Dissecting Cardiac Hypertrophy and Signaling Pathways
Evidence for an Interaction Between Multifunctional G Proteins and Prostanoids

Siiri E. Iismaa, Robert M. Graham

Over the past two decades, the ability to produce genetically engineered animal models has been widely used to unravel complex biological pathways involved in both physiology and disease. Although uniquely powerful and inherently elegant, these models, while often providing surprising new insights, have not uncommonly raised more questions than they have answered. A case in point is a transgenic mouse model developed to probe the in vivo role of the G protein, Gh, in cardiac signaling, and its consequences. Unlike “traditional” heterotrimeric G proteins, Gh is decidedly atypical, being a multifunctional protein with both GTPase and phospholipase C (PLC) activation, 1,2 that for Gh demonstrated a mild form of hypertrophy by a mechanism that remained elusive. Evidence for PKCe and PLC activation was lacking, and it was suggested, largely by default, that the hypertrophy in the cardiac Gh animals was due not to its signaling activity but to TGase-mediated protein crosslinking. In this issue of Circulation Research, FitzGerald and coworkers 3 have reexamined this issue using an independently developed model of cardiac-restricted Gh overexpression. Interestingly, these studies provide evidence linking two major biological systems, transglutaminases (TGs) and prostanoids, in the cardiac dysfunction and hypertrophy observed in this transgenic model, and potentially contributing to these disorders in humans.

TGs are a family of Ca\(^{2+}\)- and thiol-dependent enzymes that catalyze the covalent posttranslational modification of proteins, either by formation of an isopeptide bond between the \(\gamma\)-carboxamide group of glutamine residues and the \(\varepsilon\)-amino group of intrachain lysines or by modification of glutamine residues by polyamines. 4 Isopeptide bond formation stabilizes the resulting supramolecular structure and confers resistance to proteolysis—a reaction that is critical for hemostasis, for example, involving the crosslinking of fibrin clots by the TG, factor XIIIa. In addition to these protein-modifying transamidation reactions, TGs can also esterify and deamidate proteins, reactions critical, for example, to the barrier function of skin and to the pathophysiology of gluten-induced enteropathy (celiac disease), respectively. 4 Specialized noncatalytic actions of TGs have also been identified, such as scaffolding of the cytoskeleton to maintain membrane integrity (mediated by a catalytically inactive member of the TG family, band 4.2), cell adhesion and, as detailed below, potentially, signal transduction. 4

Of the mammalian TGs, encoded by nine distinct genes in the human genome, tissue TG (also known as TG2 or TG-C) was the first to be identified and, yet, paradoxically, despite extensive study, remains one of the least well understood. It is expressed ubiquitously in most organs and tissues, and although localized predominantly in the cytosol (80%), is also found in plasma (10% to 15%) and the nuclear membranes (5%), as well as being secreted from the cell by an unknown mechanism where it localizes to the cell surface and extracellular matrix. In 1987, Greenberg and colleagues showed that, unlike other TGs, TG2 could bind GTP and that when it did, its TGase crosslinking activity was inhibited. 4 Subsequently, we showed that a 74-kDa G protein, named Gh (h to indicate its high molecular weight compared with heterotrimeric G protein \(\alpha\)-subunits that are in the 40- to 45-kDa range), previously shown to couple specifically to \(\alpha\)-ARs, was identical to TG2. 5 Further, evidence was provided to suggest an elegant regulatory pathway whereby Gh exchanges GTP for GDP, once activated by \(\alpha\)-AR-stimulation, which, as a consequence, inactivates the TGase activity of Gh. In the GTP-bound form, Gh then activates PLC to increase inositol trisphosphate and intracellular Ca\(^{2+}\). Subsequent hydrolysis of GTP by the GTPase activity of Gh, or the increase in intracellular Ca\(^{2+}\), which inhibits nucleotide binding by Gh, terminates signaling and restores its TGase activity. Nevertheless, direct in vivo evidence for its role in cell signaling is still lacking, and null mutant mice, lacking TG2/Gh expression, although showing subtle phenotypic changes, 6 have not, as yet, confirmed its role as a mediator of receptor-coupled signaling. Additional studies have shown that Gh heterodimerizes with a 50-kDa \(\beta\)-subunit that stabilizes the GDP-bound form of Gh (the 74-kDa subunit thus being referred to as Gh\(_{\text{h}}\)); that TG2/Gh mediates activation of the \(\delta\) isoform of PLC and of maxi-K\(^{+}\) channels, as well as inhibition of adenylyl cyclase; that it supports signaling not only of certain \(\alpha\)-AR subtypes but also of certain oxytocin

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and thromboxane receptors; that the signaling function of TG2/Gh is preserved even with mutagenic inactivation of its TGase activity; that its interaction with receptors contributes to its plasma membrane-binding; and that the residues forming its nucleotide-binding pocket are distinct from those of other GTP-binding proteins. Evidence has also accumulated to suggest that intracellularly, under physiological conditions where levels of GTP are high and Ca²⁺ concentrations are below the submillimolar amounts required for its TGase activity, TG2/Gh is a latent enzyme that cannot mediate crosslinking. By contrast, in pathological states when GTP biosynthesis falls and intracellular Ca²⁺ increases, the TGase activity of TG2/Gh is activated, allowing it to crosslink proteins, a response implicated in processes such as apoptosis.

Evidence for the involvement of TG2/Gh in cardiac disease is the finding of increased expression but impaired α₁-AR/Gh coupling, in hearts from patients with ischemic but not dilated cardiomyopathy, and of markedly increased TG2/Gh expression in hearts from rats with decompensated pressure-overload hypertrophy.

