Protein Kinase D Links Gq-coupled Receptors to cAMP Response Element-binding Protein (CREB)-Ser133 Phosphorylation in the Heart*

Received for publication, December 3, 2007, and in revised form, March 6, 2008. Published, JBC Papers in Press, March 31, 2008, DOI 10.1074/jbc.M709861200

Nazira Ozgen1, Maria Obreztchikova1, Jianfen Guo1, Hasne Alouardighi‡, Gerald W. DornII, Brenda A. Wilson‡, and Susan F. Steinberg‡2

From the 1Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032, the 2University of Cincinnati, Cincinnati, Ohio 45229, and the 3Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Many growth regulatory stimuli promote cAMP response element-binding protein (CREB) Ser133 phosphorylation, but the physiologically relevant CREB-Ser133 kinase(s) in the heart remains uncertain. This study identifies a novel role for protein kinase D (PKD) as an in vivo cardiac CREB-Ser133 kinase. We show that thrombin activates a PKCa-PKD pathway leading to CREB-Ser133 phosphorylation in cardiomyocytes and cardiac fibroblasts. α1-Adrenergic receptors also activate a PKCa-PKD-CREB-Ser133 phosphorylation pathway in cardiomyocytes. Of note, while the epidemical growth factor (EGF) promotes CREB-Ser133 phosphorylation via an ERK-RSK pathway in cardiac fibroblasts, the thrombin-dependent EGFR transactivation pathway leading to ERK-RSK activation does not lead to CREB-Ser133 phosphorylation in this cell type. Adenoviral-mediated overexpression of PKCa (but not PKCe or PKCa) activates PKD; PKCa and PKD1-S744E/S748E overexpression both promote CREB-Ser133 phosphorylation. Pasteurella multocida toxin (PMT), a direct Goq agonist that induces robust cardiomyocyte hypertrophy, also activates the PKD-CREB-Ser133 phosphorylation pathway, leading to the accumulation of active PKD and Ser133-phosphorylated CREB in the nucleus, activation of a CRE-responsive promoter, and increased Bcl-2 (CREB target gene) expression in cardiomyocyte cultures. Cardiac-specific Goq overexpression also leads to an increase in PKD-Ser744/Ser748 and CREB-Ser133 phosphorylation as well as increased Bcl-2 protein expression in the hearts of transgenic mice. Collectively, these studies identify a novel Goq-PKCa-PKD-CREB-Ser133 phosphorylation pathway that is predicted to contribute to cardiac remodeling and could be targeted for therapeutic advantage in the setting of heart failure phenotypes.

Extracellular ligands stimulate cardiac growth and differentiation by activating a network of protein kinases that phosphorylate transcription factors and alter gene expression. Many of these mechanisms are resurrected in the damaged or failing heart in an attempt to compensate for contractile dysfunction. Our previous studies focused on the cellular actions of thrombin, a serine protease that is generated at sites of cardiac injury and proteolytically activates protease-activated receptor-1 (PAR-1).3 A G protein-coupled receptor that activates a spectrum of effectors that contribute to cardiac fibroblast proliferation and cardiomyocyte hypertrophy (1). Certain aspects of PAR-1 signaling are cell-specific; PAR-1 activates ERK primarily via an epidermal growth factor receptor (EGFR) transactivation pathway in cardiac fibroblasts and a distinct pathway that does not require EGFR kinase activity in cardiomyocytes. Of note, the PAR-1 signaling pathway in cardiomyocytes triggers a form of cellular remodeling that resembles the changes observed in dilated cardiomyopathies (with pronounced cell elongation and relatively little increased cell width). This hypertrophic phenotype is morphologically distinct from the concentric hypertrophy induced by α1-AR agonists such as norepinephrine (NE) or Pasteurella multocida toxin (PMT, a direct Goq agonist); NE and PMT induce very pronounced increases in overall cell size in association with enhanced sarcomeric organization and atrial natriuretic factor expression (2).

cAMP response element-binding protein (CREB) is a bZip transcription factor that forms homo- or heterodimers with itself or with other CREB/ATF family members and binds to specific DNA elements (termed cAMP response elements or CREs) within the regulatory regions of CREB target genes. CREB has been implicated in the maintenance of normal ventricular structure and function; cardiac-specific overexpression of dominant-negative CREB leads to dilated cardiomyopathy and interstitial fibrosis (3). CREB also has been implicated in the electrophysiological remodeling that accompanies pacing-induced cardiac memory in dogs (4). CREB is regulated via phosphorylation at Ser133, which activates CREB-dependent gene transcription by recruiting a coactivator (CREB-binding protein, or CBP) to the promoters of CREB target genes.

3 The abbreviations used are: PAR, protease-activated receptor; PKD, protein kinase D; PKC, protein kinase C; EGF, epidermal growth factor; CREB, cAMP response element-binding protein; PMT, Pasteurella multocida toxin; ERK, extracellular signal-regulated kinase; HA, hemagglutinin; WT, wild type; RSK, ribosomal S6 kinase; AR, adrenergic receptor; PKA, cAMP-dependent protein kinase; MSK, mitogen- and stress-activated protein kinase; MAPK, mitogen-activated protein kinase; MOI, multiplicity of infection; NE, norepinephrine; PMA, phorbol 12-myristate 13-acetate.

6 This work was supported, in whole or in part, by National Institutes of Health Grants HL77860, HL-67101, and HL-28958. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

7 These authors contributed equally to this study.

8 To whom correspondence should be addressed: College of Physicians and Surgeons, Columbia University, 630 West 168 St., New York, NY 10032. Tel.: 212-305-4297; Fax: 212-305-8780; E-mail: sfs1@columbia.edu.
PKD Is a Gαq- and PKCd-activated CREB-S133 Kinase

Whereas cAMP-dependent protein kinase (PKA) was the first enzyme implicated as a CREB-Ser133 kinase, CREB-Ser133 phosphorylation by other enzymes (including calcium/calmodulin-dependent kinase, p90 kDa ribosomal S6 kinase (RSK), mitogen- and stress-activated protein kinase 1 (MSK1), and AKT) also has been reported (5). The physiologically relevant CREB kinase(s) in the heart remains uncertain. ERK/p38-MAPK pathways (converging on RSK, MSK1, and/or PKA) have been implicated in α1-AR and endothelin-1 receptor-dependent CREB-Ser133 phosphorylation (6, 7), whereas phosphoinositide-3 kinase and MEK/ERK are reported to link insulin-like growth factor-1 to CREB-Ser133 phosphorylation and protection from apoptosis in cardiomyocytes (8). Because stimulus and cell-specific differences in CREB-Ser133 phosphorylation mechanisms are quite common, other mechanisms may predominate in other pathophysiologic contexts.

PMA treatment increases CREB-Ser133 phosphorylation in many cell types, but the role of PKC isoforms (and their downstream effectors) in CREB-Ser133 phosphorylation remains uncertain. Whereas CREB was originally described as a brain nuclear phosphoprotein that is directly phosphorylated in vitro by PKC (5), the general consensus in the literature is that PKCs increase CREB-Ser133 phosphorylation indirectly by activating ERK-RSK or ERK/p38-MAPK-MSK pathways (9). This study identifies PKD as an alternative PKCd-activated CREB-Ser133 kinase in the heart.

