Review

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Turning on cGMP-dependent pathways to treat cardiac dysfunctions: boom, bust, and beyond

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cGMP inhibits hypertrophy, decreases fibrosis, and protects against cardiac ischemia–reperfusion (I/R) injury. Gene-targeting studies have not defined a clear role for its major downstream effector, cGMP-dependent protein kinase I (cGKI), in cardiac hypertrophy, but do implicate cGMP–cGKI signaling in fibrosis and I/R injury. No direct cGKI activators have advanced to clinical trials, whereas cardiac trials of agents that modulate cGMP via particulate or soluble guanylyl cyclases (GCs) and phosphodiesterase 5 (PDE5) are ongoing. Here we review concerns arising from preclinical and clinical studies that question whether targeting the cGMP pathway remains an encouraging concept for management of heart dysfunction. So far, trial results for GC modulators are inconclusive, and sildenafil, a PDE5 inhibitor, although cardioprotective in mouse models, has not shown positive clinical results. Preclinical cardioprotection observed for sildenafil may result from inhibition of PDE5 in non-cardiomyocytes or off-target effects, possibly on PDE1C. On the basis of such mechanistic considerations, re-evaluation of the cellular localization of drug target(s) and intervention protocols for cGMP-elevating agents may be needed.

Introduction

In the past few years there has been great interest in the possibility of treating several types of cardiac dysfunction, including hypertrophy, fibrosis, I/R, and dystrophic injury, with drugs that target one or more steps in the cGMP regulatory cascade (see Glossary). In general, much of this interest has been generated by the favorable effects of agents that raise cGMP as observed in preclinical models. However, not all studies in different gene-target mouse models that alter this cascade could easily reproduce the findings from pharmacological alteration of the cGMP pathway. In addition, neither the molecular mechanism for cardioprotection by cGMP nor the cell type involved in this protection is yet clear. Here we give a short synopsis of selected studies utilizing genetically engineered mouse models on the role of cGMP in relation to guanylyl cyclases (GCs), cGMP-dependent protein kinase I (cGKI), and cGMP-regulated phosphodiesterases (PDEs) in normal and pathological cardiovascular function [1–5]. We also briefly review outcomes of several recent clinical trials on cGMP-elevating agents [6–9]. It is our hope that this analysis will help in guiding future experiments on the potential cardioprotective action of cGMP and its effectors on myocardial infarction (MI) and right heart dysfunction with pulmonary arterial hypertension (PAH), and perhaps in less common cardiomyopathies such as Duchenne muscular dystrophy (DMD) [10,11]. This discussion may also be useful in evaluating potential clinical avenues for future cGMP-based therapies for cardiac disease. Owing to space limitations, much of the older work in this area is not extensively discussed, but has been excellently covered elsewhere [12–14].

The second messenger cGMP is generated from GTP by two families of GCs, each of which is present in all major heart cell types. The particulate GCs (pGCs) are plasma membrane receptors specifically activated by natriuretic peptides (NPs), and the soluble GCs (sGCs) are receptors activated by nitric oxide (NO) or carbon monoxide (CO) [15,16]. Cardiac cGMP signals largely through two classes of target proteins in heart, (i) cGKI and (ii) the cGMP-regulated phosphodiesterases (PDE2, PDE3, and PDE5) [17,18]. In addition, cGMP may directly activate cAMP-dependent protein kinases (cAKs), although at relatively high concentrations [19], or indirectly modulate cAKs via cGMP-regulated PDE2 and PDE3 that can hydrolyze both cAMP and cGMP [20,21]. Owing to these multiple options for cellular signal transduction, the molecular mechanisms for the antihypertrophic and antifibrotic responses elicited by NO/CO or the NPs have been difficult to determine with assurance.

