Dual Actions of the Goq Agonist Pasteurella multocida Toxin to Promote Cardiomyocyte Hypertrophy and Enhance Apoptosis Susceptibility

Abdelkarim Sabri, Brenda A. Wilson, Susan F. Steinberg

Abstract—Previous attempts to delineate the consequences of Goq activation in cardiomyocytes relied largely on molecular strategies in cultures or transgenic mice. Modest levels of wild-type Goq overexpression induce stable cardiac hypertrophy, whereas intense Goq stimulation induces cardiomyocyte apoptosis. The precise mechanism(s) whereby traditional targets of Goq subunits that induce hypertrophy also trigger cardiomyocyte apoptosis is not obvious and is explored with recombinant Pasteurella multocida toxin (rPMT, a Goq agonist). Cells cultured with rPMT display cardiomyocyte enlargement, sarcomeric organization, and increased atrial natriuretic factor expression in association with activation of phospholipase C, novel protein kinase C (PKC) isoforms, extracellular signal–regulated protein kinase (ERK), and (to a lesser extent) JNK/p38-MAPK. rPMT stimulates the ERK cascade via epidermal growth factor (EGF) receptor transactivation in cardiac fibroblasts, but EGF receptor transactivation plays no role in ERK activation in cardiomyocytes. Surprisingly, rPMT (or novel PKC isoform activation by PMA) decreases basal Akt phosphorylation; rPMT prevents Akt phosphorylation by EGF or IGF-1 and functionally augments cardiomyocyte apoptosis in response to H2 O2. These results identify a Goq-PKC pathway that represses basal Akt phosphorylation and impairs Akt stimulation by survival factors. Because inhibition of Akt enhances cardiomyocyte susceptibility to apoptosis, this pathway is predicted to contribute to the transition from hypertrophy to cardiac decompensation and could be targeted for therapy in heart failure. (Circ Res. 2002;90:850-857.)

Key Words: Goq ■ protein kinase C ■ Akt ■ hypertrophy ■ apoptosis

Myocardial hypertrophy is an adaptive response to stresses that increase cardiac work. The increase in tissue mass diminishes systolic wall stress and improves contractile performance in the short term. However, compensated hypertrophy generally progresses to decompensated cardiac failure with chamber dilatation and contractile dysfunction. Because cardiac failure represents a major public health problem with substantial mortality, many laboratories have invested considerable effort to understand the regulatory determinants that contribute to the development of hypertrophy and its transition to heart failure. Recent research has focused on the family of Goq-coupled G protein–coupled receptors (GPCRs), which induce a hypertrophic phenotype with many common molecular and morphological features when stimulated by agonists or overexpressed in the hearts of transgenic mice.1 Goq-coupled GPCRs actions generally are attributed to effector pathways that emanate from αq subunits, which individually have been implicated in cardiomyocyte hypertrophic growth responses (including protein kinase C [PKC], mitogen-activated protein kinase [MAPK] cascades, and tyrosine kinases). However, the precise cellular actions of Goq proteins are not precisely delineated by studies of Goq-coupled GPCRs for 2 major reasons: (1) many GPCRs that couple to Goq also activate other G protein classes, perhaps explaining subtle differences in resultant hypertrophic phenotypes2;3; (2) activation of heterotrimeric Goq liberates βγ dimers (in addition to αq subunits), with independent signaling functions to downstream effector pathways. The prediction that Goq activates only a subset of the signaling machinery recruited by agonist-occupied GPCRs is borne out by experimental evidence that Goq subunits alone are not sufficient to induce the entire spectrum of changes characteristically induced by hypertrophic agonists acting at their cognate GPCRs.1

