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Phospholipase A_2 mediates immediate early genes in cultured renal epithelial cells: Possible role of lysophospholipid

YASUO KOHJIMOTO, THOMAS W. HONEYMAN, JULIE JONASSEN, KATHRYN GRAVEL, LORI KENNINGTON, and CHERYL R. SCHEID

Department of Physiology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

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Background. Exposure to high levels of oxalate induces oxidant stress in renal epithelial cells and produces diverse changes in cell function, ranging from cell death to cellular adaptation, as evidenced by increased DNA synthesis, cellular proliferation, and induction of genes associated with remodeling and repair. These studies focused on cellular adaptation to this oxidant stress, examining the manner by which oxalate exposure leads to increased expression of immediate early genes (IEGs). Specifically, our studies assessed the possibility that oxalate-induced changes in IEG expression are mediated by phospholipase A_2 (PLA_2), a common pathway in cellular stress responses.

Methods. Madin-Darby canine kidney (MDCK) cells were exposed to oxalate in the presence or absence of PLA_2 inhibitors: mepacrine and arachidonyl trifluoromethyl ketone ($AACOCF_3$). Expression of IEG (c-*jun*, *egr*-1, and c-*myc*) mRNA was assessed by Northern blot analysis. PLA_2 activity was determined by measuring the release of [^{3}H]arachidonic acid (AA) from prelabeled cells.

Results. Oxalate exposure (1 to 1.5 mmol/L) induced time- and concentration-dependent increases in IEG mRNA. Treatment with mepacrine resulted in a 75 to 113% reduction of oxalate-induced c-*jun*, *egr*-1, and c-*myc* mRNA, while $AACOCF_3$ caused a 41 to 46% reduction of oxalate-induced c-*jun* and *egr*-1 mRNA. Of the two major byproducts of PLA_2, only lysophosphatidylcholine (20 μmol/L) increased c-*jun* and *egr*-1 mRNA. In contrast, AA (25 μmol/L) attenuated the oxalate-induced increase in c-*jun* and *egr*-1 mRNA, presumably by inhibiting PLA_2 activity.

Conclusions. These findings suggest that PLA_2 plays a major role in oxalate-induced IEG expression in renal epithelial cells and that lysophospholipids might be a possible lipid mediator in this pathway.

Oxalate, a common constituent and a major risk factor for kidney stones, is an end product of metabolism that is excreted by the kidney [1]. This dicarboxylate was long considered to be an inert metabolic byproduct; however, recent studies from our laboratory [2–4] and those of others [5–7] demonstrated that oxalate exposure can induce oxidant stress, leading to death of renal epithelial cells. Not all cells die in response to oxalate, however, even at high oxalate concentrations. Rather, the majority of cells exhibit adaptive responses, including increased expression of immediate early genes (IEGs; for example, c-*jun*, *egr*-1, c-*myc*), increased DNA synthesis/increased cellular proliferation [8, 9], and increased expression of genes associated with remodeling and repair (for example, osteopontin and clusterin) [10]. The present studies examined the mechanisms underlying these adaptive responses to oxalate, focusing on biochemical pathways that link oxalate exposure to increased expression of IEGs, since these gene products coordinate a variety of cellular responses, including entry into the cell cycle (and hence DNA synthesis and proliferation) and/or programmed cell death [11–13].

One biochemical pathway that has been implicated in cellular responses to stress involves phospholipase A_2 (PLA_2), a family of enzymes that hydrolyzes membrane phospholipids, producing active byproducts [arachidonic acid (AA) and lysophospholipids] that directly or indirectly alter a number of cell functions [14–16], including the transcription of specific genes [17–20]. The PLA_2 pathway is activated in various types of renal injury, including those involving increased oxidant stress [21, 22]. Moreover, recent studies in our laboratory demonstrated that oxalate exposure can increase AA release from cultured renal epithelial cells by the action of PLA_2 [23]. Therefore, it is possible that this enzyme mediates the observed functional changes in these cells following oxalate exposure. The present studies assessed this possibility by determining whether agents that block PLA_2 activity will also block oxalate-induced changes in IEG expression in renal epithelial cells and by determining whether PLA_2 byproducts (AA and/or lysophospholipids) can mimic the effects of oxalate on IEG expression. The

Key words: MDCK cells, arachidonic acid, oxalate, kidney stones, programmed cell death, cell stress response.

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results from these studies support the role of PLA_2 in the oxalate-induced increase in IEG expression, and suggest the involvement of lysophospholipid, one of the byproducts of PLA_2 activity, as a mediator of this response.

METHODS

Materials

Dulbecco's modified Eagle medium (DMEM) and antibiotics (penicillin/streptomycin) were obtained from GIBCO BRL (Grand Island, NY, USA). Fetal bovine serum (FBS), AA, and mepacrine were purchased from Sigma Chemical (St. Louis, MO, USA). Arachidonyl trifluoromethyl ketone ($AACOCF_3$) was from Biomol Research Laboratories (Plymouth, PA, USA). [5,6,8,9,11,12,14,15-^{3}H(N)]AA ([^{3}H]AA; 60 to 100 Ci/mmol) and [^{32}P]dCTP were obtained from DuPont New England Nuclear (Cambridge, MA, USA). Lysophosphatidylcholine (LPC; 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine) and lysophosphatidic acid (LPA; 1-acyl-2-hydroxy-sn-glycero-3-phosphate) were from Avanti Polar Lipids (Birmingham, AL, USA). All other chemicals were of highest grade available.

