Lysophosphatidic acid rapidly induces protein kinase D activation through a pertussis toxin-sensitive pathway

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Paolucci, Lina, James Sinnett-Smith, and Enrique Rozengurt. Lysophosphatidic acid rapidly induces protein kinase D activation through a pertussis toxin-sensitive pathway. Am. J. Physiol. Cell Physiol. 278: C33–C39, 2000.—Protein kinase D (PKD) is a serine-threonine protein kinase with distinct structural features and enzymological properties. Herein we demonstrate that lysophosphatidic acid (LPA) induces rapid PKD activation in mouse Swiss 3T3 and Rat-1 cells. LPA induced PKD activation in a concentration-dependent fashion with maximal stimulation (7.6-fold) achieved at 5 µM. Treatment of Swiss 3T3 cells with the protein kinase C (PKC) inhibitors GF-I, Ro-31–8220, and Go-7874 completely abrogated PKD activation induced by LPA at concentrations that did not inhibit PKD activity when added directly to the in vitro kinase assays. PKD activation induced by LPA was attenuated markedly and selectively by prior exposure of either Swiss 3T3 or Rat-1 cells to pertussis toxin (PTx) in a concentration-dependent manner. In contrast, treatment with the protein tyrosine kinase inhibitor genistein, the MEK inhibitor PD-098059, or the phosphoinositide 3-kinase inhibitor wortmannin did not affect PKD activation in response to LPA. These results provide the first example of PTx-sensitive and PKD-dependent PKD activation and identify a novel G1-dependent event in the action of LPA.

lysophosphatidic acid; protein kinase C; G protein-coupled receptor; signal transduction; protein phosphorylation

LYSOPHOSPHATIDIC ACID (LPA), a major bioactive lipid of serum, elicits a broad spectrum of biological responses, including platelet activation, smooth muscle contraction, changes in neuronal cell shape, and induction of cell proliferation and differentiation (14). LPA binds to a seven-transmembrane domain receptor(s) and activates several heterotrimeric G proteins, which are responsible for transducing LPA signals into multiple biological responses (5, 15). LPA stimulates Ras activation, leading to stimulation of Raf, MEK, and the ERKs via a pertussis toxin (PTx)-sensitive pathway that involves the βγ subunits of G, (2, 3, 11, 12, 26), whereas the α subunit of this trimeric G protein mediates inhibition of adenylyl cyclase activity (5, 15). LPA induces PTx-insensitive stress fiber formation, assembly of focal adhesions, and tyrosine phosphorylation of focal adhesion proteins (20) via activation of G13 (6, 16). LPA also stimulates phospholipase C (PLC)-mediated polyphosphoinositide breakdown that leads to generation of inositol 1,4,5-trisphosphate and diacylglycerol, the second messengers responsible for Ca2+ mobilization from intracellular stores and activation of protein kinase C (PKC), respectively. These PLC-dependent responses are thought to be mediated by PTx-insensitive G proteins of the Gα family (14, 15). It is also recognized that some of the downstream responses induced by LPA, including the activation of transcription factors NF-κB (21) and serum response factor (1) are elicited by interaction of complementary pathways activated by Gαq, Gαi, and Gα12.

The PKC family consists of multiple related isoforms, i.e., conventional PKCs (α, β1, β2, and γ); novel PKCs (δ, ε, η, and θ) and atypical PKCs (ζ and ι), all of which possess a highly conserved catalytic domain (17). Protein kinase D (PKD) (25), also named PKCµ (9), is a serine-threonine protein kinase with distinct structural, enzymological, and regulatory properties. In particular, PKD can be rapidly activated in intact cells through a phosphorylation-dependent mechanism (29). Treatment of intact cells with biologically active phorbol esters (29), bradykinin (13), or neuropeptide agonists, including bombesin, endothelin, and vasopressin (30), induces PKD activation that persists during cell disruption and immunoprecipitation. Several lines of evidence, including the use of selective PKC inhibitors and cotransfection of PKD with constitutively active mutants of PKCε and η, indicate that PKD is activated by phosphorylation in living cells through a PKC-dependent signal transduction pathway (13, 29, 30). More recently, Ser744 and Ser748 have been identified as critical phosphorylation sites in the activation loop of the kinase catalytic domain of PKD (8). These findings reveal an unsuspected connection between PKCs and PKD and imply that PKD can function downstream of PKCs in signal transduction.