Strategies more directly targeted to Goq subunits have been used to resolve their role in cardiomyocyte hypertrophy. Initial studies demonstrated that microinjection of inhibitory antibodies to Goq(11) in rat cardiomyocytes cultures or cardiac-restricted overexpression of a Goq inhibitory peptide in mice (which prevents signal transmission at the receptor-Goq subunit interface) interferes with the acquisition of features of cardiac hypertrophy in response to α1-adrenergic receptors or...
pressure overload hypertrophy. Subsequent studies involved overexpression of \( \alpha_q \) subunits in cardiomyocyte cultures or expression of a \( \alpha_q \) transgene in mouse myocardium. These studies provided unanticipated evidence that modest increases in wild-type \( \alpha_q \) expression induce stable cardiac hypertrophy, but very intense \( \alpha_q \) stimulation (with very high levels of wild-type \( \alpha_q \) proteins or constitutively activated \( \alpha_q \) mutants) induces dilated cardiomyopathy, with functional decompensation and cardiomyocyte apoptosis. These observations suggest that hypertrophy and apoptosis may represent different phases of the same process, initiated by a common \( \alpha_q \)-activated biochemical signal. However, few traditional \( \alpha_q \) targets simultaneously promote hypertrophy and trigger cardiomyocyte apoptosis. One notable exception is PKC, which displays isoform-selective signaling to MAPK cascades, apoptosis, and cardioprotection. Akt, a serine/threonine protein kinase that mediates cardioprotection by receptor tyrosine kinases, also is activated by certain GPCRs (including in cardiomyocytes), but the role of \( \alpha_q \) in this process is disputed; Murga et al identify a \( \alpha_q \)-dependent pathway for Akt activation, whereas BommaKanti et al report Akt inhibition by \( \alpha_q \). Finally, \( \alpha_q \) promotes cardiomyocyte apoptosis in genetically engineered systems, where intense stimulation by exogenous proteins may alter the stoichiometry and/or targeting of \( \alpha_q \) relative to physiologically relevant binding partners in the plasma membrane. The relevance of studies on exogenous (frequently constitutively activated) proteins to physiological signaling by endogenous \( \alpha_q \) in cardiomyocytes remains uncertain.

*Pasteurella multocida* toxin (PMT) is a potent mitogen for a variety of cell types. PMT is internalized via receptor-mediated endocytosis and acts intracellularly to activate signaling pathways, including phosphoinositide hydrolysis, mobilization of intracellular calcium, translocation of PKC, activation of the extracellular-regulated kinase [ERK] cascade, and tyrosine phosphorylation of focal adhesion kinase. The actions of recombinant PMT (rPMT) are inhibited in *Xenopus* oocytes by antibodies directed against the \( \alpha \) subunit of \( G_q \) or \( G_q \)-antisense RNA and in HEK293 cells by overexpression of the C-terminal peptide inhibitor of \( G_q(14,15) \). These results identify the free monomeric \( G_q \) subunit as the target of rPMT’s actions. Recent studies in fibroblasts deficient in either \( \alpha_q \) or \( \alpha_1 \) further localize rPMT’s actions to \( \alpha_q \) (not \( \alpha_q \)). Accordingly, this study uses rPMT as a pharmacological probe to elucidate the biochemical and functional consequences of endogenous \( \alpha_q \) activation in cardiomyocytes.

**Materials and Methods**

Cultures of neonatal rat ventricular myocytes and cardiac fibroblasts were prepared and assays of inositol phosphate accumulation were performed according to methods described previously. Immuno-blotting was according to methods published previously or manufacturer’s instructions with antibodies for total or phosphorylated (activated) signaling proteins from the following sources: phospho-ERK1/2, total and phospho-p38 MAP kinase, phospho-c-Jun-NH2-terinal kinase (JNK), total and phospho-Akt, phospho-PKC-Pan, and phospho-PKC-\( \delta \)-Thr-505 (Cell Signaling Technology); ERK1/2 (Santa Cruz Biotechnology); PKC-\( \alpha \) and PKC-\( \delta \) (Biosource International); and PKC\( \varepsilon \) (Dr. Dorian Fabbro, CIBA-Geigy, Basel, Switzerland). Each panel in

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![Figure 1](image)

**Figure 1.** rPMT activates PLC. Media was supplemented with 3 \( \mu \)Ci/mL \([\text{H}]\)myoinositol for 96 hours, with 400 ng/mL rPMT for the indicated intervals at the end of this interval (A) or the indicated concentrations of rPMT (B, left) or 400 ng/mL rPMT (B, right) during the final 24 hours. This was followed by 30 minutes in the presence of 10 mmol/L LiCl (alone or plus 10 \( \mu \)mol/L NE; B, right). Results are mean of 3 replicates from a single experiment (representative of 3 separate experiments using different batches of rPMT; A) or mean \( \pm \)SEM from 3 independent experiments (using a single rPMT preparation; B). Levels of IP2 and IP3 increased in parallel in all experiments.

