Interleukin 1α Causes Rapid Activation of Cytosolic Phospholipase A_2 by Phosphorylation in Rat Mesangial Cells

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Abstract

We have shown previously that interleukin 1 (IL-1) stimulates eicosanoid production in glomerular mesangial cells (MC) by de novo synthesis of a 14-kD, group II phospholipase A_2 (PLA_2). IL-1–stimulated prostaglandin E_2 synthesis precedes expression of this enzyme, suggesting that another PLA_2 isoform must be more rapidly activated. In the presence but not absence of calcium ionophore, [^{3}H]arachidonate release is increased significantly as early as 5 min after addition of IL-1, and IL-1 concurrently stimulates a Ca^{2+}-dependent phospholipase activity, which was characterized as the cytosolic form of PLA_2 ($cPLA_2$). IL-1 does not alter either $cPLA_2$ mRNA expression or mass in serum-stimulated MC, suggesting that $cPLA_2$ activity is increased by a posttranslational modification. IL-1 treatment for 30 min doubles ^{32}P incorporation into immunoprecipitable $cPLA_2$ protein, concordant with the increase in enzyme activity. Immunoblot analysis of extracts derived from IL-1–treated (30 min) cells demonstrates a decreased mobility of $cPLA_2$, and treatment of MC lysates with acid phosphatase significantly reduces cytokine-activated $cPLA_2$ activity, further indicating that IL-1 stimulates phosphorylation of the enzyme. IL-1 treatment (24 h) of serum-deprived MC doubled $cPLA_2$ mRNA, protein, and activity. In summary, IL-1 increases $cPLA_2$ activity in a biphasic, time-dependent manner both by posttranslational modification and de novo synthesis. We consider $cPLA_2$ activation a key step in IL-1–stimulated synthesis of pro-inflammatory, lipid mediators, and an integral event in the phenotypic responses induced in target cells by this cytokine. (*J. Clin. Invest.* 1994. 93:1224–1233.) Key words: arachidonic acid • cellular signaling • cytokine • eicosanoid metabolism • inflammation

Introduction

IL-1 is a potent pro-inflammatory cytokine that activates functional responses and biochemical processes in target cells (1). The release of arachidonic acid–derived lipid mediators is a characteristic of chronic and acute inflammation, and IL-1 stimulates eicosanoid synthesis in vitro (1). The molecular mechanisms of IL-1–stimulated arachidonate metabolism have not been defined completely, but receptor-activated, phospholipase A_2 (PLA_2)[1]-mediated release of arachidonic acid from the sn-2 position of membrane phospholipids is rate-limiting in eicosanoid biosynthesis (2).

Mammalian PLA_2s, until recently, have been classified into two groups (I and II) based on position of cysteine pairs and sequence similarities to families of 14-kD PLA_2 isozymes secreted in snake venoms (3). Mammalian pancreatic PLA_2 is a group I enzyme. Mammalian group II enzymes are synthesized in many tissues and abundantly secreted into inflammatory exudates. Both groups I and II secretory PLA_2's ($sPLA_2$) require millimolar calcium concentrations for half-maximal enzyme activity and do not demonstrate preference for the fatty acid present in the sn-2 position of phospholipids. Recently a 97-kD cytosolic form of PLA_2 ($cPLA_2$) has been identified in glomerular mesangial cells and the human monocyte cell line U937 (4–6). This enzyme translocates to membrane vesicles in response to nanomolar changes in free Ca^{2+} concentration and selectively hydrolyzes arachidonyl-phospholipids in membrane vesicles (7). The primary structure of $cPLA_2$ lacks any identity with either cloned $sPLA_2$, and its biochemical characteristics suggest that this form of PLA_2 regulates intracellular arachidonic acid release (4–7).

Enhanced prostaglandin synthesis characterizes glomerular inflammation, and recent evidence suggests that IL-1 is important in the pathogenesis of glomerular injury (8, 9). Mesangial cells are the most abundant source of prostaglandins within the glomerulus, and in culture, express a phenotype that mimics their characteristics within an inflammed glomerulus. We believe IL-1–stimulated mesangial cells to be an appropriate in vitro model system to define the molecular basis for the enhanced arachidonate availability that characterizes glomerular injury.

We have demonstrated previously that IL-1 specifically stimulates mesangial cells to synthesize a group II (nonpancreatic type) $sPLA_2$ (10). IL-1–dependent induction of type II $sPLA_2$ mRNA expression increased coordinately with PLA_2 activity and PGE_2 synthesis. However, three observations reported in this study suggested that a PLA_2 distinct from this $sPLA_2$ isoform also must be activated by IL-1 stimulation. First, PGE_2 synthesis occurred before 6–10 h of IL-1 stimulation, when $sPLA_2$ mRNA is first detected. Second, IL-1 did not

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1. *Abbreviations used in this paper:* AA-$COCF_3$, the trifluoromethyl ketone analogue of arachidonic acid which inhibits $cPLA_2$ activity; AA-$COCH_3$, an arachidonate analogue devoid of inhibitory activity for $cPLA_2$; MAP, mitogen-activated protein; PLA_2, phospholipase A_2; similarly, $cPLA_2$ and $sPLA_2$, cytosolic form of and 14-kD secretory PLA_2, respectively; PLAP, PLA_2-activating protein.

increase basal [^{3}H]arachidonic acid release but did potentiate vasopressin-stimulated arachidonate release. Because $cPLA_2$ has been shown to be regulated by vasopressin (4), we reasoned that this PLA_2 may be primed by IL-1 for enhanced, agonist-stimulated arachidonic acid release. Third, although phosphatidylcholine is hydrolyzed poorly by group II $sPLA_2$, IL-1 increased hydrolysis of arachidonic acid from phosphatidylcholine, a finding consistent with enhanced $cPLA_2$ activity. Taken together, these data suggest that IL-1 directly or indirectly activates $cPLA_2$. We now report that IL-1 rapidly increases $cPLA_2$ activity by changing the phosphorylation state of the enzyme, and in longer incubations, can increase catalytic activity by inducing $cPLA_2$. $cPLA_2$ activation and enhanced arachidonate availability is integral to the glomerular synthesis of pro-inflammatory eicosanoids stimulated by IL-1.

Methods

Materials. Recombinant human IL-1α (IL-1, 3 × 10^8 U/mg) was kindly provided by Dr. Peter Lomedico (Hoffmann-LaRoche, Nutley NJ). The endotoxin content and specific activity have been previously published (10). L-α-1-palmitoyl, 2-arachidonyl, [1-^{14}C]arachidonyl-phosphatidylethanolamine (60 Ci/mmol), and arachidonic acid (5, 6, 8, 9, 11, 12, 14, 15, ^{3}H(N), 100 Ci/mmol) were purchased from Du-Pont New England Nuclear (Boston, MA). L-α-1-stearoyl, 2-arachidonyl, [1-^{14}C]arachidonyl-phosphatidylcholine (56 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). ^{32}[P]-orthophosphate (carrier free), *trans* ^{35}S-label (L-methionine, [^{35}S], 1,163 Ci/mmol), phosphate-free RPMI 1640 and methionine, cysteine-free RPMI 1640 medium were obtained from ICN Biomedicals, Inc. (Irvine, CA).

Cell culture. Well-characterized mesangial cells were grown from collagenase-treated rat glomeruli in RPMI 1640 medium containing 8.5% FBS and 8.5% calf serum, 15 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin and fungizone, 5 μg/ml of insulin and transferrin, and 5 ng/ml of sodium selenite as previously described (10). The cells were used in passages 3–8, and as indicated in the specific methods or in the results were either maintained in 17% serum, acutely serum-deprived (2 h) or were held for 24–48 h in 0.5% FBS.