In the present study, we examined the effect of the multifunctional agonist LPA on the regulation of PKD activity in intact Swiss 3T3 and Rat-1 cells, which have been used extensively as model systems to elucidate the biological effects of this bioactive lipid. We report for the first time that LPA induces a rapid PKC-dependent PKD activation in these cells. Surprisingly, treatment of the cells with PTx markedly and selectively attenuated PKD activation in response to LPA.
Our results identify a novel PTx-sensitive event in the action of LPA and provide the first example of a G-dependent pathway leading to PKD activation in any cell type.

**MATERIALS AND METHODS**

Cell culture. Stock cultures of Swiss 3T3 cells and Rat-1 cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO₂ and 95% air at 37°C. For experimental purposes, cells were plated in 100-mm dishes at 5 × 10⁵ cells/dish in DMEM containing 10% FBS and used after 6–8 days when the cells were confluent and quiescent.

Immunoprecipitation. Quiescent cultures of cells, treated as described in the individual experiments, were washed and lysed in 50 mM Tris-HCl, pH 7.6, 2 mM EGTA, 2 mM EDTA, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, and 1% Triton X-100 (lysis buffer A). Cell lysates were clarified by centrifugation at 15,000 g for 10 min at 4°C. PKD was immunoprecipitated at 4°C for 2–4 h with the PA-1 antipeptide antiserum (1:100), as previously described (29). The immune complexes were recovered using protein A coupled to agarose.

Kinase assay of PKD. The kinase activity of PKD was determined in an in vitro kinase assay by mixing 20 μl of PKD immunocomplexes with 10 μl of a phosphorylation mixture containing (final concentration) 10 μM [γ-32P]ATP (specific activity 400–600 cpm/pmol), 30 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, and 1 mM dithiothreitol. After 10 min of incubation at 30°C, the reaction was stopped by washing with 200 μl of kinase buffer and then adding an equal volume of 2× SDS-PAGE sample buffer (200 mM Tris-HCl, pH 6.8, 2 mM EDTA, 0.1 M Na₃VO₄, 6% SDS, 10% glycerol, and 4% 2-mercaptoethanol), followed by SDS-PAGE analysis (27, 29). The gels were dried, and the 110-kDa radioactive band corresponding to autophosphorylated PKD was visualized by autoradiography. Autoradiographs were scanned in a ScanJet 6100C/T (Hewlett Packard), and the labeled band was quantified using the National Institutes of Health image software program.

Phospholipase B treatment of LPA and FBS. LPA and lysophosphatidates bound to albumin in serum are inactivated by treatment with phospholipase B (PLB). Confluent and quiescent Swiss 3T3 cells were washed with DMEM and subsequently stimulated with DMEM supplemented with either 5% FBS or 5 μM LPA for 10 min and then lysed. Parallel cultures were also challenged with either LPA or FBS, which had been treated with PLB (as described in MATERIALS AND METHODS) for 10 min and then lysed. All lysates were immunoprecipitated with PA-1 antiserum. To determine PKD activity, immunoprecipitates were incubated with [γ-32P]ATP in phosphorylation mixture and products of the reaction were further analyzed by SDS-PAGE and autoradiography. All other experimental details were as described in MATERIALS AND METHODS. Autoradiograms were scanned to quantify phosphoprotein in terms of peak area. Values correspond to autophosphorylation of PKD expressed over the unstimulated value. Results shown are means ± SE of 2 independent experiments.

Measurement of intracellular calcium concentration. Intracellular calcium concentration ([Ca²⁺]ᵢ) was measured with the fluorescent indicator fura 2. Confluent and quiescent cultures of Swiss 3T3 cells, grown on 9 × 22 mm coverslips, were washed twice with DMEM and then incubated for 10 min in DMEM containing 1 μM fura 2-AM at 37°C. The cultures were then washed twice with Hanks’ buffered salt solution, pH 7.2, supplemented with NaHCO₃ (35 mM), CaCl₂ (1.3 mM), MgCl₂ (0.5 mM), MgSO₄ (0.4 mM), and 0.1% bovine serum albumin (calcium buffer). The coverslips were then incubated for a further 15 min in calcium buffer and transferred to a quartz cuvette containing 2 ml of the same buffer, and fluorescence was monitored using a Hitachi F-2000 fluorospectrophotometer with dual excitation wavelengths of 340 nm (λ1) and 380 nm (λ2) and an emission length of 340 nm (λ3) and an emission wavelength of 510 nm (λ4). Intra-
wavelength of 510 nm while the cells were continually stirred at 37°C. [Ca\(^{2+}\)] was determined using the equation

