

# Heart Failure

Subjects: [Biophysics](#)

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Heart failure (HF) is marked by dampened cardiac contractility. A mild therapeutic target that improves contractile function without desensitizing the  $\beta$ -Adrenergic system during HF may improve cardiac contractility and potentially survival. Inhibiting PKC $\alpha$  activity may fit the criteria of the therapeutic target with milder systemic effects that still boosts contractility in HF patients. PKC $\alpha$  activity has been observed to increase during HF. This increase in PKC $\alpha$  activity is perplexing because it is also accompanied by the up-regulation of a molecular braking mechanism.

myocardial infarction

translocation

lipid

contractile dysfunction

$\beta$ -adrenergic system desensitization

## 1. Introduction

Heart failure (HF) is a progressive condition and is marked by reduced output which in turn, evokes an increase in catecholamines and other neuroendocrine factors [1]. These factors can activate the reactive signaling pathways thus negatively influencing the heart and further down-regulating its functional performance [2]. Through enhancing the cardiac output, the persistent negative feedback loop could be disrupted as it may lead to a secondary reduction in neuroendocrine drive. Inotropes seem to be chemical agents of choice for this as they can selectively enhance the cardiac contractility [3][4][5][6][7][8]. Unfortunately, these agents are associated with high mortality rates, possibly due to the desensitization of the entire  $\beta$ -adrenergic system [4][5][6][7][8]. Thus, in many clinical settings, the safety of traditional inotropes is controversial [4][5][6][7][8]. Therefore, there is a critical need for heart failure research to focus on finding therapeutics that are milder alternatives to positive inotropes and do not come with a high risk of  $\beta$ -adrenergic system desensitization [8][9][10][11][12]. The overall clinical need for selectively enhancing cardiac contractility as a therapeutic strategy remains unresolved. Enhancing just the precise amount of contractility within physiological limits may increase the survival propensity of patients during HF.

Clinically, diastolic heart failure (DHF) and systolic heart failure (SHF) are the most common forms of heart failure (HF) disease. These syndromes are marked by a progressive loss in contractility, ejection failure, ventricular chamber dilation, ventricular wall thinning, increased peripheral vascular resistance and dysregulated fluid homeostasis [1][2][3][4]. The propensity towards these forms of cardiac insufficiency could be linked to the activation of PKC $\alpha$  molecule in DHF & SHF conditions [11][12]. The PKC $\alpha$  molecule act as a nodal integrator of cardiac contractility and may act as a refined and milder alternative of positive inotropes to effectively boost contractility in HF [11][12][13][14][15][16][17][18]. Clinical evidence suggests the positive inotropes worsens the prognosis in individuals with somewhat stable HF as they are susceptible to enhance contractility beyond safety limits [4-8]. Inhibiting

PKC $\alpha$  may provide a more refined and milder target possibility which might augment cardiac contractility within the physiological limits. Observations show that loss of PKC $\alpha$  activity could lead to a 20-30% increase in contractility during HF [10][11][12][13][14][15][16][17][18]. Experimental observations in angiotensin II (Ang II)-stimulated cardiomyocytes show that, in part, HF associated contractile dysfunction can be regulated through the G $\alpha$ q/DAG/PKC $\alpha$ /DGK $\zeta$  signaling cascade [19][20][21]. PKC $\alpha$  belongs to the conventional protein kinase C (cPKC) family of serine/threonine protein kinases. These kinases are canonically activated by Ca<sup>+2</sup> and lipid signaling [11]. PKC $\alpha$  is the most prominent member of the PKC family and is expressed in mouse, human and rabbit heart tissue [10][11][12]. Previous observations have linked PKC $\alpha$  to impaired left ventricular filling and ejection during heart failure. PKC $\alpha$  is necessary and sufficient to induce ventricular systolic and diastolic dysfunction [10][11]. Pharmacological and genetic inhibition of PKC $\alpha$  clearly improves contractility during heart failure, attenuating the extent of damage and disease [10][11]. Prolonged activation of PKC $\alpha$  may lead to serious malignant outcomes by inducing systolic and diastolic dysfunction [9][10][11][12][13][14][15][16][17].

