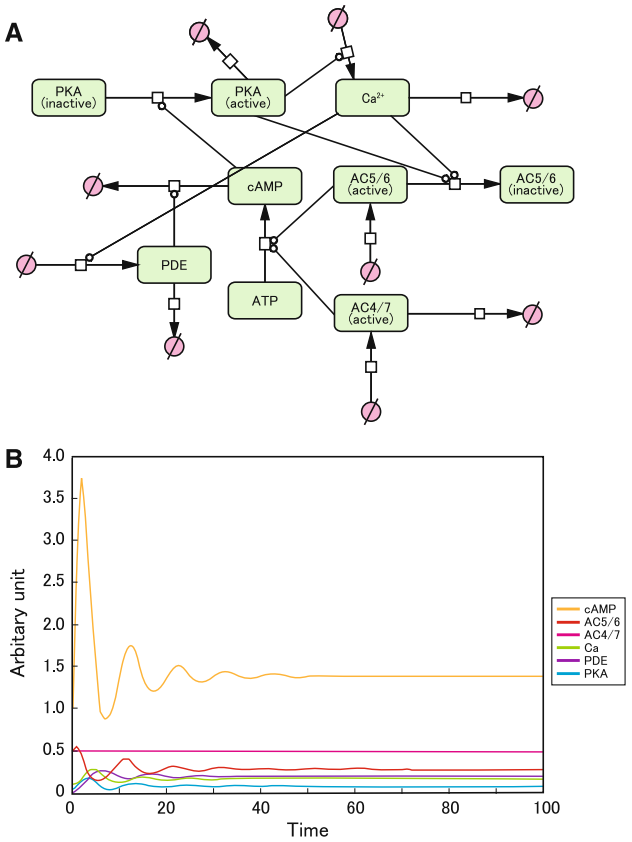

Fig. 4 Incorporation of Ca^{2+}-mediated inhibition of type 5/6 AC into the model. **a** Graphical notation used by CellDesigner to depict β-AR signaling molecules: cardiac AC subtypes (AC5/6), non-cardiac AC subtypes (AC4/7), cAMP, Ca^{2+}, and PKA. A negative feedback loop was not observed. **b** Computational oscillation loop of β-AR signaling molecules: cAMP, Ca^{2+}, AC5/6, PDE, and PKA did not form a continuous negative feedback loop, and then faded. However, essentially no oscillation occurred. The intracellular concentration of cAMP at baseline (0 min) was taken as 1 (arbitrary units)

Incorporation of Ca^{2+}-mediated inhibition of type 5/6 AC isoforms into the model

We then examined the model incorporating both Ca^{2+}-inhibitable (AC5/6) and non-inhibitable AC isoforms (AC2, 3, 4, 7, and 9) (Fig. 4a). Studies with transgenic mouse models in vivo have demonstrated that AC2, 3, 4, 7, and 9 contribute significantly to the total AC activity in the heart [2, 27], but, unlike AC5/6, are not subjected to PKA-mediated inhibition [13]. Oscillations of cAMP, Ca^{2+}, PKA, PDE, and AC5/6 were observed, and then faded. However, essentially, no oscillation of AC4/7 occurred (Fig. 4b). Thus, when both Ca^{2+}-inhibitable and Ca^{2+}-non-inhibitable AC isoforms coexist, continuous oscillation is not usually observed for cellular cAMP and Ca^{2+}.

The formulas and values used to generate this oscillation model are shown in online Supplemental methods and Supplemental Table 3.

Discussion

Control systems in vivo are dynamic and complex, and it is very difficult to predict systems behavior on the basis of biochemical studies of individual molecules. However, use of systems biology tools, for example CellDesigner, makes it feasible to simulate complex biochemical networks flexibly [28–30]. In this study, this software enabled us to study Ca^{2+} and cAMP oscillations under different conditions, e.g., in the presence or absence of Ca^{2+}-inhibitable and Ca^{2+}-non-inhibitable AC isoforms and other regulatory molecules, in silico, without the need for experimental assays [25].