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**Results**

**Agnost Properties of rPMT in Cardiomyocytes**

Consistent with evidence that rPMT gains access to cells slowly, rPMT promotes inositol phosphate accumulation in cardiomyocytes, but with slow kinetics. The response is detectable at 1 hour and increases progressively for at least 48 hours (Figure 1A). Figure 1B shows that maximal activation of inositol phosphate accumulation at 24 hours typically is with 400 ng/mL toxin; the magnitude of the response is comparable to that induced by a 30-minute challenge with the \( \alpha_1 \)-adrenergic receptor agonist norepinephrine (NE; with individual batches of rPMT displaying some variability).

rPMT-dependent activation of phospholipase C (PLC) also leads to the formation of diacylglycerol, the endogenous activator of PKC. There is general consensus that neonatal rat ventricular myocytes coexpress calcium-sensitive PKC\( \alpha \)
by short-term stimulation with NE/PMA or long-term stimulation by agonist-occupied GPCRs) does not lead to detectable changes in PKC isoforms partitioning between soluble and particulate fractions (or abundance) at 30 minutes to 2 hours (data not shown). However, persistent rPMT stimulation (400 ng/mL, 24 hours) leads to a substantial reduction in the abundance of PKCδ and PKCe in the soluble fraction; however, PKCδ and PKCe abundance in the particulate fraction is preserved (Figure 2A). Reduced amounts of PKCδ and PKCe, which disproportionately partition with the particulate fraction, recapitulates the changes detected in cardiomyocyte cultures exposed to NE for 24 hours or in ventricles from mice that overexpress Gαs.6,18 PKCα and PKCe abundance and subcellular distribution are not altered by rPMT or NE (Figure 2A and data not shown). These results provide evidence that persistent Gαs stimulation by rPMT or NE results in the activation of only novel PKCδ and PKCe isoforms. rPMT-dependent downregulation of novel PKC isoforms occurs over a dose range that parallels the concentration-response relationship for rPMT-dependent stimulation of PLC (data not shown).

Western blot analysis with anti–phospho-PKC antibodies provided independent evidence of PKC activation by rPMT. Recent studies identify PKC phosphorylation at conserved sites in the C-terminus (the turn and hydrophobic motif, corresponding to Thr641 and Ser660 in PKCβII) as a mechanism to control the maturation and allosterically regulate the activity of PKC isoforms.21 Anti–phospho-PKC-Pan, an antibody directed at the conserved hydrophobic motif in conventional and novel PKC isoforms (but not atypical PKC isoforms, where the Ser phospho-acceptor site is replaced by the acidic Glu), identifies major bands that comigrate with PKCδ, PKCa, and PKCe (approximately 78-, 82-, and 96-kDa, respectively; Figure 2A, bottom). Immunoreactivity is completely stripped by acid phosphatase treatment (data not shown) and drastically reduced by pharmacological downregulation of PKC protein with phorbol 12-myristate 13-acetate (PMA) (Figure 2A); however, PKCa and PKCe (approximately 78- and 96-kDa, respectively) are detectable in the particulate fraction of rPMT-treated cardiomyocytes, along with the bulk of PKCe protein. Consistent with evidence that the hydrophobic motif is a site of autophosphorylation in cPKC isoforms,21 PKCe phosphorylation requires intact PKC kinase activity and is prevented by GF109203X (an inhibitor of cPKC/nPKC isoforms). PKCδ phosphorylation at the hydrophobic motif also is detected in the particulate (but not soluble) fraction of quiescent and stimulated cardiomyocytes.

