Protein kinase G type I in cardiac myocytes: unmasked at last?

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This editorial refers to ‘Stress-dependent dilated cardiomyopathy in mice with cardiomyocyte-restricted inactivation of cyclic GMP-dependent protein kinase I’, by S. Frantz et al., on page 1233

Increasing evidence over the past decade has suggested that elevations in myocardial cGMP may protect against adverse ventricular remodelling. cGMP is produced in different cell types comprising cardiac muscle, including vascular smooth muscle and endothelial cells, fibroblasts, monocytes/macrophages, and cardiac myocytes (CMs) themselves, all of which participate in remodelling. In the latter, cGMP is produced either from particulate guanylate cyclase (pGC) contained within GC-A [natriuretic peptide receptor type A (NPR-A) activated by atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)] and GC-B [(NPR-B) activated by C-type natriuretic peptide (CNP)], or from soluble GC (sGC) activated by NO and CO. Given the diffusible nature of NO, CM sGC can theoretically be activated by NO from autocrine sources [endogenously expressed nitric oxide synthases (NOS)], paracrine sources (i.e. mainly from endothelial and inflammatory cells), and, possibly, exogenous NO.

cGMP-dependent protein kinase type I (cGKI) is part of a family of cGMP-activated protein kinases (PKs) encoded by two different genes; one encoding cGKIα and cGKIβ (through alternative splicing), and the other encoding cGKII, a membrane-bound PK classically detected in the intestinal brush border.1 Together with cGMP-modulated phosphodiesterases (PDEs), cGKs represent the main effectors of cGMP signalling in cardiovascular (and other) tissues. As such they modulate the function of key proteins involved in excitation–contraction (EC) coupling (Figure 1), contractility, cell survival, metabolism, and CM hypertrophic remodelling (Figure 2). Recent studies have highlighted the functional compartmentation of cGMP pools within CM, each susceptible to activate a specific subset of cGK and effectors; this would be in line with the identification of cGK anchoring proteins2 (akin to the PKA-associated A kinase anchoring proteins family) that would ensure directed signalling; cGMP-dependent signalling is controlled both by hydrolysis through a subset of PDEs (mainly PDE1,4,5 in CMs) and by retrocontrol of pGC and sGC activity through cGK.3,4

The importance of myocardial cGMP for remodelling was inferred from the phenotype of transgenic mouse models where some of these signalling elements had been deleted or overexpressed, e.g. genetic deletion of GC-A (or overexpression of a dominant negative GC-A) in CMs exacerbated pathological remodelling and functional deterioration after aortic banding,5,6 whereas CM-specific knock-in of GC-A on a global GC-A null background normalized CM size in the face of maintained systolic hypertension.7 Likewise, CM-specific overexpression of endothelial nitric oxide synthase (eNOS) protected from post-myocardial infarction (MI) remodelling and functional deterioration,8 as did CM-specific knock-in of eNOS on a NOS3 null background in banded mice;9 conversely, in systemic NOS3-deficient mice, exacerbated left ventricular (LV) remodelling and functional deterioration post-MI or trans-aortic constriction (TAC) was observed even after correction of hypertension10,11—provided the haemodynamic stress does not reach a level susceptible to producing eNOS uncoupling.12 This is in line with early experiments in cultured CMs demonstrating an inhibition of hypertrophy by exogenous administration of exogenous NO, natriuretic peptides, and the cGMP analogue, 8-bromo-cGMP.13

The identity of the downstream effector(s) for this cGMP-mediated protection from cardiac remodelling (i.e. cGMP-modulated PDEs vs. cGKs), however, remained somewhat elusive. Indirect evidence suggested the involvement of cGKI, i.e. pharmacological PDE5 inhibition correlated with an increase in activity of cGK and protection from remodelling.15 Conversely, conditional, CM-specific genetic overexpression of PDE5 correlated with attenuated cGK activity, together with exacerbated remodelling (both of which were reversed upon PDE5 suppression);16 and protein effectors clearly identified as cGK targets, such as regulator of G-protein signalling (RGS)-2/417,18 and transient receptor potential cation channel 6 (TRPC6),19–21 were demonstrated as key mediators of the protection from remodelling by PDE5 inhibition and GC-A overexpression.