DGK $\zeta$  is another key functional effector that signals during G $\alpha$ q-induced heart failure [15]. Previous research has shown that cardiac-specific overexpression of DGK $\zeta$  suppresses remodeling and fibrosis in the left ventricle (LV) independent of hemodynamic regulation. Thus, overexpression of DGK $\zeta$  under these conditions rescues angiotensin-induced congestive heart failure [15]. Previous data indicate that overexpression of DGK $\zeta$  also improves survival after MI [16]. Human heart failure is linked to increased PKC $\alpha$  activity [14]. This linkage is surprising, as PKC $\alpha$  activity in most cell types is exquisitely regulated [22][23]. PKC $\alpha$  activity in cardiomyocytes is also tightly regulated through a molecular braking mechanism. As soon as PKC $\alpha$  is activated, the molecular brakes in place to check this increase in PKC $\alpha$  concentration should also kick in, acting on the common activator. This molecular braking mechanism begs the question: why is PKC $\alpha$  activity increased and maintained during heart failure despite the concurrent activation of a braking mechanism? Here, I propose an explanation based on data analyzing a local DAG signaling cascade that may be responsible for the increase in PKC $\alpha$  activity during HF.

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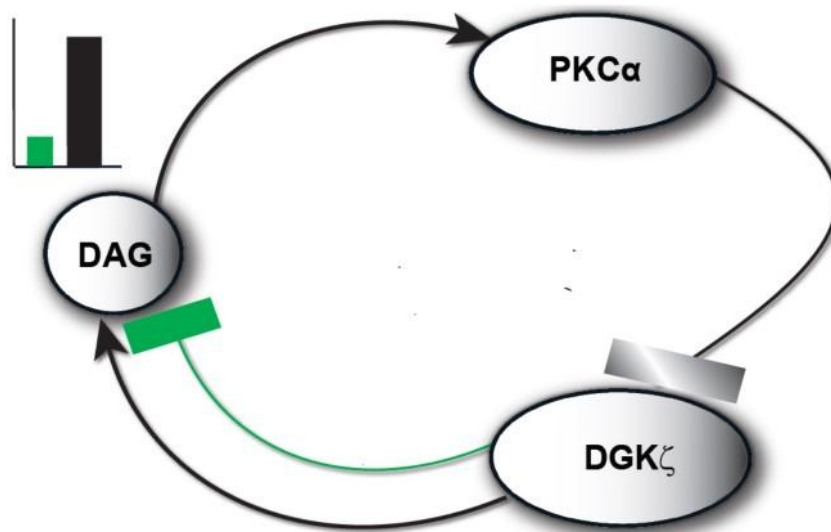
Here, I propose DAG signaling homeostasis during Ang II-induced heart failure can be regulated through a molecular loop between the positive and negative effector molecules PKC $\alpha$  and DGK $\zeta$ . This proposed molecular loop may improve understanding of the molecular mechanisms involved in the complex spatiotemporal organization of DAG-PKC $\alpha$ - DGK $\zeta$  signaling during post-MI cardiac remodeling events [29-34] [22][23][24][25][26][27][28]. Using a computational model, this study shows the proposed molecular loop has a dual regulatory character. During basal conditions, a net negative feedback loop may prevail and regulate local DAG concentration. Interestingly, upon stimulation conditions, a positive feedback effect on DAG signaling is observed. This positive

feedback can possibly explain the link between persistently high DAG levels and malignant outcomes. The transition from negative to positive feedback depends on the local biosynthesis rate of DAG and, in turn, on the mutual interactions between positive and negative DAG effector molecules.