![Figure 2](image_url)

**Figure 2.** rPMT selectively activates nPKC isoforms. Cardiomyocytes were incubated in culture medium without or with NE (10 μmol/L), rPMT (400 ng/mL), or PMA (100 nmol/L) for 24 hours (A) or 5 minutes (B) as indicated. Soluble and particulate extracts (A) or whole cell extracts (B) were separated by SDS-PAGE followed by Western blotting with antibodies specific for individual PKC isoforms. PKC phosphorylated at the hydrophobic motif (with P-PKC-Pan), or PKCδ phosphorylated at the activation loop or hinge region (with P-PKCδ-Thr-505 and P-PKCδ-Tyr-311, respectively) as indicated. The band designated PKCα on immunoblots with P-PKC-Pan corresponds to the major immunoreactive species recognized by the antibody to total PKCα; the identity of the band with slower mobility in the particulate fraction (that disappears in cells subjected to PMA for 24 hours) is less certain. Electrophoresis was performed according to conditions described by Bornancin and Parker24 (A, bottom), to improve protein separation and detect minor difference in protein mobility; NE- and rPMT-dependent shifts in total PKCδ mobility also were detected in separate experiments that used these conditions and lower amounts of protein loading (data not shown). GF109203X was included at 5 μmol/L for 30 minutes before stimulation where indicated.
rPMT reduces PKCδ mobility, without increasing PKCδ phosphorylation at the hydrophobic motif. This result is most consistent with rPMT increasing PKCδ phosphorylation at a site that is distinct from the hydrophobic motif. However, antibodies that specifically recognize PKCδ phosphorylation at Thr505 in the activation loop and Tyr311 in the hinge region ruled out rPMT-dependent phosphorylation of PKCδ at these other sites; immunoreactivity with these antibodies is confined to the particulate fraction of control cardiomyocytes, lost during PKCδ downregulation by PMA, and fails to increase in cells treated with rPMT. Because PKCδ undergoes complex regulatory phosphorylations at other Ser/Thr or Tyr residues (which can decrease its mobility), rPMT-dependent phosphorylation of PKCδ remains a viable mechanism to explain the reduced PKCδ mobility after rPMT treatment (although other modifications that reduce PKCδ mobility also are possible). Collectively, these experiments identify persistent PKC activation (with selectivity for novel PKC isoforms) in rPMT treated cardiomyocytes.

The MAPK cascades that lie downstream in Goq-dependent pathways in cardiomyocytes were next studied. Figure 3 shows that rPMT induces dose-dependent increases in signaling through the ERK1/2, JNK, and p38-MAPK cascades, as detected by an increase in the phosphorylation of the terminal kinase of each pathway. For each, activation was observed with slow kinetics (detectable at 1 hour and increasing progressively for 24 hours). Immunoblot analyses with antisera that recognize total (phosphorylated and nonphosphorylated) ERK1/2, p38 MAPK, and JNK show that rPMT does not significantly alter the expression of these proteins (Figure 3 and data not shown). This indicates that the protracted kinetics for MAPK activation by rPMT is not due to changes in terminal kinase protein expression. ERK1/2 activation by rPMT is quite robust (comparable to acute stimulation with PMA), whereas JNK and p38-MAPK activation by rPMT is more modest (relative to the strong JNK and p38-MAPK activation by sorbitol).

rPMT displays potent growth stimulatory properties in cells with proliferative potential. Figure 4 shows that rPMT also induces the hallmarks of cardiomyocyte hypertrophy. rPMT mimics the effects of NE to promote myofibrillar organization, increase ANF mRNA expression, promote cellular enlargement, and enhance [3H]phenylalanine incorporation.