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[Ca^{2+}] \text{N} = K_d \left( \frac{R - R_{\text{min}}}{R_{\text{max}} - R} \right) \times \frac{F_{\text{min}} \lambda_2}{F_{\text{max}} \lambda_2}
\]

where \(R\), \(R_{\text{min}}\), and \(R_{\text{max}}\) are the ratios of the emission at 510 nm following excitation at 340 nm and 380 nm, \(F_{\text{max}}\) is the fluorescence after the addition of 40 µM digitonin, \(F_{\text{min}}\) is the fluorescence after the Ca\(^{2+}\) in the solution has been chelated with 25 mM EGTA. The value of dissociation constant (\(K_d\)) used was 224.

Phospho-p42 \(\text{mapk (ERK-2)}\) and p44 \(\text{mapk (ERK-1)}\) were bands detected by enhanced chemiluminescence Western blotting ECL reagents (Amersham).

Materials. \([\gamma-\text{32P}]\)ATP (370 MBq/ml) was from Amersham International. GF-I (also known as GF-109203X or bisindolylmaleimide I), Gö-7874, Ro-31–8220, U-73434, and PTx were from Calbiochem. LPA, phorbol 12,13-dibutyrate (PDB), wortmannin, rapamycin, PD-098059, genistein, and PLB were from Sigma. The monodonal anti-phospho-ERK-1 and ERK-2 antibody (0.2 µg/ml, E10, Bio-Labs). Immunoactive p42 \(\text{mapk (ERK-2)}\) and p44 \(\text{mapk (ERK-1)}\) were bands detected by enhanced chemiluminescence Western blotting ECL reagents (Amersham).

RESULTS

LPA induces PKD activation in Swiss 3T3 and Rat-1 cells. Quiescent Swiss 3T3 fibroblasts have proved to be a useful model system for elucidating signal transduction pathways in the action of multiple agonists, including LPA (19). To examine whether LPA induces PKD activation, confluent and quiescent cultures of these cells were stimulated with 5 µM LPA for 10 min and lysed. The extracts were immunoprecipitated with the PA-1 antibody raised against a peptide composed of the carboxy terminal amino acids of PKD. The immunocomplexes were incubated with \([\gamma-\text{32P}]\)ATP and then analyzed by SDS-PAGE and autoradiography to examine the level of autophosphorylation. As illustrated in Fig. 1, stimulation of Swiss 3T3 cells with LPA induced a marked PKD activation that was maintained during cell disruption and immunoprecipitation. In 25 independent experiments, addition of 5 µM LPA to cultures of Swiss 3T3 cells induced a 7.6 ± 0.47 (means ± SE) -fold increase in PKD activity.

Addition of serum to quiescent cultures of Swiss 3T3 cells also induces a rapid and marked activation of PKD (Fig. 1A), in agreement with previous results (29). LPA is one of the major components in serum that stimulates proliferation in a variety of cell types (14). To determine whether LPA contributes to mediate serum stimulation of PKD activation, we next examined the effect of PLB treatment on either LPA or serum. PLB inactivates LPA by hydrolyzing the ester bond linking fatty acid to the 1-position of the glycerol backbone of lysophospholipids (10). As shown in Fig. 1A, PKD activation by either serum or LPA was abolished by prior incubation of these agents with active PLB. These results suggest that LPA is a major factor in serum contributing to PKD activation.

PKD activation was a rapid consequence of the addition of LPA to Swiss 3T3 cells (Fig. 1B). An increase in PKD activity was detectable within 1 min and reached a maximum after 5 min of LPA stimulation. LPA induced PKD activation in a concentration-dependent fashion with half-maximal and maximal stimulation achieved at 1 µM and 5 µM, respectively (Fig. 1B, inset).

Cultures of Rat-1 cells have also been used as a model system to examine LPA signaling (14). Treatment of Rat-1 cells with increasing concentrations of LPA induced a striking increase in PKD activity (Fig. 1C). The maximal effect, achieved at 2.5–5 µM, was equivalent to that induced by addition of either 200 nM PDB or fresh medium containing 10% FBS. The results presented in Fig. 1 demonstrate that LPA induces PKD activation in both Swiss 3T3 cells and Rat-1 cells.