[^{3}H]arachidonate release. Basal and stimulated arachidonate release was determined as previously described (10), with some modifications. Cells in six-well clusters were labeled for 20 h with 0.5 μCi/ml [^{3}H]arachidonic acid in RPMI 1640 medium with 17% serum. The labeling media was aspirated and the cells washed and serum-deprived for 2 h at 37°C in Hepes (10 mM)-buffered RPMI (Hepes-RPMI) containing 0.1% BSA. The experiment was initiated by the addition of vehicle or IL-1 for 1, 4, or 20 min before the addition of the Ca^{2+} ionophore, A23187 (0.25 μM) (Calbiochem Corp., San Diego, CA). This A23187 concentration was chosen to cause only a minimal change in [^{3}H]arachidonate release (Fig. 1) and to mimic a receptor-mediated increase in cytosolic Ca^{2+} (M. B. Ganz, personal communication). Ionophore was added for an additional 1 min after the cells were incubated with IL-1 for 1 and 4 min, and for 10 min after the cells had been exposed to IL-1 for 20 min for total incubation times of 2, 5, and 30 min, respectively. As positive controls, cells were also incubated with PMA for 10 min followed by an additional 10 min in the presence of ionophore. In some experiments, the cells were preincubated for 2 h with a control or $cPLA_2$-inhibitory arachidonate acid analogues before stimulation with IL-1 or PMA (11). All reactions were terminated by removal of the incubation media and the addition of 0.4 ml of cold (4°C) methanol containing 10 μg unlabeled arachidonic acid to the monolayer. The cells were scraped, and this suspension along with an additional 0.4 ml methanol rinse was combined with the incubation supernatants and acidified with formic acid (0.2%, vol/vol). Chloroform/methanol (1:1.2, vol/vol, 0.6 ml) was added to the extraction tube followed by the addition of 0.6 ml of chloroform. After separating the organic and aqueous phases by centrifugation, the organic phase was dried under N_2 and resolubilized in ethanol. Free arachidonate was separated from cellular phospholipids using TLC. [^{3}H]arachidonic acid release was expressed as a percentage of total [^{3}H]arachidonate incorporated into the mesangial cell monolayer (determined by total TLC plate radioactivity).

Cell-free extracts and PLA_2 assay. At the time of the experiment, subconfluent cells were serum-restricted by replacing growth medium

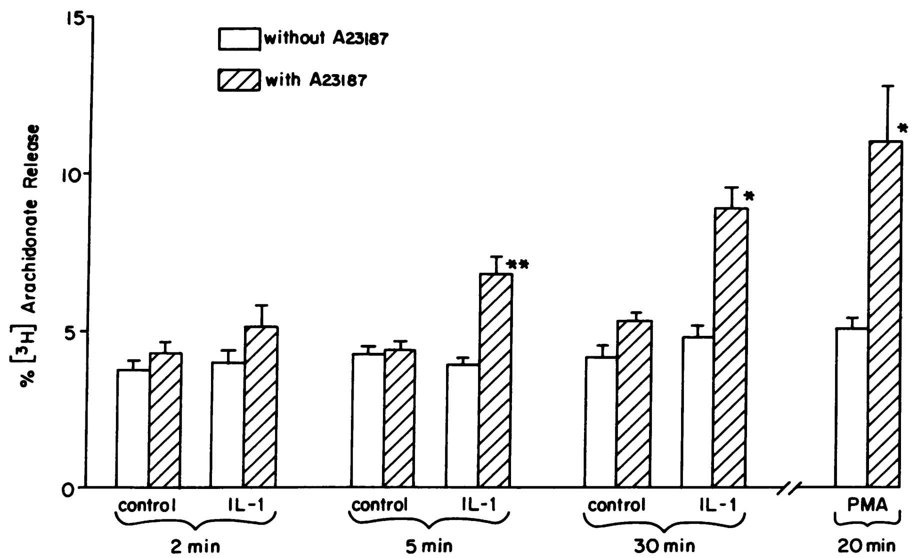

Figure 1. IL-1 rapidly stimulates [^{3}H]-arachidonate release. Subconfluent rat mesangial cells were labeled with [^{3}H]-arachidonic acid (0.5 μCi/well) for 20 h, washed with RPMI media containing 0.1% BSA, and incubated in this media for an additional 2 h. The cells were then stimulated for the indicated times with vehicle alone (control), IL-1 (50 ng/ml), or PMA (300 nM). Cells were exposed to IL-1 for 1 and 4 min before the addition of A23187 (0.25 μM) for an additional 1 min. In the longest incubations, mesangial cells were incubated for 10 and 20 min with PMA and IL-1, respectively. A23187 was subsequently added for 10 min. The times in the figure indicate the total exposure time to IL-1 or PMA in the absence and the presence of ionophore. [^{3}H]arachidonate in cells and supernatants was quantified by TLC after chloroform-methanol extraction. Results are the mean±SEM for triplicate experiments of triplicate wells (n = number of separate experiments; $^*P < 0.05$, $^{**}P < 0.025$ compared to the control value). In the absence and presence of IL-1, ionophore-stimulated [^{3}H]arachidonate release at 2 min was 4.4±0.6% and 5.1±0.8% release ($n = 4$, ionophore- vs. ionophore- and IL-1-stimulated, respectively, P = NS); at 5 min was 4.5±0.4% vs. 6.9±0.9% release ($n = 3$, ionophore- vs. ionophore- and IL-1-stimulated, respectively, $P < 0.01$); at 30 min was 5.3±0.2% and 8.8±0.7% release ($n = 3$, ionophore- vs. ionophore- and IL-1-stimulated respectively, $P < 0.01$). After 10 min, ionophore-stimulated [^{3}H]arachidonate release was 5.3±0.2% vs. 11.0±2.9% in the absence and presence of PMA, respectively ($n = 3$, $P < 0.005$).

with Hepes-RPMI for 2 h before incubation with IL-1, 12-tetradecanoyl phorbol myristate 13-acetate (Calbiochem Corp.) or vehicle-control at the indicated doses. These stimuli were added directly to the preincubation medium, and the cells were incubated at 37°C in the presence of 5% CO_2, 95% air for the indicated times. In some experiments, cells were treated with heated IL-1 (90°C, 60 min) or native IL-1 in the presence of polymyxin B (10 μg/ml, Sigma Chemical Co., St. Louis, MO) to determine if LPS contaminating the reagents accounted for the effects of the cytokine. Treatment of cells with polymyxin B alone did not alter $cPLA_2$ activity in cell-free extracts (not shown). Three 100-cm dishes of cells were used for each condition to assay PLA_2 activity in 200,000-*g* supernatants of cell lysates as previously described (4, 10). Briefly after the incubation period, the media was removed. The cells were washed and scraped at 4°C into a buffer containing 250 mM sucrose, 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM EGTA, and the protease inhibitors, pepstatin A (1 μg/ml), leupeptin (1 μg/ml), and PMSF (0.1 mM). The cells were homogenized using a Dounce homogenizer (25 strokes) and spun at 500 *g* for 5 min to remove the nuclei. The homogenate was centrifuged at 200,000 *g* for 1 h at 4°C using a model Ti-70.1 rotor (Beckman Instruments, Inc., Fullerton, CA). Protein content in the supernatant was determined (protein assay, Bio-Rad Laboratories, Richmond, CA). Samples from each condition were matched for protein concentration by appropriate dilution and assayed for PLA_2 activity. Unless otherwise indicated, [1-^{14}C]arachidonyl-phosphatidylcholine was used as substrate (final concentration, 15 μM). The assay was initiated by adding mesangial cell extract containing 1.5–7 μg of protein to an assay buffer containing 5 mM $CaCl_2$ and 50 mM Hepes, pH 7.5 (final concentrations). In some experiments, control and $cPLA_2$-inhibitory arachidonic acid analogues (11), in the indicated concentrations, were added for 5 min at 37°C to the cellular lysates in assay buffer. The assay then was initiated by the addition of [^{14}C]phosphatidylcholine. Reactions proceeded for 30 min at 37°C and were terminated by addition of 40 μl of 2% acetic acid in ethanol containing 10 μg of unlabeled arachidonic acid. The reaction mixtures and appropriate standards were spotted onto heat-activated LK 5 DF silica gel TLC plates (Whatman Inc., Clifton, NJ) and developed with ethyl acetate/iso-octane/acetic acid/water (55:75:8:100). Lipids were visualized by I_2 staining. Free arachidonate was scraped and quantified by liquid scintillation counting. Results were expressed as picomoles of arachidonate released per minute per milligram of protein.