EXPERIMENTAL PROCEDURES

Materials—All antibodies were from Cell Signaling Technology with the following exceptions: anti-PKCδ (Santa Cruz Biotechnology), PKCa (Upstate Biotechnology), and PKCe (BD Transduction).

Cell Culture—Cardiomyocytes were isolated from the hearts of 2-day-old Wistar rats by a trypsin dispersion procedure using a differential attachment procedure to enrich for cardiomyocytes followed by irradiation as detailed in previous publications (10, 11). The final yield of cells was typically 2.5–3 × 10⁶ per neonatal heart. Cells were plated on protamine sulfate-coated culture dishes at a density of 5 × 10⁶ cells/100-mm dish. Experiments were performed on cultures grown for 5 days in MEM (Invitrogen) supplemented with 10% fetal calf serum and then serum-deprived for a subsequent 24-h interval. Nonmyocyte fibroblast-like cells, derived from the cells that adhered to the plastic culture dishes during the preplating step, also were prepared according to methods described previously for some experiments (12). Primary cardiac fibroblast cultures were maintained in MEM supplemented with 10% fetal calf serum for 6 days and then serum-deprived for 24 h prior to experimental protocols.

Adenoviral Infections—Cardiomyocytes were infected with adenoviral constructs that drive expression of HA-tagged PKD1-S744E/S748E (a PKD construct with phosphomimetic substitutions in the activation loop) generously provided by Drs. Terry Rogers and William Randall, University of Maryland), wild-type (WT) or kinase-dead (KD) PKCd (K436R substitution at the catalytic domain ATP binding site), WT-PKCe, WT-PKCa, or β-galactosidase (β-gal) as a control. For studies depicted in Figs. 5 and 6, infection was performed on cultures grown for 5 days in MEM supplemented with 10% fetal calf serum (with protein extracts prepared 48 h following infections) according to the methods described previously (13). This protocol was modified for studies depicted in Fig. 4. Here, infections were performed on day 1 cultures, with protein extracts prepared on day 4 (a modification of the protocol that leads to high levels of transgene expression for both PKD and PKC isoforms) at 10–50-fold lower MOIs, without any detectable differences in the signaling responses pertinent to this study.

Immunoblotting Studies—Immunoblotting was performed on cell extracts according to the methods described previously or the manufacturer’s instructions (14). In each figure, each panel represents the results from a single gel (exposed for a uniform duration); detection was with enhanced chemiluminescence. All results were replicated in at least four experiments on separate culture preparations.

Immunoblot analysis was performed on nuclear and cytosolic fractions in some experiments. Nuclear fractions were pelleted by subjecting cell lysates recovered in a Tris (10 mM, pH 7.4) buffer containing EGTA (1 mM), sucrose (250 mM), sodium pyrophosphate (10 mM), phenylmethylsulfonyl fluoride (1 mM), aprotinin (10 μg/ml), leupeptin (10 μg/ml), Na₃VO₄ (1 mM), NaF (100 mM), calyculin A (0.1 μM), and pepstatin (5 μM) to centrifugation for 5 min at 800 × g. The supernatant from this centrifugation was then cleared of heavy and light membranes by a second centrifugation at 10,000 × g for 1 h; the supernatant from this second centrifugation was used as the cytosolic fraction.

Immunoblotting studies on ventricles from transgenic mice (FVB/N background) that overexpress Gαq under the control of the full-length mouse α-myosin heavy chain promoter (Tg-Gαq) and littermate controls were performed according to methods published previously (15).

Luciferase Assays—pCRE-luciferase (Stratagene) and Renilla luciferase (Promega) vectors were introduced into cardiomyocytes using a nucleofector kit (Amaxa Inc) according to the manufacturer’s instruction. Cre-luciferase signals were normalized to luminescence from Renilla (to eliminate interassay variability and control for agonist-dependent changes in transfection efficiency or cell viability) using a dual-luciferase reporter assay system (Promega).

RESULTS

Thrombin Promotes CREB-Ser133 Phosphorylation via PKD (and Not the EGFR Transactivation-ERK-RSK Pathway) in Cardiac Fibroblasts—The initial studies examined whether RSK and its downstream effector CREB mediate the cellular actions of the PAR-1 in cardiac fibroblasts (under conditions where PAR-1 stimulates the MEK-ERK cascade primarily through an EGFR transactivation pathway, Ref. 14). Fig. 1A shows that thrombin, EGF, and PMA activate ERK and RSK; ERK and RSK phosphorylation/activation are tracked using an immunoblotting approach with phosphorylation site-specific antibodies (PSSAs) that recognize the activated forms of ERK1/2 (ERK1 dually phosphorylated at Thr202/Tyr204 or ERK2 dually phosphorylated at Thr185/Tyr187) and RSK (RSK phosphorylated at Thr237/238). Thrombin activates ERK and RSK with similar kinetics; both responses are blocked by the MEK
inhibitor U0126 and the EGFR inhibitor AG1478 (Fig. 1, B and E). Control experiments show that AG1478 also abrogates direct EGF-dependent ERK and RSK activation (Fig. 1B). In contrast, PMA-dependent ERK and RSK activation is markedly attenuated by U0126; PMA responses are not blocked by AG1478 (Fig. 1B).

Because RSK might act as a mitogen-activated CREB-Ser133 kinase, we examined whether PAR-1 promotes CREB-Ser133 phosphorylation in cardiac fibroblasts. Fig. 1A shows that thrombin, EGF, and PMA increase CREB-Ser133 phosphorylation. Thrombin-dependent CREB-Ser133 phosphorylation is rapid (detectable at 2 min) and sustained (for at least 30 min of stimulation, Fig. 1A). This contrasts with thrombin-dependent signaling to ERK and RSK, which wanes by 10–30 min of stimulation. EGF-dependent CREB-Ser133 phosphorylation persists in U0126- and AG1478-treated cultures; AG1478 and U0126 also do not inhibit PMA-dependent CREB-Ser133 phosphorylation (Fig. 1, B and E). These results indicate that thrombin promotes CREB-Ser133 phosphorylation via an ERK-RSK-independent pathway in cardiac fibroblasts. While our previous studies implicated p38-MAPK as another downstream target of thrombin-activated PAR-1, CREB-Ser133 phosphorylation also is not blocked by SB203580 (a p38 MAPK inhibitor, data not shown).

We previously demonstrated that thrombin does not increase cAMP accumulation in cardiac fibroblasts (17). Hence, thrombin-dependent CREB-Ser133 phosphorylation is not likely to be mediated by PKA. Rather, thrombin increases intracellular calcium and could activate a calcium-dependent CREB-Ser133 kinase (14,
PKD Is a Gαq- and PKCδ-activated CREB-S133 Kinase

Therefore, the role of calcium-dependent enzymes was considered in experiments with BAPTA, a calcium chelator that can be used to clamp intracellular calcium at low levels (including during stimulation by thrombin). The observation that BAPTA does not block thrombin-dependent ERK and CREB-Ser133 phosphorylation (under conditions where BAPTA completely abrogates ionomycin-dependent increases in ERK and CREB phosphorylation), effectively excludes a role for a calcium-dependent enzyme such as CaMK or calcium-sensitive PKC isoforms as thrombin-activated CREB-Ser133 kinases (data not shown).