First, we confirmed that the original oscillation model of cAMP and Ca^{2+} developed by Cooper in 1995 [12] can be effectively simulated by use of CellDesigner [23–25]. When the signaling pathway contained only Ca^{2+}-inhibitable AC isoforms (AC5/6), we found that stable and spontaneous oscillations occurred.

Inclusion of PDE-mediated cAMP hydrolysis or PKA-mediated inhibition of AC5/6 into Cooper's model did not seem to change the oscillation behavior, but altered the amplitude to a greater or lesser extent [13, 20–22, 26].

Inclusion of PKA-mediated inhibition of AC5/6 induced a decrease of the amplitude by approximately 13 % (Fig. 3), whereas inclusion of PDE-mediated cAMP hydrolysis increased the amplitude by approximately 1.6-fold (Supplemental Fig. 2), compared with those of Cooper's original model (Fig. 1).

Phosphorylation of the L-type Ca^{2+}-channel increases the Ca^{2+} concentration, and might form an ascending loop. In turn, cardiac AC isoforms (AC5/6) are inhibited by Ca^{2+} and this might form a descending loop. Importantly, AC5/6 are inhibited by PKA, which might reduce the amplitude of the oscillation loop [13, 22]. Conversely, PDE, in association with Ca^{2+}-mediated inhibition, forms the descending phase of cAMP and Ca^{2+} oscillation in cardiac myocytes [31, 32]. These data, together with our current findings, indicate that PKA may have both positive and negative regulatory effects on the amplitude of the cAMP and Ca^{2+} oscillation loop, whereas PDE may have a positive regulatory effect on the amplitude [31, 32].

In contrast, when AC isoforms were not Ca^{2+}-inhibitable, no oscillation occurred. Interestingly, when both Ca^{2+}-non-inhibitable and Ca^{2+}-inhibitable AC isoforms were included in the model, oscillation occurred, but decayed very rapidly. Inclusion of PDE-mediated cAMP hydrolysis did not change this behavior. Therefore, our simulations indicate that for stable and spontaneous oscillation, the presence of Ca^{2+}-inhibitable AC isoforms and the absence of Ca^{2+}-non-inhibitable AC isoforms are both required.

Further studies will be required to incorporate the effects of newly identified AC5/6-associated proteins, including Snapin, a SNAP25-binding protein, and PAM, a protein associated with Myc, on the cAMP and Ca^{2+} oscillations, because the findings of this study show that Ca^{2+}-inhibitable AC isoforms (AC5/6) are essential for the oscillations of cAMP and Ca^{2+} [2, 13, 15].

Because the heart expresses seven AC isoforms [1, 7], including both Ca^{2+}-inhibitable and Ca^{2+}-non-inhibitable isoforms, continuous cAMP and Ca^{2+} oscillation in cardiac myocytes may not always occur, on the basis of the above findings (Fig. 4). Studies using AC5-deficient mice from our laboratory have shown that nearly half of the AC activity within the heart may be because of Ca^{2+}-non-inhibitable AC isoforms [1, 2, 7, 9, 33, 34]. However, microenvironments in which only Ca^{2+}-inhibitable AC5/6 are accumulated, such as lipid rafts or caveolae, are believed to exist [15, 16]. Indeed, it has been reported that Ca^{2+}-sensitive AC isoforms (AC1, 5, 6, and 8) and their associated proteins, such as PKA, A-kinase anchoring proteins (AKAPs), anchored PDEs, non-anchored PDE, and transient receptor potential (TRP) 1/3, are present in lipid rafts in many cell types, including cardiac myocytes, whereas the Ca^{2+}-insensitive AC 2, 4, and 7 are excluded from the rafts [15]. Because intracellular cAMP and Ca^{2+} mediate a diverse array of cellular functions, oscillation of cAMP and Ca^{2+} concentration might be involved in receptor-mediated signal transduction, not only in excitable cells, for example cardiac myocytes, but also in non-excitable cells [35]. Further, the occurrence or disturbance of cAMP and Ca^{2+} oscillations might contribute to the development of cardiac dysfunction or arrhythmia.

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Conflict of interest The authors declare no potential conflicts of interest.

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