**ERK Activation by rPMT Does Not Require EGF Receptor Kinase Activity in Cardiomyocytes**

GPCR-dependent activation of ERK can follow a Ras-independent pathway involving PKC isoforms or a Ras-dependent pathway involving receptor or nonreceptor tyrosine kinases, depending on cell type and particular GPCR. Seo et al recently reported that rPMT stimulation of ERK is via a Ras-dependent (PKC-independent) pathway that involves EGF receptor transactivation in HEK293 cells. To determine whether this pathway mediates rPMT actions in cardiomyocytes, studies were performed in the presence of the EGF receptor-specific tyrosinostatin AG1478. Figure 5A shows that AG1478 effectively blocks EGF receptor-mediated ERK phosphorylation in cardiomyocytes, but induces only a very minor reduction in ERK phosphorylation by rPMT. As controls, similar studies were performed in cardiac fibroblasts. Here, rPMT-mediated activation of ERK displays a significant requirement for EGF receptor kinase activity; it is markedly inhibited by AG1478 (Figure 5B).

**Figure 3.** rPMT promotes the activation of ERK1/2, JNK, and p38-MAPK cascades. Incubations were for 24 hours in the presence of the indicated concentrations of rPMT or 5 minutes with PMA (100 nmol/L) or sorbitol (0.5 mmol/L). Cell lysates were subjected to SDS-PAGE and Western blotting with specific anti–phospho-ERK1/2, anti–p38 MAPK, or anti–JNK antibodies; stripped blots were subsequently analyzed for total ERK1/2 or p38 MAP kinase protein (A and B). Anti–P-p38-MAPK recognizes activated forms of both α and β isoforms of p38-MAPK (although only α-p38-MAPK is recognized by the antibody against total protein). Top, Representative autoradiograms (with each lane from a single gel exposed for the same duration). Bottom, Quantification of experiments from separate cultures (n=3).

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These results indicate that EGF receptor transactivation is necessary for Gq/q activation of ERK in cardiac fibroblasts, but plays little to no role in Gq/q signaling to ERK in cardiomyocytes.

rPMT or Activation of PKC Isoforms Negatively Regulates Akt; rPMT Enhances Susceptibility to H2O2-Induced Apoptosis
Mechanisms for Akt activation by receptor tyrosine kinases are well established. In contrast, efforts to delineate the mode and mechanism(s) of Akt regulation by GPCRs have yielded seemingly conflicting evidence, with Goq activation variably reported to activate or inhibit Akt.12,13 Given the importance of Akt as a mediator of cell survival (and recent evidence that intense Goq signaling enhances cardiomyocyte apoptosis), the studies next examined whether PKC isoforms might represent a mechanism whereby Gq/q subunits negatively regulate Akt. Figure 7 shows that α1-adrenergic receptor activation with NE leads to a very modest increase in Akt phosphorylation. In contrast, selective activation of the PKC limb of the phosphoinositide signaling pathway with PMA leads to a marked reduction in basal Akt phosphorylation (79 ± 6%, n = 5, P < 0.05). PMA-dependent inhibitory modulation of Akt phosphorylation is completely abrogated by GF109203X (a relatively nonselective inhibitor of phorbol ester-sensitive PKCα, PKCδ, and PKCe), under conditions where PMA-dependent activation of ERK is effectively abrogated.18 In contrast, PMA-dependent inhibition of Akt phosphorylation is not influenced by Go6976, a selective inhibitor of cPKC (−α, −β, and −γ). The MEK inhibitor U0126

Figure 6A shows that exposure to rPMT for 24 hours leads to reduced basal Akt phosphorylation (71 ± 5%, n = 4, P < 0.05); this effect is sustained for at least 48 hours (Figure 6B). Of note, EGF and IGF-1 increase Akt phosphorylation in control cultures, but both responses are severely blunted in rPMT-treated cultures. Reduced levels of phospho-Akt in rPMT-treated cultures do not result from a change in Akt protein expression; total Akt immunoreactivity is similar in control and rPMT-treated cultures. Decreased signaling through the Akt pathway could render cardiomyocytes vulnerable to stresses that induce apoptosis. Indeed, Figure 6C shows that rPMT induces a small increase in the number of TUNEL-positive cells in cardiomyocyte cultures grown in serum-free conditions for 48 hours. Although this effect of rPMT to augment basal apoptosis is not statistically significant, apoptosis induced by H2O2 is significantly augmented by rPMT.