Extract fractionation. As described (4, 12), protein-matched extracts from control, IL-1- and PMA-stimulated cells were applied to a prepacked 5 × 50-mm Mono-Q HR 5/5 anion exchange column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) preequilibrated with 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, and 50 mM Hepes, pH 7.5. The column was washed with equilibration buffer. PLA_2 activity was eluted with a linear salt gradient (0.15–1.0 M NaCl). 0.5-ml fractions were collected and assayed for PLA_2 activity.

Measurement of $cPLA_2$ mRNA levels. IL-1–induced changes in $cPLA_2$ mRNA levels were assessed by Northern analysis of total cellular RNA (20 μg) as described (13) with the following modifications. A gel-purified cDNA fragment which encoded the 2.9 kb human cytosolic PLA_2 (7, kindly provided by Dr. James D. Clark, Genetics Institute, Inc., Cambridge, MA), was labeled with [α-^{32}P]dCTP (Dupont/NEN, Boston, MA) by nick translation. Filters were hybridized with ^{32}P-labeled $cPLA_2$ probe overnight at 42°C in 5× SSPE (1× SSPE: 180 mM NaCl, 10 mM $Na_2HPO_4 \cdot 7H_2O$, and 1 mM EDTA), 50% formamide, 5× Denhardt's solution, 1% SDS, 10% dextran sulfate, and 200 μg/ml denatured salmon sperm DNA. The blot was subsequently washed with 2× SSPE at room temperature for 30 min, 2× SSPE, 2% SDS at 50°C for 30 min and a final wash in 0.1× SSPE, 1% SDS at 52°C. After autoradiography for 15 h, bound probes were stripped from the blots by boiling and the filter was sequentially rehybridized with ^{32}P-labeled cDNA probes for rat nonpancreatic secretory PLA_2 (10, 14), murine IL-6 (14), and rat GAPDH (14). Autoradiographic densities were quantified by scanning densitometry (Scan Maker, Microtek, Torrence, CA) and appropriate software (Adobe Photoshop, Adobe Systems, Mountainview, CA; and Scan Analysis, Biosoft, Cambridge, UK). $cPLA_2$ mRNA abundance was corrected for background and normalized for changes in GAPDH transcript abundance.

Protein phosphorylation and ^{35}S labeling of $cPLA_2$ and immunoprecipitation. Subconfluent mesangial cells in 100-mm plates were incubated for 1 h in serum-free, phosphate-free RPMI 1640 media, supplemented with 12.5 mM Hepes, pH 7.0, and $NaHCO_3$ (1.1 mg/ml). [^{32}P]orthophosphate (0.35 mCi/10-cm dish) was added for an additional 2 h. The cells were stimulated with vehicle or IL-1 (50 ng/ml) for the final 30 min of labeling, washed twice with ice cold PBS, and incubated for 10 min at 4°C in 0.4 ml of lysis buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 30 mM sodium pyrophosphate, 0.2 mM sodium orthovanadate, and 1.0% NP-40). Extracts from three 10-cm dishes were combined and spun at 200,000 *g* for 60 min. The resulting supernatants from control and stimulated mesangial cell lysates were matched for protein, and cleared with protein A-Sepharose (Pharmacia). The samples (1 ml) were then incubated overnight at 4°C with excess (10 μl) rabbit antisera against $cPLA_2$ or preimmune serum (7, kindly provided by Dr. James D. Clark). Immunocomplexes were precipitated using protein A-Sepharose, washed, and analyzed on 8% SDS-polyacrylamide gels. The ^{32}P-labeled proteins were detected by autoradiography using intensifying screens. In some experiments, the extent of phosphorylation was quantitated using storage phosphor technology and image analysis (15). The dried gel was placed on the photostimulatable storage phosphor imaging plate in a Molecular Dynamics (Sunnyvale, CA) exposure cassette for 2 d at room temperature and subsequently scanned by a Molecular Dynamics model 400A phosphor imager using a 10 mW helium-neon laser. Luminescence at 390 nm was collected, digitalized and stored. A digital image of the original incident radiation detected by the storage phosphor plates was generated, and bands of interest were quantified using Molecular Dynamics image quant software. Storage phosphor imaging plates have a large linear dynamic range and high sensitivity that allows accurate quantitative results (15). In some experiments, PLA_2 activity was quantified in cell lysates (110 μl) before and after $cPLA_2$ was immunoprecipitated using specific and preimmune antisera (3 μl).

To assess cytokine-induced changes in de novo protein synthesis, cells were washed, depleted of methionine for 30 min and transferred to fresh methionine-, cysteine-free RPMI media containing [^{35}S]-methionine (0.5 mCi/10-cm dish) for 1 h. IL-1 (50 ng/ml) was added for an additional 1 h. Cell-free lysates were prepared and immunoprecipitated, and immunoprecipitates were separated by electrophoresis as described above. ^{35}S-labeled proteins were imaged by fluorography at −70°C after soaking the gels with a fluor amplifying reagent (Amersham Corp.).

Immunoblot analysis of $cPLA_2$. 2 h before the experiment, cells maintained in complete medium were placed in RPMI media containing 10 mM Hepes, and treated with the indicated agonists. In some experiments, cells were serum-deprived for 24 h and then treated with IL-1 (10 ng/ml) or vehicle for an additional 24 h. Cells were then washed with PBS containing 4 mM EDTA, PMSF, leupeptin, and pepstatin A, and lysed as previously described (10). The samples (80 μg protein) were loaded on 8% SDS-polyacrylamide gel. To observe a phosphorylation-stimulated shift in $cPLA_2$ mobility (16), electrophoresis was continued until the 49-kD prestained protein marker (ovalbumin) had migrated to the end of the gel (a distance of 17 cm). The proteins were electrophoretically transferred (90 min at 85 V 4°C) to nitrocellulose membrane in a buffer containing 15% MeOH, 25 mM Tris, 192 mM glycine, 0.05% SDS, and 0.5 mM Na_3VO_4. The blot was blocked at 22°C for 2 h in PBS containing 0.2% Tween-20, 5% nonfat milk, and 0.5 mM Na_3VO_4. $cPLA_2$ protein was detected by incubation with a rabbit polyclonal antibody (1:2,000 dilution) against a recombinant fragment of human $cPLA_2$ (17) for 1 h followed by a 1 h incubation with a peroxidase-labeled, anti–rabbit IgG antibody (1:2,000 dilution, Kirkegaard & Perry, Gaithersburg, MD). The primary antibody cross-reacts with rat $cPLA_2$. Immunolocalized proteins were detected using a chemiluminescent detection system (ECL, Amersham Corp.).

All blots were stained subsequently with India ink to verify that proteins were equivalent and transferred.

Results

IL-1 stimulates [^3H] arachidonate release from intact cells. We first determined if IL-1 activated a PLA_2 before the previously described induction at 6–10 h of the group II $sPLA_2$. Enzyme activity was initially assessed by measuring intracellular and extracellular [^{3}H]arachidonic acid release from equilibrium-labeled glomerular mesangial cells in the presence or absence of the calcium ionophore A23187. Calcium ionophore was included because IL-1 does not increase cytosolic Ca^{2+} (18) and most PLA_2s are Ca^{2+} dependent. IL-1 alone in incubations as long as 30 min did not significantly increase [^{3}H]arachidonate release. In contrast, cells exposed to IL-1 followed by ionophore showed enhanced [^{3}H]arachidonate release (Fig. 1). The small increase in arachidonic acid release detected at 2 min was followed by a statistically significant increase at 5 and 30 min. As a positive control, cells were also incubated with the phorbol ester PMA in the absence or presence of ionophore (Fig. 1). PMA is known to activate $cPLA_2$ in vitro and "primes" intact cells for enhanced arachidonate metabolism (4, 19). Primed stimulation is the synergistic enhancement of cellular responses, when combinations of stimuli are utilized. PMA alone had little effect on mesangial cellular [^{3}H]-arachidonate release but synergized with A23187 to stimulate a twofold increase in cytosolic arachidonate release (Fig. 1). These data suggest that IL-1, similar to PMA, primes cells for enhanced arachidonate release either by modifying a Ca^{2+}-dependent PLA_2 or by activating a PLA_2 modulatory protein. Because IL-1 primes mesangial cells within 5 min for enhanced ionophore-stimulated arachidonate release, a PLA_2 that is distinct from the nonpancreatic group II $sPLA_2$ must be activated.

