Protein Kinase G I and Heart Failure
Shifting Focus From Vascular Unloading to Direct Myocardial Antiremodeling Effects

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The cyclic GMP (cGMP) signaling pathway is a ubiquitous second messenger system that regulates diverse cellular functions. The cGMP-dependent protein kinase G (PKG) is a primary direct cGMP effector in multiple cell types. cGMP-PKG signaling plays critical roles throughout the cardiovascular system, although the best understood function of PKG is as an inducer of vascular smooth muscle relaxation, leading to arteriolar vasodilation (see Münzel et al1 for an extensive review on the subject). However, during the past decade, multiple studies have revealed important functions of cGMP and PKG signaling in the myocardium, and particularly in the attenuation of pathological cardiac hypertrophy and remodeling. This has created great interest in targeting this pathway to treat heart failure. Multiple cGMP-generating compounds have now been investigated for the treatment of heart failure. Although these agents target different upstream components of the cGMP pathway, they all ultimately function as PKG I (PKGI) activators (Figure). Because of the rapid induction by PKG of vascular relaxation, most clinical trials to date have studied these agents in acute decompensated heart failure (ADHF). However, more recent clinical studies, informed by basic data, have begun to explore a role for these agents as chronic myocardial antiremodeling therapies, which, ironically, are being limited in many cases by undesired excessive vasodilation and hypotension resulting from PKG activation in the vasculature.

The purpose of this review, therefore, is 3-fold. First, we will review the upstream PKGI activation pathways. Second, we will examine the basic science studies that revealed the antiremodeling function of this pathway, both in isolated cardiac myocytes (CMs) and in vivo. Third, we will review the heart failure clinical trials of PKG activating agents, both in ADHF and also as emerging chronic antiremodeling therapies. Throughout, we will focus on the evidence from basic and clinical studies supporting exploration of CM-specific PKGI substrates that might serve as novel antiremodeling therapeutic targets to circumvent the undesired hypotension resulting from PKGI-induced vasodilation.

Molecular Evidence That PKGI Inhibits Pathological Cardiac Hypertrophy and Remodeling

Overview of PKGI Activation in the Cardiovascular System

The Figure outlines the cGMP/PKG signaling pathway. cGMP is synthesized by guanylate cyclase (GC), either in the form of soluble GC (sGC) or particulate GC (pGC). sGC and pGC are in turn activated by upstream NO or natriuretic peptides (NP), respectively. As the names imply, sGC resides in the cytosol, where it is stimulated by NO diffusing directly across the plasma membrane. pGC, on the contrary, is a membrane-bound receptor (also termed the NP receptor) activated by extracellular NPs, including atrial NP and B-type NP (BNP). NP activation of pGC induces cGMP synthesis on the inner plasma membrane.

Phosphodiesterases (PDEs), which catabolize cGMP, provide a second layer of regulation of intracellular cGMP levels. At least 9 PDEs exist in the cardiovascular system.2 Most of the PDEs catabolize both cGMP and cAMP, although cGMP-selective PDEs, such as PDE5, also exist. cGMP also inhibits PDE5 in a negative feedback manner.2 cGMP directly activates a number of molecules, including PDEs and ion channels. However, PKGI represents the best characterized cGMP downstream signaling effector.3 Two PKG genes, PKGI and PKGII, exist but only PKGI is expressed in the cardiovascular system,3 and this review therefore focuses on PKGI. The PKGI gene expresses as 2 separate isoforms: PKGIα and Iβ, which are splice variants of the same gene and differ only in sequence of their N-terminal leucine zipper (LZ) interaction domain.3 These LZ domains mediate PKGI binding to specific substrates, many of which contain similar LZ domains, thus allowing LZ–LZ cointeraction. The difference in sequence between the Iα and Iβ LZ domains permits these different isoforms to interact with and phosphorylate unique sets of kinase substrates. As we will describe, much of our understanding of the role of these kinases in the
cardiovascular system has arisen from identifying the different LZ-dependent interacting proteins and kinase substrates of PKGIα and PKGIβ. PKGIα and PKGIβ are each highly expressed in various cardiovascular tissues, including vascular smooth muscle cells (VSMC), cardiac and vascular fibroblasts, endothelium, and CMs.1,4 Many prior studies of PKGI focused on understanding its mechanism of inducing VSMC relaxation and arterial dilation. These studies have been reviewed extensively.1 Whole-body PKGI knockout (KO) leads to abnormal vascular relaxation.5 And, selective mutation of the PKGIα LZ interaction domain in mice also produces hypertension and vascular dysfunction.6 To date, multiple PKGIα and PKGIβ LZ-dependent substrates have been identified in VSMC,1 further supporting a critical role of the specific PKGIα and PKGIβ LZ domains in regulating cardiovascular function.

**In Vitro Studies in CMs**

In contrast with the vasculature, the potential regulatory role of the PKGI signaling pathway on cardiac function attracted the interest of investigators only relatively recently, and these studies are summarized in Table 1. Initial studies tested the effect of upstream PKGI activators in isolated CMs subjected to pathological neurohumoral stimulation. These studies primarily examined CM [3H]-labeled amino acid incorporation and fetal gene re-expression as markers of hypertrophy and pathological remodeling, respectively. NO, which activates PKGI by stimulating sGC synthesis of cGMP, inhibited nor-epinephrine-induced CM hypertrophy and fetal gene expression in rat neonatal CMs29 and mediated the antihypertrophic effects of angiotensin-converting enzyme inhibition in the same culture system.7,8 NPs also inhibited both norepinephrine and angiotensin II (AngII)–induced hypertrophy in neonatal rat CMs.16,29 In addition, atrial NP, BNP, and C-type NP blocked AngII-induced hypertrophy and fetal gene expression in adult CMs.17 Many of these studies16,17,29 identified identical effects with cGMP administration, supporting that both NPs and NO inhibit hypertrophy and remodeling by inducing elevation of cGMP in the CM.

While the CM studies described above therefore suggested PKGI as the logical mediator of these effects, gene transfer of PKGI in neonatal CMs confirmed a specific role of PKGI in inhibiting CM hypertrophy induced by α-adrenergic activation.32,33 These studies also demonstrated the requirement of PKGI in mediating the NO antihypertrophic effect13 and in inhibiting calcineurin-mediated CM hypertrophy. Taken together, these studies in isolated CMs revealed antihypertrophic functions of upstream cGMP regulators as well as cGMP and demonstrated PKGI to be the downstream mediator of these effects in the CM. This evidence led to the exploration of the PKGI activation pathway in in vivo models of cardiac remodeling and heart failure.

**In Vivo Investigations of the PKGI Activation Pathway in Cardiac Remodeling and Heart Failure: NO Synthase**

Although cell culture experiments identified a direct role of PKGI in regulating pathological CM hypertrophy, initial in vivo studies investigated the role of upstream components of the cGMP-PKG signaling system. Multiple genetic deletion models of NO synthase 3 (NOS3; the enzyme that synthesizes NO in the CM, among other tissues) support a role of NO signaling in antagonizing cardiac hypertrophy and remodeling. Mice with whole-body deletion of NOS3 develop chronic hypertension and left ventricular hypertrophy (LVH).14 Although LV systolic function of the NOS3 KO mice is initially preserved, their LVs develop fibrosis, apoptosis, CM death, and decreased β-adrenergic responses, as the mice age, supporting a functionally important consequence of NOS3 disruption.15 Two separate studies demonstrated increased LVH in NOS3 KO mice, compared with wild-type controls, in response to LV pressure overload by transaortic...
Table 1. Basic Studies of PKGI in Cardiac Hypertrophy and Remodeling

