Identification of calcium-independent phospholipase A$_2$γ in mitochondria and its role in mitochondrial oxidative stress

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Oxidant-induced cell death is a mediator of pathologies associated with ischemia-reperfusion, toxicants, and inflammation in many different organs. Reactive oxygen species (ROS) react with several intracellular targets including DNA, proteins, and lipids. Lipid peroxidation changes the biophysical properties of membrane phospholipids, which can affect ion permeability (17) and membrane fluidity (8). Glycerophospholipids in cell and organelle membranes contain two fatty acyl chains linked to the glycerol backbone at the sn-1 and sn-2 positions. The sn-2 fatty acids have a higher degree of unsaturation than sn-1 fatty acids making them a primary target of ROS in biological membranes. Phospholipase A$_2$ enzymes specifically catalyze the cleavage of the sn-2 bond of phospholipids and have been hypothesized to selectively cleave oxidized fatty acids from the sn-2 position to preserve membrane integrity (41).

There are more than 20 isoforms of PL A$_2$ with different characteristics including Ca$^{2+}$ requirement and subcellular localization. Six and Dennis (35) classified PL A$_2$ enzymes into 14 groups based on their nucleotide sequence. Most PL A$_2$ in these groups are relatively small proteins (12–19 kDa), require millimole amounts of Ca$^{2+}$ for activity, and use a histidine for catalysis. These groups (I-III, V, and IX-XIV) have historically been called secreted PL A$_2$ (sPL A$_2$). The remaining groups consist of Ca$^{2+}$-dependent cytosolic PL A$_2$ (cPL A$_2$; group IVA and B), platelet-activating factor acetylhydrolases (PAF-AH; groups VII and VIII), and Ca$^{2+}$-independent PL A$_2$ (iPL A$_2$, group VI and IVC).

cPL A$_2$γ (group IVC) contains sufficient nucleotide homology to cPL A$_2$α to be classified as a group IV enzyme, but lacks the Ca$^{2+}$-dependent lipid binding domain and is anchored to endoplasmic reticulum, golgi apparatus, and mitochondrial membranes (38, 40). iPL A$_2$β (group VIA) is predominately cytosolic, but one of the several splice variants of iPL A$_2$β associates with crude membrane fractions (22). iPL A$_2$γ (group VIB) appears to be exclusively membrane bound (20, 24, 25, 44). The 63-kDa isoform of iPL A$_2$γ has been localized in peroxisomal membranes of rat liver cells (44) and higher molecular weight isoforms have been identified in microsomes of rabbit renal proximal tubular cells (RPTC) and ventricular myocytes (20) and in rat heart mitochondria (25).

One method used to identify the isoform(s) of PL A$_2$ responsible for an observed activity is to determine the sensitivity of the activity to different PL A$_2$ inhibitors (10, 15, 20). iPL A$_2$β is sensitive to myeloy arachidonyl fluorophosphonate (MAFP), arachidonyl trifluoromethylketone (AACOCF$_3$), and S-BEL, but not R-BEL at low micromolar concentrations (2, 15, 23). iPL A$_2$γ is sensitive to R-BEL, but not MAFP or S-BEL at low micromolar concentrations (15, 20). cPL A$_2$α is inhibited by MAFP and AACOCF$_3$, but not BEL (38). Finally, PAF-AH is potently inhibited by MAFP and not inhibited by BEL at concentrations up to 20 μM (18).

Recent studies showed that mitochondrial PL A$_2$ activity in different tissues and species is Ca$^{2+}$ independent (6, 7, 13, 25, 40, 43). In rat liver mitochondria, the iPL A$_2$ activity was completely inhibited by BEL but not by inhibitors of sPL A$_2$ or cPL A$_2$ (6). In rabbit ventricular myocytes, mitochondrial iPL A$_2$ expression was demonstrated in the inner mitochondrial membrane (43). Several studies have utilized a commercially available iPL A$_2$β antibody to demonstrate iPL A$_2$ expression in mitochondria (6, 7, 43). Recently, Gross et al. (25) reported that iPL A$_2$γ is expressed in rat heart mitochondria based on immunoblot analysis with an iPL A$_2$γ antibody. Finally, immunohistochemical analysis revealed that cPL A$_2$γ colocalizes with mitochondria in immortalized mouse lung fibroblasts (40). In summary, iPL A$_2$γ, iPL A$_2$β, and...
cPLA$_2$$\gamma$ are reportedly localized to mitochondria in different tissues, but the presence, identity, and role of a mitochondrial iPLA$_2$ in the kidney has not been examined.