Given recent evidence that certain PKC isoforms interact in a functionally relevant manner with Akt in tumor cells, and that rPMT leads to prominent PKC isoform activation in cardiomyocytes, the studies next examined whether PKC isoforms might represent a mechanism whereby Gq/q subunits negatively regulate Akt.
also did not influence PMA-dependent repression of Akt phosphorylation (under conditions where PMA-dependent activation of ERK was completely abrogated); these results indicate that nPKC isoform(s) repress Akt phosphorylation through a mechanism that does not require activation of the ERK cascade. Short-term incubations with GF109203X and Go6976 appeared to be well-tolerated. In contrast, chelerythrine (a general inhibitor of all PKC isoforms) and rottlerin (a commonly used PKC inhibitor) impaired cardiomyocyte viability in association with a marked increase in the phosphorylation of Akt (as well as changes in the phosphorylation of a host of other signaling kinases) and were avoided in this study; this is consistent with recent literature that impugns the selectivity/efficacy of several compounds touted to be useful PKC inhibitors.25,26 A pharmacological approach to explore the role of PKC isoforms in the inhibitory modulation of Akt by rPMT also was not informative, because prolonged incubations with PKC inhibitors alone (ie, the controls required to study rPMT’s actions) reduced cardiomyocyte viability and/or markedly altered Akt phosphorylation. This could reflect a PKC requirement for cell viability or alternatively a nonspecific toxic effect of these compounds that becomes manifest with prolonged incubations; it precludes conclusions from long-term experiments in neonatal cardiomyocyte cultures with pharmacological PKC antagonists. Hence, although these studies cannot exclude a PKC-independent mechanism that contributes to rPMT repression of Akt phosphorylation, the observations that nPKC isoforms are activated in rPMT-treated cardiomyocytes and that nPKC isoforms mediate Akt repression by PMA supports the provisional conclusion that nPKC isoforms contribute to inhibitory modulation of Akt by rPMT.

Discussion

Previous studies using molecular strategies to identify the signaling properties of Gαq in cardiomyocytes provided unanticipated evidence that Gαq induces a continuum of responses, from compensated hypertrophy to decompensated heart failure, as the stimulus strength increases or is maintained over prolonged intervals. Although this suggests that hypertrophy and apoptosis may be initiated by a common Gαq-activated biochemical signal, few molecular signals fulfill this requirement. Studies reported herein, using rPMT to identify signals emanating from endogenous cardiomyocyte Gαq proteins, emphasize a role for PKC isoforms in this...
process. The observation that Goq stimulation by rPMT activates PLC, nPKC isoforms, and MAPK cascades (ERK, with lesser activation of JNK and p38-MAPK) and that these pathways act as potent signals for cardiomyocyte hypertrophy was anticipated. However, the evidence that Goq stimulation by rPMT (or direct activation of nPKC isoforms by PMA) represses Akt phosphorylation and prevents its recruitment by ligands that activate receptor tyrosine kinases was unexpected. These results suggest a novel model that places PKC pivotal upstream in pathways that both promote hypertrophy and render cardiomyocytes susceptible to apoptosis (Figure 8). This model provides an appealing explanation for the characteristic progression from hypertrophy to cardiac decompensation observed in clinical syndromes of heart failure in humans. It would explain studies in transgenic mice where modest levels of Goq overexpression generally are tolerated, but stresses (pregnancy, parturition, or aortic banding) result in a lethal dilated cardiomyopathy; stresses or the insults that characterize the progression of most cardiac diseases would be poorly tolerated by cells that cannot recruit the Akt pathway.