<table>
<thead>
<tr>
<th>PKG Activating Molecule</th>
<th>Experimental System</th>
<th>Hypertrophic Stimulus</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>Isolated CMs</td>
<td>AngII</td>
<td>Decreased [3H] phenylalanine incorporation.</td>
<td>7*</td>
</tr>
<tr>
<td>NO</td>
<td>Isolated CMs</td>
<td>PE</td>
<td>Potentiation by NO of antihypertrophic ACE inhibitor effect.</td>
<td>8*</td>
</tr>
<tr>
<td>NOS3</td>
<td>Whole-body NOS3 deletion</td>
<td>MI</td>
<td>Increased LVH, chamber dilation, systolic and diastolic dysfunction 28 d post-MI.</td>
<td>9</td>
</tr>
<tr>
<td>NOS3</td>
<td>Whole-body NOS3 deletion</td>
<td>TAC</td>
<td>Increased LVH after TAC.</td>
<td>10,11</td>
</tr>
<tr>
<td>NOS3</td>
<td>Whole-body NOS3 deletion</td>
<td>TAC</td>
<td>Reduced LVH and LV remodeling after TAC.</td>
<td>12</td>
</tr>
<tr>
<td>NOS3</td>
<td>CM-restricted NOS3 transgene on whole-body NOS3 deletion background</td>
<td>TAC</td>
<td>Presence of CM-restricted transgene ameliorated TAC-induced LVH, LV fibrosis, and LV systolic dysfunction.</td>
<td>13*</td>
</tr>
<tr>
<td>NOS3</td>
<td>Whole-body NOS3 deletion</td>
<td>Basal</td>
<td>Hypertension, progressive LVH.</td>
<td>14,15</td>
</tr>
<tr>
<td>BNP</td>
<td>Isolated CMs and endothelial cells</td>
<td>AngII</td>
<td>Decreased [3H] phenylalanine.</td>
<td>16</td>
</tr>
<tr>
<td>ANP, BNP, CNP</td>
<td>Adult CMs</td>
<td>AngII</td>
<td>ANP, BNP, CNP, and 8Br-cGMP inhibited AngII-induced [3H] phenylalanine incorporation.</td>
<td>17*</td>
</tr>
<tr>
<td>NPR-A</td>
<td>Whole-body NPR-A deletion</td>
<td>Basal</td>
<td>Systemic hypertension, LVH, and sudden cardiac death.</td>
<td>18</td>
</tr>
<tr>
<td>NPR-A</td>
<td>Whole-body NPR-A deletion</td>
<td>Basal (aging)</td>
<td>Severe LVH; LV systolic and diastolic function.</td>
<td>19</td>
</tr>
<tr>
<td>NPR-A</td>
<td>Whole-body NPR-A deletion</td>
<td>Basal, TAC</td>
<td>Persistent LVH when BP normalized; increased LVH in response to TAC.</td>
<td>20</td>
</tr>
<tr>
<td>NPR-A</td>
<td>CM-restricted NPR-A overexpression on WT or whole-body NPR-A KD</td>
<td>Basal</td>
<td>Restoration of CM NPR-A on NPR-A whole-body deletion background reduced basal LV and CM hypertrophy.</td>
<td>21*</td>
</tr>
<tr>
<td>NPR-A</td>
<td>CM-restricted NPR-A deletion</td>
<td>Basal, TAC</td>
<td>Increased basal LV mass. Markedly increased TAC-induced LV hypertrophy and functional impairment.</td>
<td>22*</td>
</tr>
<tr>
<td>NPR-A</td>
<td>CM-restricted overexpression of NPR-A guanylate cyclase catalytic domain</td>
<td>Iso, TAC</td>
<td>Decreased isoproterenol and TAC-induced LVH. Attenuated TAC-induced LV systolic dysfunction.</td>
<td>23*</td>
</tr>
<tr>
<td>NPR-A; NOS3</td>
<td>CM-restricted NPR-A deletion on whole-body NOS3 deletion background</td>
<td>Basal</td>
<td>Markedly exacerbated LVH with no increase in systemic hypertension.</td>
<td>24*</td>
</tr>
<tr>
<td>PDE5</td>
<td>Sildenafil (PDE5 inhibitor)</td>
<td>TAC</td>
<td>Sildenafil reduced and reversed TAC-induced LV hypertrophy and remodeling. Sildenafil decreased hypertrophic gene expression in neonatal mouse CMs.</td>
<td>25*</td>
</tr>
<tr>
<td>PDE5</td>
<td>Sildenafil</td>
<td>Doxorubicin</td>
<td>Sildenafil reduced doxorubicin-induced apoptosis and LV contractile dysfunction.</td>
<td>26</td>
</tr>
<tr>
<td>PDE5</td>
<td>CM-restricted PDE5 overexpression</td>
<td>MI</td>
<td>Increased LV hypertrophy, remodeling, contractile dysfunction, and diastolic dysfunction 10 d post-MI.</td>
<td>27*</td>
</tr>
<tr>
<td>PDE5</td>
<td>CM-restricted PDE5 overexpression and doxycycline-controlled PDE5 transcriptional repression</td>
<td>TAC</td>
<td>Increased TAC-induced cardiac remodeling, LV fibrosis, hypertrophy, and functional impairment in PDE5 overexpressors.</td>
<td>28*</td>
</tr>
<tr>
<td>ANP, NO, cGMP</td>
<td>Isolated CMs</td>
<td>NE</td>
<td>NO, ANP, and cGMP each inhibited NE-induced [3H] accumulation in CMs and fibroblasts.</td>
<td>29*</td>
</tr>
<tr>
<td>cGMP</td>
<td>Isolated CMs</td>
<td>Ang II</td>
<td>Inhibitors and activators of sGC demonstrated requirement of sGC for mediating bradykinin inhibition of AngII-induced [3H] phenylalanine incorporation in CMs.</td>
<td>30*</td>
</tr>
<tr>
<td>sGC</td>
<td>CM-specific sGC transgene</td>
<td>Mdx model</td>
<td>CM-restricted sGC rescued the contractile deterioration in isolated working hearts from Mdx mice. Further rescue with addition of sildenafil.</td>
<td>31*</td>
</tr>
<tr>
<td>PKGI</td>
<td>Isolated CMs</td>
<td>PE</td>
<td>cGMP, NO, and adenoviral PKGI inhibited; (1) PE-induced NFAT nuclear translocation; (2) CM hypertrophy.</td>
<td>32*</td>
</tr>
</tbody>
</table>
| PKGI                   | Isolated CMs        | PE | Gene transfer of PKGI promoted NO donor or cGMP complete inhibition of PE-mediated CM hypertrophy. | 33*        | (Continued)
constriction (TAC).10,11 Conversely, a separate study instead observed decreased hypertrophy and LV dysfunction in NOS3 KO mice.12 The authors demonstrated that oxidative stress after TAC led to NOS uncoupling and resultant synthesis of reactive oxygen species. Oxidative stress decreased in NOS3 KO hearts after TAC, presumably attributable to absence of NOS3. In this study, however, pharmacological reversal of NOS uncoupling restored protection against pathological cardiac remodeling, suggesting that NO synthesis by NOS3 does inhibit pathological cardiac remodeling in vivo.

NOS3 also protects against remodeling after experimental myocardial infarction (MI).9 Importantly, normalization of hypertension in the NOS3 KO mice failed to prevent their increased MI-induced remodeling, which supports a myocardial intrinsic role of NOS3 in this process. Further support for a CM-intrinsic role of NOS3 came from an elegant study of whole-body NOS3 KO mice harboring CM-restricted transgenic NOS3 expression.13 After TAC, NOS3 restoration in the CM rescued the adverse cardiac remodeling of the NOS3 KO, identifying a critical function of NOS3 in the CM in limiting the pathological response to LV pressure overload. Thus, multiple lines of evidence reveal a critical requirement of NOS3 in limiting the cardiac remodeling response. Furthermore, these studies indicate that NOS3 exerts this effect through a specific function in the CM. Although NOS3 presumably inhibits LVH and remodeling through NO-dependent cGMP synthesis and resultant PKGI activation, none of the above-mentioned studies tested whether cGMP, or PKGI, mediates the NOS3 effect in vivo.