PKC inhibitors block certain thrombin-activated signaling responses, but the PKC isoforms activated by PAR-1 in cardiac fibroblasts have never been identified. PKCδ activation is associated with the phosphorylation of a conserved activation loop residue (Thr505), a modification that increases the activity of the membrane-targeted allosterically activated enzyme (13). Fig. 1, A and C show that PKCδ is recovered from resting cardiac fibroblast cultures without Thr505 phosphorylation; thrombin and PMA promote PKCδ-Thr505 phosphorylation. Agonist-dependent PKCδ-Thr505 phosphorylation is blocked by GF109203X (a non-selective inhibitor of all PKC isoforms), but not by Gö6976 (an inhibitor that selectively blocks conventional PKC (cPKC) isoforms, but does not block novel PKC (nPKC) isoforms such as PKCδ and PKCe). These results indicate that PKCδ-Thr505 phosphorylation requires nPKC activity. The evidence that PKCδ is a downstream component of the thrombin-activated signaling pathway provides a rationale to consider a role for PKCs in the thrombin-dependent CREB-Ser133 phosphorylation pathway. Fig. 1, A and C show that GF109203X and Gö6976 block thrombin- and PMA-dependent CREB-Ser133 phosphorylation. Whereas GF109203X also attenuates thrombin and PMA-dependent ERK activation, ERK activation is not inhibited by Gö6976. Collectively, these results identify two distinct CREB-Ser133 phosphorylation pathways (schematized in Fig. 1F). Ligand-activated EGFRs promote CREB-Ser133 phosphorylation via the ERK-RSK pathway. Whereas PAR-1 also activates ERK-RSK via an EGFR transactivation pathway, this does not lead to CREB-Ser133 phosphorylation. Rather, thrombin-dependent CREB-Ser133 phosphorylation is via a distinct ERK-RSK-independent pathway that is inhibited by GF109203X or Gö6976.

While cPKC isoforms are directly inhibited by both GF109203X and Gö6976, this pharmacologic profile also is a signature of the cellular actions of PKD, a family of three structurally related nPKC-activated serine-threonine kinases that are directly inhibited by Gö6976 (18). PKD1 preferentially phosphorylates peptides with a +1 position hydrophobic amino acid and a −5 position leucine (relative to the phosphoryl-erine, Ref. 19). CREB-Ser133 is flanked by a sequence conforming to an optimal PKD consensus phosphorylation motif (Leu-Ser-Arg-Arg-Pro-Ser133-Tyr) and has recently been identified as a PKD substrate (20), providing a rationale to consider a role for PKD as a PKC-activated CREB-Ser133 kinase.

PKD activation is via translocation to membranes and phosphorylation at Ser744/Ser748 in the activation loop; PKD-Ser744/Ser748 phosphorylation is generally attributed to nPKC isoforms and is followed by PKD-Ser916 autophosphorylation. Fig. 1A shows that thrombin and PMA increase in PKD-Ser744/Ser748 and PKD-Ser916 phosphorylation; these agonists also induce a modest PKD electrophoretic mobility shift (indicative of PKDphosphorylation). PKDionactivatedbyEGF. Thrombin-dependent PKD activation is detected at 5 min and persists for at least 30 min of continuous stimulation. The activated form of PKD in thrombin- and PMA-treated cultures is resolved as a doublet. Similar molecular heterogeneity for PKD has been identified in other studies with these antibodies and both cardiac and non-cardiac preparations (21, 22). This could represent a single PKD isoform with distinct phosphorylation patterns (and therefore electrophoretic mobilities) or different PKD isoforms, because the 115,000 Da PKD1 and 105,000 Da PKD2 proteins are coexpressed in many tissues, including the heart, and both would be recognized by these antibodies (22, 23). In either case, thrombin- and PMA-dependent PKD phosphorylations are fully blocked by GF109203X, but not by AG1478 or U0126 (Fig. 1D). These results implicate PKD as a PKC-activated component of the PAR-1 signaling pathway.

While immunoblotting studies with anti-PKD1 protein antibodies show activation-dependent PKD1 electrophoretic mobility shifts (Fig. 1A), it should be noted that most commercially available anti-PKD1 protein antibodies (including the reagent used in this study) provide imperfect loading controls because they were raised against a synthetic peptide corresponding to a C-terminal sequence flanking the Ser916 autophosphorylation site; previous studies have convincingly demonstrated that these antibodies preferentially recognize the inactive form of PKD1 that is not phosphorylated at this C-terminal epitope (24, 25). Therefore, immunoblotting for PKCδ protein (which band shifts, but is not influenced by PKCδ phosphorylation) also is included as a loading control in Fig. 1A.

Thrombin Activates a PKD-dependent Pathway Leading to CREB-Ser133 Phosphorylation in Cardiomyocytes—Our previous studies showed that certain PAR-1 signaling responses (particularly those mediated by the EGFR transactivation pathway) vary substantially according to cell context; the EGFR transactivation pathway is prominent in cardiac fibroblasts, but not in cardiomyocytes. Because PAR-1 activates PKD and promotes CREB-Ser133 phosphorylation via a mechanism that does not require EGFR kinase activity in cardiac fibroblasts, we tested the hypothesis that PAR-1 acts in a similar manner to stimulate PKD and CREB in cardiomyocytes (and that this PKD-CREB-Ser133 phosphorylation pathway also would be activated by other hypertrophic stimuli, including the α1-AR agonist NE).

Fig. 2, A and B show that thrombin, NE (at concentrations ≅10−10 M), and PMA activate PKCδ and PKD in cardiomyocytes (detected as increased PKCδ-Thr505, PKD-Ser744/Ser748, and PKD-Ser916 phosphorylation); PMA markedly slows PKD migration in SDS-PAGE, whereas effects of thrombin and NE to slow PKD mobility are more modest (and detected more variably between experiments). Thrombin-dependent responses are detected at 5 min and wane by 20–30 min of continuous stimulation, whereas NE-dependent responses are sustained for at least 30 min. Of note, NE-dependent PKCδ/PKD activation is mediated by an α1-AR-dependent pathway that is blocked by prazosin (α1-AR blocker), but not by propranolol.
\(\beta\)-AR activation with isoproterenol does not lead to PKC\(\delta\) or PKD phosphorylation. PKD is resolved as a Ser\(^{916}\)-phosphorylated doublet in PMA- and thrombin-treated cardiomyocytes (similar to the findings in cardiac fibroblasts, compare Figs. 1A and 2A). In contrast, NE selectively activates a single molecular form of PKD. Similar agonist-dependent differences in PKD activation (with certain stimuli leading to the accumulation of only certain molecular forms of PKD) have been identified in other cell types. Although the significance of this finding remains uncertain, Fig. 2C shows that all measures of PKD activation (including the agonist-dependent increases in PKD-Ser\(^{744}/748\) or -Ser\(^{916}\) phosphorylations and the PMA-dependent PKD band shift) are blocked by GF109203X (i.e. are mediated by PKC).

