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Phospholipase A_2 Activation and Increases in Specific Prostaglandins in the Oxidatively Stressed *14CoS/14CoS* Mouse Hepatocyte Line

*Ming Yu, Gordon A. Jamieson, Jr., George D. Leikauf and Daniel W. Nebert**

CENTER FOR ENVIRONMENTAL GENETICS AND DEPARTMENT OF ENVIRONMENTAL HEALTH, P.O. BOX 670056, UNIVERSITY OF CINCINNATI MEDICAL CENTER, CINCINNATI, OHIO 45267-0056, USA

ABSTRACT. This laboratory has previously shown that increases in the expression of several genes in SV40-transformed hepatocyte cultures derived from the untreated newborn c^{14CoS}/c^{14CoS} mouse, and in newborn mouse liver—when compared with the c^{ch}/c^{ch} wild-type—are associated with enhanced levels of reactive oxygenated metabolites (ROMs) and reduced glutathione (GSH). We show here that, in contrast to the *ch/ch* wild-type levels, the oxidatively stressed *14CoS/14CoS* liver cell line displays 2- to 5-fold increases in: 1) phospholipase A_2 (PLA_2) enzyme activity, 2) Ca^{2+}-dependent Group II secreted PLA_2 mRNA levels, 3) arachidonic acid release, and 4) arachidonic acid metabolites co-eluting with prostaglandins D_2, E_2, and $F_{2\alpha}$. These findings suggest that the cyclooxygenase-2 (COX2) pathway, and possible involvement of the "inflammatory" and/or "acute phase response" signal transduction pathways, might be activated during the endogenous ROM-mediated oxidative stress response in *14CoS/14CoS* cells. BIOCHEM PHARMACOL **55**;2: 193–200, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. [*Ah*] gene battery; Ah receptor; *14CoS/14CoS* mutant mouse line; oxidative stress; fumarylacetoacetate hydrolase; melittin; serum response; calcium ionophore A23187; arachidonic acid cascade; prostaglandins $F_{2\alpha}$, D_2, and E_2; cyclooxygenase-2; phospholipase A_2

The c^{14CoS}‡ mutation in the mouse represents a 3,800-kb deletion on Chr 7 around the *c* locus. The c^{14CoS}/c^{14CoS} homozygote dies during the first day post partum for unknown reasons, whereas the c^{ch}/c^{ch} wild-type and the c^{ch}/c^{14CoS} heterozygote remain viable [1, 2]. Compared with the *ch/ch* wild-type and the *ch/14CoS* heterozygote, the untreated *14CoS/14CoS* homozygote exhibits an oxidative stress response [2].

In contrast to the *ch/14CoS* heterozygote or the *ch/ch* wild-type mouse, SV40-transformed hepatocyte cultures and newborn liver from the *14CoS/14CoS* mouse display elevations in: 1) the mRNA levels of three *gadd* genes; 2) the enzyme activities of superoxide dismutase, glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase; 3) NADPH and GSH concentrations; and 4) the mRNA levels and enzyme activities of four genes in the mouse aromatic hydrocarbon-responsive [*Ah*] battery [3]. The oxidative stress response in the *14CoS/14CoS* mouse is the result of an absence of the *Fah* gene located within the deleted region of Chr 7 [4, 5]. Absence of the FAH enzyme, part of the tyrosine degradation pathway, causes an accumulation of ROMs, thereby leading to endogenous ROM-mediated oxidative stress in untreated *14CoS/14CoS* newborn liver [5, 6].

A possible role for the AHR in the oxidative stress response, within the context of the [*Ah*] gene battery, has been suggested [6]. The mouse [*Ah*] gene battery comprises at least six dioxin-inducible genes: two Phase I cytochrome P450 genes, *Cyp1a1* and *Cyp1a2*, and four Phase II genes. The four Phase II genes include: *Nmo1*, *Ahd4*, *Ugt1a6* and *Gsta1*. All six genes are up-regulated by AHR ligands such as dioxin or benzo[a]pyrene. In contrast, all four [*Ah*] Phase II genes—but not *Cyp1a1* or *Cyp1a2*—are induced by electrophile-mediated oxidative stress.

3-Methylcholanthrene, a ligand for the AHR, was re-

* Corresponding author: TEL: 513-558-0154; FAX: 513-558-0925; E-mail: dan.nebert@uc.edu