Cell culture

Madin-Darby canine kidney (MDCK) cells (CCL 34, passages 53 to 90) were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and were maintained in DMEM supplemented with 2% FBS, 4.5 g/L D-glucose, and antibiotics (100 U/mL penicillin + 100 mg/mL streptomycin) at 37°C under 5% CO_2/95% air. In preparation for mRNA experiments, cells were plated in six-well culture plates, grown to 80% confluence, and then made quiescent by incubation in fresh DMEM containing 0.25% FBS for 48 hours. Cells reached confluence during this period, although growth rates were slowed markedly. Such conditions also reduced the basal expression of IEG mRNA, which is normally high in serum-containing media, such that we could reliably detect an induction of IEG in response to various stimuli. For studies on the release of AA, cells were grown to approximately 80% confluence in 12-well culture plates prior to labeling with [^{3}H]AA.

Experimental protocol

Various treatments were performed by exchanging the medium for serum-free DMEM containing the agent of interest and continuing the incubation for a predetermined period at 37°C under an atmosphere of 5% CO_2/95% air. Where indicated, sodium oxalate was added at a concentration of 1 or 1.5 mmol/L (total), which provides free oxalate levels of 350 and 550 μmol/L, and produces relative super-saturation levels (RSS) for calcium oxalate of 24.9 and 35.9, respectively. Estimates of free oxalate and RSS were obtained using the EQUIL program [24]. Stock solutions (10 mmol/L) of AA, LPC, and $AACOCF_3$ were prepared in dimethyl sulfoxide. Stock solutions (10 mmol/L) of dibucaine and A23187 were prepared in ethanol. Drugs were diluted to obtain concentrations as indicated in Table 1 and the figure legends. Corresponding vehicle controls were included for comparison. In the experiments testing the effect of PLA_2 inhibitors, cells were incubated with inhibitors for 30 minutes before and during oxalate exposure.

Northern blot analysis

Total RNA was extracted using RNeasy Mini Kits (Qiagen, Valencia, CA, USA) according to the manufacturer's directions and fractionated on denaturing 1% agarose gels (10 μg total RNA per lane). Separated RNA was transferred to nitrocellulose membranes (Hybond-C extra; Amersham Pharmacia Biotech, Piscataway, NJ, USA) by capillary blotting and fixed by baking at 80°C for two hours. Blots were prehybridized for at least two hours at 42°C, hybridized overnight to [^{32}P]dCTP-labeled cDNA probes prepared by random primer labeling, and then washed stringently prior to image analysis. The murine c-*jun* cDNA probe (a gift from Dr. Paul Dobner, University of Massachusetts Medical School, Worcester, MA, USA) hybridized with two mRNAs (2.7 and 3.2 kb) in MDCK cells. The murine *egr*-1 cDNA probe (obtained from ATCC) was hybridized with a 3.3 kb mRNA. The murine c-*myc* cDNA probe (a gift from Dr. Nancy Phillips, University of Massachusetts Medical School) was hybridized with a 2.3 kb mRNA. DNA-RNA hybrids were detected by phosphorimage analysis, using a Bio-Rad Molecular Imager (Hercules, CA, USA). Quantitative analysis was performed using Multi Analyst program (Bio-Rad). All estimates of mRNA abundance were corrected for differences in RNA loading and transfer efficiency by normalization to the abundance of 18S RNA on the blot, which was determined by scanning densitometry of the photographic negative of the ethidium-bromide–stained RNA. All experiments were performed at least three times.

Measurement of arachidonic acid release

[^{3}H]arachidonic acid release was determined as described previously [23]. Subconfluent cultures of MDCK cells in 12-well plates were incubated for 12 to 18 hours with 0.2 mCi/mL (2 to 3.3 nmol/L) of [^{3}H]AA in serum-free DMEM, a period that allowed cells to reach confluence and to incorporate 94.0 ± 0.5% of added radioactivity. The cells were then washed twice with phosphate-buffered saline (PBS; pH 7.4) and once with DMEM containing 0.2% fatty acid-free bovine serum albumin (BSA) prior to the addition of various agents. Cells were then exposed to the agent of interest for a predetermined time. The medium was collected and centrifuged to remove any floating cells, and the radioactivity in 400 μL of superna-

Table 1. Effect of exogenous arachidonic acid (AA) on oxalate- and A23187-induced immediate early genes (IEG; c-*jun* and *egr*-1) mRNA and [^{3}H]AA release in MDCK cells

Treatment	mRNA abundance[a] (% control)		[^{3}H]AA release[c] % control
	c-*jun*	*egr*-1	
Control + vehicle	100.0	100.0	100.0 ± 11.1
AA 25 *μmol/L*	79.9 ± 10.6	74.2 ± 3.8[b]	129.9 ± 8.2
Oxalate 1 *mmol/L* + vehicle	326.0 ± 60.2	183.9 ± 16.7	256.1 ± 29.5
AA 25 *μmol/L*	177.8 ± 44.5[b]	60.9 ± 25.1	129.1 ± 10.9[d]
A23187 3 *μmol/L* + vehicle	647.5 ± 209.6	270.3 ± 11.8	468.2 ± 52.0
AA 25 *μmol/L*	291.1 ± 151.1	72.7 ± 29.1	304.9 ± 14.7[e]

[a]Quiescent cultures of MDCK cells were treated with oxalate (1 mmol/L) or A23187 (3 μmol/L) for 2 hr in the presence or absence of AA (25 μmol/L). Total RNA was extracted and subjected to Northern blot analysis for c-*jun* and *egr*-1. The abundance of mRNA was quantified and expressed relative to 18S RNA abundance. All values were then normalized relative to control for each experiment. Data are presented as means ± SE of 3 independent experiments. Exogenous AA decreased control and oxalate- and A23187-induced increases in c-*jun* and *egr*-1 mRNA, although the differences were not significant by one-way ANOVA performed on 6 treatment groups