In Vivo Investigations: sGC and pGC

Although upstream NOS3 has been studied extensively in vivo, the specific role of downstream NO-activated sGC has been less well characterized, compared with pGC. However, CM-restricted transgenic overexpression of sGC rescued the cardiac contractile and metabolic abnormalities in a mouse model of Duchenne cardiomyopathy.31 Genetic manipulation of the NP receptor pGC-A (also termed NPR-A) has provided extensive evidence that the cGMP system attenuates cardiac remodeling in vivo, through a myocardial-specific role. Initial exploration of the pGC-A whole-body KO mouse revealed hypertension, as well as LVH and sudden death.18 As the mice age, the LVH becomes more severe and significant LV systolic and diastolic dysfunction ensues.19 These seminal studies revealed a critical role of NP-pGC signaling in global cardiac remodeling, but did not address the possibility that the severe cardiac phenotype of the pGC-A KO mice arose solely from hypertension.

However, multiple ensuing studies revealed the CM-specific role of pGC-A in attenuating the cardiac response to LV pressure overload. First, although pGC-A KO mice develop hypertension, the normalization of their blood pressure (BP) failed to reverse the LVH observed.20 Conversely, TAC produced exaggerated LVH and contractile dysfunction in the pGC-A KO mice.21 In addition, mice with CM-restricted deletion of pGC-A developed basal LVH and marked TAC-induced LVH with systolic deterioration.22 CM-restricted transgenic overexpression of pGC attenuated both the basal LVH of whole-body pGC-A KO mice,23 and, when expressed on the wild-type background, inhibited TAC and isoproterenol-induced LVH.24

In Vivo Investigations: PDE5

Multiple lines of evidence reveal that NOS3 and pGC inhibit pathological cardiac remodeling in vivo. Although NO and GCs both increase intracellular cGMP, none of the studies described above proved a requirement of cGMP or its downstream effectors in inhibiting LVH in vivo. Interestingly, the majority of evidence specifically identifying cGMP as an anti-remodeling signaling molecule comes from investigations of PDE5, a cGMP-selective PDE. PDE5 is normally expressed at low levels in the myocardium but its expression increases in the heart in response to mechanical stress, such as LV pressure overload.27 In a groundbreaking translational study, Takimoto et al28 demonstrated that the PDE5 inhibitor sildenafil (Viagra) strongly inhibits TAC-induced LVH and remodeling. In the same study, the investigators made the arguably even more important observation that sildenafil administration after TAC nearly completely reversed cardiac remodeling.25 Sildenafil...
treatment increased myocardial PKG activity, suggesting that sildenafil inhibition of cGMP catalysis by PDE5 increased intracellular cGMP, leading to increased PKGI activation. However, the study did not specifically demonstrate a direct requirement of either cGMP or PKGI for the sildenafil effect in vivo. Subsequent studies demonstrated that PDE5 inhibition with sildenafil also attenuates LV dysfunction and CM apoptosis in other experimental models, such as cardiotoxic doxorubicin administration. These pharmacological studies both identified cGMP as an in vivo antiremodeling molecule and also provided the proof of concept for using PDE5 inhibitors, which are safe and available for human patients, as chronic antiremodeling agents.

Subsequent work in genetically manipulated mouse models confirmed the deleterious proremodeling effect of PDE5, thus further supporting the potential merits of PDE5 inhibition in treating heart failure. This was first demonstrated in a translational study that identified increased CM PDE5 expression in hearts from humans with ischemic or dilated cardiomyopathy, compared with normal hearts. In the same study, the authors showed that transgenic overexpression of PDE5 solely in the CM predisposed to accelerated cardiac remodeling, LVH, and CM functional decrement after surgical MI. A similar model, in which PDE5 expression was genetically enhanced in the CM but could be reversed by doxycycline, demonstrated increased TAC-induced cardiac remodeling in PDE5 transgenic mice, and further that inhibition of PDE5 expression with doxycycline produced a recovery of LV function and remodeling. Importantly, this study also demonstrated marked reduction of myocardial PKGI activity in hearts of PDE5 transgenic mice, providing strong correlative evidence for a role of PKGI in this process.

In Vivo Investigations: PKGI as an Antiremodeling Molecule

The mouse models and pharmacological studies described above revealed a central role of both upstream cGMP generators, as well as of cGMP regulation by PDE5, in inhibiting the CM hypertrophic and remodeling program. However, confirming the role of PKGI specifically in this process had been difficult because of lack of both selective PKGI modulating drugs and viable in vivo models of PKGI disruption. For example, mice with whole-body deletion of PKGI die early in life, which precludes full analysis of their adult cardiac phenotype. And, in fact, the relatively low basal expression of both PDE5 and PKGI in the CM has led some to question the function of PKGI in the heart. This notion was tested in a study of mice harboring smooth muscle expression of PKG/β on a PKGI whole-body null background. This PKG/β expression is sufficient to rescue the lethal phenotype of whole-body PKGI KO. In the β rescue mouse, the degree of LVH induced by either TAC or isoproterenol infusion did not differ compared with wild-type controls. Because these mice lack PKGI in the CM, the authors interpreted their study to indicate that PKGI does not function in the CM to inhibit LVH. Importantly, these studies examined only a single time point and measured only LVH, but not LV function.

Two separate animal models of PKGI disruption recently provided direct evidence for the role of PKGI in attenuating cardiac remodeling in vivo. First, mice with CM-restricted deletion of PKGI develop a dilated cardiomyopathy phenotype in response either to chronic AngII infusion or to TAC. Interestingly, like the PKGIβ rescue mice, these mice displayed a normal cardiac response to isoproterenol infusion. This study provided the first direct evidence for a CM-specific role of PKGI signaling in maintaining LV function in response to LV pressure overload.

The specific role of the PKGIα, the predominant myocardial PKGI isoform, was examined in separate studies using the whole-body PKGIα LZ mutant (LZM) knockin model. These LZM mice harbor discrete mutations in the PKGIα LZ domain, yielding a kinase with retained activity but disrupted binding to LZ-dependent substrates. The LZM mice develop adult-onset hypertension and abnormalities of vascular relaxation, resulting, in elderly LZM mice, in severe, pathological LVH. The LZM mice were subjected to TAC in the prehypertensive state, a time at which their LV size and function is normal. In response to TAC, the LZM mice developed LV systolic and diastolic dysfunction detectable as early as 2 days post-TAC, followed by increased LVH, LV remodeling, and markedly increased congestive heart failure (CHF) mortality. And, unlike wild-type mice, the LZM LVs failed to respond to the antihypertrophic effect of sildenafil administration. Therefore, these studies not only revealed the requirement of PKGIα for attenuation of pressure overload–induced cardiac remodeling, but also further identified the critical function of the PKGIα LZ domain in this process. This suggests that PKGIα LZ-dependent binding partners and kinase substrates in the myocardium mediate cGMP-PKGI antiremodeling effects. As discussed later, these substrates, if identified, could serve as novel therapeutic targets.

Recent work has extended the study of PKGI to humans. Biopsies of patients with heart failure revealed significantly decreased PKGI activity in myocardium of patients with heart failure with preserved LV ejection fraction (HFPEF), compared with patients with heart failure with reduced ejection fraction (HFrEF) or aortic stenosis. Remarkably, in the same study, administration of PKG to isolated CMs from patients with HFPEF reduced resting passive CM tension, suggesting a mechanism by which PKGI promotes diastolic function. Most importantly, this study extends the relevance of cGMP-PKGI signaling to human disease.

Potential Antiremodeling Mechanisms Regulated by PKGI

A large body of evidence now supports that PKGI signaling attenuates LVH and cardiac remodeling. Less is understood, however, about the cellular mechanisms regulated by PKGI in the heart to counteract these processes. In addition, the downstream PKGI kinase substrates that mediate the PKGI antiremodeling effect remain incompletely understood. Both in vivo and in vitro studies suggested that PKGI regulates processes ranging from CM apoptosis to individual CM hypertrophy. Animal studies support that PKGI may normally function to preserve LV contractility in the setting of acute pressure overload or LV stress, regulate diastolic function, or preserve intracellular calcium homeostasis. CM-restricted deletion of either PKGI or of PDE5 also leads to marked LV...
fibrosis after TAC, suggesting that PKGI activity in the CM actually regulates the extracellular matrix composition of the local microenvironment. Finally, PKGIr promotes angiogenesis in vivo, a process that may normally prevent the development of pathological LV remodeling. However, it remains unknown which of these multiple processes normally mediates the adaptive effect of PKGI on the remodeling process.