Prostanoids, consisting of the prostaglandins (PGs) and thromboxanes (TXs), are arachidonic acid (AA) metabolites synthesized in response to various physiological and pathophysiological stimuli (see Figure). They are quickly released from cells where, locally, they mediate diverse and often opposing physiological actions, such as vasoconstriction (thromboxane A₂, TxA₂) and vasodilatation (prostacyclin, PGI₂). These responses are mediated by specific G protein-coupled receptors, termed IP, TP, and FP (for PGI₂, TxA₂, and PGF₂α, respectively). Cyclooxygenase (COX), the rate-limiting enzyme in prostanoid biosynthesis, exists as two isoforms encoded by different genes, which have similar enzymatic properties but differ with respect to cellular localization, expression, and regulation. COX-1 is constitutively expressed and is the only functionally active isoform in normal cardiomyocytes, whereas COX-2 can be induced by cytokines, growth factors, and hormones. Its expression is increased in ischemic human myocardium and in dilated cardiomyopathy, and deletion of the COX-2 gene may cause myocardial fibrosis. The products of COXs are the transient endoperoxide intermediates, PGG₂ and PGH₂, whose further conversion to various prostanoids, including PGI₂, TxA₂, and PGF₂α, is specific to a given cell or tissue. TxA₂ and PGI₂, synthesized mainly by platelets and the vascular endothelium, respectively, play key, yet opposing roles in circulatory homeostasis and hemostasis. Moreover, perturbations in the levels of prostanoids, their synthases or their receptors, have been implicated in various cardiovascular disorders. There are two alternatively sliced TP isoforms, α and β, with the former being the physiologically relevant isoform for vasoinhibitory responses. In heart, the major COX-2–derived eicosanoid is PGI₂, which is protective against oxidative stress by a mechanism involving activation of extracellular-regulated kinases (ERK1/2), although TxA₂ and PGF₂α are also produced. Oxidant stress, as occurs with ischemia/reperfusion, heart failure, and atherosclerosis, leads to oxidative modification of AA and formation of free radical–catalyzed products called isoprostanes (iPs). Formed initially in situ in the phospholipid domain of cell membranes, they are then cleaved by phospholipases, circulate in esterified and unesterified forms, are excreted in the urine and, importantly, may act as incidental ligands for prostanoid receptors.

Initial evidence linking prostanoids and transglutaminases was the finding that TxA₂/PGF₂α receptors copurified with a high molecular weight (80 to 85 kDa) G protein. Subsequently, it was shown that TP and TG2/Gh can be coinmuno precipitated from platelets and vascular tissue and, in expression systems, that the α but not β isoform of TP can signal via TG2/Gh to increase intracellular calcium and inositol phosphates. The study by FitzGerald and coworkers shows that cardiac G₉ overexpression results in the hemodynamic, genetic, and ultrastructural changes of left ventricular hypertrophy and decompensation, which in older animals leads to premature death. Consistent with COX-2 activation and increased lipid peroxidation, careful evaluation of key components of the prostanoid biosynthetic pathway and their receptors demonstrated increased cardiomyocyte expression of COX-2, TxA synthase, IP, FP, and IP, increased urinary metabolites of PGI₂, and TxA₁, and increased urinary excretion of F₂-isoprostanes. COX-2 inhibition suppressed both TxA₂ and PGI₂ biosynthesis, indicating that this isoform is the dominant source of these mediators. Moreover, COX-2 inhibition, TP antagonism, or suppression of lipid peroxidation rescued the phenotype, and IP activation improved cardiac function. In contrast, FP activation exacerbated the phenotype.

![Phospholipids](image-url)
A parsimonious interpretation of these findings is that enhanced TP signaling due to TG2/Gh overexpression is the primary and initiating event underlying the observed phenotype (see Figure). ERK1/2 activation as a result of the enhanced TP signaling would account for the hypertrophy, as well as for sustained PLA2 activation and COX-2 induction, and as a result of the latter, enhanced TXA2 production. Coincident oxidant stress would contribute to the phenotype via regulation of COX-2 expression and/or by iPa activation, which itself can induce hypertrophy via TP stimulation. Apoptosis, in this paradigm, may be a direct result of enhanced TPα signaling\(^1\) or may be secondary to TP-mediated vasoconstriction causing myocardial ischemia. However, a major unresolved issue in this study\(^3\) and that of Small et al.,\(^1\) is whether or not the TGase activity of TG2/Gh \(\) contributes to the phenotype, either as a direct result of increased protein crosslinking and apoptosis, or possibly due to latent transforming growth factor-β activation by TG2/Gh.\(^4\) Thus, although TGase activity was shown to be enhanced with Ca\(^++\) activation of myocardial lysates from the TG2/Gh overexpressors,\(^1,3\) unfortunately evidence for increased intracellular TGase activity in vivo, or in intact cardiomyocytes, was not sought. Indeed, it is quite possible that intracellular TGase activity was not increased despite TG2/Gh overexpression, or may even have been suppressed, due to enhanced TP activation causing increased GTP exchange by TG2/Gh and, thus, suppressing its enzymatic activity. Resolution of this issue is important and should be eminently feasible in this model. In either case, however, the evidence presented for enhanced TG2/Gh-mediated TP signaling is compelling\(^3\) and as such provides strong support for the notion that TG2/Gh is an important receptor-coupled signaling molecule, at least in situations where its expression is increased. The significance of this study,\(^3\) therefore, is to provide evidence for a previously underappreciated role of prostanoids in cardiac signaling and pathophysiology, as well as in vivo data linking prostanoids with the receptor signaling activity of the Gh family of multifunctional G proteins.

Finally, this study\(^3\) highlights the therapeutic potential of antioxidants in cardiac disease.

**References**


**Key Words:** prostanoids ■ tissue transglutaminase ■ cyclooxygenase ■ thromboxane ■ cardiac failure
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