Thrombin, NE, and PMA also promote CREB-Ser\(^{133}\) phosphorylation (Fig. 2A). NE-dependent CREB-Ser\(^{133}\) phosphoryl-

**FIGURE 2.** NE and thrombin activate a PKC\(\delta\)-PKD-CREB-Ser\(^{133}\) phosphorylation pathway in cardiomyocytes. **A,** cardiomyocytes were treated with NE (10 \(\mu M\)) or thrombin (1 \(nM\)) for the indicated intervals, or with PMA (300 \(nM\)) for 20 min. **B,** cardiomyocytes were pretreated for 45 min with vehicle, prazosin (0.1 \(\mu M\)), or propranolol (0.1 \(\mu M\)) and then subjected to stimulation with isoproterenol (0.1 \(\mu M\), 5 min), the indicated concentrations of NE (for 5 min), or PMA (300 \(nM\), 10 min). **C–E,** inhibitor treatments were according to protocols described in the Fig. 1 legend; stimulations were for 5 min with 1 \(nM\) thrombin, 10 \(\mu M\) NE, 300 \(nM\) PMA, 100 \(nM\) EGF, or 0.1 \(mM\) \(H_2O_2\). Immunoblotting was on cell extracts according to “Experimental Procedures,” with data quantified in **E** (\(n=4\)). Of note, PKD is detected in thrombin-treated cardiomyocytes as a doublet with the anti-PKD-pSer\(^{916}\) PSSA and as a single band with the anti-PKD1 protein antibody; similar molecular heterogeneity for PKD has been identified in previous studies (including cardiac preparations, Refs. 21, 22). This could be attributable (at least in part) to the distinct molecular forms of PKD that are identified by the anti-PKC-pSer\(^{744}/748\) antibody (which in theory recognizes activated forms of PKD1, PKD2, and PKD3), the anti-PKD-pSer\(^{916}\) antibody (which should recognize activated PKD1 and PKD2, but not PKD3, an isoform that lacks this phosphorylation site) and the Cell Signaling Technologies anti-PKD1 antibody (which may cross-react to a variable degree with other PKD isoforms, but preferentially recognizes the inactive, not phosphorylated, form of the enzyme; see text).
PKD Is a $G_{q_1}$- and PKCδ-activated CREB-Ser133 Kinase

**CARDIOMYOCYTES**

A. 

| PKD-pS744/S748 | PKD-pS748 | PKD | CREB-pS133 | CREB | pERK | ERK | Bcl-2 |
|----------------|-----------|-----|-------------|------|------|-----|-------|
| PMT: -          | +         |      | -           | -    | -    | -   | -     |
| PMT: +          | -         |      | -           | -    | -    | -   | -     |

B. 

![Image](373x26 to 400x38)

**FIGURE 3.** PMT activates PKD, promotes CREB-Ser133 phosphorylation, and increases Bcl-2 expression in cardiomyocytes. A, cardiomyocytes were treated for 24 h with vehicle or 400 ng/ml PMT (without or with vehicle, 5 μM Gö6976, or 5 μM U0126 in B). Immunoblotting was on cell extracts, according to "Experimental Procedures," with the results quantified in C (n = 4).

C. 

![Image](49x505 to 408x733)

**FIGURE 4.** Ad-PKD-S744E/S748E overexpression activates a CREB-Ser133 phosphorylation pathway that is further augmented by PMA in cardiomyocytes. Day 1 cardiomyocyte cultures were infected with adenoviruses that drive expression of PKD1-S744E/S748E (Ad-PKD1-SS/EE) or β-gal as a control (at an MOI of 5, unless indicated otherwise in A). Protein extracts were prepared 3 days later, with vehicle or PMA treatment (alone or in the presence of GO19203X or Gö6976, according to protocols described in the legend to Fig. 1) for the final 20 min of this interval in B. Immunoblotting was with the indicated antibodies on whole cell extracts according to "Experimental Procedures." Exposure times were optimized to visualize regulatory events on indicated antibodies on whole cell extracts according to "Experimental Procedures," with the results quantified in C (n = 4).

**Ga$q_1$ Activates PKD and Increases CREB-Ser133 Phosphorylation in Cardiomyocytes—Harrison et al.** (22) previously reported that phenylephrine activates PKD for at least 24 h in cardiac cultures. However, our preliminary studies failed to detect prolonged thrombin- or NE-dependent PKD activation under our culture conditions (perhaps resulting from differences in the culture conditions used in our laboratory, data not shown). Therefore, we performed additional studies with PMT, a $G_{q_1}$ agonist that induces pronounced cardiomyocyte hypertrophy in association with a robust increase in nPKC and ERK activity that is sustained for at least 24–48 h (2). Fig. 3A shows that PMT increases PKD-Ser744/Ser748, PKD-Ser916, and CREB-Ser133 phosphorylation. While PMT also activates ERK, PMT-dependent CREB-Ser133 phosphorylation is markedly inhibited by Gö6976 (which does not inhibit ERK) and only modestly reduced by U0126 (under conditions that markedly inhibit PMA-dependent CREB phosphorylation, Fig. 3, B and C). PMT-dependent CREB-Ser133 phosphorylation is completely abrogated by combined treatment with Gö6976 and U0126. These results implicate a PKD-dependent pathway (with only a minor contribution from the MEK-ERK-RSK pathway) in PMT-dependent CREB-Ser133 phosphorylation. The physiologic significance of this mechanism is suggested by the further observation that PMT markedly increases the expression of Bcl-2, a known CRE-responsive gene product (4.2 ± 0.3-fold over basal, n = 5, Fig. 3A).

**PKD Overexpression Leads to CREB-Ser133 Phosphorylation in Cardiomyocytes—We used an adenoviral overexpression strategy with Ad-PKD1-S744E/S748E (a PKD construct that harbors phosphomimetic substitutions in the activation loop) to explore the role of PKD1 as an in vivo CREB kinase. Fig. 4A shows that Ad-PKD1-S744E/S748E at an MOI of 0.2–5 plaque-forming units (pfu)/cell leads to a dose-dependent increase in PKD1-S744E/S748E overexpression and CREB-Ser133 phosphorylation in resting cardiomyocytes cultures. Similar results
PKD Is a Goαq- and PKCδ-activated CREB-S133 Kinase

FIGURE 5. PKCδ activates a PKD-CREB-Ser133 phosphorylation pathway in cardiomyocytes. Adenoviral-mediated gene transfer was used to overexpress PKD1-S744E/S748E (PKD1-SS/EE), WT-PKCδ, KD-PKCδ, WT-PKCε, WT-PKCα, or β-gal as a control. Infections (MOI 50) were initiated on culture day 2, with protein extracts prepared 48 h following infection as described under “Experimental Procedures.” Cultures were treated with vehicle or 400 ng/ml PMT for the final 24 h in A; treatment was with vehicle or 10 μM NE for the final 10 min in B. Immunoblotting was with the indicated antibodies on cell extracts according to the “Experimental Procedures.” Note, endogenous PKD and PKC are not detected in A because conditions are optimized to detect the heterologously overexpressed enzymes; PKD and PKCα are readily detected with increased protein loading and longer exposure times (i.e. the conditions used in Figs. 2 and 3). These results were replicated in six separate experiments.