Several studies have implicated PKC-mediated phosphorylation as a mechanism for activation of membrane-bound iPLA$_2$ in the heart and kidney (11, 28, 37). Membrane-associated iPLA$_2$ activity in ventricular myocytes and human coronary artery endothelial cells is increased in a diacylglycerol-dependent, Ca$^{2+}$-independent fashion suggesting the involvement of a novel PKC isoform (28, 37). In ventricular myocytes, PKC$\epsilon$ is the only novel isoform detected in the membrane fraction (37). Endoplasmic reticulum iPLA$_2$$\gamma$ activity in RPTC is increased by phorbol 12-myristate 13-acetate (PMA) treatment, which mimics the effect of diacylglycerol (11). To date, no studies have examined the regulation of mitochondrial iPLA$_2$ activity by PKC.

We suggested that iPLA$_2$$\gamma$ acts to repair or prevent lipid peroxidation which protects cells from oxidative stress. This hypothesis is supported by the finding that inhibition of iPLA$_2$$\gamma$ activity in RPTC potentiates oxidant-induced lipid peroxidation and necrotic cell death (10). Furthermore, doxorubicin- and tert-butyl hydroperoxide (TBHP)-induced cardiomyocyte death is enhanced by inhibition of iPLA$_2$ with BEL (27, 39). In this study, we determined that RPTC possess mitochondrial iPLA$_2$ activity and identified the isoform responsible for the activity. Several potential mechanisms for regulation of mitochondrial iPLA$_2$ activity were examined and the role for mitochondrial iPLA$_2$ in oxidant-induced mitochondrial lipid peroxidation and swelling was investigated.

**MATERIALS AND METHODS**

**Materials.** Female New Zealand White rabbits (1.5–2.0 kg) were purchased from Myrtle’s Rabbitry (Thompson Station, TN). R- and S-BEL were generously provided by B. S. Cummings (University of Georgia, Athens, GA) (20) or purchased from Cayman Chemical (Ann Arbor, MI). The iPLA$_2$$\beta$ antibody was purchased from Cayman Chemical. The PKC$\epsilon$-specific inhibitor PKCe VI-1 was purchased from Biomol (Plymouth Meeting, PA). All other chemicals and materials were obtained from Sigma (St. Louis, MO) or reported previously (10, 11, 23).

**Isolation of renal cortical mitochondria.** Rabbits were euthanized by intravenous injection of 75 mg/kg pentobarbital sodium and kidneys were removed by blunt dissection using a protocol approved by the Medical University of South Carolina IACUC. Human cadaveric kidneys (International Bioresearch Solution, Tucson, AZ) that were rejected for transplant were used within 24 h of removal from the donor according to the Medical University of South Carolina IRB guidelines. Kidney cortex tissue was collected and placed on ice in mitochondrial isolation buffer containing (in mM): 270 sucrose, 5 Tris$\cdot$HCl, 1 EGTA (pH 7.4). Rabbit and human renal cortical mitochondria (RCM) were isolated by differential centrifugation and purified by Percoll density gradient separation where noted (3). Mitochondrial inner membrane fractions were isolated from Percoll-purified mitochondria as described by our laboratory (3).

**Immunoblot analysis.** We contracted Aves Labs (Tigard, OR) to generate the anti-rabbit iPLA$_2$$\gamma$ antibody using the rabbit-iPLA$_2$$\gamma$-specific peptide sequence, CENIPLDESRNEKLDQ (20). Resultant antisera was affinity purified and used as the primary antibody. Equal amounts of mitochondrial and cytosolic protein (25 $\mu$g) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were incubated with the iPLA$_2$$\gamma$ or iPLA$_2$$\beta$ antisera at a dilution of 1:1,000. Bound antibodies were visualized by chemiluminescence detection on a ChemiImager 5500 imager (Alpha Innotech, San Leandro, CA).