PKC mediates LPA-stimulated PKD activation. Next, we determined the role of PKCs in PKD activation induced by LPA. Quiescent cultures of Swiss 3T3 cells were treated with various concentrations of GF-I (also known as GF-109203X or bisindolylmaleimide I), a potent inhibitor of phorbol ester-sensitive isoforms of PKC (24) but not PKD (29, 30), before PDB stimulation. As shown in Fig. 2, treatment of the cells with GF-I potently blocked PKD activation induced by subsequent addition of LPA, in a concentration-dependent fashion. In contrast, GF-I added directly to the in vitro kinase assay, even at the concentrations (0.5–2.5 µM) required to abrogate LPA-mediated PKD activation in intact 3T3 cells, did not inhibit PKD activity.

To substantiate the results obtained with GF-I, we examined whether other inhibitors of PKC, including Ro-31–8220 and Gö-7874, also prevent PKD activation in response to LPA. As illustrated by Fig. 2, treatment of intact 3T3 cells with increasing concentrations of Ro-31–8220 and Gö-7874 for 1 h before stimulating with LPA profoundly inhibited PKD activation. Importantly, neither Ro-31–8220 nor Gö-7874 reduced PKD activity when added directly to the in vitro kinase assay at identical concentrations to those required to block PKD activation in vivo. Thus the results shown in Fig. 2 imply that GF-I, Ro-31–8220, and Gö-7874 do not inhibit PKD activity directly but interfere with LPA-mediated PKD activation in intact cells by blocking PKC.

LPA induces PKD activation via PLC. To determine whether LPA induces PKC-dependent PKD activation
through a PLC-dependent pathway, Swiss 3T3 cells were treated with the aminosteroid U-73122, an inhibitor of PLC, prior to stimulation with LPA. As shown in Fig. 3A, U-73122 markedly reduced PKD activation in response to the subsequent addition of LPA in a concentration-dependent fashion. Maximal inhibition of LPA-stimulated PKD activation was achieved at 2.5 µM. The inhibitory effect of U-73122 was selective because this agent, at similar concentrations, did not interfere with PKD activation induced by PDB. Furthermore, U-73343, an inactive analog of U-73122, did not affect LPA stimulation of PKD activation when added at an identical concentration (Fig. 3B).

Treatment with the protein tyrosine kinase inhibitor genistein, which prevents LPA-mediated Ras activation (26), or the MEK inhibitor PD-098059, which prevents ERK activation, did not affect PKD activation in response to LPA (see Fig. 3B). Similarly, inhibition of the phosphoinositide 3-kinase with wortmannin or of the phosphoinositide 3-kinase downstream target p70 ribosomal S6 kinase (p70S6K) with rapamycin did not affect the increase in PKD activity induced by LPA (Fig. 3B). In addition, pretreatment with 250 nM AG-1478, a selective inhibitor of epidermal growth factor (EGF) receptor tyrosine kinase transactivation (2, 3), did not interfere with LPA-induced PKD activation (results not shown). These results demonstrate the specificity of the PKC and PLC inhibitors and indicate that neither Ras-Raf-ERK, phosphoinositide 3-kinase, nor EGF receptor tyrosine kinase are involved in the signaling pathway(s) that mediates LPA-induced PKD activation.

LPA stimulates PKD activation via a PTx-sensitive pathway. LPA activates several heterotrimeric G proteins including Gq and Gi, which are responsible for transducing LPA signals into multiple biological responses (6, 15). PLC-mediated polyphosphoinositide breakdown, leading to rapid Ca2+ mobilization and PKC activation, is thought to be mediated by PTx-insensitive G proteins of the Gi family, at least in rodent cell lines (14). We verified that treatment of Swiss 3T3 cells with 30 ng/ml PTx for 3 h, a condition known to promote ADP ribosylation and inactivation of Gi in these cells (22), did not interfere with the rapid and transient increase in [Ca2+] induced by LPA (Fig. 4A). In contrast, a similar treatment with PTx mark-