The opinions expressed in this article are not necessarily those of the Editors of the European Heart Journal or of the European Society of Cardiology.1

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Direct genetic evidence implicating cGKI in vivo has been limited so far. About a year ago, a study by Lukowski et al. reported the phenotype of mice with smooth muscle knock-in of cGKI on a global cGKI null background (which is otherwise lethal, due to severe gastrointestinal dysmotility). When submitted to isoproterenol (ISO) infusion or TAC, these mice showed LV remodelling virtually identical to the controls. However, this study left some unanswered questions, i.e. there was no functional...
assessment of LV function in stressed mice, so it is unsure whether the ISO or TAC stimuli were of sufficient intensity to induce remodelling susceptible to evolve towards heart failure (where the protective benefit of cGKI would have been manifested); there was also no direct assessment of cGKI activation (in the control animals), or of some of the putative cGKI-targeted pathways regulating pathological remodelling under the same stress; and the genetic ‘rescue’ model itself may bear some inherent limitations for the interpretation of the phenotype. In addition, others have reported that mice expressing a mutant cGKIα (that is unable to bind effectors) are no longer protected by sildenafil (preferential PDE5 inhibitor) from TAC-induced remodelling and LV function deterioration, in contrast to wild-type controls,23 again pointing to a key role for cGKI in anti-remodelling effects of cGMP.

The study now reported by Frantz et al.24 resolves some of these questions. It uses a model of CM-specific cGKI knockout submitted to different types of stress inducing remodelling, i.e. ISO, but also angiotensin II (AII) (coupled to Gq, remodelling pathways) and TAC, some of which (AII and TAC) were harsh enough to induce LV structural and functional deterioration. cGKI-deficient mice suffered more structural and functional damage with AII and TAC than their wild-type controls. This was associated with alterations of Ca^{2+} handling [decreased sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a)/phospholamban (PLB) ratio, and decreased whole Ca^{2+} transient at baseline and under acute β-adrenergic stimulation]. Interestingly, knockout animals developed more fibrosis, in parallel with stronger myocardial expression of the pro-fibrotic cytokine connective tissue growth factor (CTGF).

The authors claim that their results confirm that CM cGKI has no influence on myocardial ‘hypertrophy’; at first glance, their gravidometric data [heart weight to body weight ratio (HW/BW)] would support such a conclusion; however, with their protocol of ISO infusion, it is unclear whether the ‘hypertrophy’ observed is actually part of an adverse myocardial remodelling, or merely corresponds to physiological hypertrophy, as suggested by the absence of associated functional deterioration (LV function actually slightly increases); therefore, as discussed above, the β-adrenergic stress imposed may have been too mild to recruit the pathological signalling pathways cGKI has been proposed to inhibit. With AII, hypertension is observed and, although LV function is conserved in wild-type animals, it deteriorates in the knockout animals; with TAC, LV function decreases in both groups, but more so in knockout mice. Again, simple gravidometric data show no difference between genotypes, but echocardiographic measurements clearly indicate LV eccentric remodelling; so although LV mass may be unchanged, this remodelling was most probably associated with dilatation and thinning of the LV wall (which were not measured). In this setting, cardiac myocyte transverse dimensions may be unchanged (as we see here), yet cardiac myocyte length is increased, as crudely indicated by the increased maximal myocyte length of isolated myocytes from AngII-treated knockout animals. Furthermore, the molecular signature of hypertrophy is clearly more pronounced in knockout animals following both AngII and TAC, as indicated by the more marked activation of the foetal gene program. This underlines the confusion associated with the use of ‘hypertrophy’ as a definition of whole LV remodelling, as recently discussed in a position paper of the European Society of Cardiology Working Group on Myocardial Function.25