[b]$P < 0.05$ vs. corresponding vehicle using a Student's *t*-test for paired data

[c]Subconfluent cultures of MDCK cells were incubated for 12 to 18 hours with 0.2 μCi/mL (2 to 3.3 nmol/L) of [^{3}H]AA in serum-free DMEM. After labeling, cells were washed twice with PBS and once with DMEM containing 0.2% fatty acid-free BSA. Cells were then exposed to oxalate (1 mmol/L) or A23187 (3 μmol/L). After 1 hour, the medium was collected, centrifuged, and counted for [^{3}H]AA released by cells, and the adherent cells were solubilized with 1% Triton X-100 and counted to determine [^{3}H]AA associated with cells. The release of [^{3}H]AA into the medium was expressed as a percentage of total incorporated (cell-associated plus released). All of the values were then normalized relative to controls for each experiment. Simultaneous addition of AA significantly decreased oxalate- and A23187-induced [^{3}H]AA release. Data are means ± SE of 3 independent experiments performed in triplicate

[d]$P < 0.01$

[e]$P < 0.05$ vs. corresponding vehicle using a Student's *t*-test for unpaired data

tant was measured in a liquid scintillation counter (LS 6500; Beckman Instruments, Fullerton, CA, USA). Adherent cells were solubilized with 1 mL of 1% Triton X-100, and the radioactivity in 400 μL of the cell lysate was determined. The release of [^{3}H]AA into the medium was expressed as a percentage of total label incorporated (cell-associated plus released).

Statistical analyses

Statistical evaluation of the effects of various agents on IEG expression was performed using one-way analysis of variance and the SuperANOVATM software package (Abacus Concepts Inc., Berkeley, CA, USA). Prior to data analysis, mRNA data were corrected for 18S RNA abundance. Within each Northern blot, data were normalized to their respective control group (that is, cells in serum-free media for 1, 2, or 4 h). Variance around the arithmetic means was found not to be normally distributed; therefore, data were transformed to their natural logarithms prior to statistical analysis to stabilize this variance [25]. Comparisons among treatment groups were made using the Duncan multiple comparison procedure [26]. Following statistical analyses, logarithmically transformed data were converted back to their antilogarithms and then plotted as histograms using arithmetic scales. [^{3}H]AA release data were normalized relative to control for each experiment, and statistical evaluation was performed using Student's *t*-test for unpaired data.

RESULTS

Oxalate increases expression of immediate early genes in MDCK cells

These studies examined the expression of three IEGs: c-*jun*, *egr*-1, and c-*myc*, in quiescent cultures of MDCK cells after a one-, two-, or four-hour exposure to oxalate. As shown in the representative Northern blot in Figure 1, exposure to oxalate (1 to 1.5 mmol/L) increased the expression of c-*jun* and egr-1 mRNA at one, two, and four hours, and c-*myc* mRNA at four hours. Quantitative analyses of mRNA data from at least three experiments of this type are shown in Figure 2. Note that the oxalate-induced increase in c-*jun* expression was maximal at two hours, with 1 and 1.5 mmol/L oxalate producing 1.9- and 3.0-fold increases, respectively. *Egr*-1 mRNA induction was also maximal at the same time, with 1 and 1.5 mmol/L oxalate producing 2.2- and 3.4-fold increases, respectively. Oxalate-induced changes in c-*myc* expression were only apparent at four hours, however, with 1 and 1.5 mmol/L oxalate producing 1.6- and 2.5-fold increases, respectively. Thus, oxalate exposure induced time- and concentration-dependent increases in c-*jun*, *egr*-1, and c-*myc* mRNA in MDCK cells. Based on these findings, subsequent experiments examined c-*jun* and *egr*-1 expression at two hours and c-*myc* expression at four hours after the addition of 1 mmol/L oxalate, a concentration that elicits submaximal induction of each gene.

PLA_2 inhibitors reduce oxalate-induced IEG expression

Previous studies demonstrated that oxalate exposure increases [^{3}H]AA release from MDCK cells by a process dependent on PLA_2 and suggested that this enzyme is responsible, at least in part, for oxalate toxicity [23]. To determine whether PLA_2 is also involved in regulating gene expression in response to oxalate, we determined whether treatment with PLA_2 inhibitors would block the oxalate-induced changes in IEG expression. Two different inhibitors were used, mepacrine, a general