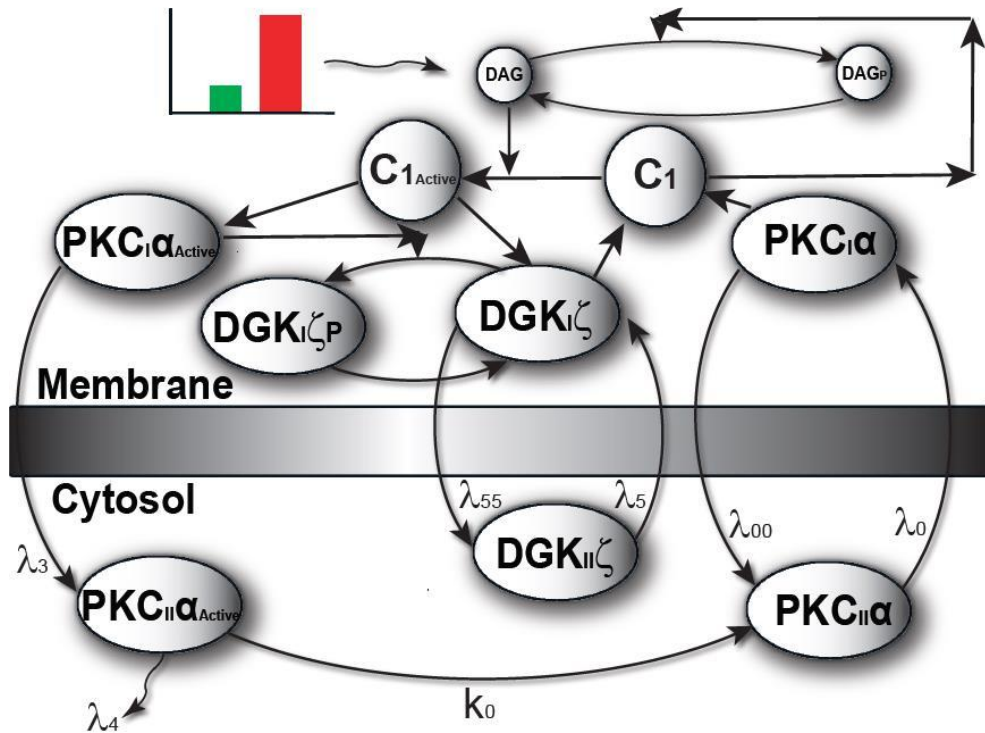
## 2. Results and Discussions

### 2.1 Local DAG Signaling Regulation in Cardiomyocytes

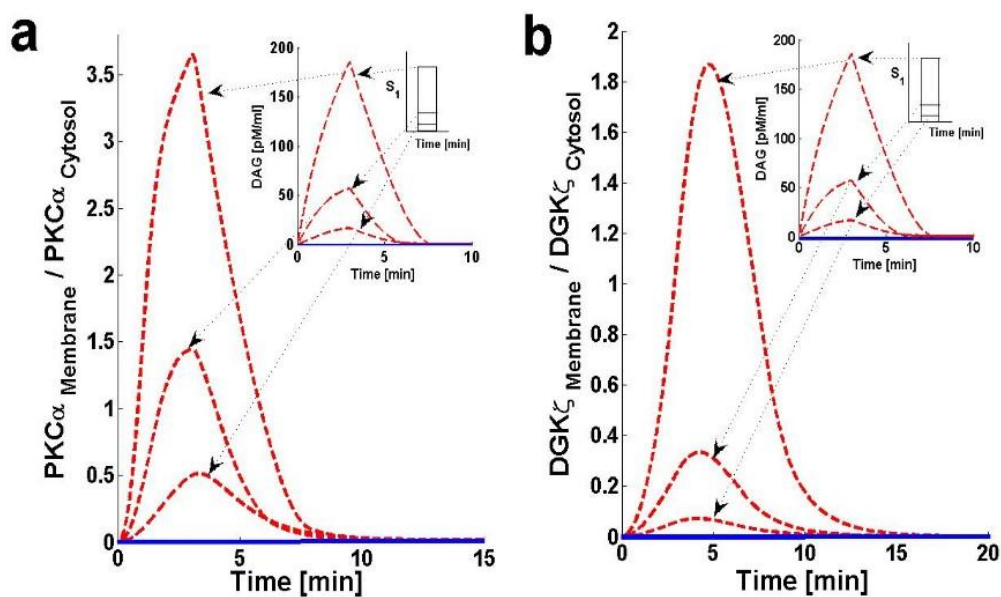
The model I propose in **Figure 1 & 2**, describe local regulation of DAG homeostasis in cardiomyocytes. The model is composed of two molecular components: 1. PKC $\alpha$ , the target protein of DAG signaling, can exist in one of four states: cytosolic dormant (PKC $_{II}\alpha$ ), inactive membrane (PKC $_I\alpha$ ), active membrane (PKC $_I\alpha^A$ ) or active cytosolic (PKC $_{II}\alpha^A$ ). 2. DGK $\zeta$ , the attenuator protein of DAG signaling, can be in one of three states: cytosolic (DGK $_{II}\zeta$ ), active membrane (DGK $_I\zeta$ ), or phosphorylated/inactive membrane (DGK $_I\zeta_P$ ). Both these components migrate to the plasma membrane in a DAG-dependent manner. Once in the plasma membrane, DGK $\zeta$  forms complex C $_1$  with PKC $\alpha$ . These two components interact in a closed loop and regulate DAG homeostasis in a negative feedback loop.