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Fig. 2. Protein kinase C (PKC) inhibitors GF-I, Ro-31-8220, and G6-7874 prevent PKD activation by LPA. Confluent and quiescent Swiss 3T3 cells were washed with DMEM and incubated for 1 h with different concentrations (µM) of selective PKC inhibitors GF-109203X (GF-I), Ro-31-8220 (Ro), or G6-7874 (Go) as indicated (top, Pretreat). Control cells received an equivalent amount of solvent (−). Cultures were subsequently stimulated with 5 µM LPA for 10 min, lysed, and extracts were immunoprecipitated with PA-1 antiserum. PKD activity was determined by in vitro kinase assay carried out in absence (−) or in presence (+) of indicated concentrations of GF-I, Ro-318220, G6-7874 added directly to incubation mixture (bottom, in vitro). Control PKD immunoprecipitates received equivalent amount of solvent (−). Reactions were analyzed by SDS-PAGE and autoradiography. Results shown are representative of 3 independent experiments.

Fig. 3. Phospholipase C (PLC) inhibitor U-73122 abrogates PKD activation in Swiss 3T3 cells. A: confluent and quiescent Swiss cells were washed with serum-free medium and then incubated with DMEM containing increasing concentrations of selective PLC inhibitor U-73122 (0.5, 1, and 2.5 µM) for 1 h. Control cultures received equivalent amount of solvent (−). Cells were subsequently stimulated with 200 nM phorbol 12,13-dibutyrate (PDB) or 5 µM LPA for 10 min and lysed. Lysates were immunoprecipitated with PA-1 antiserum, and PKD activity was determined by in vitro kinase assay as described in MATERIALS AND METHODS, followed by SDS-PAGE and autoradiography. B: confluent and quiescent cells were washed and treated with 2.5 µM Ro-31-8220 (Ro), 2.5 mM U-73122, 2.5 mM U-73343, 100 nM wortmannin (War), 20 nM rapamycin (Rap), 25 µM PD-098059 (PD), 50 µM genistein (Gen), or an equivalent volume of solvent for 1 h. Cells were then incubated with 5 µM LPA for 10 min. Cultures were lysed, extracts were immunoprecipitated with PA-1 antiserum, and immunocomplexes of PKD were subjected to in vitro kinase assay, SDS-PAGE, and autoradiography. Results shown are means ± SE of 3 independent experiments.
PKD activation in response to LPA requires functional PKC and PLC (Figs. 2 and 3), suggesting the involvement of a Gq-mediated pathway. However, these results do not exclude the contribution of additional signaling inputs. To test this possibility, quiescent cultures of Swiss 3T3 cells were treated with increasing concentrations of PTx for 3 h and then challenged with 5 µM LPA for 10 min. Surprisingly, prior exposure of the cells to PTx markedly attenuated the increase in PKD activity induced by LPA in a concentration-dependent manner (Fig. 4B). In 14 independent experiments, treatment with different preparations of PTx (30 ng/ml for 3 h) reduced LPA-stimulated PKD activation to 22.0 ± 2.6% of the untreated control. In other experiments, we found that treatment with PTx also decreased PKD activation in response to serum (to 50.3 ± 4.2% of the untreated control, n = 4) in agreement with the conclusion that LPA is a major factor in serum that stimulates PKD activation.

PTx inhibited PKD activation in response to LPA in a selective fashion. As shown in Fig. 5A, prior exposure of parallel cultures to PTx for 3 h did not interfere with PKD activation induced by either PDB, which directly activates PKC and thereby PKD (29), or bombesin, which induces polyphosphoinositide hydrolysis and PKC activation through a seven transmembrane domain receptor coupled to PTx-insensitive Gq (7). In contrast, similar treatment with PTx markedly inhibited LPA-induced PKD activation in parallel cultures (Fig. 5A). Thus the results shown in Figs. 4 and 5A indicate that PTx attenuates LPA-induced PKD activation in a selective fashion.

To determine whether the Gq-dependent pathway leading to PKD activation is also functional in other cells, we examined the effects of LPA, FBS, and PDB in Rat-1 cells pretreated with or without 100 ng/ml PTx. As shown in Fig. 5B, and consistent with the results obtained with 3T3 cells, prior exposure of Rat-1 cells to PTx markedly attenuated PKD activation in response to either LPA or FBS but did not interfere with PKD activation induced by PDB.