**Mitochondrial swelling.** After incubation with cis-parinaric acid and washing, isolated mitochondria were suspended at a concentration of 0.4 mg protein/ml in swelling buffer (150 mM KCl, 20 mM Tris$\cdot$HCl, pH 7.4) in a 96-well plate and incubated with diluent, inhibitors, or antioxidants at room temperature for 10 min. Ferrous sulfate dehydrate (Sigma) to achieve a final Fe$^{2+}$ concentration of 10 $\mu$M or diluent (swelling buffer) was then added to initiate oxidative stress (14). Mitochondrial swelling was measured using a SpectraMax 190 spectrophotometric plate reader (Molecular Devices, Sunnyvale, CA) as the loss of optical density at 540 nm over time as previously described (14). Swelling buffer was deoxygenated immediately before use to prevent immediate oxidation of Fe$^{2+}$ to Fe$^{3+}$ (14).

**Measurement of cis-parinaric acid oxidation.** Lipid peroxidation in isolated mitochondria was measured using the fluorescent lipid, cis-parinaric acid as described previously (21, 29), with modifications. Isolated mitochondrial samples were suspended in swelling buffer containing (in mM): 100 Tris$\cdot$HCl, 1 EGTA (pH 7.4). The mitochondria were pelleted by centrifugation, the supernatant discarded, and the mitochondria were resuspended (0.4 mg protein/ml) in deoxygenized swelling buffer. Mitochondria were added to a 96-well plate and incubated with diluent, inhibitors, or antioxidants at room temperature for 10 min and then treated with 10 $\mu$M Fe$^{2+}$. Lipid peroxidation was measured as the loss of cis-parinaric acid fluorescence (excitation 320 nm, emission 405 nm) over time using a Fluoroskan Ascent fluorescent plate reader (Thermo Labsystems, Franklin, MA).

**Isolation of rabbit RPTC, culture conditions, and inhibitor treatment.** Rabbit RPTC were isolated using the iron oxide perfusion method and grown under improved conditions as previously described (30). Confluent monolayers were treated with R- or S-BEL or diluent control for 30 min before harvesting and isolation of mitochondria. RPTC mitochondria and cytosol were isolated by differential centrifugation as described previously (20).

**Measurement of iPLA$_2$ activity.** PLA$_2$ activity was determined under linear reaction conditions in mitochondria as described previously (10, 11, 20). Activity was measured using synthetic (16:0, [3H]18:1) plasmenyl- or phosphatidylcholine (100 $\mu$M) in the presence (1 mM CaCl$_2$) and absence of Ca$^{2+}$ (4 mM EGTA). For PLA$_2$ activity inhibition studies, mitochondrial samples were incubated with solvent control [DMSO < 0.1% (vol/vol)], racemic, R-, or S-BEL, MApF, or AACOCF$_3$ for 10 min before the addition of the phospholipid substrate to start the reaction.

**Protein determination.** Protein determination was performed using the bicinchoninic acid assay method as described by Sigma.

**Statistical analysis.** Mitochondria, cytosol, or RPTC isolated from one rabbit or human kidney represent one experiment ($n = 1$). The appropriate ANOVA was performed for each data set using SigmaStat statistical software. Individual means were compared using Fisher’s protected least significant difference test with $P \leq 0.05$ being considered indicative of a statistically significant difference between mean values.

**RESULTS**

Rabbit RCM were assayed for PLA$_2$ activity using synthesized plasmenylcholine and phosphatidylcholine phospholipids with palmitic acid (16:0) at the sn-1 position and [3H]oleic acid (18:1) at the sn-2 position. Percoll-purified RCM and RCM inner membrane possess PLA$_2$ activity that is not dependent on Ca$^{2+}$ and has a preference for plasmenylcholine (Fig. 1A). The effect of pH on iPLA$_2$ activity in Percoll-purified RCM is presented in Fig. 1B. A significant increase in plasmenylcholine cleavage is observed as the pH decreases from 8 to 7.5,
activity in human kidney cortex. In summary, RCM possess iPLA2 activity that prefers plasmenylcholine over phosphatidylcholine, the activity is differentially affected by pH, and human kidney cortex mitochondria exhibit a similar level of iPLA2 activity as the ER and mitochondrial inner membrane in rabbit kidney cortex.