**Figure 1.** The regulatory molecular loop between the target (PKC $\alpha$ ) and attenuator (DGK $\zeta$ ) of local DAG signaling.



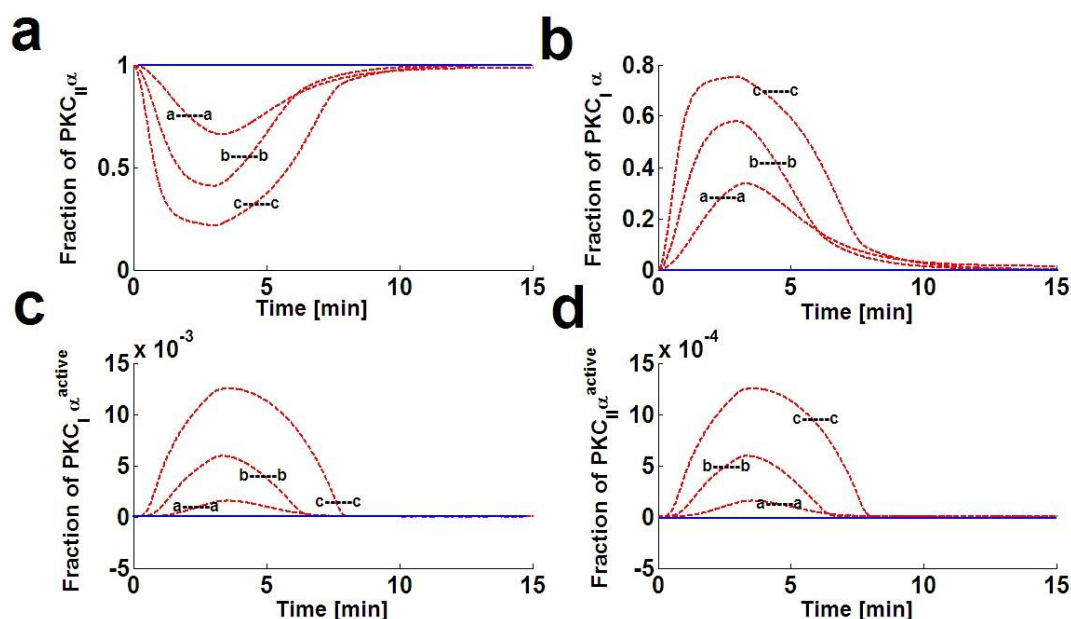
**Figure 2.** The two-compartment regulatory model of local DAG signaling in cardiomyocytes. Here, one of the compartments is cytosol, whereas the other compartment is the plasma membrane.