In vitro, the trifluoromethyl ketone analogue of arachidonic acid (AA-$COCF_3$) is a slow binding, but specific and potent inhibitor of $cPLA_2$ (11). We tested the effects of this compound in intact [^{3}H]arachidonate-labeled cells. A 2-h preincubation with AA-$COCF_3$ completely inhibited IL-1–stimulated but not basal [^{3}H]arachidonate release (Table I). Another arachidonate analogue, in which the trifluoromethyl ketone group is substituted with a -$COCH_3$ group (AA-$COCH_3$) and which is devoid of inhibitory activity, had no effect on IL-1–stimulated [^{3}H]arachidonate release (Table I). Two inferences can be drawn from these data. First, IL-1 specifically activates $cPLA_2$ to stimulate arachidonic acid release in intact cells. Second, basal [^{3}H]arachidonic acid release may result from a $cPLA_2$-independent mechanism since AA-$COCF_3$ had no effect in vehicle-stimulated cells (Table I), but almost completely inhibits $cPLA_2$ activity in the in vitro phosphatidylcholine vesicle assay using cell-free extracts (see below).

IL-1 stimulates $cPLA_2$ activity. Increased intracellular arachidonate may result from either activation of acylhydrolases and/or inhibition of acyltransferases (2). Since we and others have shown that IL-1 increases acyltransferase activity (20, 21), we focused on IL-1–stimulated deacylation reactions in cell-free extracts. Mesangial cells were stimulated for 2 h with IL-1 (10 ng/ml) or vehicle in the absence of serum. As a positive control, extracts of cells stimulated with PMA (300 nM) for the final 10 min of serum deprivation, were assayed as shown in Table II. Both IL-1 and PMA stimulated PLA_2 activity in subconfluent mesangial cells. IL-1 consistently increased activity by twofold or greater. As previously reported (4), PMA gave a two- to threefold increase in PLA_2 activity. PLA_2 activity in extracts of cells incubated with IL-1 increased progressively from 10 to 60 min (Fig. 2 *A*). At 30 min, IL-1 stimulated PLA_2 activity at concentrations between 1.0 and 10 ng/ml, and maximal activation was observed at 50–100 ng/ml (Fig. 2 *B*). LPS contamination of reagents did not account for these results. $cPLA_2$ activity in extracts of control cells and cells treated with heated IL-1 was similar (not shown). Addition of polymyxin B, a binder of LPS, with IL-1 did not affect $cPLA_2$ activity assayed in the phosphatidylcholine vesicle assay.

These results clearly show that IL-1–treated mesangial cells rapidly activate an enzymatic activity which releases arachidonate from the sn-2 position of exogenous [^{14}C]-phosphatidylcholine in a standard assay for $cPLA_2$ activity. To characterize the IL-1–stimulated acylhydrolase activity, protein-matched cell extracts from control, IL-1–, and PMA–stimulated mesangial cells were fractionated on a Mono Q HR 5/5

Table I. [^3H]Arachidonic Acid Release in the Presence and Absence of a Fatty Acid Analogue Inhibitory for $cPLA_2$

Condition	[^{3}H]Arachidonic acid released
	cpm
Inactive FA analogue (AA-$COCH_3$)	
Vehicle	10,781±486
IL-1	17,653±548
Inhibitory fatty acid analogue (AA-$COCF_3$)	
Vehicle	9,889±913
IL-1	10,809±1078

Mesangial cells were labeled with [^{3}H]arachidonic acid as described in Fig. 1. The cells were deprived serum for 2 h in the presence of the inhibitory fatty acid or the inactive fatty acid analogue. (Final concentrations, 25 μM.) Cells were then stimulated with vehicle or IL-1 (20 ng/ml) for 20 min. A23187 (0.25 μM) was subsequently added for 10 min. [^{3}H]arachidonate release was quantified as described in Fig. 1. Results are the mean±SD of triplicate wells representative of duplicate experiments. Total ^{3}H incorporation was the same in control and IL-1–stimulated cells in both treatment protocols.

Table II. Stimulation of $cPLA_2$ Activity in Rat Mesangial Cells by IL-1 and PMA

Condition	PLA_2 activity
	pmol/min per mg
Control	238.9±27.9
IL-1	497.8±99.4*
PMA	664.3±92.8‡

Subconfluent cultures of mesangial cells were incubated in serum-free media for 2 h, with or without IL-1 (10 ng/ml). In some cultures, PMA (300 nM) was added for the final 10 min of the serum-free incubation. Cell-free extracts were prepared by homogenization and ultracentrifugation as described in Methods, matched for protein, and assayed for PLA_2 activity. Results are expressed as mean values±SEM for triplicate determinations in four individual experiments. * $P < 0.025$, ‡ $P < 0.005$ compared to the control value.

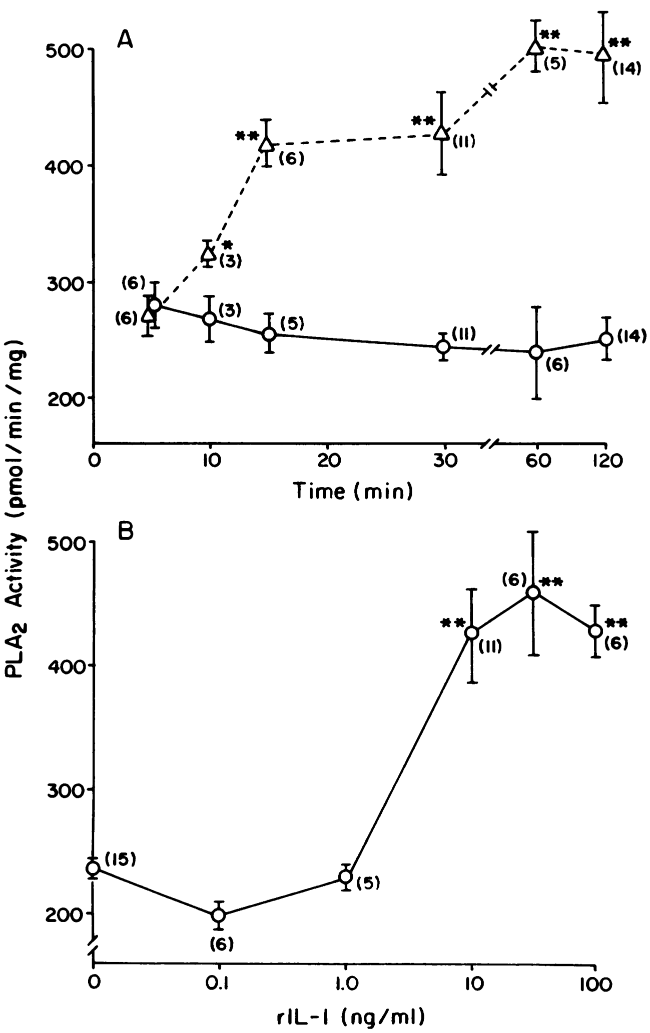

Figure 2. Time course and dose dependence of $cPLA_2$. (*A*) IL-1 stimulates $cPLA_2$ activity in a time-dependent manner. Mesangial cells were incubated in serum-free media for 2 h before the addition of IL-1 (10 ng/ml). Cell-free extracts were prepared from IL-1–treated or control cells at the indicated times, matched for protein, and assayed for PLA_2 activity. Data are presented as the mean value±SEM of two to five individual experiments assayed in duplicate or triplicate (n = number of determinations). $^{**}P < 0.001$, compared to the concurrent control value. (*B*) IL-1 stimulates $cPLA_2$ activity in a concentration-dependent manner. IL-1 at the indicated concentrations was added to mesangial cells incubated in serum-free media for 2 h. After 30 min, the cells were harvested and cell-free extracts prepared. The protein-matched samples were then assayed for PLA_2 activity. Results shown are the mean value±SEM from two to five individual experiments. $^{**}P < 0.001$ compared to the control values (n = number of determinations).

anion exchange column. PLA_2 activity was eluted with a NaCl gradient. > 95% of enzyme activity bound to the column. As shown in Fig. 3, the PLA_2 activity from each extract comigrated as a single peak, which was eluted with 0.4–0.45 M NaCl. This elution profile is consistent with that previously described for $cPLA_2$ in U937 and mesangial cells (4–6, 12). PLA_2 activity in fractionated extracts from both IL-1– and PMA-treated cells maintained higher specific activities than control extracts. IL-1–stimulated $sPLA_2$ is eluted with 1.2 M NaCl (M. Konieczkowski and J. R. Sedor, unpublished results). No PLA_2 activity was detected in fractions eluted with this salt concentration. Recoveries in all cases were > 75%.