DISCUSSION

LPA promotes a broad range of biological responses and multiple molecular events in target cells (15). Consistent with the stimulation of multiple signaling pathways, LPA has been shown to bind to several heptahelical receptors (5) and activate several heterotrimeric G proteins, including Gq, Gi, and G12 in Swiss 3T3 cells (6) and in Rat-1 cells (14). The results presented here demonstrate that LPA rapidly induces PKD activation in intact Swiss 3T3 and Rat-1 cells and thus identify a novel molecular response in LPA action. Our results also suggest that LPA is a major factor in serum that mediates PKD activation in these cell types.

Treatment of the cells with the PKC inhibitors GF-1, Ro-31–8220, and Gö-7874 before stimulation with LPA strikingly prevented PKD activation. Importantly, these PKC inhibitors did not reduce PKD activity when added directly to the in vitro kinase assays, even at concentrations higher than those used in intact cells to...
block LPA-induced PKD activation. Furthermore, the PLC inhibitor U-73122 selectively prevented PKD activation by LPA. We conclude that LPA-induced PKD activation is downstream to PLC and PKC in Swiss 3T3 cells.

LPA-induced PLC and PKC activation is thought to be mediated by LPA receptor coupling to PTx-insensitive G\(_{q}\) (14). In line with this hypothesis, inositol phosphate production and Ca\(^{2+}\) mobilization in response to LPA is not prevented by treatment with PTx in rodent cell lines, including Swiss 3T3 cells. In addition, LPA has been shown to stimulate phosphorylation of the Rac exchange factor Tiam-1 via a PTx-insensitive PKC-dependent pathway in these cells (4).

Although LPA induces PLC and PKC activation through G\(_{q}\) and PKD activation in response to LPA is downstream to PKC, it could not be excluded that other signaling inputs also contribute to PKD activation induced by LPA. A surprising feature of our results is that PKD activation in response to LPA is attenuated markedly and selectively by prior treatment of either Swiss 3T3 cells or Rat-1 cells with low concentrations of PTx. These results indicate that the G\(_{q}\) pathway is not sufficient to promote PKD activation in response to LPA in these cells and identify for the first time the involvement of an additional G\(_{i}\)-dependent pathway leading to PKD activation in any cell type.

Interestingly, the concentration of LPA required for stimulation of PKD activation in Swiss 3T3 cells (EC\(_{50}\) 1 \(\mu\)M) is similar to that needed for activation of the transcription factors NF-\(\kappa\)B (21) and serum response factor (1). Recent evidence indicates that LPA leads to the activation of these transcription factors through parallel signal transduction pathways. For example, the stimulation of NF-\(\kappa\)B by LPA is mediated by G\(_{i}\) and G\(_{q}\) pathways (21), and the activation of the serum response factor is mediated by cooperative effects between G\(_{i}\) and G\(_{q}\) pathways (1). We propose that LPA-induced PKD activation, which precedes the activation of these transcription factors, is also mediated by complementary pathways initiated by G\(_{i}\) and G\(_{q}\).

It is well established that treatment with PTx almost completely blocks LPA-induced mitogenesis in a variety of cell types. LPA signaling through PTx-sensitive G\(_{i}\) activates the Ras-Raf-ERK kinase cascade (2, 3, 11, 12, 26) and phosphoinositide 3-kinase activity in cultured fibroblasts (18). Recently, Takeda et al. (23) demonstrated that LPA induces phosphoinositide 3-kinase-dependent activation of PKC\(_{z}\) via G\(_{i}\). The results presented here identify PKD activation as a novel PTx-sensitive early molecular response in the action of LPA, which can be dissociated from either Ras-Raf-ERK or phosphoinositide 3-kinase signaling pathways. In addition, our previous results showed that cotransfection of PKD with a constitutively active form of PKC\(_{z}\) does not lead either to PKD activation (29) or to the formation of stable molecular complexes between PKC\(_{z}\) and PKD (28). The role of PKD in the PTx-sensitive biological responses induced by LPA warrants further experimental work.

In conclusion, our results demonstrate that LPA induces PKC-dependent PKD activation in Swiss 3T3 cells. In contrast to the model of PKD regulation by neurotide agonists through G\(_{q}\) (30), we propose that LPA stimulates PKD activation through both G\(_{i}\) and G\(_{q}\) in these cells. Our results identify a novel PTx-sensitive molecular response in the action of LPA and demonstrate for the first time the involvement of a G\(_{i}\)-dependent pathway leading to PKD activation in any cell type.

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