**Figure 3.** Membrane to cytosol (M/C) ratio of target & attenuator molecules of DAG signaling.

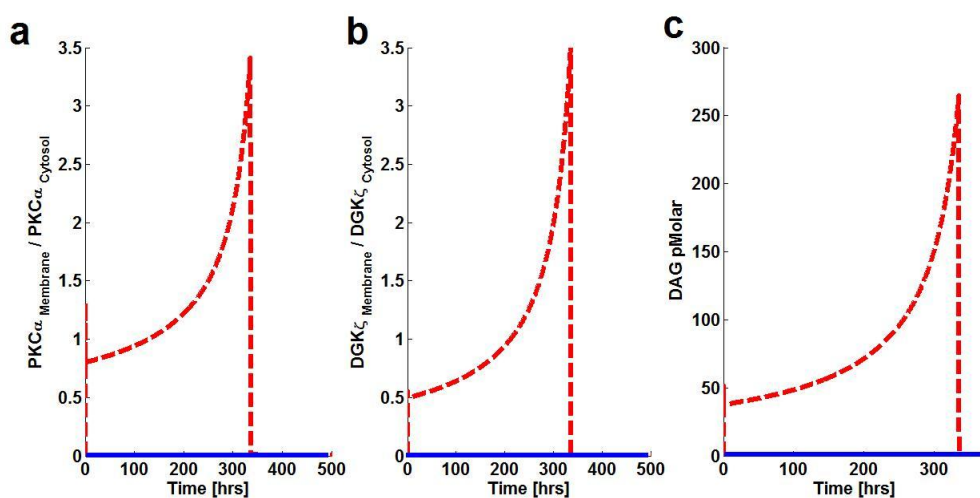
Using the computation model, I next determined the translocation characteristics of both the target and attenuator molecules of DAG signaling in cardiomyocytes (**Figure 3**). In the model, translocation characteristics were determined by measuring the membrane to the cytosol (M/C) ratio of target and attenuator molecules. The M/C ratio describes the relative distribution of PKC $\alpha$  and DGK $\zeta$  molecules in the membrane and cytosolic

compartments. For PKC $\alpha$ , this ratio is an indirect index of activation. According to the proposed model, the M/C ratio for DGK $\zeta$  could be indirectly linked to kinase deactivation. A higher M/C ratio indicates high rates of molecular migration from the cytosol to the plasma membrane. Ang II-induced biosynthesis of DAG was implemented using a three-minute pulse, as described in the previous section. The strength of the pulse is described by an arbitrary parameter  $S_1$ . Here, the parameter  $S_1$  is set at three arbitrary levels: 0.5, 2 and 6. In the absence of a pulse (**Figure 3, solid lines**) there is no *de novo* DAG biosynthesis and the system is fixed in its basal state. In the basal state, both molecules reside in the cytosol with no possibility of translocation. In the presence of a pulse, the M/C ratios of both PKC $\alpha$  (**Figure 3a, dashed line**) and DGK $\zeta$  (**Figure 3b, dashed line**) increase to their maximum levels followed by gradual clearance of signal. The temporal dynamics depicted in Figure 3 clearly show two phases of translocation. The first is an early phase in which the target and attenuator molecules of DAG signaling migrate from the cytosol to the plasma membrane. The first phase is followed by a second phase where both molecules translocate back to the cytosol. These results indicate that the migration of PKC $\alpha$  and DGK $\zeta$  from the cytosol to the plasma membrane depends on local DAG concentration at the plasma membrane. My results also show the relative distribution of different forms of PKC $\alpha$  in different compartments (**Figure 4**). These results on the relative distribution of PKC $\alpha$  in different states describe that the biological function of the PKC $\alpha$  molecule in the proposed model is regulated through a four-step cycle.<sup>11, 48</sup> The four steps within this model are translocation, activation, redistribution/re-translocation, and deactivation. The model assumes that inactive but catalytically-competent PKC $\alpha$  is stored in the cytosol. *De novo* synthesis generates a rather unstable naïve form of PKC $\alpha$  which constitutively undergoes a sequence of ordered priming and autophosphorylations. This sequence produces a mature, inactive, phosphatase-resistant, and proteasome-resistant molecule.<sup>48</sup> These phosphorylations are essential for PKC $\alpha$  stability and catalytic competence.<sup>48</sup> The PKC life cycle is complex and modulated through precise and tightly-coupled molecular events.<sup>48</sup> Evidence suggests that, before PKC becomes responsive to second messengers, it must first be phosphorylated at three conserved positions:<sup>48</sup> Thr500, Thr641, and Ser660. This model does not attempt to model *de novo* synthesis or the subsequent processes of enzyme maturation through phosphorylation. This study assumes the cytosol contains sufficient amounts of mature, second messenger-responsive PKC $\alpha$  enzyme. In these simulations, this is modeled by setting the initial conditions such that only the concentration of PKC $\alpha$  is non-negligible. The concentration of all other forms of PKC $\alpha$  is initially set at negligibly small values.