The cGMP signaling pathway: localization of its elements and their roles in cardiac dysfunction

The heart is composed of several different cell types that include cardiomyocytes (CMs), coronary vascular smooth

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Glossary

Cardiac fibroblasts (CFs) and myofibroblasts (CMFs): CFs are the most prominent non-myocyte cell type in the heart. They reside within the extracellular matrix (ECM). Among other functions, CFs together with acellular components of the ECM help to maintain the structural integrity of the heart and form a scaffold that distributes mechanical forces throughout the myocardium. However, profibrotic cytokines, elevated mechanical stress during pressure or volume overload, infarction, and neurohormonal stimulation promote CF activation, resulting in their transformation into CMFs. The CF-to-CMF transformation is an essential regenerative process after cardiac injury (e.g., a heart attack), but sustained CMF activity may lead to excessive ECM accumulation resulting in amplified scarring and functional deterioration of the myocardium.

Cardiac hypertrophy: thickening of the heart muscle caused by an increase in mass, weight, and CM volume. It develops as an adaptive response of the heart to an altered physiological demand and is common to many cardiovascular disorders such as hypertension, infarction, mutations of sarcomeric proteins and valve defects. Although both physiological heart hypertrophy, as seen in athletes who undergo sustained exercise or during pregnancy, and pathological hypertrophy of the heart have similar morphological characteristics including enlarged cardiomyocytes, only pathological hypertrophy leads to large increases in fibrosis (which is an excessive deposition of scar tissue), arrhythmias and eventually heart failure. During development of cardiac hypertrophy macroscopic changes characterized by an increment in size are observed in CMs. These changes include, but are not limited to (i) changes in the organization of the sarcomeric structures, (ii) quantitative and qualitative changes in gene expression, and (iii) an increase in the rate of protein synthesis. Several prohypertrophic and profibrotic signaling cascades are involved in the molecular pathogenesis. Clinical studies and functional analyses of genetically modified mice indicate that natriuretic peptides and their cardiac receptors exhibit antifibrotic and antihypertrophic effects via the second messenger cGMP.

Vascular smooth muscle cells (VSMCs): contractile cells of all blood vessels including the coronary arteries and veins. A smooth muscle 'specific' myosin is selectively expressed in these cells. They also express abundant amounts of cGKI, PDE5, sGC, and pGC.

Cardiomyocytes (CMs): major contractile cell type of the heart. The contractile unit consists of a highly organized chain of sarcomeres composed of two principal proteins: actin and a cardiac-specific myosin. CMs express several cell type-specific marker proteins including cardiac myosin heavy chain. Many CM-specific transgenic animals are produced using the promoter sequence from this gene. Populations of individual CMs can be isolated in highly enriched form via collagenase digestion and dispersion techniques.

cGMP: ubiquitous second messenger formed from GTP by soluble and particulate guanylyl cyclases. In the cardiovascular system most effects downstream of cGMP are mediated through activation of cGMP-dependent protein kinase type I. In addition, cGMP binds to and regulates the activity of several phosphodiesterases that degrade cAMP. High cGMP levels can stimulate the activity of cAMP-dependent protein kinase via direct cross activation.

cGMP-dependent protein kinases (cGKs): important intracellular cGMP effectors belonging to the serine-threonine family of protein kinases. Mammalian cells may express three different cGK forms encoded by two genes, *prkg1* and *prkg2*. The cGKIα and cGKIβ isoforms are both products of the *prkg1* gene. The cGKIs share many common structural features with the *prkg2*-encoded cGKII, but exhibit differences in tissue expression and function. cGKIα is the predominant isoform in the heart, where it is expressed in endothelial cells, smooth muscle cells, cardiomyocytes, and cardiac fibroblasts/myofibroblasts in most, if not all, mammalian species.

Duchenne muscular dystrophy (DMD): X-chromosome-linked recessive disorder caused by a functional deficiency of the dystrophin protein, an important structural component of all striated muscle fibers. Among many other symptoms, DMD patients develop progressive cardiac myopathy.

Ejection fraction (EF): important parameter of cardiac function that is often severely disturbed in many types of heart disease. EF represents the blood volume pumped out by the left (or right) ventricle on a single heartbeat in proportion to the blood in the respective ventricle at the end of diastole. Several noninvasive imaging techniques such as cardiac echocardiography are used in the clinic (and in experimental models) to measure EF.