Immunoblot analysis with antibodies for iPLA2β and iPLA2γ revealed immunoreactive proteins to both antibodies in rabbit RCM (Fig. 2). While the bands are similar in size, the antigenic peptides used for both antibodies are unique for their respective proteins. The protein recognized by the Cayman iPLA2β antibody is similar in size to iPLA2β reported in rabbit heart mitochondria (43) and rat liver mitochondria (6). iPLA2γ in RPTC mitochondria is the long isoform of iPLA2γ (~88 kDa), based on the nucleotide sequence analysis of Mancuso et al. (24). Consistent with the very low cytosolic PLA2 activity, no PLA2 expression was detected in RPTC cytosol.

In an effort to determine which PLA2 isoform is responsible for the iPLA2 activity in RCM, the inhibitor sensitivity profile was determined. RCM iPLA2 activity was inhibited by racemic BEL, but not MAPF or AACOCF3 (Fig. 3A). This profile is consistent with iPLA2γ, and not iPLA2β or cPLA2γ, both of which are inhibited by MAPF and AACOCF3 at concentrations lower than 10 μM (2, 23, 38). As an additional approach to confirm that iPLA2γ is responsible for the mitochondrial PLA2 activity, the effect of the R- and S-enantiomers of BEL was investigated. R-BEL selectively inhibits iPLA2γ at low micromolar concentrations while S-BEL selectively inhibits iPLA2β (15). In Percoll-purified RCM, R-BEL was effective at inhibiting iPLA2 activity while S-BEL had no effect (Fig. 3B). In mitochondria isolated from confluent RPTC, after a 30-min incubation with increasing concentrations of inhibitors or diluent control, R-BEL was significantly more effective at inhibiting iPLA2 activity than S-BEL (Fig. 3C). These results demonstrate that iPLA2γ is responsible for mitochondrial iPLA2 activity in RCM and that treatment of RPTC with R-BEL selectively decreases mitochondrial iPLA2γ activity.

The addition of ATP (1 mM) to RCM increased iPLA2 activity ~30% (Fig. 4). Previous investigators suggested that membrane-associated iPLA2γ activity is regulated by PKC-mediated phosphorylation (11, 28, 37), and the addition of the PKCγ-specific inhibitor PKCe VI–2 blocked ATP-induced increases in iPLA2 activity in RCM (Fig. 4A). To ensure that

while a significant increase in phosphatidylcholine cleavage occurs as the pH decreases from 9 to 8. Cleavage of both substrates decreases to background levels at a pH of ~6. Ca2+-independent activity was observed in the endoplasmic reticulum (ER) and inner mitochondrial membrane of kidney cortex tissue from cadaveric human kidneys (Fig. 1C). Similar to rabbit kidney (10) there was little basal cytosolic PLA2

Fig. 1. Phospholipase A2 activity in rabbit renal cortex mitochondria and human renal cortex subcellular fractions. A: cleavage of 16:0 [3H]18:1 plasmenyl- or phosphatidylcholine substrates in the presence (1 mM CaCl2) and absence (4 mM EGTA) of calcium by whole Percoll-purified rabbit RCM and mitochondrial inner membrane fractions. B: effect of pH on the cleavage of plasmenyl- or phosphatidylcholine substrates in the absence of calcium in whole Percoll-purified rabbit RCM. C: cleavage of 16:0 [3H]18:1 plasmenyl- or phosphatidylcholine substrates in the presence (1 mM CaCl2) and absence (4 mM EGTA) of calcium by human kidney cortex ER and Percoll-purified mitochondrial inner membrane and cytosolic fractions. Values are means ± SE of 3 experiments. Means with different superscripts are significantly different from each other within groups, P < 0.05.
ATP was not activating latent iPLA2 in the mitochondria, the effect of R-BEL and S-BEL (5 μM) on ATP-induced iPLA2 activity was determined. S-BEL had no inhibitory effect on iPLA2 activity in the presence or absence of ATP while R-BEL inhibited iPLA2 activity under both conditions, suggesting that the increase in iPLA2 activity in the presence of ATP is solely due to an increase in iPLA2γ activity and not the activation of a latent iPLA2β (Fig. 4B).