FIGURE 6. PMT treatment leads to the nuclear accumulation of active PKD and CREB and transactivation of a CRE-luciferase reporter in cardiomyocytes. Cardiomyocytes were treated for 24 h with vehicle or 400 ng/ml PMT or infected with adenoviruses that drive PKD1-S744E/S748E (PKD1-SS/EE) or β-gal overexpression. A and B, cytosol and nuclear fractions were subjected to immunoblotting with the indicated antibodies according to the “Experimental Procedures.” The fractionation scheme was validated by immunoblotting for PCNA, a nuclear marker recovered in nuclear but not cytosolic fractions. It is worth noting that the PMT-dependent increase in CREB protein immunoreactivity in the nuclear fraction represents a relatively minor pool of total cellular CREB protein and hence a reciprocal PMT-dependent decline in CREB protein in the cytosolic fraction (which contains high levels of CREB protein immunoreactivity) is not resolved under these conditions. Furthermore, it should be noted that protein loading was reduced 5-fold to probe for the heterologously overexpressed PKD and PKC transgenes in an active conformation at membranes.

were obtained in cardiac fibroblast cultures (data not shown). These results implicate PKD1 as an upstream component of the CREB-Ser133 phosphorylation pathway.

While the PKD1-S744E/S748E mutant has been characterized as a constitutively active enzyme (largely on the basis of studies in COS7 or HeLa cells (26, 27)), Fig. 4B provides unanticipated evidence that PMA treatment leads to a further increase in both PKD1-S744E/S748E-Cre916 and CREB-Ser133 phosphorylation in cardiomyocytes. The effects of PKD1 overexpression on PKD1-S744E/S748E-Cre916 and CREB-Ser133 phosphorylation are blocked by G66976. These PMA responses are not blocked by GF109203X, consistent with the notion that activation loop Ser → Glu substitutions bypass the PKC requirement for PKD1 activation. This is in marked contrast to PMA-dependent activation of endogenous PKD1, which is via a PKC-dependent pathway and is abrogated by GF109203X (see Figs. 1 and 2, as well as the prolonged gel exposures in Fig. 4B). These results suggest that the PKD1-S744E/S748E enzyme displays some basal level of constitutive activity in cardiomyocytes, but that maximal PKD1-S744E/S748E activity requires a lipid cofactor (which presumably releases an intramembrane autoinhibitory interaction and/or stabilizes the PKD1-S744E/S748E transgene in an active conformation at membranes).

PKD1-S744E/S748E overexpression does not lead to any compensatory changes in PKC isoform expression (or signaling via the ERK-RSK pathway, effectively excluding an alternative indirect mechanism for CREB-Ser133 phosphorylation, Fig. 5). Rather, PKCδ overexpression leads to an increase in basal and agonist-activated PKD-Ser744/Ser748, PKD-Ser916 and CREB-Ser133 phosphorylation (without changing the expression of other PKC isoforms or increasing signaling via the ERK pathway, Fig. 5, A and B). KD-PKCδ overexpression blocks NE-dependent PKD-Ser744/Ser748, PKD-Ser916, and CREB-Ser133 phosphorylation (Fig. 5B). KD-PKCδ overexpression also prevents PKD activation by PMT (data not shown). Other PKC isoforms such as PKCε or PKCα do not increase PKD or CREB-Ser133 phosphorylation in cardiomyocytes. Collectively, these results implicate PKCδ as an upstream activator of the PKD-CREB-Ser133 phosphorylation pathway in cardiomyocytes.

Because CREB-Ser133 phosphorylation is required, but not necessarily sufficient, to induce CREB-dependent gene transcription, the subcellular targeting and transactivation function of Ser133-phosphorylated CREB was explored further. Fig. 6A shows that the bulk of cellular PKD and CREB proteins are recovered in the cytosolic fraction, but small amounts of PKD and CREB are detected in the nuclei of resting cardiomyocytes. PMT treatment leads to the accumulation of active PKD (detected as a PKD protein mobility shift and increased PKD-
PKD Is a Goq- and PKCδ-activated CREB-S133 Kinase

FIGURE 7. PKD-Ser744/Ser748 and CREB-Ser133 phosphorylation and Bcl-2 protein expression are increased in Tg-Goq hearts. A, representative immunoblotting on three WT and Goq overexpressing ventricular samples. B, quantification of data with results expressed as fold stimulation in Tg-Goq relative to WT (mean ± S.E.). *, p < 0.05 by unpaired Student’s t test (n = 6).

pSer916 immunoreactivity), increased CREB protein, and enhanced CREB-pSer133 immunoreactivity in the nuclear fraction. Ad-PKD1-S744E/S748E overexpression also leads to an increase in CREB protein (and CREB-pSer133 immunoreactivity) in the nuclear fraction. Nuclear CREB-pSer133 immunoreactivity increases further when Ad-PKD1-S744E/S748E cultures are treated with PMT, which delivers additional active PKD1 to the nuclear fraction (Fig. 6B). Consistent with these findings, Fig. 6C shows that PMT transactivates a Cre-luciferase promoter. While PKD1-S744E/S748E overexpression alone leads to a modest increase in CRE-luciferase activity (that is not statistically significant), CRE-luciferase activity is significantly increased in Ad-PKD1-S744E/S748E cultures treated with PMT (compared with Ad-β-gal cultures treated with PMT). In contrast, direct activation of the cAMP/PKA pathway for forskolin leads to a modest increase in CRE activity that is not significantly augmented by PKD1-S744E/S748E overexpression under these conditions (Fig. 6C).

PKD-Ser744/Ser748 and CREB-Ser133 Phosphorylation and Bcl-2 Expression Are Increased in TG-Goq Hearts—The role of PKD as a Goq effector that promotes CREB-Ser133 phosphorylation was examined in Tg-Goq mouse hearts, which represent an in vivo correlate of the in vitro studies on PMT-treated cardiomyocytes. Fig. 7 shows that ventricular myocardium from Goq mice display elevated levels of PKD-Ser744/Ser748 and CREB-pSer133 phosphorylation as well as increased Bcl-2 expression. Whereas CREB protein also is increased in Tg-Goq hearts, the increment in CREB protein expression is relatively modest compared with the robust increase in CREB-pSer133 phosphorylation, suggesting bona fide activation of the CREB-Ser133 phosphorylation pathway in vivo in Tg-Goq hearts.

DISCUSSION

PKD has recently emerged as a biologically important enzyme that is activated by certain hypertrophic stimuli and contributes to ventricular remodeling (22). Studies to date have focused on the role of PKD as a class II HDAC kinase. PKD-dependent HDAC5-Ser259/Ser498 phosphorylation creates a docking site for 14-3-3 proteins that escort HDAC5 from the nucleus to the cytosol; this neutralizes the repressive effects of class II HDACs on MEF2-dependent transcription and effectively inhibits the HDAC5 anti-hypertrophic actions (28). PKD also has been characterized as a cTnI kinase that reduces myofilament calcium sensitivity by phosphorylating sites on cTnI generally viewed as targets for PKA (29). This study identifies an additional function for PKD as a CREB-Ser133 kinase that links the Goq-PKCδ pathway to CREB-Ser133 phosphorylation in both cardiac fibroblasts and cardiomyocytes.