Goq11-PCRs employ divergent pathways to initiate signaling via the ERK cascade. This heterogeneity has been attributed to differences in the identity of the GPCR as well cell type–specific differences in endogenous signaling machinery. Recent studies identify transactivation of EGF receptors as a prominent mechanism leading to ERK activation by Goq11-PCRs (and rPMT) in experimental model systems. Studies reported herein identify a similar pathway for rPMT activation of ERK in cardiac fibroblasts. However, consistent with previous evidence that signaling in cardiac fibroblasts and cardiomyocytes differs, studies reported herein establish that ERK activation by Goq subunits does not require EGF receptor kinase activity in cardiomyocytes. These studies establish the utility of rPMT as a reagent to define the signaling properties and cellular actions of endogenous Goq subunits in cardiomyocytes and emphasize the need for caution when applying results obtained in model systems to the heart.

There is recent interest in growth factor–dependent pathways leading to PI-3K/Akt activation as a mechanism for cell survival, proliferation, and differentiation. While growth responses typically reflect a dynamic balance between stimulatory and inhibitory pathways, mechanisms that curtail Akt activation are less well understood and there is only very limited information on Akt regulation by phorbol ester–sensitive PKC isoforms. To date, most of the studies have focused on the atypical PKCζ, which coimmunoprecipitates with Akt and attenuates its phosphorylation in CHO and breast cancer cells. A physical association between certain phorbol ester–sensitive PKC isoforms (PKCα and PKCδ) and the PH-domain of Akt has been reported, although the importance of this interaction (or the conditions that favor this pathway) has been questioned. Other studies identify a range of effects of phorbol ester–sensitive PKC isoforms on Akt, with PKCα overexpression increasing Akt activity (and reducing apoptosis) in myeloid cells, evidence interpreted as implicating PKCδ in the repression of IGF-1–dependent activation of Akt in PC12 cells, and a kinase-deficient mutant of PKCε (but not wild-type PKCε or kinase-dead PKCα or PKCζ) inhibiting Akt phosphorylation/activation by insulin, heat shock, or H2O2 in L6 myotubes and CHO cells. On the basis of these divergent PKC isoform-dependent effects on Akt, the consequences of PKC activation on Akt phosphorylation in cardiomyocytes could not be predicted. Studies reported herein demonstrate that persistent Goq stimulation with rPMT targets to the selective activation of nPKC isoforms, which repress Akt phosphorylation in cardiomyocytes. The relative importance of individual PKC isoforms in the cellular actions of rPMT could not be resolved with available pharmacological PKC inhibitors because these agents alone impair cell viability in association with changes in the phosphorylation state of various signaling kinases during long-term treatment protocols. The interpretation of studies that use a molecular strategy might be more straightforward; such studies are ongoing, given the potential profound clinical importance of a PKC–dependent pathway for Akt repression that negatively impacts on cardiomyocyte survival.

PKC is a well-recognized mediator of ischemic preconditioning and is generally viewed as cardioprotective. This study suggests that PKC isoforms fulfill a more broad range of cellular functions with very distinct biological consequences. PKC is highly protective when acutely activated in the course of ischemia/reperfusion injury. However, the evidence that PKC represses Akt indicates that prolonged PKC activation (as typically occurs in heart failure with elevated catecholamine levels) also represses survival pathways and would be deleterious to the natural history of heart failure. Evidence that cardioprotection (ischemic preconditioning) and enhanced apoptosis (negative regulation of Akt) reflect the actions of distinct PKC isoforms would provide strong impetus to consider agents targeted to individual PKC isoforms as therapeutic modalities to halt the progression of clinical heart failure syndromes. This would be consistent with recent evidence for PKC isoform–selective regulation of MAPK cascades, modulation of cardiac injury during ischemic insults, and induction of apoptosis. Moreover, this

Figure 8. Schematic of rPMT signaling pathways in cardiomyocytes. rPMT induces sustained Gq stimulation, which stimulates nPKC isoforms; nPKC stimulates ERK (and to a lesser extent JNK and p38–MAPK) and negatively regulates the Akt survival pathway.
type of interplay between receptor tyrosine kinases and PKC activators (that positively and negatively regulate Akt, respectively) could have more broad clinical implications that extend to the pathogenesis and therapy of other diseases, including insulin-resistant diabetes.

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References

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