A prominent finding in this study is the genotype influence on the development of myocardial fibrosis, which most probably participated in LV functional deterioration. Several mechanistic explanations appear plausible, based on previous literature: (i) cGMP/cGKI

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**Figure 3** Modulation of myocardial remodelling through cGMP-dependent protein kinase type I (cGKI) in multiple cell types. In cardiac myocytes, cGKI inhibits hypertrophy, protects from apoptosis, and reduces the paracrine actions of connective tissue growth factor (CTGF) on fibroblasts to reduce fibrosis. In addition, cGKI in endothelial cells acts downstream of natriuretic peptide receptor type A (NPRA) to promote angiogenesis,42 and in fibroblasts cGKI has been shown to inhibit profibrotic pathways, which would be additive to the reduction of paracrine CTGF production. Finally, cGKI expressed in cardiac progenitors may promote differentiation to cardiac myocytes thereby enhancing regeneration.
was shown to prevent expression of the profibrotic CTGF in other models of fibrosis and, accordingly, myocardial CTGF was increased in cGKI null mice in the study of Frantz et al. (see Figure 3); (ii) cGKI was previously shown to mediate cardiac myocyte survival and its deletion may have promoted myocyte loss in the face of cardiac stress, as well as subsequent reparative fibrosis (although the authors report no difference in CM apoptosis between genotypes in the AngII model, no apoptosis data is reported in the harsher TAC model where, possibly, differences may have been highlighted); and (iii) in addition to enhancing myocyte loss, cGKI deletion may have hampered myocyte regeneration. Indeed, haemodynamic stress can activate cardiac stem cells, which, upon differentiation, would have activated α- myosin heavy chain–Cre-mediated recombination and loss of cGKI; that, in turn, would have deprived them of the autocrine/paracrine effect of NO to enhance stem cell differentiation, which we found to be dependent on cGMP in adult cardiac stem cells.

The authors also used their model to test the differential effect of ANP or CNP on Ser16 phosphorylation of PLB through cGKI; they conclude that CNP, but not ANP, results in cGKI-mediated phosphorylation of PLB and subsequent modulation of Ca²⁺ handling and contractility. As CNP and its target receptor, GC-B, are up-regulated in the failing heart, CNP could participate in the preservation of contractility and myocardial structure under cardiac stress. This would be in line with previous demonstrations of functional compartmentation of cGMP pools, as discussed above. On the other hand, cGKI deletion does not seem to affect the contractile response to ISO (at the single dose of 10μM); however, these experiments were performed at room temperature, which could have hampered optimal activation of the NOS-dependent cGMP pool (and erased differences between genotypes).

Some questions remain unanswered but should be resolved in subsequent studies. Paramount to the relevance in human cardiac diseases is whether cGKI expression is altered in the pathological heart, i.e. in the course of remodelling. Very little is known of endogenous NOS with tetrahydrobiopterin, or (e)NOS activation by specific G-protein-coupled receptor activation, such as β3-adrenoceptors. The study by Frantz et al. will undoubtedly revive the interest for the clinical testing of each of these pharmacological approaches.

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14. Rosenkranz AC, Woods RL, Dusting GJ, Ritchie RH. Antihypertrophic actions of NCT00763867), as well as synthetic natriuretic peptides (although currently being tested in clinical trials (RELAX trial). Preferential inhibitors of cGMP-hydrolysing PDEs (e.g. PDE5) are elevated endogenously in heart failure—is more disputable); other strategies to restore the endogenous sGC-dependent pool of cGMP include the use of direct activators of sGC, recoupling of endogenous NOS with tetrahydrobiopterin, or (e)NOS activation by specific G-protein-coupled receptor activation, such as β3-adrenoceptors. The study by Frantz et al. will undoubtedly revive the interest for the clinical testing of each of these pharmacological approaches.


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