Similarly, the specific cardiac cell type(s) in which PKGI functions to attenuate LVH and remodeling remains unknown. Potential cells through which PKGI may function in the heart, in addition to cardiomyocytes, include the cardiac fibroblast and possibly cardiovascular stem cells. And, the studies described above on CM-specific deletion or restoration of PKGI or its upstream regulators (NOS3, pGC-A, PDE5) provide strong support for a critical role of this pathway in the CM.

Because PKGI likely functions in the CM to inhibit remodeling, further understanding the specific PKGI kinase substrates in the CM might reveal novel antiremodeling molecules. As discussed below, the identification of CM-specific PKGI substrates could be of great interest because many clinical trials of PKGI activators have been limited by hypotension caused by activation of PKGI in the vasculature. To date, there has not been a systematic exploration of the specific PKGI kinase substrates in the heart. In addition, the majority of potential PKGI antiremodeling targets have not been rigorously demonstrated to be required PKGI effectors in the heart, but rather have been inferred from those identified in other tissues. Of note, a number of LZ-dependent PKGI substrates initially discovered in the VSMC are expressed in the myocardium as well, raising the possibility that they also function as PKGI effectors in the regulation of cardiac remodeling. For example, the regulator of G protein Signaling 2 and RhoA both bind PKGI through the LZ domain and function as PKGI effectors in the VSMC. And, both are also expressed in the myocardium. PKGI regulation of RhoA and regulator of G protein Signaling 2 appears to inhibit experimentally induced cardiac remodeling. However, because RhoA and regulator of G protein Signaling 2 are highly expressed both in the CM and VSMC as well as in ubiquitous tissues, these molecules may prove difficult to exploit as myocardial-specific antiremodeling therapeutic targets, given their roles in vasodilation.

Other candidate PKGI effectors in the myocardium include the transient receptor potential cation channel 6, leading to nuclear localization of the transcription factor, nuclear factor of activated T cells (NFAT), thereby preventing NFAT induction of pathological fetal gene re-expression. Upstream regulation of NFAT by PKGI may occur through PKGI phosphorylation of transient receptor potential cation 6, leading to decreased calcineurin activation of NFAT, and by activation of c-Jun N-terminal kinase (JNK) signaling that phosphorylates NFAT and prevents its nuclear localization.

In summary, basic investigations have implicated NO, GCs, and cGMP as inhibiting pathological CM in vitro and in vivo. PKGI, the downstream cGMP signaling effector, has recently been identified as an antiremodeling molecule and as being required for the antiremodeling effects of sildenafil in vivo. Although this pathway likely functions through the CM to inhibit hypertrophy, the downstream PKGI substrates identified to date are largely expressed both in CMs and in the vasculature. We hypothesize that, in addition to these ubiquitous substrates, PKGI may bind and regulate CM-specific substrates through interactions mediated by its LZ domain. As discussed below, many studies to date of PKGI activating agents in heart failure have been limited by hypotension, presumably caused by vascular PKGI activation. Therefore, identifying CM-specific PKG antiremodeling substrates might circumvent this current limitation.

Clinical Investigations of PKGI Activating Drugs

Acute Decompensated Heart Failure

As depicted in the Figure, existing pharmacological agents target multiple steps of the PKGI activation pathway. Table 2 summarizes the important clinical trials of these agents in the acute and chronic treatment of heart failure. Initial clinical investigations studied the vasodilating properties of these compounds in ADHF. And, the vasodilating effects of PKGI activators also provided the rationale for their use as afterload reducing agents in chronic heart failure. However, more recent trials have also begun to examine the chronic effects of these agents on myocardial properties attributable to their potential CM-specific effects.

NO Donors: Nitrates and Nitroprusside in ADHF

NO donors were among the first vasodilators used to treat ADHF. NO activates sGC which increases cGMP synthesis, leading to PKGI activation. The most well-characterized NO donors include isosorbide dinitrate (ISDN) and sodium nitroprusside (SNP). Seminal studies in the 1970s demonstrated that both of these agents reduce pulmonary capillary wedge pressure (PCWP), systemic vascular resistance (SVR), and systolic BP (SBP) in patients with heart failure and thereby increase cardiac output (CO). However, while ISDN and SNP decrease PCWP equally, via venodilation, SNP affects SVR and CO to a greater extent. Clinical studies in the 1970s established the hemodynamic efficacy of nitroglycerin (both ISDN and topical nitrates) in acute heart failure. Although generally small (<20 patients) and uncontrolled, these studies did reveal a significant lowering of PCWP and, to a lesser extent, SBP in the setting of acute heart failure.

During the same period, early phase trials of intravenous (IV) SNP also demonstrated its acute unloading effects in patients with decompensated heart failure. Similar to studies of nitrates, these small, single-center experiences lacked placebo controls. IV SNP infusion acutely improved CO and decreased mean arterial pressure and PCWP in 18 patients with intractable heart failure. A single-center experience comparing SNP with nitrates in patients with acute heart failure identified that SNP decreased PCWP to a similar extent as nitrates, but had additive effects on mean arterial pressure and SVR reduction. The use of SNP has remained limited, chiefly...
attributable to the risk of serious hypotension and metabolite toxicity. Most centers restrict the administration of SNP to patients with heart failure in the intensive care unit setting. Recently, the Cleveland Clinic SNP experience has been published, describing the careful continuous infusion protocol used at that center in patients with ADHF and decreased CO. Although not a randomized controlled trial, this study demonstrated a positive association between SNP administration and oral vasodilator therapy at hospital discharge. Furthermore, patients receiving SNP experienced decreased all-cause mortality extending 80 months after initiation of SNP, compared with case controls not receiving SNP.

Despite the limitations of the trials of NO donors in ADHF, these agents remain important options for acutely decreasing LV filling pressures. And, the groundbreaking studies of these agents provided much of the original rationale for the general implementation of vasodilator therapy in both acute and chronic heart failure. As expected, systemic hypotension arising from NO activation of cGMP-PKG signaling in the vasculature continues to limit the use of these agents in ADHF.

**sGC Activators in ADHF**

Although NO donors have proven efficacious as unloading agents in acute heart failure, NO activation of sGC can be diminished in certain disease states. For example, in vivo oxidative stress alters sGC structure, which impairs GC activation by NO, but permits preserved basal GC activity. Therefore, NO donors may be incompletely effective when downstream sGC becomes oxidized and rendered resistant to NO activation. Recently developed sGC activators can stimulate GC activity even of the oxidized enzyme. One such sGC activator, cinaciguat, has been examined in ADHF. Phase I safety and dose finding studies of cinaciguat in patients with ADHF and PCWP >18 mm Hg demonstrated potential reduction of PCWP, pulmonary artery pressure, central venous pressure, SVR, and pulmonary vascular resistance, with a concomitant increase in CO and decrease in SBP after 6-hour IV infusion. However, a phase IIb controlled trial in a similar patient population demonstrated similar unloading effects, but was terminated early because of excessive hypotension in the cinaciguat arm. The study did achieve the targeted primary end point of PCWP reduction, and no increase in mortality was observed in the cinaciguat group. Thus, although cinaciguat offers the theoretical benefit of activating sGC more efficiently than NO, its routine applicability in treating ADHF remains unclear.