As a complementary approach to verify that the IL-1–stimulated PLA_2 activity was $cPLA_2$, we incubated IL-1–stimulated extracts for 30 min at room temperature with either a rabbit polyclonal antiserum against human $cPLA_2$ (7) or preimmune antiserum. The specific antiserum recognizes rat $cPLA_2$ epitopes. After immunoprecipitation, residual supernatant PLA_2 activity was undetectable in control or IL-1–stimulated mesangial cell extracts. In contrast, PLA_2 activity was similar before and after immunoprecipitation with preimmune serum in extracts of IL-1–stimulated cells (control extracts, 141.1±12.7 pmol/min per mg; untreated IL-1–stimulated extracts, 238.5±13.6 pmol/min per mg; and residual $cPLA_2$ activity in IL-1–stimulated extracts pretreated with preimmune sera, 310.2±21.5 pmol/min per mg, $n = 2$).

The effects of several PLA_2 inhibitors on $cPLA_2$ activity were tested in the phosphatidylcholine vesicle assay. The specific $cPLA_2$ inhibitor (11), $AA\text{-}COCF_3$, completely inhibited IL-1–stimulated $cPLA_2$ activity. In contrast, a control arachidonate analogue, $AA\text{-}COCH_3$, which is devoid of inhibitory activity (11), had no effect. $cPLA_2$ activity was 721.7±57.7, 720.8±33.8, and 95.0±9.2 pmol/mg per min (mean±SEM, $n = 3$) in vehicle (DMSO)-, $AA\text{-}COCH_3$ (10 μM)-, and $AA\text{-}COCF_3$ (10 μM)-treated extracts of IL-1–stimulated mesangial cells. Compounds that effectively inhibit $sPLA_2$ activation, less effectively inhibited $cPLA_2$ activity in extracts of both control and IL-1–stimulated cells. Aristolochic acid (150 μM), quinacrine (100 μm), and 7, 7-dimethyl-5,8-eicosadienoic acid (5 μM) inhibited IL-1–stimulated activity by 36±4.1%, 28±2.8%, and 18±0.3%, respectively. A qualitatively similar pattern of inhibition was observed when these compounds were added to vehicle-stimulated cells (not shown). These data provide further evidence that IL-1 specifically can activate $cPLA_2$.

Other biochemical characteristics of the cytokine-activated $cPLA_2$ were determined. Unlike the IL-1–induced $sPLA_2$ activity (10), the PLA_2 activity rapidly stimulated by IL-1 hydrolyzed arachidonate from both [^{14}C]phosphatidylcholine and [^{14}C]phosphatidylethanolamine (data not shown). A pH (pH 4.5–9.0) dependence analysis of the IL-1–stimulated $cPLA_2$ demonstrated little activity below pH 7.0 and peak activity at an alkaline pH (approximately pH 9.0). Significant PLA_2 activity could not be measured in the extracts in the absence of Ca^{2+} of either vehicle- or IL-1–stimulated mesangial cells (data not shown). These characteristics are similar to those previously reported for both the mesangial cell and kidney enzymes (4, 12, 22).

Regulation of $cPLA_2$ by IL-1. The preceding data suggest that IL-1 causes a stable modification of $cPLA_2$ since the cytokine-activated enzyme stimulation was maintained after cell disruption, ultracentrifugation, and column fractionation. We explored two potential mechanisms which could result in a stable enhancement of $cPLA_2$ activity: (*a*) de novo synthesis of $cPLA_2$ or a stimulatory protein such as PLA_2-activating protein, and (*b*) a posttranslational modification of $cPLA_2$ which increases enzyme activity.

As shown in Fig. 4, Northern analysis demonstrated $cPLA_2$ mRNA in both control and cytokine-treated mesangial cells maintained in 17% serum. In four separate experiments, IL-1 had no consistent effect on $cPLA_2$ mRNA expression or activity (not shown) in incubations as long as 24 h, but induced expression of $sPLA_2$ at 24 h (Fig. 4) and IL-6 at 1 and 2 h (not

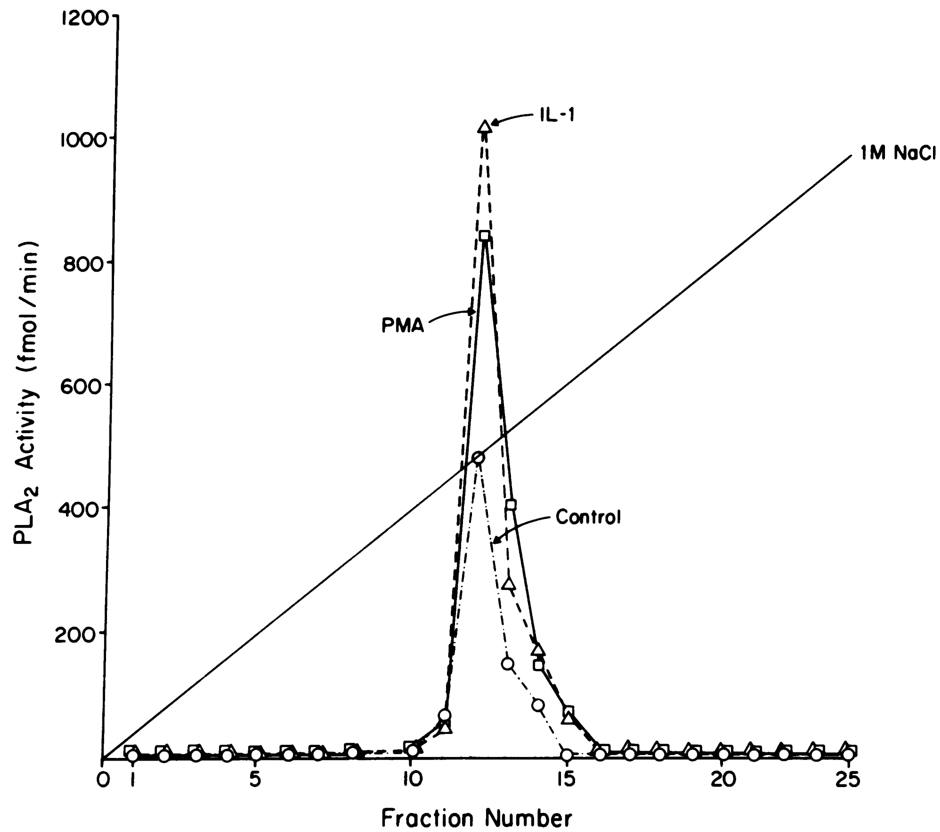

Figure 3. Characterization of PLA_2 activity from PMA and IL-1–treated rat mesangial cells by Mono-Q anion exchange chromatography. Cells were stimulated with PMA and IL-1 as described in Table I. Protein-matched, cell-free extracts were prepared and fractionated using a linear 0.15–1.0 M NaCl gradient. Each 0.5 ml fraction was assayed for PLA_2 activity. Peak PLA_2 activity coeluted at fraction 11 for each treatment. After fractionation, activity in IL-1– and PMA-stimulated extracts was greater than control samples.

shown). Moreover, IL-1 did not induce $cPLA_2$ protein when newly synthesized proteins in vehicle- and IL-1-stimulated (1 h) mesangial cells were radiolabeled with [^{35}S]methionine and enzyme levels were analyzed by immunoprecipitation (data not shown). Taken together, these data demonstrate that the rapid stimulation of $cPLA_2$ activity in serum-stimulated cells by IL-1 did not result from increased enzyme mass.