**Figure 4.** Dynamical characteristics of all four isoforms of PKC $\alpha$ . These temporal dynamics corresponds to results presented in figure 3 (relative distribution of PKC $\alpha$  and DGK $\zeta$  in the membrane and cytosol compartments in response to Ang-II like stimulation). These results show that in the basal conditions all the PKC $\alpha$  resides in the dormant form in cytosol i.e., PKC $_{II\alpha}$ . However, on stimulation, the enzyme is distributed into all four forms i.e., PKC $_{II\alpha}$ , PKC $_I\alpha$ , PKC $_{II\alpha}^{Active}$ , and PKC $_I\alpha^{Active}$ . The extent and duration of this distribution is directly dependent on the Ang-II like stimulation and hence, on DAG. Here, three different levels of stimulation are used (Figure 3). The symbol a—*a* represents a pulse strength of 0.5, b—*b* represents a pulse strength of 2.0, and c—*c* represents the pulse strength of 6.0. Higher levels of the pulse (c—*c*) correspond to higher levels of DAG generation.

Experimental data indicate prolonged activation of PKC $\alpha$  in cardiomyocytes. The model proposed in this work is also calibrated against these results as shown in figure 5.



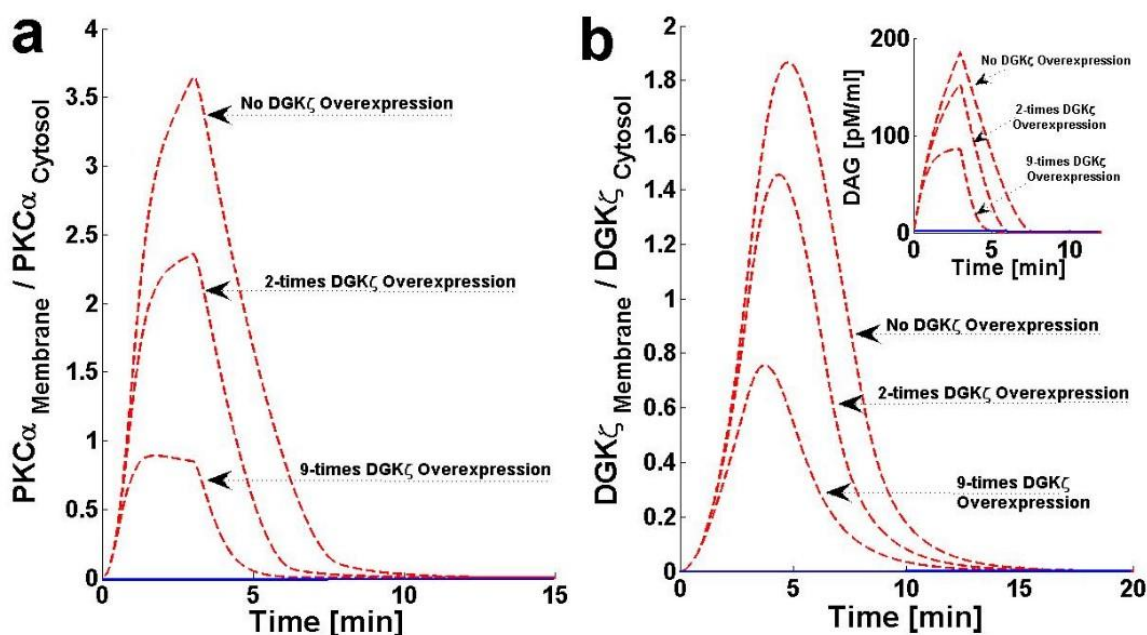
**Figure 5.** The effect of prolonged duration of stimulation on Membrane to cytosol (M/C) ratio of target & attenuator molecules of DAG signaling. These results show that prolonged pulse-like (duration of, 14 days) stimulation leads