**NPs: Activating the pGC Natriuretic Receptor-A in ADHF**

Compared with activators of sGC, NPs that activate pGC have been more extensively studied in ADHF. These agents increase pGC enzymatic activity, thereby increasing intracellular cGMP, leading to PKGI activation and induction of vasodilation. To date a number of NPs have been tested in ADHF. Unlike NO-sGC agents, NPs have been evaluated for a broad range of clinical end points, in addition to hemodynamic unloading. Ularitide, a synthetic form of the renal-generated NP urodilatin, was shown in a phase II trial to improve dyspnea, PCWP, and cardiac index in patients with ADHF after a 24-hour infusion. However, both the low- and high-dose arms of the study induced 5.0% and 12% rates of hypotension, respectively. Other NPs under preliminary investigation include CD-NP, a fusion of Dendroaspis NP with C-type NP, which seems to increase glomerular filtration rate while also activating myocardial NP receptors.

The human recombinant atrial NP compound carperitide reduces PCWP in patients with ADHF when administered as a bolus dose. Carperitide remains in clinical use in Japan for treatment of ADHF. In addition, more recent studies investigated the effect of carperitide on LV remodeling after acute anterior MI. Two separate trials conducted in Japan demonstrated improved LV ejection fraction (EF) at 1 month or at 6 to 12 months after acute carperitide infusion in patients receiving reperfusion therapy for anterior MI. It remains unknown whether carperitide improves remodeling through direct myocardial antiremodeling effects, or rather through reduction of infarct size and reperfusion injury, leading to a secondary improvement in LV remodeling.

Synthetic BNP (nesiritide) represents the most extensively studied NP to date in ADHF. The initial multicenter, randomized controlled trial of IV nesiritide in ADHF tested a 6-hour infusion of 2 doses. In addition to improving PCWP at 6 hours, nesiritide improved dyspnea score, fatigue, and global clinical status indices compared with placebo. In the same study, nesiritide infusion induced increased hypotensive events. These unloading effects led, in the early 2000s, to the widespread use of nesiritide for ADHF. However, a meta-analysis of selected nesiritide studies suggested an association between nesiritide administration and increased mortality. This concern prompted the design of a large-scale clinical trial of nesiritide in ADHF, the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial, which compared nesiritide with placebo at 6 and 24 hours after drug infusion, as well as 30 days after hospital discharge. Importantly, the ASCEND-HF study detected no increase in 30-day mortality in the nesiritide arm, although conversely there was no mortality reduction with nesiritide. Nesiritide treatment modestly improved dyspnea at 6 and 24 hours after initiation, compared with placebo. As in prior studies, nesiritide significantly increased hypotension compared with placebo. The findings of the ASCEND-HF study tempered enthusiasm for nesiritide in ADHF. The study at least provided reassuring evidence that this agent does not induce excess mortality. Based on the results of the ASCEND-HF study, nesiritide is not currently considered a first treatment for ADHF. As discussed below, however, emerging studies are now shifting focus toward testing the efficacy of nesiritide as a chronic antiremodeling agent, rather than as an acute unloading agent in ADHF.

**PDE5 Inhibitors: Acute Hemodynamic Effects in Patients With Heart Failure**

NO donors, sGC activators, and NPs all induce vasodilation via direct activation of GCs, leading to synthesis of cGMP and direct PKGI activation. PDE5 inhibitors activate PKGI indirectly by inhibiting cGMP breakdown. PDE5 inhibitors induce PKGI-mediated vasodilation in corpus cavernosum venules, leading to their well-established benefit in erectile dysfunction. This vasodilating property has identified these...
Table 2. Clinical Studies of PKGI Activating Drugs in Heart Failure

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of PKG Activation</th>
<th>Study Design</th>
<th>Results</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isosorbide dinitrate</td>
<td>NO donor</td>
<td>-12 patients</td>
<td>1. Decreased PCWP</td>
<td>1. Nitrates included oral isosorbide or nitroglycerine ointment</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-HF and high PCWP</td>
<td>2. Decreased BP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroprusside, nitrates</td>
<td>NO donor</td>
<td>-Single-center experience with vasodilators in acute HF</td>
<td>1. Each agent decreased PCWP and MAP</td>
<td></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Each agent increased CO</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3. SNP induced more decrease in MAP and more increase in CO, compared with nitrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>NO donor</td>
<td>-Single-center experience with SNP in ICU setting, titrated to MAP goals</td>
<td>1. Increased oral vasodilators at hospital discharge</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Decreased mortality after hospital discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>NO donor</td>
<td>-18 patients with intractable CHF</td>
<td>1. Decreased PCWP</td>
<td>1. No placebo arm</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-IV infusion 24–72 h</td>
<td>2. Increased CO</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. Decreased MAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinaciguat (BAY 58–2667)</td>
<td>Soluble guanylate cyclase (sGC) activator</td>
<td>-Multicenter phase II</td>
<td>1. Reduced PAP and PCWP from baseline</td>
<td>1. Nonrandomized, uncontrolled</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Study; 6-h infusion study</td>
<td>2. Increased responder rate defined as reduction of PCWP</td>
<td>2. 10% rate of serious hypotension</td>
<td></td>
</tr>
<tr>
<td>Cinaciguat</td>
<td>sGC activator</td>
<td>-Placebo-controlled, multicenter phase IIb</td>
<td>1. Decreased PCWP in cinaciguat vs placebo</td>
<td>1. Terminated early because of hypotension events in cinaciguat group</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-ADHF and LVEF ≤40%</td>
<td>2. SBP decreased in cinaciguat group vs placebo group</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>-8-h infusion</td>
<td></td>
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<tr>
<td>Ularitide</td>
<td>Renal secreted natriuretic peptide</td>
<td>-Phase II randomized controlled trial</td>
<td>Improved PCWP, dyspnea score, and cardiac index</td>
<td>Drug discontinued because of hypotension in 5.0% and 12.7% of low and high dose</td>
<td>60</td>
</tr>
<tr>
<td>Carperitide</td>
<td>Recombinant human ANP activation of GC via NPR</td>
<td>-Single center</td>
<td>1. Improved LVEF recovery at 1 mo post-MI in carperitide group</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-24-h infusion of carperitide vs IV nitroglycerine after reperfusion of anterior STEMI</td>
<td>2. Prevention of LV chamber enlargement at 1 mo post-MI in carperitide group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carperitide</td>
<td>Recombinant human ANP</td>
<td>-Multicenter, randomized, placebo controlled, single blind</td>
<td>1. Reduced creatinine kinase elevation</td>
<td>Severe hypotension in 10.5% of treatment group, vs 0.3% in placebo</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3-d infusion after reperfusion for anterior STEMI</td>
<td>2. Improved LVEF 6–12 mo post-MI</td>
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</tr>
<tr>
<td>Nesiritide</td>
<td>Synthetic BNP activation of GC via NPR</td>
<td>-Multicenter, randomized, placebo controlled</td>
<td>Improved PCWP, dyspnea score, fatigue, and global clinical status</td>
<td>Increased hypotension in both low- and high-dose nesiritide groups</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-6-h infusion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nesiritide</td>
<td>Synthetic BNP</td>
<td>-Multicenter, phase II randomized, placebo-controlled infusion for 1–7 d</td>
<td>Modest improvement in dyspnea at 6 and 24 h. No differences in all-cause mortality at 30 d.</td>
<td>11% absolute increase in hypotensive events in nesiritide group</td>
<td>64</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibitor (inhibits cGMP catalysis)</td>
<td>-Class III systolic HF, LVEF ≤35%.</td>
<td>Improved peak VO2, decreased PASP</td>
<td>1. Hemodynamic and exercise improvements only observed in patients with pre-existing pulmonary hypertension (PASP &gt;25);</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Hemodynamic and exercise analysis 60 min after oral sildenafil dose</td>
<td>2. Patients taking nitrates excluded from enrollment.</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
### Table 2. Continued