We previously reported that PAR-1 activates ERK via distinct mechanisms (that differ in their requirements for EGFR activity) in cardiac fibroblasts and cardiomyocytes. However, studies reported herein identify a grossly similar PAR-1-dependent pathway leading to PKD activation and CREB-Ser133 phosphorylation (that does not require EGFR kinase activity) in both cell types. Similarly, while our previous studies identified morphologically distinct forms of hypertrophy in cardiomyocytes treated with PAR-1 and α1-AR agonists, studies reported herein identified the PKCδ-PKD-CREB-Ser133 phosphorylation pathway as a common effector for both PAR-1 and α1-ARs in cardiomyocytes. These results suggest that the PKD-CREB-Ser133 phosphorylation pathway does not underlie the distinct forms of cardiomyocyte hypertrophy observed in NE- and thrombin-treated cultures. However, it would be imprudent to entirely dismiss PKD as a stimulus-specific cardiac effector, because NE and thrombin activate distinct molecular forms of PKD which are presumed to reflect either different PKD isoforms or distinct activation modes/phosphorylation patterns for a single PKD isoform. This molecular heterogeneity in PKD activation could lead to the recruitment of different downstream effectors and the induction of distinct hypertrophic phenotypes. In this regard, PKD is pivotally positioned to control both the forward and backward reaction kinetics of lysine acetylation through the phosphorylation of both CREB (which recruits CBP/p300, a CREB-binding protein with histone acetyl transferase activity) and HDAC5. Agonist-specific differences in the balance of PKD signaling to CREB/CBP/p300 versus HDAC5 could potentially underlie stimulus-specific differences in chromatin remodeling and hypertrophic phenotypes and are the focus of ongoing studies.

Whereas this study focuses primarily on the PKD-CREB-Ser133 phosphorylation pathway in cardiomyocytes, studies in cardiac fibroblasts also are included to emphasize that CREB-Ser133 phosphorylation may be regulated by distinct signaling mechanisms that are recruited in a stimulus-specific manner. Specifically, we show that direct activation of EGFRs (by EGF)
PKD Is a Gαq- and PKCδ-activated CREB-S133 Kinase

leads to the activation of a MEK-ERK-RSK pathway that promotes CREB-Ser133 phosphorylation in cardiac fibroblasts, whereas thrombin (which transactivates EGFRs and increases signaling via the MEK-ERK-RSK pathway) increases CREB-Ser133 phosphorylation via an ERK-RSK-independent pathway involving PKCδ and PKD. A similar PKC-PKD pathway also links PAR-1 to CREB-Ser133 phosphorylation in cardiomyocytes. The disconnect between thrombin-dependent ERK-RSK activation and CREB-Ser133 phosphorylation is surprising given previous evidence that a PKCδ-ERK-RSK pathway links B cell receptor activation to CREB-Ser133 phosphorylation in B cells (30) and evidence that a RSK-2 gene mutation underlies the defect in EGF-dependent CREB-Ser133 phosphorylation in the Coffin-Lowry Syndrome (31)). Our findings could be explained by differences in ERK-RSK pathway compartmentalization in EGF- and thrombin-activated cells. ERKs localize primarily to the cytosol of resting cells; ERK/RSK must shuttle to the nucleus to phosphorylate transcription factors and influence proliferation/differentiation gene programs. Thrombin is reported to activate a pool of ERK that is confined (at least in the initial hours of stimulation) to the cytosol in endothelial cells (32). Studies of the related protease-activated receptor PAR-2 suggests a mechanism, implicating β-arrestin as a scaffold that is recruited to PAR-2 and traps ERK as part of a multiprotein complex in the cytosol (33). In contrast, direct EGFR activation leads to the accumulation of active ERK and RSK in the nucleus where they are positioned to activate the transcription machinery. According to this formulation, stimulus and/or cell-specific differences in ERK/RSK subcellular localization would dictate the biologic importance of RSK as a CREB-Ser133 kinase. In this regard, our studies implicate PKD as the predominant CREB-Ser133 kinase in NE-, thrombin-, PMA-, and PMT-treated cardiomyocytes; our findings do not exclude a potential role for other enzymes (such as PKA, RSK, or CaMK) in the CREB-Ser133 phosphorylation pathway activated by other stimuli (including β-AR activation, calcium overload, or oxidative stress).

Our studies provide novel evidence that PKCδ lies upstream from both PKD activation and CREB-Ser133 phosphorylation in cardiomyocytes. PKD activation has been attributed to various nPKC isoforms in other cellular contexts. For example, PKCe activates heterologously overexpressed PKD in Swiss 3T3 cells (18), PKCγ cooperates with heterotrimeric G protein βγ subunits to activate PKD in the Golgi (18), and PKCδ links thrombin and angiotensin II receptor stimulation to PKD activation in vascular smooth muscle cells (34, 35); PKCδ also cooperates with Abl to stimulate a PKD-activated NF-κB pathway during oxidative stress (27). Our results also implicate PKCδ in the PKD-CREB-Ser133 phosphorylation pathway in the heart. Collectively, these results suggest that PKD might be activated by nPKCs in a stimulus- and/or cell-specific manner as a result of: 1) differences in the subcellular localization patterns of agonist-activated PKC and PKD isoforms (perhaps due to differences in their C1 domain phospholipid requirements, Ref. 37) and/or 2) mechanisms that regulate PKD-nPKC isoform interactions. For example, the PKD-PH domain is reported to form stable complexes with PKCγ (but not PKCe, Ref. 38), and there is recent evidence that the PKCδ C2 domain (which functions as a phosphotyrosine binding domain with a unique specificity) interacts with a consensus recognition motif in PKD (39, 40). Mechanisms that control PKD partnering with individual nPKC isoforms are likely to influence PKD targeting to subcellular microdomains and lead to functionally important differences in PKD signaling to downstream cytosolic (cTnI) and nuclear (CREB, class II HDACs, and NF-κB) targets.

PMA promotes CREB-Ser133 phosphorylation in many cell types, but the role of individual PKC isoforms and their downstream effectors as CREB-Ser133 kinases remains disputed. The early studies from Yamamoto et al. (5) used in vitro kinase assays and two-dimensional tryptic maps to show that PKC and PKA phosphorylate CREB at a similar serine-containing peptide (presumably Ser133). Subsequent studies suggested that PKCα might act as a direct CREB-Ser133 kinase in depolarized skeletal muscle cells (although it is important to note that the role of PKCα as a CREB-Ser133 kinase is based largely on pharmacologic studies with GF109203X and Go6976 that did not consider a potential role for PKD (41)). Other laboratories have focused on RSK and/or MSK as PMA-activated CREB-Ser133 kinases that vary in importance according to the inciting stimulus and cell type (7, 30). However, a model that focuses exclusively on MSK or RSK as the physiologically relevant PMA-activated CREB-Ser133 kinases has become increasingly untenable with observations that PMA-dependent CREB-Ser133 phosphorylation persist in MSK1/2 double knock-out fibroblasts and in cells treated with the selective RSK inhibitor BI-D1780 (9, 42). Our results reconcile some of these discrepancies by implicating that PKD acts as a novel PMA-activated CREB-Ser133 kinase that links PKCδ to CREB-Ser133 phosphorylation in the heart.