Guanylyl cyclases (GCs): synthesize cGMP. Two classes can be distinguished according to their subcellular localization and specific ligands. Particulate GCs (pGCs) include pGC-A and pGC-B, which are plasma membrane receptors for atrial, brain, or C-type natriuretic peptides. The second cyclase class is nitric oxide (NO)-stimulated soluble GCs (sGCs), which can be activated by NO or carbon monoxide. It is generally believed that, at least in the heart (in cardiomyocytes), the cGMP pools generated by pGC-A/B and sGC differ from each other, providing a mechanistic basis for their different roles in pathophysiology.

Ischemia–reperfusion (I/R) injury: main pathology underlying many diseases presenting with high mortality and morbidity, such as myocardial infarction and stroke. Ischemia occurs when blood flow to a certain area of tissue is stopped, which results in duration-dependent injury to the cells. The best strategy for preventing tissue death from ischemia is to induce rapid and complete restoration of blood flow. However, it is now accepted that although necessary, this reperfusion drives detrimental events contributing significantly to acute mortality as well as long-term morbidity of patients presenting with an ischemic event. The degree and extent of the injury that can be seen several days to weeks later can be ameliorated by agents that increase cGMP.

Natriuretic peptides (NPs): bind to the membrane-bound guanylyl cyclase receptors pGC-A and pGC-B. Atrial (ANP), brain (BNP), and C-type natriuretic peptide (CNP) are encoded by three different genes that give rise to precursor proteins, which are further processed to mature and (more) active cyclic peptides of 28, 32, and 22 amino acids, respectively. The main sources for ANP and BNP are cardiomyocytes, whereas CNP is mainly produced by the endothelium. Among many other functions, ANP and BNP increase natriuresis and decrease vascular resistance, thereby reducing blood volume, pressure, and afterload. The major NP receptors on cardiomyocytes are pGC-A and pGC-B, which are selectively activated by ANP/BNP and CNP, respectively.

Phosphodiesterases (PDEs): large family of enzymes encoded by 11 gene families in mammals. PDEs hydrolyze and thus negatively regulate both cAMP and cGMP. The differential expression and localization of PDEs and their unique combinations in a given cell type provide much of the molecular basis for modulation of the spatiotemporal dynamics of cyclic nucleotide signaling in the heart. PDE5 is a major controller of cGMP breakdown in many cell types, including VSMCs, ECs, CFs/CMFs, and platelets.

muscle cells (VSMCs), and endothelial cells (ECs). Under conditions of stress or disease, many cardiac fibroblasts (CFs) are transformed into cardiac myofibroblasts (CMFs), which express many markers similar to VSCMs. Analyses of each of these cell types isolated from mouse, rat, rabbit, and human hearts have indicated that each of the GC forms mentioned above and cGKI are expressed in each cell type but at different levels [1,16,22,23]. The cellular expression pattern of different cGMP–PDE subtypes is more complex and differs somewhat between cells.

pGC activators as cardioprotective agents: NP mimetics

The first notion that the cGMP pathway might be a suitable target for treatment of cardiac dysfunction came from work reporting on disruption or overexpression of the NP receptor pGC-A [24,25]. Mice deficient in pGC-A were mildly hypertensive and showed increased cardiac hypertrophy and fibrosis that appeared to be greater than that attributable to the modest increase in blood pressure measured in these animals [24,25]. Importantly, CM-specific ablation of pGC-A phenocopied the cardiac hypertrophy seen in global pGC-A mutants [26], whereas CM-restricted overexpression of pGC-A attenuated remodeling on an otherwise pGC-A negative background [27]. These studies focused attention on CMs as an important site of cGMP action.

It recently became clear that brain NP (BNP), produced by ventricular CMs and by the brain, and C-type NP (CNP), synthesized mostly in ECs including the coronary endothelium, are likely to be particularly important for regulation of cardiac hypertrophy and fibrosis [28,29]. The selective BNP analog nesiritide has undergone clinical trials for treatment of acute decompensated heart failure [30,31]. To date, these trials have not given positive results, at least in part because of the downregulation and decreased sensitivity of the receptors for BNP that occur during heart failure [32]. Much of this apparent desensitization may be due to the high levels of inactive proBNP peptides observed during heart failure. In fact, serum levels of these peptides are used as a marker to assess treatment efficacy.

It was also shown that CNP, a ligand for pGC-B, efficiently stimulates cGMP production in ventricular CMs