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of PKG Activation</th>
<th>Study Design</th>
<th>Results</th>
<th>Comments</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>- Patients with pre-existing CHF, EF ≤40% - Exercise capacity, endothelial function, and DLCO 60 min after single dose of sildenafil</td>
<td>Improvements in PASP, endothelial function, and DLCO</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>- Analysis of LVAD patients 90 min after sildenafil administration</td>
<td>Significant reduction on pulmonary arterial pressure</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>- Analysis of acute (60 min) and chronic (4 wk) effects of sildenafil in outpatients with CHF</td>
<td>1. Acute: Decreased PASP 2. Chronic: Decreased PASP; improved peak exercise capacity, ventilator efficiency, and gas exchange</td>
<td>Patients taking nitrates excluded from enrollment</td>
<td>68</td>
</tr>
<tr>
<td>Chronic administration</td>
<td>Isosorbide dinitrate</td>
<td>- 16-patient, randomized, double-blind, crossover trial - 8-wk treatment with each agent</td>
<td>1. Improved exercise duration 2. Decreased heart failure events</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>Isosorbide dinitrate</td>
<td>NO donor</td>
<td>- 30-patient, randomized, double blind, placebo controlled - 12-wk administration</td>
<td>1. First dose: Acute reduction of PCWP, SBP, PASP, PVR, SVR; no change in exercise capacity 2. At 12 wk: Reduced PCWP, PVR, PASP; improved exercise capacity; improved clinical status</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Isosorbide dinitrate and hydralazine</td>
<td>NO donor (isosorbide dinitrate)</td>
<td>- Men with HF and decreased LVEF - Placebo controlled, double blind - Treatment with placebo, prazosin, or isosorbide dinitrate/hydralazine</td>
<td>Decreased overall mortality in hydralazine/nitrate group compared with placebo or with prazosin</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>Isosorbide dinitrate and hydralazine</td>
<td>NO donor (isosorbide)</td>
<td>- Self-described black patients Class III or IV symptoms and: LVEF &lt; 35% or LVEF &lt;45% with LV chamber dilation</td>
<td>At 18 mo, 4% reduction in mortality in treatment group; improved composite end point of mortality, first CHF hospitalization, and quality of life, in treatment group</td>
<td>1. Terminated early because of improved mortality in treatment group 2. Combined PKG activator (NO donor isosorbide), and non-PKG activator vasodilator hydralazine</td>
<td>72</td>
</tr>
<tr>
<td>Nesiritide</td>
<td>Synthetic BNP activation of GC via NPR</td>
<td>- Phase IIIB, randomized, double blind, placebo controlled, multicenter - Class III and IV symptoms; multiple hospitalizations within past 12 mo; LVEF ≤40% - 1 or 2 weekly outpatient infusions</td>
<td>No difference observed in all-cause mortality</td>
<td>Hypotensive events in 32.4% of nesiritide treated patients, vs 18.0% in placebo groups</td>
<td>73</td>
</tr>
<tr>
<td>Subcutaneous BNP</td>
<td>Activation of GC via NPR</td>
<td>- Class II and III stable CHF, LVEF ≤35% - 8 wk of BNP subcutaneously twice daily</td>
<td>Improved (reduced) LV systolic and diastolic volumes. Reduction of LV mass index; improved Minnesota Living with Heart Failure Score</td>
<td>12.5% of patients excluded from study for hypotension with test dose; an additional 12.5% of patients experienced hypotensive symptoms during study</td>
<td>74</td>
</tr>
</tbody>
</table>
agents as treatments for primary pulmonary hypertension (PH). In patients with CHF, LV dysfunction leads to increased PCWP, which can produce secondary PH. Right ventricular dysfunction arising from secondary PH predicts increased mortality in patients with heart failure. Therefore, the earliest studies of sildenafil in heart failure primarily evaluated its effects on pulmonary hemodynamics and ventilatory parameters. These clinical studies of PDE5 inhibitors in CHF have recently been reviewed extensively and are summarized in Table 2. Briefly, these studies all detected acute favorable effects of PDE5 inhibition on pulmonary hemodynamics and ventilation-related parameters. Interestingly, however, these findings have not led to trials of these agents as acute unloading agents in ADHF. Rather, subsequent studies have primarily focused on the chronic effects of these agents in CHF.

### Summary of PKGI Activators in ADHF

In summary, NO, sGC, pGC, and PDE5 activators have all been investigated for their efficacy as acute vasodilators, a shared property arising from their elevation of cGMP levels and PKGI activity in the vasculature. To date, nitrates remain the most widely used PKGI activators in ADHF. GC activators (SNP, nesiritide, and cinaciguat) all induce favorable effects on LV filling pressures, likely attributable to PKG-mediated vasodilation. However, this vasodilation frequently leads to hypotension and has proven a severe limitation in many of the above-mentioned trials. Finally, small trials of PDE5 inhibitors have suggested acute, favorable effects selectively in the pulmonary, rather than systemic circulation. With the exceptions of cinaciguat and nesiritide, acute trials of other PKGI activators have generally been small. Therefore, despite

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**Table 2. Continued**

<table>
<thead>
<tr>
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<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCZ-696</td>
<td>Inhibition of neprylisin degradation of NPs</td>
<td>-Chronic HFPEF -36-wk treatment with LCZ-696 or valsartan -Phase II, randomized, controlled</td>
<td>-Reduction of NT-proBNP in LCZ-696 at 12 wk -Improved NYHA class at 36 wk</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>-Symptomatic HF and pulmonary hypertension -12-wk treatment with sildenafil</td>
<td>Improved 6 min walk distance, peak VO₂, and Quality of Life Indices</td>
<td>Patients taking nitrates excluded from enrollment</td>
<td>76</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>-Patients with stable, class II or III CHF, EF ≤45% -Treatment with sildenafil for 6 mo</td>
<td>Improved PASP, exercise ventilation and aerobic efficiency, and endothelial function at 3 and 6 mo</td>
<td>Patients taking nitrates excluded from enrollment</td>
<td>77</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>-Chronic CHF (LVEF &lt;40%) and diastolic dysfunction on echocardiography -1 y treatment with sildenafil</td>
<td>Improved LVEF, LV mass index, quality of life, and peak VO₂ at 1 y</td>
<td>Patients taking nitrates excluded from enrollment</td>
<td>78</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>-Chronic HFPEF, with concomitant secondary PH</td>
<td>1. Reduced PASP (primary end point) 2. Improved indices of RV systolic function 3. Reduction of LV mass index 4. Reduction of PCWP, despite lack of effect on systemic blood pressure</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5 inhibition</td>
<td>-24 wk sildenafil administration -Multicenter, randomized, placebo controlled</td>
<td>No improvement detected for primary end point (peak VO₂)</td>
<td>Patients taking nitrates excluded from enrollment 9% rate of hypotension in sildenafil arm</td>
<td>80</td>
</tr>
</tbody>
</table>

ADHF indicates acute decompensated heart failure; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; BP, blood pressure; CHF, congestive heart failure; CO, cardiac output; DLCO, diffusion capacity of carbon monoxide; EF, ejection fraction; HF, heart failure; HFPEF, heart failure with preserved left ventricular ejection fraction; ICU, intensive care unit; IV, intravenous; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; MI, myocardial infarction; NPR, natriuretic peptide receptor; NT-proBNP, N-terminal prohormone of BNP; NYHA, New York Heart Association; PAP, pulmonary artery pressure; PASP, pulmonary artery systolic pressure; PCWP, pulmonary capillary wedge pressure; PDE5, phosphodiesterase 5; PH, pulmonary hypertension; PKGI, protein kinase G I; PVR, pulmonary vascular resistance; RV, right ventricular; SBP, systolic blood pressure; SNP, sodium nitroprusside; STEMI, ST-segment–elevation myocardial infarction; SVR, systemic vascular resistance; and VO₂, peak oxygen consumption.
the wide variety of targets available for PKGI activation, the specific role for PKGI activation in ADHF remains undetermined. The ongoing multicenter, randomized controlled Renal Optimization Strategies Evaluation in Acute Heart Failure (ROSE HF) study (http://clinicaltrials.gov/ct2/show/NCT01132846), comparing placebo, dopamine, and low-dose nesiritide in patients with ADHF and renal impairment, may provide insight into the efficacy of acute vasodilation in this specific patient population. It might also prove illustrative to test the effects of acute PDE5 inhibition in ADHF, although to our knowledge such studies have not been designed to date.

