The Role of cGMP-Dependent Protein Kinase in Controlling Cardiomyocyte cGMP

Sharron H. Francis

The role of cGMP as a second messenger in physiological and pathophysiological processes is widely appreciated. The successes of drugs that target the cGMP-signaling pathway (nitrovasodilators and PDE5 inhibitors [sildenafil, tadalafil, vardenafil]) have inspired interest in other cardiovascular benefits that might derive from a better understanding of this pathway. Elevation of cGMP in cardiomyocytes or intact heart is associated with a negative inotropic effect, blunting and/or reversal of cardiac hypertrophy, protection against ischemia/reperfusion injury, and changes in apoptosis. Activation of the cGMP-dependent protein kinase (PKG) and phosphorylation of target proteins is involved in each of these processes, although the precise mechanisms that bring about these effects are not fully understood. cGMP activation of PKG in cardiomyocytes lowers cellular calcium, which can reduce contractility and counter calcineurin-mediated dephosphorylation/activation/nuclear translocation of NFAT (nuclear factor of activated T cells), which promotes expression of a cadre of prohypertrophic genes. PKG-mediated phosphorylation of an unknown protein increases opening of mitochondrial K+/ATP channels, thereby diminishing damages resulting from ischemia/reperfusion or myocardial infarction. Moreover, cGMP elevation suppresses β-adrenergic signaling in the heart and is associated with activation of PKG phosphorylation of troponin (Tn). PKG activation increases the GTPase activity of RGS2 (regulator of G protein–coupled channels), thereby diminishing damages resulting from ischemia/reperfusion or myocardial infarction. Moreover, cGMP elevation suppresses β-adrenergic signaling in the heart and is associated with activation of PKG phosphorylation of troponin (Tn).

Several groups have provided evidence for spatially and functionally distinct cellular pools of cGMP. In this issue of Circulation Research, Castro et al document surprising new complexities involved in modulating cGMP level in cardiomyocytes in response to atrial natriuretic peptide (ANP) or nitric oxide (NO). Distinct cGMP pools are generated by action of the particulate guanylyl cyclase (pGC), which is located on the plasma membrane and activated by ANP, and the cytosolic NO-stimulated GC (NO-GC). Fischmeister and colleagues have previously shown that the cGMP pool near the plasma membrane is controlled primarily by the action of the cGMP-hydrolyzing phosphodiesterase (PDE)2, whereas PDE5 action limits the cytosolic cGMP pool (Figure). The biological effects of both pools are apparently mediated by activation of PKG.

The report by Castro et al demonstrates that PKG activation in response to ANP activation of pGC elicits a strong feed-forward mechanism that further enhances cGMP production in the subsarcolemmal pool (Figure). The protein target of PKG that elicits this effect is unknown. Notably, this is the first feed-forward effect to be defined for cGMP signaling in any tissue. Surprisingly, it appears that there is little activation of PDE2 activity through cGMP binding to its allosteric sites, which should counter the effect, and the mechanism for terminating the feed-forward signal is not determined. Moreover, the mechanism whereby PDE2 is selectively localized to this cGMP pool is unknown.

In contrast, increased cGMP production by NO-GC elicits the opposite effect on cGMP levels by activating a negative-
feedback regulation of cytosolic cGMP; this is mediated by activation of PKG, which phosphorylates and activates PDE5. The resulting increased cGMP breakdown blunts further elevation of cGMP and lowers cytosolic cGMP. In the absence of PDE inhibitors, there is modest increase in cGMP in response to NO. Allosteric cGMP binding in PDE5 and phosphorylation by PKG increase catalytic activity and are important in negative-feedback regulation of cGMP in several tissues.1,21,22 Surprisingly, in the present report, only a role for PDE5 phosphorylation is indicated.18 Allosteric cGMP binding in PDE5 increases the rate of phosphorylation by PKG but is not required for phosphorylation or PDE5 activation.18 However, the allosteric site has higher affinity for cGMP than does the catalytic site; presumably, both sites on a PDE5 molecule would bind nearby cGMP based on their respective affinities for cGMP. However, there may be unknown influences that impact PDE5 regulatory mechanisms in intact cells. By all accounts, PDE5 in cardiomyocytes is very low and localized to z-bands2,5; this localization is sensitive to PKGI action and sustained NO-GC activity, which would be predicted to foster allosteric cGMP binding by PDE5, as well as phosphorylation by PKGI. Curiously, it appears that the cytosolic cGMP pool in cardiomyocytes is confined by PDE5, which is low in abundance and not free to diffuse in that pool. The results reported by Castro et al18 provide exciting insights into the contrasting mechanisms controlling cGMP signaling in the heart, but there is still controversy in the field regarding the effects of PKGI and PDE5 in cardiomyocytes.23–25 The conflicting results could have several explanations. However, compelling results from numerous studies (including the present study) support a role for cGMP and PKG action in cardiac function.

These new findings18 raise many questions regarding cGMP signaling in the heart and use of therapies that target this pathway. Although the GCs and PDEs that define cellular cGMP pools are confined to particular regions, the localization of PKG is unclear. Are PKGs that mediate the effects in these regions persistently localized therein or recruited following cGMP elevation? What mechanism provides for PKG localization/recruitment? Is the same PKGI isoenzyme involved in each pool? PKGIα and PKGIβ differ in affinity for cGMP, as well as in substrates in some instances, which could influence signaling.1,26 What is the role of the relatively abundant PDE1, which participates in controlling cGMP levels in some region of the cardiomyocyte and effects biologically meaningful changes?7,27? How is pGC activated by PKG, and what mechanism provides for termination of this feed-forward process? What is the role of PKGI, PDE5, and cGMP signaling in normal cardiomyocytes because there are minimal effects on cardiac function when PKGI is absent or when PDE5 is blocked in individuals taking PDE5-selective inhibitors26,29,29? Acute effects of PDE5 inhibition in cardiomyocytes8 and in studies using mice and humans are modest.13 Will chronic use of PDE5 inhibitors alter regulation of the cGMP pools and/or roles of PDEs 1, 2, and 5 in cardiac functions? If cGMP signaling is primarily cardioprotective against stressors, eg, ischemia/reperfusion or pressure overload, how is this regulated? Lastly, species differences in proteins involved in signaling pathways and changes that occur when cells are cultured present a challenge to extrapolating findings to functions in human tissues. However, this elegant piece of work by Castro et al provides a significant advance in understanding cGMP signaling and opens new avenues for investigation of this complex pathway.

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None.

References

Non-standard Abbreviations and Acronyms

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<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANP</td>
<td>atrial natriuretic peptide</td>
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<tr>
<td>NFAT</td>
<td>nuclear factor of activated T cells</td>
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<tr>
<td>NO-GC</td>
<td>nitric oxide–stimulated guanylyl cyclase</td>
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<tr>
<td>pGC</td>
<td>particulate guanylyl cyclase</td>
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<tr>
<td>PKGI</td>
<td>cGMP-dependent protein kinase I</td>
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<tr>
<td>RGS</td>
<td>regulator of G protein–coupled signaling</td>
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<td>TnI</td>
<td>troponin I</td>
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