To investigate the role of iPLA2γ in oxidant-mediated mitochondrial damage, RCM were treated with Fe2+ to initiate oxidant stress and mitochondrial swelling (Fig. 5) (14). Mitochondrial swelling induced by 10 μM Fe2+ was completely blocked by the antioxidants butylated hydroxyanisole (25 μM BHA; Fig. 5A) and N,N′-diphenyl-1,4-benzenediamine (DPPD; 5 μM, data not shown). Pretreatment of mitochondria with R-BEL (8 μM) accelerated the onset of Fe2+-induced mitochondrial swelling, whereas pretreatment with S-BEL (8 μM) had no effect (Fig. 5A). The time required for Fe2+ to induce maximal mitochondrial swelling in the presence of R- and S-BEL was compared with the time required for Fe2+ alone to induce maximal swelling. Pretreatment with R-BEL decreased the time required for Fe2+ to initiate mitochondrial swelling by ~120 s, while S-BEL had no effect (Fig. 5B). Pretreatment with cyclosporine A (CsA; 1 μM) had no effect on Fe2+-induced mitochondrial swelling (data not shown), suggesting the Fe2+-induced swelling is not the result of mitochondrial permeability transition (MPT) pore opening. In summary, Fe2+ induced RCM swelling that is mediated by oxidative stress, but not the result of MPT, and specific iPLA2γ inhibition accelerated the oxidant-induced swelling.

The fluorescent lipid cis-parinaric acid was used to measure the rate of lipid peroxidation under the same conditions as Fig. 5A. cis-Parinaric acid incorporates into membranes and loses...
fluorescence as it becomes oxidized (21, 29). Treatment with Fe$^{2+}$ resulted in an increased rate of cis-parinaric acid oxidation compared with control mitochondria. The Fe$^{2+}$-induced oxidation rate was further increased by R-BEL pretreatment; however, pretreatment with S-BEL had no effect (Fig. 6). BHA and DPPD significantly inhibited cis-parinaric acid oxidation in a concentration-dependent manner (Fig. 6 and data not shown, respectively). Similar to Fe$^{2+}$-induced swelling, iPLA$_2\gamma$ inhibition significantly accelerated the rate of oxidant-induced lipid peroxidation in RCM.

Previous studies in RPTC demonstrated that oxidants directly inactivate ER-iPLA$_{2\gamma}$ (12). To determine the effect of oxidative stress on mitochondrial iPLA$_2\gamma$ activity, RCM were incubated with diluent control or 10 $\mu$M Fe$^{2+}$ and assayed for iPLA$_2$ activity over time. Similar to ER-iPLA$_2\gamma$, oxidant treatment significantly inhibited iPLA$_2\gamma$ activity in RCM (Fig. 7).

DISCUSSION

In this study, we demonstrate the novel finding that human and rabbit RCM possess iPLA$_2$ activity. The preference of RCM iPLA$_2$ for plasmenylcholine phospholipid substrates over phosphatidylcholine substrates is similar to iPLA$_2\gamma$ in microsomes of RPTC and rabbit ventricular myocytes (10, 20). The localization of kidney mitochondrial iPLA$_2$ activity to the inner mitochondrial membrane is in agreement with a previous report in rabbit heart mitochondria (43). While immunoblot analysis results suggested that iPLA$_{2\gamma}$ and iPLA$_{2\beta}$ are localized to RPTC mitochondria, inhibitor sensitivity analysis re-

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Fig. 5. Effect of iPLA$_2$ inhibition on Fe$^{2+}$-induced mitochondrial swelling. Freshly isolated rabbit RCM were pretreated with diluent control, R- or S-BEL 25 nmol/mg (8 $\mu$M), or the antioxidant 25 $\mu$M BHA and then exposed to 10 $\mu$M Fe$^{2+}$. Mitochondrial swelling was measured as the loss of optical density at 540 nm (A). The effect of iPLA$_2$ inhibitors on the time required for Fe$^{2+}$ to induce swelling is presented in B. Values are means ± SE of 3 experiments. Means with different superscripts are significantly different from each other, $P < 0.05$.