To assess whether the cytokine-stimulated $cPLA_2$ activity resulted from de novo synthesis of a PLA_2-activating protein, cycloheximide was added for 30 min or 2 h before the addition of IL-1 (50 ng/ml) for a subsequent 30 min incubation. Cycloheximide (2 μg/ml) pretreatment had no effect on IL-1–stimulated $cPLA_2$ activity. The percent stimulation of enzyme activity by IL-1 above the control value was 239±78 and 211±37 (mean±SEM, $n = 2$) in the absence and presence of cycloheximide, respectively.

We next determined whether IL-1–stimulated $cPLA_2$ activity resulted from a post-translational modification, and hy-

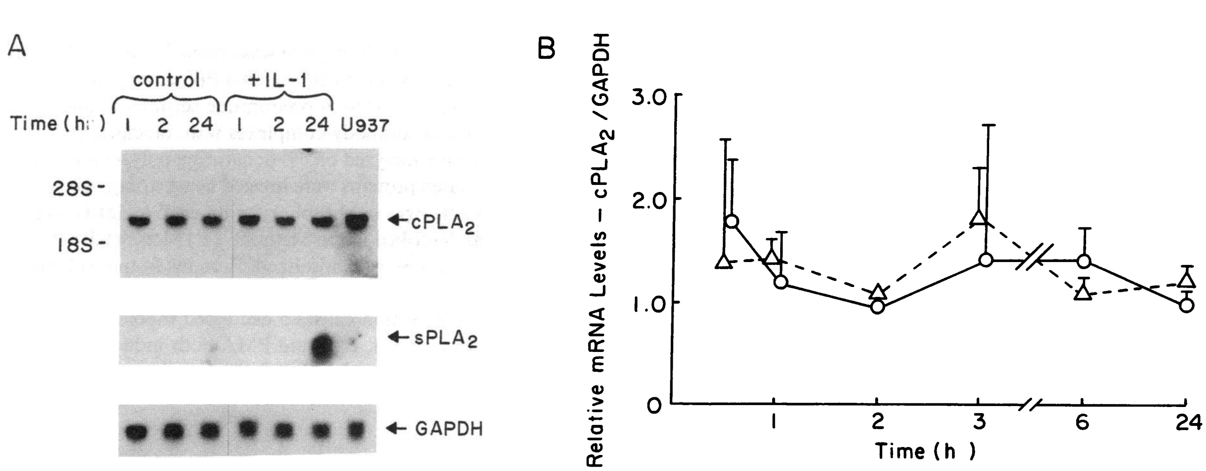

Figure 4. IL-1 does not increase $cPLA_2$ mRNA levels in mesangial cells maintained in serum. Mesangial cells were incubated with IL-1 (10 ng/ml) or vehicle and the cells harvested for RNA at the indicated times. Total cellular RNA (20 μg) was analyzed for $cPLA_2$ message by Northern analysis as described in Methods. The filter was then rehybridized using probes for the 14-kD nonpancreatic secretory PLA_2 ($sPLA_2$), as a positive control for IL-1-stimulated transcript induction, and rat GAPDH (1.2 kb) to assess integrity and sample equivalence. $cPLA_2$ mRNA expression was corrected for changes in GAPDH transcript abundance. A representative Northern analysis is shown in *A* and mean (±SEM) change in relative $cPLA_2$ mRNA abundance in vehicle (△)- and IL-1 (○)-stimulated cells is shown from four separate experiments in *B*.

pothesized that IL-1 altered enzyme activity by phosphorylation for two reasons. First, $cPLA_2$ contains consensus phosphorylation sequences for several kinases (7, 16); second, IL-1 activates serine-threonine-kinases (23, 24). Mesangial cells were labeled in the absence of serum with [^{32}P]orthophosphate for 2 h and stimulated with IL-1 (50 ng/ml) for an additional 30 min. $cPLA_2$ was immunoprecipitated from the high-speed supernatants of control and IL-1–treated cells using polyclonal $cPLA_2$ antiserum or preimmune sera. As shown in Fig. 5 *A*, a specific protein was precipitated by the anti–$cPLA_2$ antiserum but not preimmune serum in both the control and IL-1–treated cells. The specific band was 97 kD, corresponding to the molecular mass of phosphorylated $cPLA_2$. ^{32}P incorporation into this band was nearly twofold greater than control in response to IL-1, a finding demonstrated in duplicate experiments. This IL-1–induced increase in $cPLA_2$ phosphorylation correlated with the magnitude of enzyme activation by IL-1 in whole cells at 30 min and is consistent with the idea that protein phosphorylation may regulate $cPLA_2$ activity. Others have demonstrated that agonist-induced phosphorylation and activation of $cPLA_2$ is associated with decreased mobility in SDS-polyacrylamide gels (16). Immunoblot analysis demonstrated that treatment of mesangial cells with IL-1 for 30 min caused shift in $cPLA_2$ mobility from a more rapidly to a more slowly migrating species (Fig. 5 *B*). As previously described (16), PMA similarly induced a shift in $cPLA_2$ mobility.

We next determined if phosphorylation of $cPLA_2$ by IL-1 resulted in increased enzyme activity (Table III). $cPLA_2$ activity was twofold higher in high-speed supernatants derived from IL-1–stimulated cells compared to vehicle-treated cells. In two separate experiments, treatment of the extracts with human acid phosphatase (0.4 IU) significantly reduced IL-1–stimulated enzyme activity but had no significant effect on enzyme activity in control cells. Taken together, these data suggest that IL-1 enhances $cPLA_2$ by phosphorylation. Basal $cPLA_2$ activity either is not dependent on phosphorylation or results from a phosphorylation event which is insensitive to acid phosphatase.

Mesangial cells used in the preceding experiments were either serum stimulated or acutely serum deprived (2 h). For two reasons, we next assessed IL-1–induced $cPLA_2$ expression in cells that were held for 24–48 h in 0.5% FBS. First, we have demonstrated that growth factors in serum attenuate induction of $sPLA_2$ mRNA activity (14). Second, serum contains glucocorticoids and dexamethasone prevents IL-1 induction of $cPLA_2$ in other cell types (25, 26). Fig. 6 depicts the results of a representative Northern and immunoblot analysis of $cPLA_2$ expression in control and IL-1-stimulated, serum-deprived cells. IL-1 increased $cPLA_2$ mRNA in 24-h incubations (Fig. 6 *A*) but not in incubations shorter than 6 h (not shown). Relative $cPLA_2$ mRNA levels, corrected for changes in GAPDH mRNA, were 2.4±0.4-fold (mean±SEM, n = 2) higher in IL-1-stimulated cells when compared to mesangial cells only exposed to vehicle. IL-1 concordantly increased $cPLA_2$ protein 1.7±0.1-fold (mean±SEM, n = 2) (Fig. 6 *B*). Under these conditions, $cPLA_2$ mobility is similar in vehicle- and IL-1–treated cells, suggesting that the amounts of hormones/growth factors contained in 0.5% of serum can maintain $cPLA_2$ in its phosphorylated state. Consistent with this hypothesis, we have noted that mesangial cells must be completely serum-deprived to maximally down-regulate $cPLA_2$ activity (M. Konieczkowski and J. R. Sedor, unpublished results). $cPLA_2$ enzyme activity was similarly doubled in serum-deprived mesangial cells treated with IL-1 for 24 h (385.6±13.2 and 620.3±15.2 pmol/min per mg, extracts from control and IL-1–treated cells, mean±SEM, triplicate determinations). This finding is consistent with our previous results using the phosphatidylcholine vesicle assay (10). Although IL-1 markedly induces $sPLA_2$ by 24 h, phosphatidylcholine vesicles are a poor substrate for