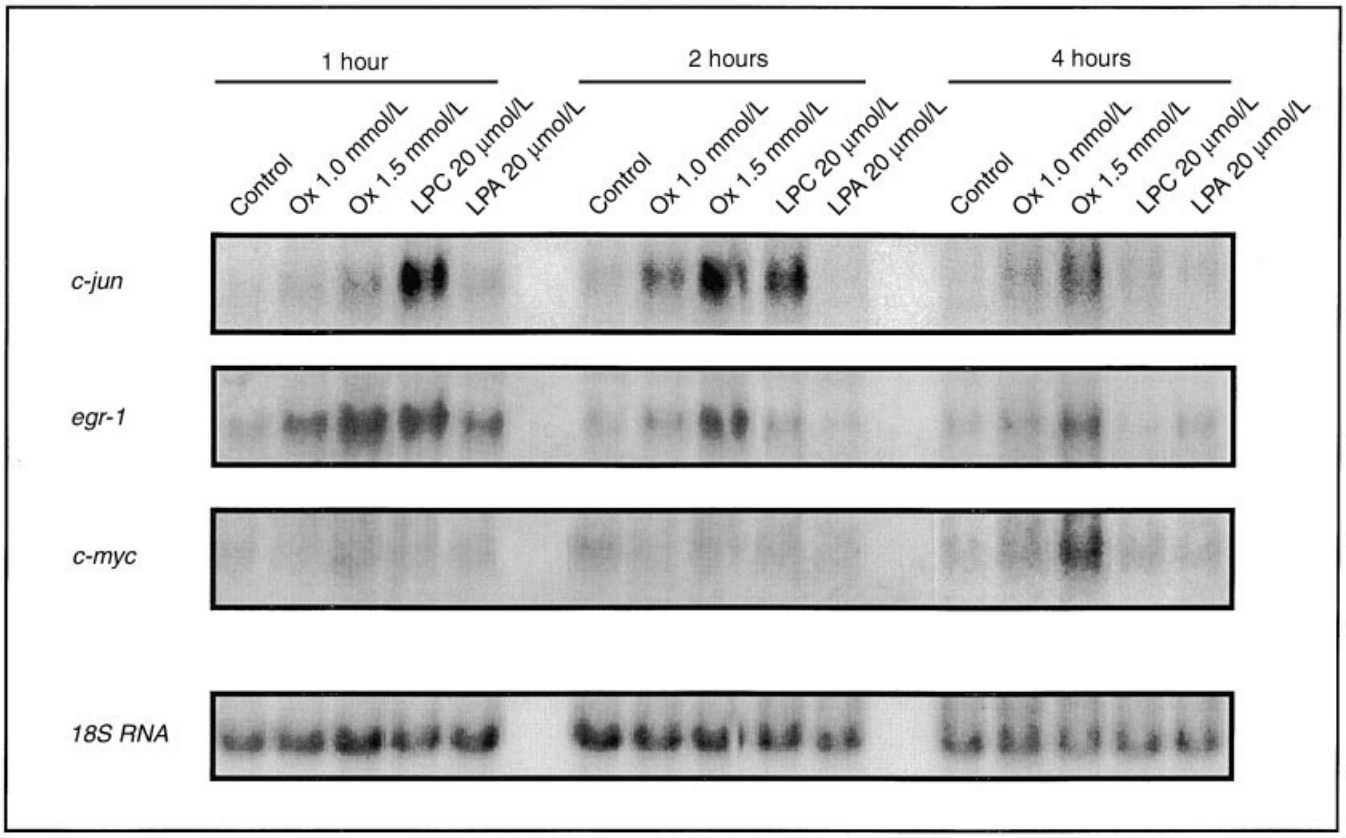

Fig. 1. Northern blot analysis of total RNA for immediate early genes (IEGs; c-*jun*, *egr*-1 and c-*myc*) following exposure of MDCK cells to oxalate, lysophosphatidylcholine (LPC), and lysophosphatidic acid (LPA). Quiescent cultures of MDCK cells were exposed to oxalate (1 to 1.5 mmol/L), LPC (20 μmol/L), or LPA (20 μmol/L) for various times, as indicated at the top of the panel. Total RNA was extracted and fractionated on a denaturing 1% agarose gel (10 μg total RNA per lane) and then transferred to nitrocellulose membrane. The blot was hybridized with [^{32}P]cDNA probes for c-*jun*, *egr*-1, and c-*myc*. Oxalate exposure induced time- and concentration-dependent increases in three IEGs. LPC also increased c-*jun* and *egr*-1 mRNA, while LPA did not. Ethidium-bromide stained 18S RNA from the same blot is also shown. The blot is representative of four independent experiments.

PLA_2 inhibitor, and $AACOCF_3$, a selective inhibitor for cytosolic PLA_2 ($cPLA_2$) [27], and cells were incubated with concentrations of inhibitors known to inhibit oxalate-induced [^{3}H]AA release in this cell line [23]. As shown in the representative Northern blot in Figure 3, both $AACOCF_3$ (10 μmol/L) and mepacrine (25 μmol/L) markedly reduced oxalate-induced c-*jun* and *egr*-1 mRNA expression at two hours. $AACOCF_3$ was not used in studies on c-*myc* expression, since this gene is induced more slowly in MDCK cells (4 hours vs. 1 to 2 hours for c-*jun* and *egr*-1; Fig. 1) and since $AACOCF_3$ is reportedly converted to an inactive byproduct [$AACH(OH)CF_3$] on prolonged incubation with living cells [27]. Thus, studies on c-*myc* expression employed only mepacrine as a PLA_2 inhibitor. The results showed that treatment with mepacrine abolished the oxalate-induced increase in c-*myc* mRNA at four hours. Neither $AACOCF_3$ alone nor mepacrine alone had any effect on basal expression of the three IEGs. Quantitative analyses demonstrated that treatment with $AACOCF_3$ attenuated the oxalate-induced increases in c-*jun* and *egr*-1 mRNA by 41 to 46%, while treatment with mepacrine reduced oxalate effects on all three IEGs by 75 to 113% (Fig. 4). These findings suggest that PLA_2 plays a role in oxalate-induced IEG expression in MDCK cells.