This study used an Ad-PKD1-S744E/S748E overexpression strategy to explore PKD signaling function in cardiomyocytes. This construct has been widely used in the literature as a constitutively active PKD1 enzyme based upon studies in COS7 and HeLa cells, where PKD-dependent PKD1-S744E/S748E phosphorylation is essential for PKD1 activation in response to diverse stimuli and activation loop Ser744/Ser748 phosphorylation (or Ser → Glu substitution) is sufficient to induce near maximal in vivo PKD1 activation (although it is interesting to note that even the early studies exposed a modest 10–15% increase in PKD1-S744E/S748E activity in phorbol ester-treated COS7 cells, Refs. 26, 27). Of note, more recent studies have exposed cell-specific differences in basal PKD1-S744E/S748E activity, showing that PKD1-S744E/S748E requires antigen receptor stimulation, treatment with phorbol esters, or deletion of the PH domain for optimal activation in lymphocytes (21). Studies reported herein show that the PKD1-S744E/S748E enzyme also is further activated by PKD in cardiomyocytes. In each case, this appears to reflect a requirement for lipid cofactors that bind to the C1 domain, localize PKD1 properly at membranes, and may play an additional role to release the PKD1 catalytic domain from N-terminal autoinhibition (independent of the DAG requirement for PKC-mediated PKD1 activation loop phosphorylation). Wood et al. (21) have speculated that endogenous DAG levels are sufficient to activate PKD1-S744E/S748E in model cell systems (such as COS7 and HeLa cells), but that endogenous DAG levels are too low (or
improperly compartmentalized) to activate PKD1 in quiescent lymphocytes. Our results identify a similar scenario in cardiomyocytes, emphasizing the importance of studies that identify the molecular determinants of PKD1 activation in highly differentiated cell types. Model-specific differences in PKD-S744E/S748E basal activity could explain the discrepancy in the PKD1 cardiac function noted in the previous literature. Specifically, Harrison et al. (22) reported that PKD1-S744E/S748E overexpression is sufficient to target pathological cardiac remodeling in the mouse heart, but the Ad-PKD1-S744E/S748E construct does not stimulate hypertrophic growth in cardiomyocyte cultures. Whereas these results were interpreted as evidence that PKD1 signaling is sufficient to stimulate cardiac hypertrophy only in the context of the whole animal, our studies could suggest an alternative explanation. It is possible that the Ad-PKD1-S744E/S748E construct is constitutively active and induces hypertrophy in vivo, but this construct requires lipid cofactors to be activated and promote hypertrophy in cardiomyocyte cultures. Alternatively, the balance of PKD signaling to CREB, HDAC5, and potentially other cellular effectors may differ between the in vivo and in vitro models and lead to distinct cardiac phenotypes.

During the course of our studies, Johannessen et al. (43) identified an effect of PKD (both PKD1 and PKD2) to act as an in vitro CREB-Ser133 kinase. These investigators also showed that heterologously overexpressed PKD1-S744E/S748E interacts with CREB, activates CREB-mediated transcription, increases expression of Nur77 (a prototypical CREB-responsive gene), and increases expression of inducible cAMP early repressor (ICER, a CRE-binding protein that lacks a transactivation domain and therefore functions as an endogenous CRE antagonist (43). Of note, studies by Johannessen et al. focused almost exclusively on the signaling functions of heterologously overexpressed PKD1-S744E/S748E in model cell types. In particular, PKD1-S744E/S748E-dependent changes in endogenous gene transcription were examined in HEK293 cells to take advantage of the high transfection efficiency of this cell. Whereas these previous studies provided tentative evidence that PKD1-S744E/S748E induces Nur77 expression via a mechanism that requires CREB (that can be attenuated by dominant-negative CREB variants), the additional observation that dominant-negative CREB variants do not fully abrogate Nur77 expression suggests that PKD1 may also regulate Nur77 expression via additional CREB-independent mechanisms (that could involve class II HDACs or NF-kB, which also are regulated by PKD and have been implicated as transcriptional activators of Nur77, Refs. 36, 44–45). Johannessen et al. also comment in their discussion about problems encountered using an siRNA approach to implicate PKD1 in CREB-dependent changes in Nur77 expression, noting that an siRNA approach that completely depleted cellular PKD1 did not reduce agonist-dependent CREB-Ser133 phosphorylation. This could be due to functional redundancy between different PKD enzymes (which has been encountered in other systems, Ref. 36) or contributions from other signaling molecules with CREB kinase activity (such as PKC isoforms, Msk1, or RSK). In this context, our studies were performed in a more physiologically relevant cardiomyocyte context and implicate PKD in a stimulus-specific pathway that triggers CREB-Ser133 phosphorylation and enhanced expression of Bcl-2 (a CREB-responsive gene that, to the best of our knowledge, is not regulated by class II HDACs or NF-kB). Of note, activation of the PKD-CREB-Ser133 phosphorylation pathway did not lead to a detectable increase in ICER expression in cardiomyocytes (data not shown), emphasizing the cell-specific nature of PKD-CREB signaling and the importance of studies that define PKD signaling in a cardiomyocyte context.

Finally, these studies identify PKD and CREB-Ser133 phosphorylation as downstream targets of the cardiac Goq signaling pathway. Of note, Goq activation has been linked to both hypertrophy and pro-apoptotic mechanism in cardiomyocytes, depending upon the stimulus strength or duration. In this regard, PKD has emerged as a regulator of chromatin remodeling that promotes cardiac hypertrophy by phosphorylating class II HDACs; PKD also plays a dual role to induce apoptosis (via ASK and JNK) or cell survival (via the activation of NF-kB) in other cell types. The observation that PKD activation by Goq leads to CREB-Ser133 phosphorylation and the induction of CRE-responsive genes such as Bcl-2 (which would contribute to agonist-dependent cell survival) suggests that PKD-activated effectors may influence the evolution of cardiac hypertrophy and heart failure phenotypes and that interventions that alter the balance of PKD signaling to CREB and other downstream effectors may have therapeutic benefit.