Investigations of PKGI Activators as Chronic Therapies in CHF

Although PKGI activators have been in use as chronic treatments for CHF, their investigation has intensified recently as a result of basic studies that identified important functions of PKGI in the CM. As will be detailed below, chronic PKGI activation in CHF was initially proposed to confer benefit in CHF solely through the reduction of vascular afterload. But, more recent investigations have evaluated PKGI activating compounds as antiremodeling molecules, attributable to their potential direct myocardial effects, as opposed to only vascular unloading. In addition, despite this potential new niche for these drugs, their chronic administration remains frequently associated with concerns of hypotension.

Nitrates as Chronic Therapy for CHF

The first evaluation of a PKGI activating agent in chronic heart failure came from a study of ISDN in the 1970s.69 This groundbreaking 8-week, double-blinded, randomized, crossover trial compared ISDN with placebo in 16 patients with New York Heart Association class III and IV heart failure. The nitrate arm experienced a significant reduction of cardiac events (comprising CHF hospitalization, pulmonary embolism, MI, worsening CHF symptoms, arrhythmia, and electrocardiogram changes). Importantly, initial discontinuation of nitrate therapy also correlated with significantly increased composite risk of death, heart failure hospitalization, worsening symptoms, or new arrhythmia. Although a small and relatively short duration study, this investigation suggested that the benefits of nitrates in patients with heart failure extended past their acute vasodilating effects. A subsequent investigation in 30 patients with moderate to severe chronic CHF demonstrated improved PCWP and exercise capacity after 12 weeks of ISDN administration, compared with placebo recipients.70 In this study, the initial dose of ISDN decreased SVR, pulmonary vascular resistance, and PCWP, but by 12 weeks of administration only PCWP remained normalized.

The Veterans Affairs Vasodilator-Habitat Failure Trial (V-HeFT) extended these findings and stands as the first large-scale, randomized controlled trial for CHF therapy.71 This well-known study tested the hypothesis that chronic afterload reduction could improve the natural history of CHF. Specifically, male patients with chronic HFREF were randomized either to placebo or to afterload reduction with the α-adrenergic receptor antagonist prazosin or with a combination of ISDN and hydralazine. The combination of ISDN/hydralazine improved overall mortality compared with either placebo or prazosin. These findings provided the first evidence that chronic afterload reduction altered the natural history of heart failure. Because ISDN/hydralazine improved outcomes in CHF, whereas prazosin provided no beneficial effect, these results also suggested a selective additive benefit of ISDN/hydralazine in addition to their vasodilating properties. Hydralazine induces arterial dilation via a PKGI-independent mechanism. It remains unknown, therefore, whether the protective effects of ISDN/hydralazine arise from PKGI activation or instead from a selective effect of hydralazine. To date, there have been no randomized controlled trials testing whether ISDN alone might alter the natural history of CHF.

Nearly 20 years after V-HeFT, the African American-Heart Failure Trial (A-HeFT) examined the effect of ISDN/hydralazine therapy when added to standard therapy specifically in self-identified black patients with CHF and EF <35%.72 This study was discontinued prematurely because of a reduction in all-cause mortality with the treatment arm. Post hoc analysis of the A-HeFT study also revealed that ISDN and hydralazine therapy reversed LV remodeling, suggesting a direct myocardial effect of these agents.81 As in V-HeFT, it remains unknown whether the PKGI activating effects of nitrates, as opposed to the non-PKGI–related effects of hydralazine, drove the benefits in that study.

Therefore, PKGI activation with nitrates, in combination with hydralazine, is a well-proven therapy for reducing mortality in chronic heart failure. These trials suggest that chronic PKGI activation with NO donors interrupts and potentially reverses cardiac remodeling and the natural history of CHF. However, definitive evidence showing that ISDN alone can confer similar benefit to ISDN/hydralazine remains lacking, with the exception of the small-scale study of ISDN alone described above.

NPs: Chronic Activation of the pGC Natriuretic Receptor-A in CHF

Nesiritide, a synthetic form of BNP and a potent pGC agonist, initially received attention as an acute unloading agent in ADHF. However, basic studies also revealed a CM-specific effect of pGC activation on cardiac remodeling. Accordingly, the second Follow-Up Serial Infusions of Nesiritide (FUSION II) study sought to explore the benefit of nesiritide infusion on the chronic course of CHF.73 The FUSION II trial was a 12-month, double-blind, placebo-controlled phase II study comparing 1 or 2 weekly infusions of nesiritide with placebo in patients with New York Heart Association class III or IV CHF. The study demonstrated no difference in mortality in the nesiritide group although it was not adequately powered to detect mortality differences. However, nesiritide treatment caused a hypotension event rate of >30%, which was significantly increased compared with placebo. Thus, FUSION II suggested that chronic outpatient infusions of nesiritide might induce excessive dangerous vasodilation, presumably caused by arterial PKGI activation. In addition, although the FUSION II study did not demonstrate improvement in cardiac remodeling, it is possible that the short half-life of nesiritide reduced its potential chronic benefits on the myocardium because patients received infusions only 1 to 2 times weekly. Thus, although this phase II study proved the overall safety of intermittent
PKGI activation in patients with chronic HFREF, it also suggested that hypotension arising from vascular PKGI activation might limit the potential myocardial benefits of nesiritide.

More recently, subcutaneous nesiritide has been developed in an attempt to replace intermittent infusion with more chronic delivery of systemic NP therapy. A recent study tested the effects of chronic subcutaneous nesiritide in patients with New York Heart Association class II and III CHF and EF <35%.

Although it was only an 8-week trial, subcutaneous nesiritide treatment remarkably induced relative improvements in LV diastolic function (e/e′ ratio), LV mass index, LV chamber dimensions, and CHF symptoms. However, 12.5% (3/24) of eligible patients experienced hypotension after a subcutaneous nesiritide test dose, resulting in their exclusion from the study. The results of this study therefore highlight 2 important concepts. First, they support that chronic administration of the upstream PKGI activator BNP may directly improve LV function and morphology in patients with heart failure. Second, they add further evidence that hypotension arising from chronic upstream vascular PKGI activation may prove to be a limiting factor for the strategy of PKGI activation in chronic remodeling.

Neprilysin Inhibitors: Preventing NP Degradation in Chronic CHF

An alternative strategy to supplementing NPs in patients with CHF involves inhibiting NP breakdown by the vasopeptidase enzyme neprilysin. The use of neprilysin inhibitors in CHF has been recently reviewed extensively in this journal. Briefly, combined angiotensin-converting enzyme/neprilysin inhibitors, such as omapatrilat, showed initial promise for treatment of HFREF, although it failed to improve outcomes in phase III trials. More recently, LCZ-696, a combined inhibitor of both neprilysin and AngII receptor, has been developed. LCZ-696 consists of equal doses of the AngII receptor blocker valsartan and the neprilysin inhibitor prodrug AHU-397. In the recently completed phase II Prospective Comparison of Angiotensin Receptor Neprilysin Inhibition With Angiotensin Receptor Blockade on Management Of Heart Failure With Preserved Ejection Fraction (PARAMOUNT) trial, LCZ-696 reduced both BNP levels and left atrial size in patients with HFPEF, compared with valsartan alone. LCZ-696 also improved functional class compared with valsartan alone. Importantly, LCZ-696 did not induce excess hypotension in this study. LCZ-696 is currently under investigation in HFREF in the patients with stable chronic heart failure and reduced EF in the Prospective Comparison of Angiotensin Receptor Neprilysin Inhibitors With Angiotensin-Converting Enzyme Inhibitors to Determine Impact on Global Mortality and Morbidity in Patients With Heart Failure (PARADIGM-HF) study. In summary, inhibiting neprilysin to increase circulating NPs may provide a novel therapeutic strategy for both HFREF and HFPEF.