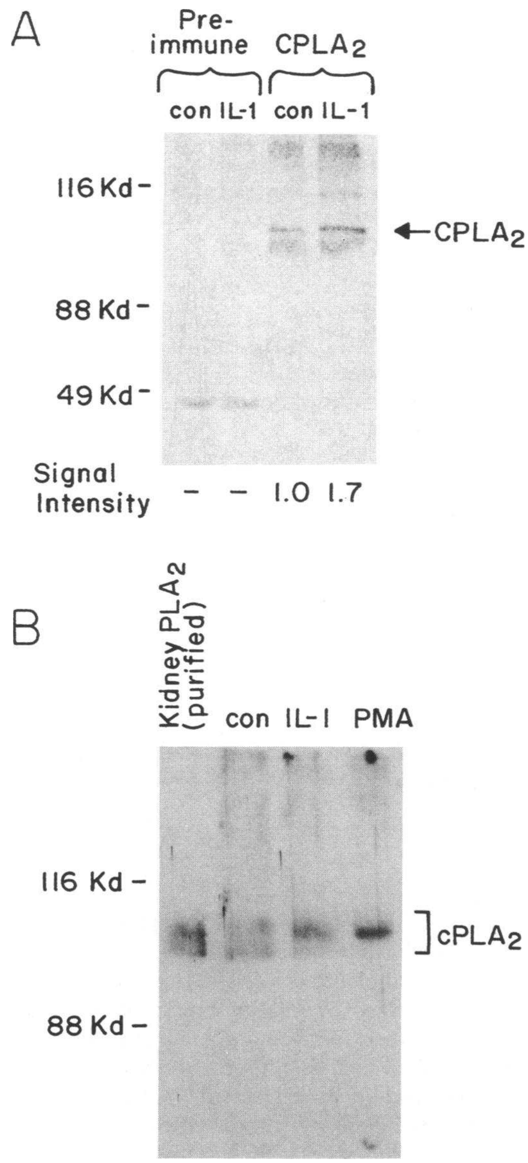

Figure 5. IL-1 stimulates the phosphorylation of $cPLA_2$. (*A*) Mesangial cells were labeled with [^{32}P]orthophosphate in the absence of serum for 2 h, and then IL-1 (50 ng/ml) was added for an additional 30 min. Cells were rapidly washed with ice-cold PBS and lysed in 1% NP-40. Lysates were incubated with preimmune serum and antiserum to $cPLA_2$. Protein–antibody complexes were precipitated with protein A-Sepharose and analyzed on 8% denaturing polyacrylamide gels. [^{32}P]phosphorylated proteins were imaged using storage phosphor technology and changes in phosphorylation were quantified using image analysis as described in the Methods. (*B*) Mesangial cells were treated with IL-1 for 30 min and lysed. Proteins from cell lysates were analyzed by immunoblotting as described in Methods. $cPLA_2$ phosphorylation and activation causes a decreased mobility in SDS-polyacrylamide gels (22). IL-1 and PMA both induced $cPLA_2$ to migrate more slowly by phosphorylating the protein.

Table III. $cPLA_2$ Activity in the Presence and Absence of Acid Phosphatase Treatment

Pretreatment	Stimulus	$cPLA_2$ activity
		pmol/min per mg
None	Control (6)	322.1±33.3
	IL-1 (6)	633.1±79.2
Acid phosphatase	Control (9)	245.3±22.6‡
	IL-1 (7)	331.1±68.3*

0.4 U of human acid phosphatase (Calbiochem Corp., 500 IU/ng) was added to 2.5–5 μg of mesangial cell extract prepared as described in Methods. The extracts and acid phosphatase were incubated for 15 min at 30°C, followed by the addition of 30 nCi [^{14}C]arachidonyl-phosphatidylethanolamine and 5 mM $CaCl_2$. The samples were incubated subsequently for an additional 30 min at 37°C, and the $cPLA_2$ activity was quantified as described. $cPLA_2$ activity is expressed as the mean±SEM for triplicate determinations in two separate experiments. IL-1 significantly stimulated $cPLA_2$ activity over control, * $P < 0.01$, IL-1 stimulated vs. IL-1 stimulated and acid phosphatase pretreated; ‡ $P > 0.1$, vehicle stimulated vs. vehicle stimulated and acid phosphatase pretreated.

this PLA_2 isoform, suggesting the changes in PLA_2 activity measured by this assay are predominantly if not exclusively a result of $cPLA_2$ activation.

Discussion

IL-1 is a potent stimulus of eicosanoid metabolism (1, 9, 10). In the present study, we have demonstrated that IL-1, in the presence of ionophore, quickly stimulates arachidonate release from intact mesangial cells. In whole-cell lysates, IL-1 rapidly activates a PLA_2 isoform which we have characterized as $cPLA_2$. This increase in cytokine-stimulated $cPLA_2$ activity does not require new protein synthesis but is calcium dependent. IL-1 doubles ^{32}P incorporation into immunoprecipitable $cPLA_2$ protein consistent with the -fold stimulation of enzyme activity in similarly timed incubations. Importantly, the IL-1–stimulated enhancement of PLA_2 activity was significantly reduced by acid phosphatase treatment. Taken together, these data suggest that IL-1–stimulated eicosanoid metabolism is mediated, at least acutely, by phosphorylation and activation of $cPLA_2$. These results support the premise, advanced by Lin et al. (16), that agonist-stimulated increases in intrinsic $cPLA_2$ activity are only apparent when a rise in cytosolic Ca^{2+} allows translocation of the enzyme to its substrate (4, 7, 27). IL-1 does not increase cytosolic Ca^{2+} in mesangial cells (18) and by itself does not increase arachidonate release (10, this paper). In contrast, arachidonic acid release stimulated by the Ca^{2+} mobilizing agonists, vasopressin (10) and ionophore (this paper), is doubled in IL-1–treated cells.

Previous studies have suggested that PLA_2 activity can be regulated by changes in its phosphorylation state. Sequence analysis of $cPLA_2$ has demonstrated consensus phosphorylation sites for the mitogen-activated protein (MAP) kinase and protein kinase C (7, 16), and $cPLA_2$ activity can be both positively and negatively regulated by agents that increase activity of certain kinases. Protein kinase C activators prime or potentiate agonist-induced arachidonate release, an effect that can be blocked in part by either protein kinase C down-regulation or PKC inhibitors (19, 28–30). EGF, similar to IL-1 and PMA, causes an increase in $cPLA_2$ activity that results from stable posttranslational modification (4, 12), and EGF receptor tyrosine kinase activity and autophosphorylation appears necessary for PLA_2 activation (31–33). One study has suggested that PLA_2 is directly activated by the EGF receptor tyrosine kinase (34). Calcium-dependent PLA_2 activity can be potentiated by the addition of either the catalytic subunit of the cAMP-dependent protein kinase or by casein kinase II and is inhibited by incubation with the Ca^{2+}/calmodulin-dependent protein kinase II (35). Lin and co-workers have reported that treatment of CHO cells, overexpressing $cPLA_2$ with ATP, A23187, PMA, and thrombin increases arachidonate release from whole cells, stimulates $cPLA_2$ activity in vitro, and concomitantly increases ^{32}P incorporation into $cPLA_2$ exclusively on serine residues (16). The protein kinase C inhibitor staurosporine prevents ATP-stimulated $cPLA_2$ phosphorylation and activation, and treatment of ATP-stimulated cell lysates with acid phosphatase eliminates agonist-induced increases in $cPLA_2$ activity. Recent in vitro data has indicated that both MAP kinase and protein