PLA_2 inhibitors also reduce A23187-induced IEG expression

To obtain further evidence that PLA_2 plays a role in IEG expression, we examined IEG expression after exposure to A23187, a known activator of $cPLA_2$ in MDCK cells [28, 29]. As shown in Figures 3 and 4, treatment of the cells with 3 μmol/L A23187, a concentration that increases [^{3}H]AA release from MDCK cells markedly increased the expression of all three IEGs (Table 1), indicating that the $cPLA_2$-activating agent, A23187, can mimic oxalate actions on IEG expression. Furthermore, the effects of A23187 on IEG mRNA, like the effects of oxalate, were attenuated by the PLA_2 inhibitors $AACOCF_3$ and mepacrine. These findings support the

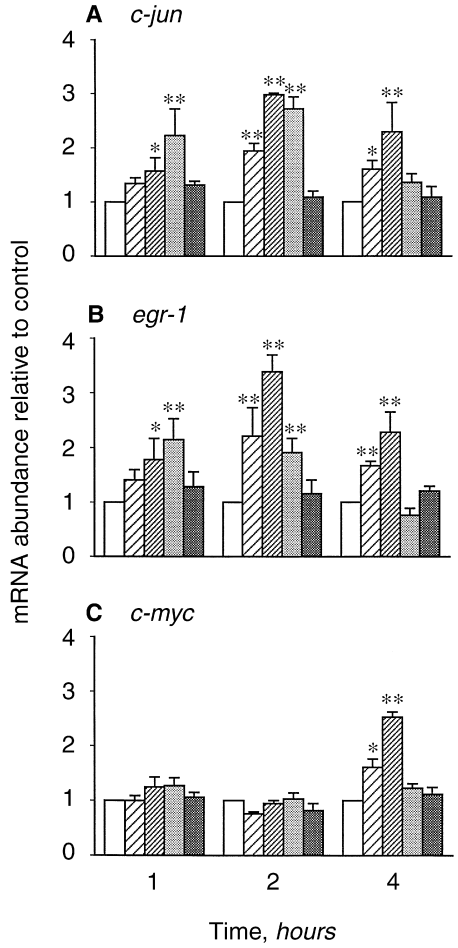

Fig. 2. Quantitative analysis of the effects of oxalate, LPC, and LPA on IEG mRNA expression. Symbols are: (□) control; (▨) oxalate 1.0 mmol/L; (▨) oxalate 1.5 mmol/L; (■) LPC 20 μmol/L; (■) LPA 10 μmol/L. MDCK cells were treated as in Figure 1. Abundance of c-*jun*, *egr*-1, and c-*myc* mRNAs was corrected for 18S RNA abundance. All values were then normalized relative to the control value (serum-free DMEM) at each time point. Oxalate-induced increases in c-*jun* and *egr*-1 mRNAs were maximum at two hours, whereas increased c-*myc* expression was apparent only at four hours. LPC-induced increases in c-*jun* and *egr*-1 mRNAs were comparable at one and two hours. LPC had no effect on c-*myc* mRNA. LPA had no effect on any IEG mRNA. Data are plotted as the mean plus one standard error of the mean for three or four independent experiments. $*P < 0.05$ vs. control; $**P < 0.01$ vs. control.

contention that PLA_2 activity plays a role in the induction of c-*jun*, *egr*-1, and c-*myc* in response to oxalate.

Exogenous arachidonic acid fails to mimic oxalate actions on IEG expression

The finding that PLA_2 activity mediates oxalate-induced IEG expression prompted us to examine which of the PLA_2 products, AA or lysophospholipid, mediates oxalate-induced IEG expression. Since AA and its metabolites have been demonstrated to induce IEG in different cell lines [17–19], we first assessed the effect of exogenous AA on IEG mRNA. In contrast to our expectation, treatment of MDCK cells with AA (25 μmol/L) did not increase c-*jun* and *egr*-1 mRNA; rather, it reduced basal levels and markedly attenuated both the oxalate-induced and the A23187-induced increases in c-*jun* and *egr*-1 mRNA (Table 1). This attenuation appeared to reflect feedback inhibition of PLA_2, as evidenced by the finding that the addition of exogenous AA (25 μmol/L) significantly reduced [^{3}H]AA release from prelabeled cells (Table 1). Thus, although oxalate-induced changes in IEG appear to involve PLA_2 activity, it does not seem likely that the effects are mediated by AA. Subsequent studies examined other possible byproducts of PLA_2 activity.

LPC mimics oxalate actions on IEG expression

Since lysophospholipids are the other byproducts of PLA_2, we determined whether oxalate-induced changes in IEG could be mimicked by one or more members of this class of lipids. Studies focused initially on the possible involvement of LPC, since phosphatidylcholine, the lipid precursor for LPC, is the most common phospholipid in cell membranes, and since studies in other systems had demonstrated that LPC (and LPA, which can be derived from LPC) can elicit various changes in cellular function, including changes in gene expression [32–38]. Thus, we examined the effect of exogenous LPC and LPA on IEG mRNA expression in MDCK cells. As shown in Figures 1 and 2, LPC (20 μmol/L) increased c-*jun* and *egr*-1 expression at one and two hours to the same extent as produced by oxalate. Unlike oxalate, however, LPC failed to increase c-*myc* mRNA expression. LPA (20 μmol/L) had no effect on mRNA levels for any of the IEG, irrespective of the time point examined. These results suggest that LPC may serve as a mediator of oxalate-induced expression of c-*jun* and *egr*-1, although regulation of c-*myc* expression appears to involve a different mechanism.

To examine the involvement of LPC in oxalate actions further, we looked at the effect of concomitant addition of oxalate and LPC on IEG mRNA. As shown in Figure 5, treatment of MDCK cells with a combination of oxalate (1.5 mmol/L) plus LPC (20 μmol/L) failed to produce a further increase in c-*jun* and *egr*-1 mRNA over that seen with oxalate alone or with LPC alone. Note that either stimulus alone did not produce a maximum induction of the IEGs, since the addition of A23187 (5 μmol/L) resulted in a greater increase in c-*jun* and *egr*-1 mRNA than was observed with either oxalate or LPC alone (Table 1). These findings suggest that oxalate and LPC use a common biochemical pathway.