PDE5 Inhibitors in Chronic CHF

The recently completed trials of PDE5 inhibitors in chronic heart failure further highlight the combined vascular and myocardial effects of PKGI activators. Rather than a limitation, the vasodilating properties of PDE5 inhibitors seemed favorable, especially because acute PDE5 inhibition in CHF seems to reduce pulmonary vascular pressures selectively while producing minimal systemic effects. Accordingly, as recently reviewed extensively, the initial chronic PDE5 inhibitor trials in CHF focused on clinical end points related to pulmonary vasodilation. For example, an initial 12-week sildenafil trial in patients with CHF included only those with secondary PH. The trial demonstrated improvements in resting and exercise-induced pulmonary vascular resistance in theildenafil group but did not detect reduction in PCWP in sildenafil-treated patients. Exercise capacity and clinical symptoms also improved in the sildenafil group. Subsequent small studies confirmed favorable effects of PDE5 inhibition on pulmonary artery systolic pressure and endothelial function at 1 month, as well as after 6 months of sildenafil administration. LVEF in these studies reportedly did not change with sildenafil treatment. Unlike nesiritide trials, PDE5 administration in these studies did not correlate with hypotension or with reduction in systemic BP. Importantly, however, all of these early sildenafil studies excluded any patients taking nitrates because of concerns for potential resultant excessive vasodilation and hypotension.

Notably, the above-mentioned trials initially focused on hemodynamic, rather than myocardial, effects of chronic PDE5 inhibition. However, a subsequent investigation tested the effects of PDE5 inhibition on chronic LV remodeling in HFREF. In this single-center, randomized, controlled trial, patients with chronic CHF, LVEF <40%, and diastolic dysfunction detected by echocardiography received sildenafil or placebo for 1 year. The sildenafil arm experienced improved relative increase in LVEF, decreased LV mass index, improved quality of life, and increased peak oxygen consumption (V̇O₂) compared with placebo. Diastolic parameters also improved in the sildenafil group. Sildenafil did not decrease SBP, although patients on nitrates were again excluded from enrollment. The apparent regression of LV remodeling and hypertrophy in the absence of reduced afterload in this study strongly suggested a direct effect of sildenafil on myocardial function and remodeling. As a relatively small study, this trial did not detect differences in mortality in the sildenafil group. Currently, the PDE5 Inhibition with Tadalafil Changes outcomes in Heart Failure (PITCH HF) trial, a multicenter, randomized controlled trial of patients with systolic heart failure and secondary PH, will test the effect of tadalafil (Cialis) on a similar patient population. This large-scale, phase III study will hopefully clarify the effects of PDE5 inhibition on mortality, pulmonary hemodynamics, and chronic remodeling in patients with HFREF.

In addition to HFREF, PDE5 inhibitors remain under investigation for the treatment of HFPEF. Unlike HFREF, no current therapies currently have been demonstrated to alter the progression and natural history of HFPEF. Many of the basic studies outlined above have supported a protective effect of PKGI on LV diastolic function. Therefore, there has been considerable enthusiasm for the strategy of activating PKGI in patients with HFPEF. The majority of sildenafil HFPEF studies to date have focused on the vasodilating properties of PDE5 inhibitors in the study design. For example, the first published controlled trial of sildenafil in HFPEF limited enrollment to patients with HFPEF and secondary PH. This single-center, randomized, placebo-controlled study
detected a sildenafil-induced reduction in pulmonary artery systolic pressure, as well as improvements in multiple indices of right ventricular systolic function. However, LV mass index and PCWP also decreased significantly in the sildenafil group, despite a lack of effect on systemic BP. This small (44 patients) study suggested not only a beneficial effect of PKGI-mediated vasodilation, but also a direct attenuating effect of sildenafil on LV hypertrophy and diastolic dysfunction.

More recently, however, a first multicenter, phase II randomized controlled trial of sildenafil in HPFPEF has been completed. The Phosphodiesterase-E-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure (RELAX) study enrolled patients with a diagnosis of CHF and EF >50. Patients received either placebo or sildenafil for 24 weeks. The primary end point of the study, change in peak VO2, at 24 weeks, did not differ between treatment groups. In addition, secondary end points, such as mortality, quality of life, and 6-minute walk test, did not differ between treatment groups. Therefore, the authors concluded that the RELAX findings do not support routine use of sildenafil for the treatment of HPFPEF. Although an appropriate conclusion, the trial does deserve further discussion. First, although a phase II study, this was a relatively small trial, with a total of only 216 patients enrolled. By comparison, other natural history CHF trials, such as Studies of Left Ventricular Dysfunction (SOLVD),92 enrolled >2000 subjects. Perhaps more importantly, however, the trial duration of 24 weeks may have been adequate to detect effects arising from rapid effects on pulmonary pressures, but likely was too brief to alter chronic LV remodeling and disease progression (by comparison, again, the SOLVD trial treated patients for an average of >40 months).92 Furthermore, patients receiving sildenafil only received the full sildenafil dose (60 mg TID) for 12 weeks, with the initial 12 weeks providing a small (20 mg TID) dose. Interestingly, sildenafil-treated patients in this study also experienced higher rates of symptomatic hypotension. The RELAX trial experience therefore highlights 2 important features of common PKGI activating agents. First, it provides an example of a trial attempting to exploit the vasodilating properties of sildenafil which might have been detected at 24 weeks, rather than the more chronic effects on LV remodeling, which might require years to detect. Second, it raises the theoretical possibility that the vasoactive side effects of PKGI activation actually limited the study, as evidenced by the significant hypotension rate in the treatment arm.

Based on the available data, PDE5 inhibitors show promise in the treatment of systolic heart failure and seem to affect pulmonary hemodynamics more potently than systemic hemodynamics. These studies also suggested a direct, afterload independent, effect of PDE5 inhibition on the failing and remodelled myocardium. The specific role for PDE5 inhibition in HPFPEF remains unclear, in light of the recent RELAX data. However, whether long-term (>6 months) administration of PDE5 inhibitors improves remodeling and disease progression in a broad population of patients with HPFPEF remains untested. Although the majority of these trials suggest that PDE5 inhibition affects SBP minimally, all of these studies excluded patients taking oral nitrates. A high percentage of CHF patients currently receive chronic nitrate therapy. Therefore, systemic PDE5 inhibition will likely remain contraindicated in many patients with CHF.

Such as other PKGI activating agents described above, PDE5 inhibitors share at least the hypothetical risk of inducing hypotension. Ongoing and future trials will further define the cardiac and vascular effects of PKGI activators in patients with chronic CHF. However, it would likely prove advantageous to identify myocardial-specific PKGI substrates and effectors that mediate the antiremodeling effect of PKGI in the myocardium. Such a strategy might identify novel therapeutic targets that could circumvent the undesired excessive hypotension arising from PKGI activation in the blood vessel.

**Summary: An Evolving Paradigm of PKGI and Its Downstream Substrates as a Myocardial Therapeutic Targets**

In summary, basic and clinical investigations have identified multiple steps of the PKGI activation pathway that inhibit cardiac hypertrophy and remodeling and that can be targeted in the treatment of CHF. The shared property of PDE5 inhibitors, NO donors, GC activators, and nephrilysin inhibitors is their cGMP-increasing effects, which activate PKGI. The rapid development of pharmacological therapies targeting this pathway has been influenced by basic science discoveries. These basic advances have also influenced the clinical scenarios in which PKGI activators are used. Although earlier trials of these agents focused predominantly on their acute hemodynamic effects, the field has begun to explore these agents as potential direct antiremodeling therapies independent of their afterload reducing effects. Ironically, although these agents initially came to clinical attention as vasodilators, it is precisely these vascular effects that, when excessive, have limited the utility of the agents as chronic therapies for patients with heart failure. PKGI has now been identified as functioning in the myocardium to inhibit cardiac remodeling. This supports future investigations to understand the currently unknown PKGI effectors expressed specifically in the myocardium and the CM. Identification of PKGI downstream antiremodeling substrates in the myocardium may identify novel therapeutic targets for chronic CHF.

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