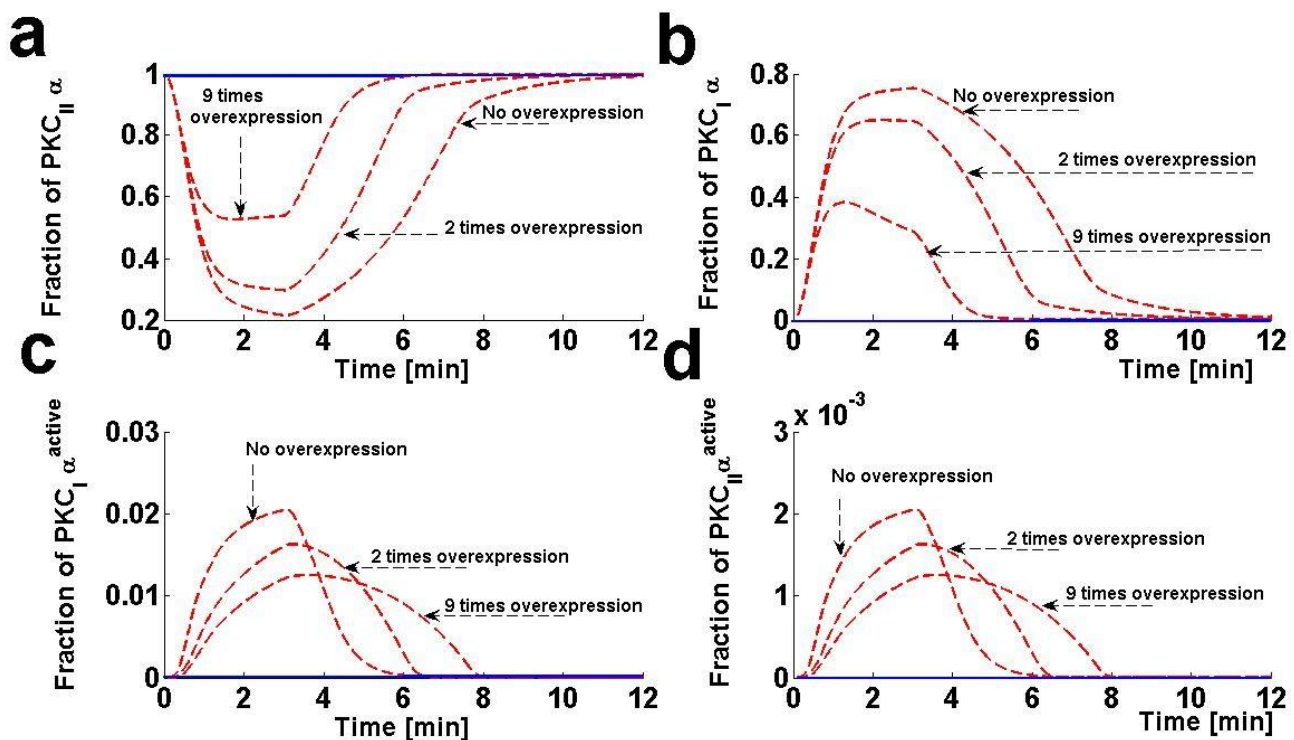
to the rapid generation of DAG and persistence. The generation of the second messenger, in turn, stimulates the translocation of both the target and attenuator molecules of DAG signaling from the cytosol to the membrane.