DISCUSSION

The present studies on renal epithelial cells derived from the distal kidney of the dog (MDCK cells) are the first to demonstrate a causal link between PLA_2 activity

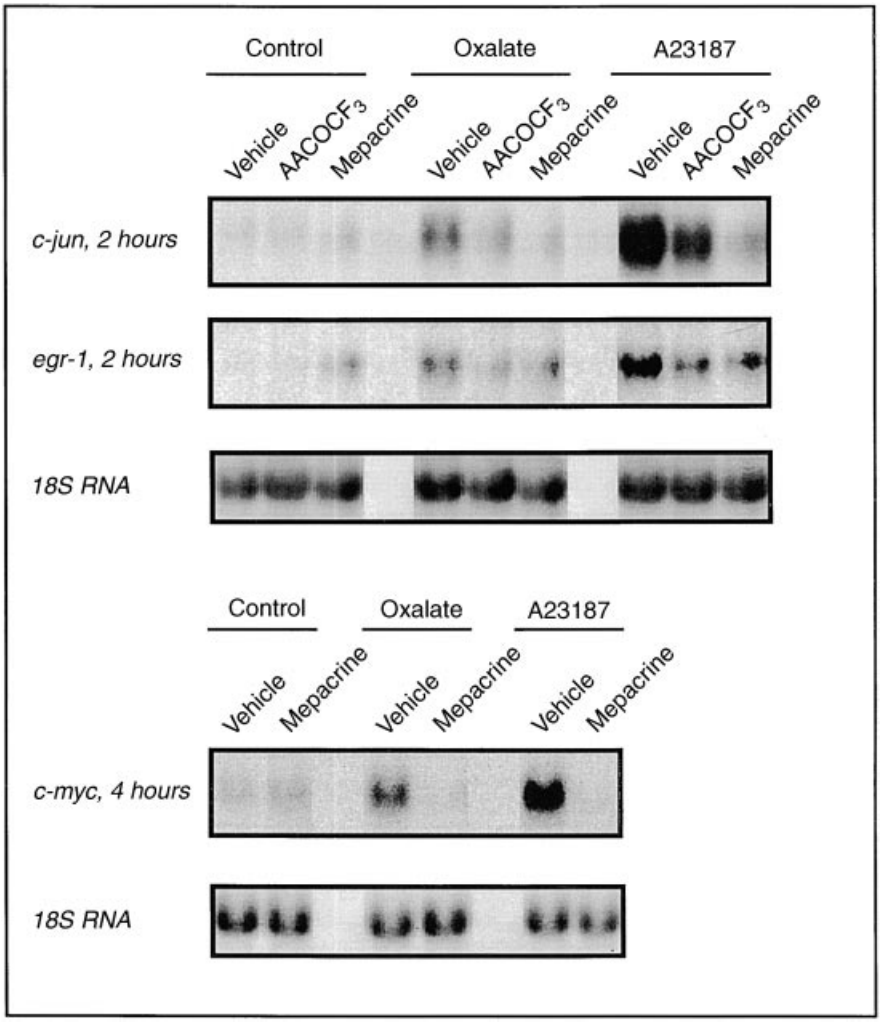

Fig. 3. Northern blot analysis of total RNA for IEGs (c-*jun*, *egr*-1, and c-*myc*) following exposure of MDCK cells to oxalate or calcium ionophore in the presence or absence of phospholipase A_2 (PLA_2) inhibitors. Quiescent cultures of MDCK cells were pretreated with either vehicle, $AACOCF_3$ (10 μmol/L), or mepacrine (25 μmol/L) for 30 minutes and then exposed to oxalate (1 mmol/L) or A23187 (3 μmol/L) for the indicated times. Total RNA was extracted and subjected to Northern blot analysis for c-*jun*, *egr*-1, and c-*myc*. Note that both inhibitors markedly reduced oxalate- and A23187-induced c-*jun* and *egr*-1 expression at two hours and that c-*myc* induction at four hours was also abolished by mepacrine. The blot is representative of four independent experiments.

and induction of several IEGs (c-*jun*, *egr*-1 and c-*myc*) following oxalate exposure. These studies also provide evidence suggesting that the lipid byproduct that mediates the observed changes in gene expression is a lysophospholipid rather than AA. Since IEG expression in turn regulates the transcription of other genes that are involved in the adaptation to cellular stress, these findings provide a possible mechanism whereby oxalate exposure leads to activation of cellular processes responsible for remodeling, repair, and replacement of renal cells. The conclusion that PLA_2 mediates oxalate-induced IEG mRNA in MDCK cells is supported by several lines of evidence. For one, the effects of oxalate on IEG expression were markedly attenuated by the PLA_2 inhibitors mepacrine and $AACOCF_3$. Second, A23187, an agent that stimulates $cPLA_2$ in MDCK cells [28, 29], mimicked oxalate actions on IEG expression, while the induction of IEG by A23187 was also attenuated by PLA_2 inhibitors. Third, LPC, a byproduct of PLA_2 action on membranes, increased the expression of two IEG mRNAs, c-*jun* and *egr*-1, in MDCK cells. Thus, the response of MDCK cells to oxalate resembles the response to other agents that modulate IEG expression via pathways involving PLA_2 [17–20].