REFERENCES

1. Steinberg, S. F. (2005) Mol. Pharmacol. 67, 2–11
2. Sabri, A., Wilson, B. A., and Steinberg, S. F. (2002) Circ. Res. 90, 850–857
3. Fentzke, R. C., Korcarz, C. E., Lang, R. M., Lin, H., and Leiden, J. M. (1998) J. Clin. Investig. 101, 2415–2426
4. Patberg, K. W., Obreztchikova, M. N., Giardina, S. F., Symes, A. J., Plotnikov, A. N., Qu, J., Chandra, P., McKinnon, D., Liou, S. R., Rybin, A. V., Shlapakova, I., Danilo, P., Jr., Yang, J., and Rosen, M. R. (2005) Cardiovasc. Res. 68, 259–267
5. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H., III, and Montminy, M. R. (1988) Nature 334, 494–498
6. Markou, T., Hadzopoulou-Cladaras, M., and Lazou, A. (2004) J. Mol. Cell Cardiol. 37, 1001–1011
7. Harrison, J. G., Sugden, P. H., and Clerk, A. (2004) Biochem. Biophys. Acta 1644, 17–25
8. Mehrhof, F. B., Muller, F. U., Bergmann, M. W., Li, P., Wang, Y., Schimitsch, W., Dietz, R., and von Harsdorf, R. (2001) Circulation 104, 2088–2094
9. Sapkota, G. P., Cummings, L., Newell, F. S., Armstrong, C., Bain, J., Frodin, M., Grauert, M., Hoffmann, M., Schnapp, G., Stegemaker, M., Cohen, P., and Alessi, D. R. (2007) Biochem. J. 401, 29–38
10. Lau, Y. H., Robinson, R. B., Rosen, M. R., and Bilezikian, J. P. (1980) Circ. Res. 47, 41–48
11. D’Angelo, D., Sakata, Y., Lorenz, J. N., Boivin, G. P., Walsh, R. A., Liggett, S. B., and Dorn, G. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8121–8126
12. Rybin, V. O., and Steinberg, S. F. (1996) Circ. Res. 78, 388–398
13. Rybin, V. O., Sabri, A., Short, J., Brazy, A. B., Molkentin, J. D., and Steinberg, S. F. (2003) J. Biol. Chem. 278, 14555–14564
14. Sabri, A., Short, J., Guo, J., and Steinberg, S. F. (2002) Circ. Res. 91, 532–539
15. D’Angelo, D., Sakata, Y., Lorenz, J. N., Boivin, G. P., Walsh, R. A., Liggett, S. B., and Dorn, G. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8121–8126
16. Deleted in proof
17. Patberg, K. W., Obreztchikova, M. N., Giardina, S. F., Symes, A. J., Plotnikov, A. N., Qu, J., Chandra, P., McKinnon, D., Liou, S. R., Rybin, A. V., Shlapakova, I., Danilo, P., Jr., Yang, J., and Rosen, M. R. (2005) Cardiovasc. Res. 68, 259–267
18. Rozengurt, E., Rey, O., and Waldron, R. T. (2005) J. Biol. Chem. 280, 13205–13208
PKD Is a Gαq- and PKCδ-activated CREB-S133 Kinase

JUNE 20, 2008•VOLUME 283 • NUMBER 25

JOURNAL OF BIOLOGICAL CHEMISTRY 17019

19. Nishikawa, K., Toker, A., Johannes, F. J., Songyang, Z., and Cantley, L. C. (1997) J. Biol. Chem. 272, 952–960
20. Johannessen, M., and Moens, U. (2007) Front. Biosci. 12, 1814–1832
21. Wood, C. D., Marklund, U., and Cantrell, D. A. (2005) J. Biol. Chem. 280, 6245–6251
22. Harrison, B. C., Kim, M. S., van Rooij, E., Papst, P. J., Vega, R. B., McAnally, J. A., Richardson, J. A., Bassel-Duby, R., Olson, E. N., and McKinsey, T. A. (2006) Mol. Cell Biol. 26, 3875–3888
23. Sturany, S., Van Lint, J., Muller, F., Wilda, M., Hameister, H., Hocker, M., Brey, A., Gern, U., Vandenheede, J., Gress, T., Adler, G., and Seufferlein, T. (2001) J. Biol. Chem. 276, 3310–3318
24. Matthews, S. A., Rozengurt, E., and Cantrell, D. (1999) J. Biol. Chem. 274, 26543–26549
25. Rennecke, J., Johannes, F. J., Richter, K. H., Kittstein, W., Marks, F., and Gschwendt, M. (1996) Eur. J. Biochem. 242, 428–432
26. Iglesias, T., Waldron, R. T., and Rozengurt, E. (1998) J. Biol. Chem. 273, 27662–27667
27. Storz, P., Doppler, H., and Toker, A. (2004) Mol. Cell Biol. 24, 2614–2626
28. Vega, R. B., Harrison, B. C., Meadows, E., Roberts, C. R., Papst, P. J., Olson, E. N., and McKinsey, T. A. (2004) Mol. Cell Biol. 24, 8374–8385
29. Haworth, R. S., Cuello, F., Herron, T. J., Franzen, G., Kentish, J. C., Gautel, M., and Avkiran, M. (2004) Circ. Res. 95, 1091–1099
30. Blois, J. T., Mataraza, J. M., Mecklenbrauker, I., Tarakhovsky, A., and Chiles, T. C. (2004) J. Biol. Chem. 279, 30123–30132
31. Trivier, E., De Cesare, D., Jacquot, S., Pannetier, S., Zackai, E., Young, L., Mandel, J. L., Sassone-Corsi, P., and Hanauer, A. (1996) Nature 384, 567–570
32. Olivot, J. M., Estebanell, E., Lafay, M., Brohard, B., Aiach, M., and Rendu, F. (2001) Circ. Res. 88, 681–687
33. Lefkowitz, R. J., Rajagopal, K., and Whalen, E. J. (2006) Mol. Cell 24, 643–652
34. Tan, M., Xu, X., Ohba, M., Ogawa, W., and Cui, M. Z. (2003) J. Biol. Chem. 278, 2824–2828
35. Tan, M., Xu, X., Ohba, M., and Cui, M. Z. (2004) Arterioscler. Thromb. Vasc. Biol. 24, 2271–2276
36. Matthews, S. A., Liu, P., Spitaler, M., Olson, E. N., McKinsey, T. A., Cantrell, D. A., and Scharenberg, A. M. (2006) Mol. Cell Biol. 26, 1569–1577
37. Oancea, E., Bezzerides, V. J., Groka, A., and Clapham, D. E. (2003) Dev. Cell 4, 561–574
38. Waldron, R. T., Iglesias, T., and Rozengurt, E. (1999) J. Biol. Chem. 274, 9224–9230
39. Benes, C. H., Wu, N., Elia, A. E., Dharia, T., Cantley, L. C., and Soltoff, S. P. (2005) Cell 121, 271–280
40. Doppler, H., and Storz, P. (2007) J. Biol. Chem. 282, 31873–31881
41. Cardenas, C., Muller, M., Jaimovich, E., Perez, F., Buchuk, D., Quest, A. F., and Carrasco, M. A. (2004) J. Biol. Chem. 279, 39122–39131
42. Wiggins, G. R., Solaaga, A., Foster, J. M., Murray-Tait, V., Cohen, P., and Arthur, J. S. (2002) Mol. Cell Biol. 22, 2871–2881
43. Johannessen, M., Delghandi, M. P., Rykx, A., Dragset, M., Vandenheede, J. R., Van Lint, J., and Moens, U. (2007) J. Biol. Chem. 282, 14777–14787
44. Darragh, J., Solaaga, A., Beardmore, V. A., Wingate, A. D., Wiggins, G. R., Peggie, M., and Arthur, J. S. (2005) Biochem. J. 390, 749–759
45. Pei, L., Castrillo, A., Chen, M., Hoffmann, A., and Tontonoz, P. (2005) J. Biol. Chem. 280, 29256–29262