## 2.2 Effects of DGK $\zeta$ Overexpression on the Translocation Characteristics of PKC $\alpha$ in Cardiomyocytes

The chance of survival after MI increases upon cardiac-specific overexpression of DGK $\zeta$ . This is due to the attenuation of post-infarction LV remodeling. The survival rate almost doubles with DGK $\zeta$  overexpression.<sup>14</sup> Observations in transgenic mice indicate, that independent of hemodynamic effects, DGK $\zeta$  overexpression attenuates angiotensin II-induced activation of DAG-PKC $\alpha$  signaling and subsequent contractile dysfunction.<sup>13-15</sup> Experimental observations show that, in wild type mouse hearts, angiotensin II induces the translocation of PKC $\alpha$  from the plasma membrane to the cytosol.<sup>13</sup> This translocation event is blocked by DGK $\zeta$  overexpression.<sup>13,15</sup> I set out to address the question of why translocation is blocked by DGK $\zeta$  overexpression by using a simple regulatory model for local DAG signaling. In this model's simulations, DGK $\zeta$  overexpression was implemented by adjusting the DGK $\zeta$ -to-PKC $\alpha$  ratio under initial conditions. In the case where DGK $\zeta$  is not overexpressed, the DGK $\zeta$  to PKC $\alpha$  ratio is set at 1. For simulations mimicking two- and nine-fold overexpression over basal levels of DGK $\zeta$ , this ratio is set at 2 and 9, respectively. Here, the effects of DGK $\zeta$  overexpression on the migration of target and attenuator molecules of DAG signaling (**Figures 6a and 6b and Figure 7**) were tracked. **Figure 6a** shows that overexpression of DGK $\zeta$  restricts PKC $\alpha$  translocation from the cytosol to the plasma membrane. At 2-fold DGK $\zeta$  overexpression, a significant reduction in the M/C ratio of PKC $\alpha$  is observed. For 9-fold overexpression, a further reduction in the M/C ratio is observed and the M/C ratio for this case is less than one. At even higher levels of DGK $\zeta$  overexpression, PKC $\alpha$  translocation to the plasma membrane is completely eliminated. These results also show that overexpression of DGK $\zeta$  only slightly influences the migration characteristics of attenuator molecules of DAG signaling (**Figure 6b**). These results indicate that the maximum value of the M/C ratio of DGK $\zeta$  decreases from 1.84 to 0.78 as the overexpression level increases from basal levels to 9-fold overexpression.



**Figure 6.** The effect of cardiac-specific overexpression of diacylglycerol kinase  $\zeta$  (DGK $\zeta$ ) on the membrane to cytosol (M/C) ratio of target and attenuator molecules of DAG signaling in cardiomyocytes.



**Figure 7.** The effect of DGK $\zeta$  overexpression on the dynamical characteristics of different forms of PKC $\alpha$  enzyme. These temporal dynamics correspond to results presented in figure 6 (relative distribution of PKC $\alpha$  and DGK $\zeta$  in the membrane and cytosol compartments in response to the DGK $\zeta$  overexpression at the pulse strength of 6.0).

In conclusion, a two-compartment model was developed for regulating DAG homeostasis in Ang II-induced heart failure. This computational model is a promising tool to study mechanisms of DAG regulation in the context of heart failure. This model may be used to identify novel therapeutic targets with the aim of improving survival and quality of life outcomes in heart failure patients.

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