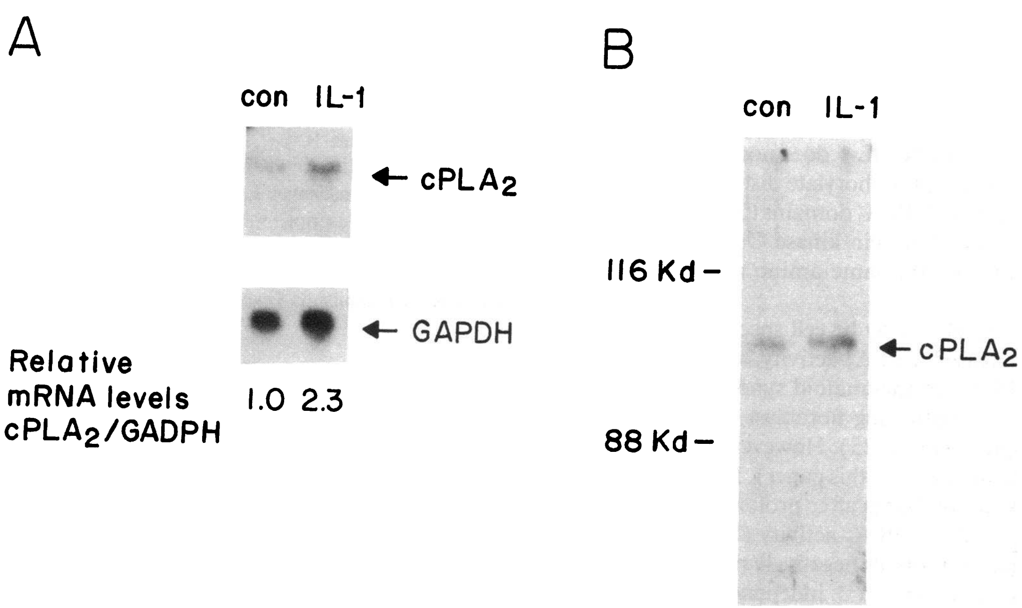

Figure 6. Northern (*A*) and immunoblot (*B*) analysis of $cPLA_2$ mRNA and protein, respectively, in vehicle and IL-1–stimulated mesangial cells. Cells were held in medium containing 0.5% FBS for 24 h and subsequently stimulated with IL-1 for 24 h. $cPLA_2$ mRNA and protein expression were determined as described in Methods.

kinase C can activate $cPLA_2$ by phosphorylation (36, 37). Collectively, these data strongly implicate posttranslational changes in $cPLA_2$ phosphorylation state as an important regulator of enzyme activity.

Since cell lysates rather than purified protein have been used as the enzyme source, our data, as well as most of the data just summarized, are also consistent with activation of a $cPLA_2$ regulatory protein by phosphorylation. PLA_2 stimulatory proteins have been isolated from mammalian cells. Best characterized is a 28-kD protein, PLA_2-activating protein (PLAP) (38). Rapid, agonist-stimulated, de novo synthesis of PLAP is the primary mechanism of PLA_2 activation by PLAP (38). However, we found that cycloheximide does not prevent IL-1–stimulated $cPLA_2$ activation. A PLAP has also been purified from Aplysia (39). This PLA_2 stimulatory activity is increased in Aplysia extracts treated with phorbol dibutyrate, and in vitro, this protein is a substrate for protein kinase C (39). Phosphorylation also inactivates or enhances the degradation of lipocortins, molecules which bind to phospholipids and prevent PLA_2 activation by "substrate depletion." However phosphorylation of lipocortin I does not correlate temporally with EGF-stimulated activation of PLA_2 in cells overexpressing the wild-type EGF receptor (33). In vitro reconstitution experiments have demonstrated that both MAP kinase and protein kinase C phosphorylate and increase $cPLA_2$-specific catalytic activity (36, 37), suggesting that direct phosphorylation of $cPLA_2$ stimulates, at least in part, increased enzymatic activity in intact cells.

IL-1–dependent protein phosphorylations have been reported previously, and IL-1 stimulates protein serine-, threonine-kinase activity in a variety of cell types. The identity of the kinase(s) involved in IL-1–mediated transmembrane signalling is still speculative. IL-1–stimulated arachidonate release in fibroblasts does not require either protein kinase C or cAMP-dependent protein kinase activity (40). Cleveland maps of phosphorylated talin in fibroblasts demonstrate distinct phosphopetides after PMA and IL-1 treatment, suggesting that the IL-1 activates kinases distinct from C kinase (41). Recently, IL-1 has been shown to rapidly stimulate MAP kinase activity (23, 24) and one study has suggested that IL-1 increases protein phosphorylation by inhibition of protein phosphatase activity (42). $cPLA_2$ is phosphorylated and activated in vitro by MAP kinase (36, 37), and we suggest that IL-1–stimulated MAP kinase phosphorylates $cPLA_2$ to increase its activity. Phosphorylation of distinct $cPLA_2$ domains may independently regulate enzyme activity. IL-1 and vasopressin synergistically increase arachidonate release; vasopressin increases protein kinase C activity in mesangial cells but IL-1 does not. In vitro protein kinase C and MAP kinase phosphorylate distinct residues within recombinantly expressed $cPLA_2$ domains (37). However other workers have suggested protein kinase C and MAP kinase identically phosphorylate the same amino acid residue (36).

Stimulation of PLA_2 activity by IL-1 may be cell specific. Similar to our findings, IL-1 increased a PLA_2 activity, with Ca^{2+} requirements similar to $cPLA_2$, in rheumatoid synovial fibroblasts (43). Treatment of a human lung fibroblast line with IL-1 results in $cPLA_2$ phosphorylation (25). However, in contrast to our findings in mesangial cells (10, this paper), IL-1 only stimulated PGE_2 synthesis in fibroblasts after prolonged (> 5 h) incubation and did not induce $sPLA_2$ activity (25). Human IL-1β added to guinea pig eosinophils negatively regulated PLA_2 activity through an indirect, Ca^{2+} independent mechanism (44). In a preliminary report, Clark and coworkers have demonstrated that de novo PLAP synthesis mediates IL-1–stimulated PLA_2 activity in EL-4 cells (45). This cell specific regulation of IL-1–stimulated PLA_2 activity suggests that arachidonic acid hydrolysis may be an important determinant in the cellular response to inflammation.

$cPLA_2$ activity clearly is regulated at multiple levels. In addition to a rapid posttranslational modification of $cPLA_2$, IL-1 induces, in 24-h incubations a small (approximately twofold) but consistent increase in $cPLA_2$ mRNA, protein, and activity. This effect was only detected in mesangial cells held in 0.5% FBS but not those cells maintained in serum. Growth factors (14) and/or glucocorticoids (25, 26) present in serum may negatively regulate IL-1–induced $cPLA_2$ expression. Long-term incubations (> 8 h) with macrophage colony-stimulating factor (46), TNF (25), epidermal growth factor (47) and PMA (47) also increases $cPLA_2$ expression. $cPLA_2$ induction by IL-1 and TNF is not surprising, in that both cytokines activate the transcriptional factors, AP-1 and NFkB, and the 5′ flanking region of the $cPLA_2$ gene contains AP-1 and kB binding elements (48).

In summary, IL-1 regulates the activity of $cPLA_2$ in a biphasic, time-dependent manner. A rapid increase in catalytic activity results from protein phosphorylation. More prolonged incubation with IL-1 can increase $cPLA_2$ mass in resting but not serum-activated cells. $cPLA_2$ activation provides one mechanism for biological events induced by this cytokine. Arachidonic acid release and enhanced eicosanoid metabolism are hallmarks of glomerular inflammation. Increased prostaglandin, HETE, and leukotriene synthesis mediate both the hemodynamic alterations and the cellular activation which characterize inflammatory injury. Recent studies also have demonstrated that these same fatty acid mediators comprise an important intracellular, transmembrane signaling system. Arachidonic acid and/or its metabolites activate GTP-binding proteins (49), regulate the activity of Ras-GTPase activating protein (50), stimulate kinase activity (51), control ion channel function (52), and can activate latent transactivating factors (53). These interactions between arachidonate and other second messenger pathways provide mechanisms by which IL-1–mediated activation of $cPLA_2$, not only results in the synthesis of proinflammatory lipid mediators, but also initiates a specific program of phenotypic responses in the mesangial cell.

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