Fig. 6. Effect of iPLA$_2$ inhibition on Fe$^{2+}$-induced mitochondrial lipid peroxidation. Freshly isolated rabbit RCM were incubated with the fluorescent lipid cis-parinaric acid and washed as described in MATERIALS AND METHODS and then pretreated with diluent control, R- or S-BEL 8 $\mu$M, or the antioxidant BHA, then exposed to 10 $\mu$M Fe$^{2+}$. The loss of fluorescence (indicative of lipid peroxidation) was followed over time (A). To determine the rate of lipid peroxidation, the difference of percent initial fluorescence in each treatment group from that of control was determined and the oxidation rate was determined by linear regression analysis. Values are means ± SE of 3 experiments (B). Means with different superscripts are significantly different from each other, $P < 0.05$.

Fig. 7. Fe$^{2+}$-mediated inactivation of iPLA$_{2\gamma}$ in RKC mitochondria. Freshly isolated rabbit RCM were treated with diluent control or 10 $\mu$M Fe$^{2+}$ and samples were taken at the indicated time points for iPLA$_2$ activity assays. Activity represents cleavage of 16:0; [H]$^{18}$:1 plasmenylcholine substrates in the presence of 4 mM EGTA. Values are means ± SE of 3 experiments. Means with different superscripts are significantly different from each other, $P < 0.05$. 

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revealed that in rabbit RCM and RPTC mitochondria iPLA2 activity is due solely to the iPLA2γ isoform and not iPLA2β or cPLA2γ.

The iPLA2 isoform in rabbit heart mitochondria and rat liver mitochondria was hypothesized to be iPLA2β based on iPLA2β immunoblot results and sensitivity of the activities to racemic BEL (6, 43). The iPLA2β antibody also detected an immunoreactive protein of ~85 kDa in the microsomal fraction (10) and in mitochondria of RPTC. This size protein is similar to those reported by others in the mitochondria (6, 43). However, the presence of iPLA2β in RPTC is not supported by RT-PCR (11) or inhibitor sensitivity analysis of the iPLA2 activity in RPTC microsomes (10, 20) and mitochondria. In contrast, iPLA2γ expression has been confirmed at the mRNA (11) and protein level (20) (Fig. 2) and the inhibitor sensitivity profile, using racemic, R- and S-BEL, MAPF and AACCOCF3, of microsomes (10, 20) and mitochondria (Figs. 3 and 4) from RPTC confirms iPLA2γ. The concentrations of MAPF and AACCOCF3 used (10 μM each) are well above the IC50 values reported for iPLA2β, cPLA2γ, and PAF-AH (2, 15, 18, 23). In summary, immunoblot analysis results prohibit ruling out iPLA2β expression in RPTC, but RT-PCR and inhibitor sensitivity of the iPLA2 activity in RPTC raise questions about the identity of the protein recognized by the iPLA2β antibody in RPTC.

Regulation of iPLA2γ activity has not been extensively studied. The pH of the environment surrounding iPLA2γ is a potential regulator of its activity. Our experiments revealed that mitochondrial iPLA2 activity increases toward plasmencolcholine substrates but is unchanged toward phosphatidylcholine substrates as pH decreases from 8 to 7.5. This is relevant because a significant portion of rabbit RPTC phospholipids is made up of plasmalogenes (plasmencolcholine and plasmencolcholine) and these phospholipids are enriched with arachidonic acid (20:4) at the sn-2 position (32). Mitochondrial matrix pH is ~8 and decreases toward 7.5 as the inner mitochondrial membrane potential is lost and by agents that increase cytosolic Ca2+ (1). During mitochondrial stress (i.e., ischemia-reperfusion, oxidative stress, Ca2+ overload), the decrease in matrix pH could increase iPLA2γ activity to cleave arachidonic acid containing plasmalogenes, which are targets of ROS in cellular membranes (19). Changes in pH may result in conformational changes of iPLA2γ, phospholipids, or both leading to the observed changes in activity.