Many different forms of mammalian PLA_2 have been identified, including secretory PLA_2 ($sPLA_2$), $cPLA_2$, and calcium-independent PLA_2 ($iPLA_2$). The form of PLA_2 that is involved in different conditions can vary, depending on the cell type involved, the type of stimulus applied, and/or the nature of the pathology [14, 15]. The PLA_2 inhibitors used in this study showed different inhibitory potency on oxalate-induced IEG expression. Mepacrine, a "generic" inhibitor that inhibits all isoforms of PLA_2, blocked oxalate-induced IEG expression to a greater extent than $AACOCF_3$, an inhibitor selective for $cPLA_2$ (75 to 113% vs. 41 to 46%, respectively). This concentration of $AACOCF_3$ completely abolished the oxalate-induced [^{3}H]AA release from MDCK cells, however. Thus, it is possible that oxalate actions by other PLA_2 isozymes and/or that these cells express other isoforms of $cPLA_2$ that are resistant to $AACOCF_3$ [30, 31]. The relative

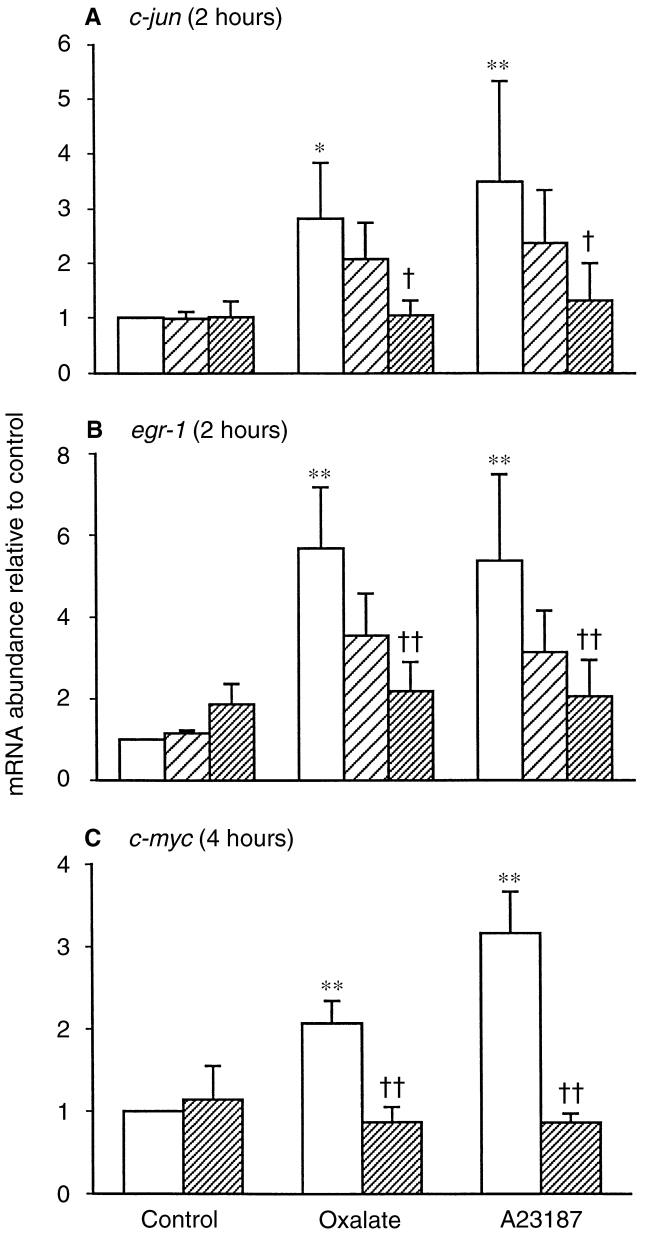

Fig. 4. Quantitative analysis of the effects of PLA_2 inhibitors on the induction of IEG mRNAs by oxalate or calcium ionophore. MDCK cells were treated as in Figure 3. Symbols are: (□) vehicle; (▨) $AACOCF_3$; (▨) mepacrine. Abundance of c-*jun*, *egr*-1, and c-*myc* mRNAs was corrected for 18S RNA abundance. All values were then normalized relative to the control value. Treatment with $AACOCF_3$ attenuated the oxalate-induced increases in c-*jun* and *egr*-1 mRNA by 41 to 46%, while treatment with mepacrine reduced oxalate effects on all three IEGs by 75 to 113%. Data were plotted as the mean plus one standard error of the mean for three to four independent experiments. $^*P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; †$P < 0.05$ vs. corresponding vehicle (oxalate or A23187); ††$P < 0.01$ vs. corresponding vehicle (oxalate or A23187).