Previous observations led to the hypothesis that microsomal iPLA2 activity in kidney, heart, and coronary artery endothelial cells is regulated by PKC-mediated phosphorylation (11, 28, 37). Our recent studies demonstrated that the microsomal iPLA2 in kidney and heart is iPLA2γ (20). In support of this hypothesis, sequence analysis of iPLA2γ revealed the presence of multiple potential serine/threonine phosphorylation sites (5). The addition of ATP to isolated RCM increased iPLA2γ activity, and the increase in activity was blocked by pretreatment with a PKCε-specific inhibitor, suggesting that PKCε regulates mitochondrial iPLA2γ activity. Our findings in conjunction with the observation by Nowak et al. (31) that PKCε is present in RPTC mitochondria and that PKCε translocation to mitochondria is increased after oxidant treatment support the hypothesis that oxidant-induced PKCε translocation may result in upregulation of mitochondrial iPLA2γ activity in response to oxidant stress in renal cells.

Previous studies from our laboratory revealed that inhibition of iPLA2 activity with BEL in rabbit RPTC-potentiated oxidant-induced lipid peroxidation and necrotic cell death (10) and inhibition of ventricular myocyte iPLA2 activity with BEL potentiated doxorubicin- and TBHP-induced cell death (27, 39). These findings led to the hypothesis that iPLA2γ prevents or repairs lipid peroxidation. In this study, we used the iPLA2γ inhibitor, R-BEL, to show that specific inhibition of iPLA2γ in isolated RCM accelerated oxidant-induced lipid peroxidation and mitochondrial swelling. These observations support our hypothesis and demonstrate that mitochondrial iPLA2γ activity protects mitochondria against oxidative stress. Our previous demonstration that iPLA2γ is expressed in ER of rabbit RPTC and ventricular myocytes (20) is consistent with the idea that iPLA2γ localized to these two organelles, which are routinely exposed to oxidants, serves as a defense mechanism against lipid peroxidation-induced organelle dysfunction.

A protective role for iPLA2β in mitochondria has recently been proposed. Seleznev et al. (34) reported that overexpression of iPLA2β in insulinoma cells and Chinese hamster ovary cells results in mitochondrial localization of the GFP-linked iPLA2β and protects against staurosporine-induced apoptosis. Staurosporine-induced apoptosis is at least partially mediated by mitochondrial ROS production and lipid peroxidation (34). The expression and localization of different iPLA2 isoforms vary greatly among different cell types and the role of lipid peroxidation repair may be determined by the available isoforms in a specific cell or organelle.

The ER iPLA2γ in RPTC is directly inactivated by diverse oxidants in a dithiothreitol-sensitive manner, implicating reduced thiols are required for iPLA2γ activity and are a target of oxidants (12). Our current results demonstrate that mitochondrial iPLA2γ also is inactivated by oxidants. Recently, iPLA2β was also shown to be inactivated by oxidants and the specific oxidant-induced damage was elucidated (36). Oxidant-induced inhibition of iPLA2 may be a form of signaling (negative regulation) to prevent excessive activity. Conversely, it may represent an additional mechanism of oxidant-induced toxicity (i.e., oxidants cause lipid peroxidation and inactivate the lipid peroxidation repair enzyme). Prevention of oxidant inactivation of iPLA2γ may provide protection to cells and organelles during oxidative stress.

In conclusion, we demonstrated that iPLA2γ is expressed and active in RPTC mitochondria, and inhibition of iPLA2γ accelerates lipid peroxidation and swelling in isolated RCM. Human kidney cortex displays significant iPLA2 activity in the inner mitochondrial membrane and ER similar to rabbit kidney cortex. Our data suggest that mitochondrial iPLA2γ activity is regulated by pH and phosphorylation by PKCε. Finally, similar to iPLA2γ in RPTC ER, mitochondrial iPLA2γ is inactivated by oxidant stress. Efforts to preserve and enhance mitochondrial iPLA2γ activity may be useful to prevent oxidative stress-induced cell death in the proximal tubular cells of the kidney and in other tissues, including the heart.

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IPLA2γ IN RENAL CELL MITOCHONDRIA