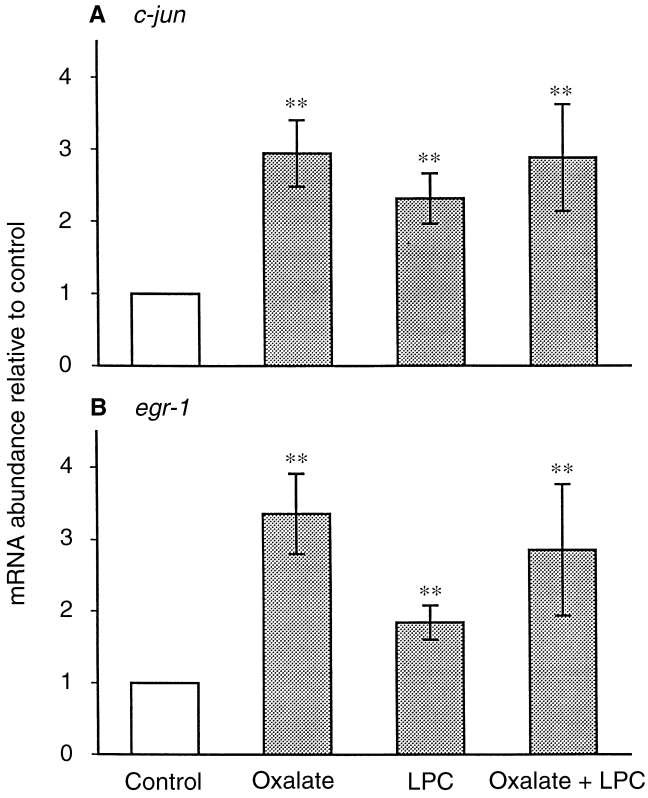

Fig. 5. Effect of simultaneous addition of oxalate and LPC on c-*jun* and *egr*-1 mRNA expression in MDCK cells. Quiescent cultures of MDCK cells were exposed for two hours to oxalate (1.5 mmol/L), LPC (20 μmol/L), or oxalate (1.5 mmol/L) + LPC (20 μmol/L). The abundance of c-*jun* and *egr*-1 mRNAs was corrected for 18S RNA abundance. All values were then normalized relative to the control value. Data for mRNA abundance from selected treatment groups in Figure 2 were replotted here, along with data from cultures exposed to a combination of oxalate and LPC. Data are plotted as the mean plus one standard error of the mean for three to seven independent experiments. $^{**}P < 0.01$ vs. control.

sensitivity of other isoforms of $cPLA_2$ to $AACOCF_3$ has not been established.

The finding that oxalate actions on c-*jun* and *egr*-1 expression could be mimicked by LPC but not by AA was somewhat surprising. The long history of studies on AA and its metabolites and recent evidence that these compounds can modulate gene expression (including the expression of several IEG) and the activity of transcription factors such as activating protein (AP-1) and nuclear factor-κB (NF-κB) [17–20] led us to predict that AA would mediate oxalate actions in MDCK cells. However, the present studies showed that exogenous AA (25 μmol/L) markedly reduced basal expression of c-*jun* and *egr*-1 mRNA and attenuated the oxalate- and A23187-induced changes in these gene products. The finding that exogenous AA also inhibited oxalate- and ionophore-induced [^{3}H]AA release from MDCK cells suggests that AA elicits a product inhibition of PLA_2. This effect resembles the response of rat proximal tubules in which the addition of somewhat higher concentrations of AA (>100 μmol/L) inhibited PLA_2 activity [39].

The possibility that LPC may serve as a signal for the regulation of gene expression has been suggested in a number of other studies on growth factor actions [32–38,

40]. Three lines of evidence support a role of LPC as a signal for oxalate-induced gene expression in renal epithelial cells. For one, the addition of exogenous LPC mimicked the effects of oxalate on c-*jun* and *egr*-1 mRNA expression. Second, LPC and oxalate appeared to use a common biochemical pathway, since simultaneous exposure to oxalate plus LPC failed to produce a further increases in c-*jun* or *egr*-1 mRNA. Finally, the response to LPC occurred more rapidly than the response to oxalate (as would be expected for a "downstream" mediator of a response). Interestingly, LPC failed to mimic the effects of oxalate on c-*myc* expression (Figs. 1 and 2), even when LPC was administered twice during four hours of incubation (data not shown). Further study is required to define the mediator(s) responsible for oxalate induction of c-*myc* mRNA in MDCK cells.

The mechanism responsible for LPC-induced IEG mRNA remains unknown. The finding that c-*jun* and *egr*-1 mRNA are both induced by LPC or oxalate is most easily explained as a consequence of some shared regulatory element in the promoter region of these two genes. For example, the binding sites for transcription factors AP-1 and Sp-1 are present in the promoter regions of both genes [11, 13]. Furthermore, the binding of these transcription factors can be increased by LPC via alterations in the activity of c-Jun NH_2-terminal kinase and/or protein phosphatase 2A [37, 38, 40]. Thus, it is possible that this sort of pathway couples the oxalate-induced formation of LPC to changes in gene expression in MDCK cells.

The precise cellular consequences of oxalate (or LPC)-induced activation of IEG via a PLA_2-dependent pathway in renal cells are unknown. In other cell types, LPC induces a variety of genes, including intercellular adhesion molecule-1 (*ICAM-1*), platelet-derived growth factor (*PDGF-A* and *-B*), heparin-binding epidermal growth factor-like growth factor, interferon γ (*IFN-γ*), and *nitric oxide synthase-III* [32–35, 37]. The transcription of some of these genes can be regulated by the protein products of the c-*jun* or *egr*-1 genes. For example, the LPC response element of the *IFN-γ* gene contains AP-1, a transcription factor in which c-Jun is a component [41]. AP-1 has also implicated in the LPC-induced expression of ICAM-1 [42], while the product of the *egr*-1 gene regulates transcription of PDGF-A and -B [43]. Thus, it is possible LPC and oxalate-induced changes in IEG expression trigger the synthesis of a variety of proteins involved in remodeling/repair or proliferation and/or in programmed cell death.

In summary, the present studies demonstrate that activation of PLA_2 and generation of LPC may play a central role in oxalate-induced expression of IEGs. These results extend our previous studies, demonstrating the involvement of PLA_2 in the toxic actions of oxalate and suggest that lipid signals produced by this pathway may mediate other cellular actions of oxalate. Further insights as to the cellular consequences of oxalate-induced changes in phospholipid metabolism may prove useful in understanding the pathogenesis of kidney diseases associated with excessive oxalate excretion, including primary hyperoxaluria, end-stage renal disease, and urolithiasis.

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Reprint requests to Cheryl R. Scheid, Ph.D., Department of Physiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655-0127, USA.
E-mail: cheryl.scheid@umassmed.edu

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