‡ *Abbreviations:* *Ahd4*, mouse gene that encodes the TCDD-inducible aldehyde dehydrogenase-3c; AHR, Ah receptor; *c*, the mouse gene encoding tyrosinase; c^{ch}, wild-type allele having no Chr 7 deletion; c^{14CoS}, allele in which there is a 3,800-kb deletion on Chr 7 that includes the *c* and *Fah* genes; Chr, mouse chromosome; *Cox2*, the gene encoding cyclooxygenase-2 (COX2; prostaglandin endoperoxide H synthase); FAH, fumarylacetoacetate hydrolase encoded by the mouse *Fah* gene; *gadd*, growth arrest- and DNA damage-inducible genes; *Gsta1*, mouse gene encoding the TCDD-inducible glutathione transferase Ya. By convention, mouse genes include lower-case letters and italics, and other mammalian genes include all capital letters italicized, whereas the cDNA, mRNA or enzyme (gene product) of all mammals including mouse are depicted in all capital letters that are not in italics; HETE, hydroxyeicosatetraenoic acid; 12-HHT, 12(S)-12-hydroxy-5,8,10-heptadecatrienoic acid; *Nmo1*, mouse gene that encodes the TCDD-inducible NAD(P)H:menadione oxidoreductase (DT diaphorase, quinone/azo dye reductase); PG, prostaglandin; ROMs, reactive oxygenated metabolites; SSC, 0.15 NaCl + 0.015 M sodium citrate, pH 7.0; TCDD or dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *Ugt1a6*, mouse gene coding for the TCDD-inducible UDP glucuronosyltransferase-1A6.

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ported to induce PLA_2 activity in mouse skin [7]. Benoxaprofen (a nonsteroidal anti-inflammatory agent that inhibits cyclooxygenase activity) blocks TCDD-induced toxicity in the chick embryo [8]. Dioxin is known to elicit oxidative stress in the intact animal [9, 10]. These observations suggested to us a possible role of PLA_2 and the arachidonic acid cascade in AHR-mediated effects within the [*Ah*] battery. The purpose of this study [11], therefore, was to compare arachidonic acid release, changes in the eicosanoid metabolite profile, and PLA_2 mRNA and enzyme activity in the *14CoS/14CoS* oxidatively stressed mutant and the *ch/ch* wild-type liver cell lines.

MATERIALS AND METHODS

Cell Culture Conditions

Development and characterization of the *ch/ch* and *14CoS/14CoS* simian virus-40 (SV40)-transformed cell lines, derived from newborn mouse liver, have been described in detail [12]. The *14CoS/14CoS* and *ch/ch* hepatocyte lines are routinely grown at 34° in 75-cm^2 flasks containing 15 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum, 0.1% gentamycin, and 26 mM $NaHCO_3$, under 95% air:5% CO_2. These two cell lines, in exponential growth (about 75% confluent), were used for all studies. The phenotypes of elevated *Nmo1*, *Ahd4*, *Ugt1a6* and *Gsta1* gene expression in the *14CoS/14CoS* line, compared with levels observed in the *ch/ch* wild-type cell line, have remained stable for more than 9 years.

Determination of Arachidonic Acid Release

Cells in 60-mm dishes were incubated with 0.2 μCi/ml of [^{3}H]arachidonic acid (218.2 Ci/mmol; New England Nuclear) for 6 hr in HEPES-buffered DMEM medium, containing 0.1% fatty acid-free BSA in an air incubator (34°). The cells were washed free of the radiolabeled medium, and assays were then performed (always using medium containing fatty acid-free BSA). The assays were initiated by the addition of fresh medium containing melittin, fetal calf serum, the calcium ionophore A23187, bradykinin, gastric release peptide, vasopressin, bombesin, epidermal growth factor, or 12-*O*-tetradecanoylphorbol 13-acetate—at concentrations over three orders of magnitude known to cause arachidonic acid release. The medium without stimulants was used for determining the basal levels of arachidonic acid release. Following a 5-min stimulation, aliquots of the medium were removed and microfuged to ensure the absence of any particulate/cellular material. An aliquot was then assayed by scintillation counting for total [^{3}H]arachidonic acid and its metabolites [13].

HPLC Analysis of Eicosanoid Metabolites

Cells were prelabeled with [^{3}H]arachidonic acid for 6 hr and then treated with melittin (2.5 μg/mL) for 5 min, and aliquots of medium were removed, as described above. Prostaglandin B_2 (50 μg/mL) was added to each medium sample as an internal standard to help quantify the recovery rates of eicosanoid metabolites. The sample was then acidified with formic acid (pH = 3.5) and extracted three times with two volumes of ethyl acetate. Samples were dried under a stream of N_2 and then quantitatively injected onto an ODS-Ultrasphere column. A gradient-HPLC elution program allowed for the identification of major cyclooxygenase and lipoxygenase products. Tentative identification of each metabolite was accomplished by co-elution of the radiolabeled products (monitored by using a flow-through liquid scintillation detector) with authentic standards (monitored by UV-absorbance with a multi-wavelength detector) [13].

Assay of PLA_2 Enzyme Activity

The untreated liver cell lines in 150-mm dishes (75% confluent) were harvested in HB-HBSS buffer (phenol-red free Hanks' balanced salt solution (HBSS), 20 mM HEPES, 7.5% $NaHCO_3$, pH 7.4). The cells were homogenized, then sonicated on ice [in 50 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonylfluoride (PMSF)], and the homogenate was centrifuged at 1,000 rpm for 10 min at 4°. An aliquot of the supernatant fraction containing 50 μg protein was used for the enzyme assay [14]. PLA_2 activity was determined by using [^{3}H]arachidonate-phosphatidylcholine as substrate (0.1 μCi/reaction) in a final concentration of 100 mM Tris-HCl buffer (pH 8.0) containing 5 mM $CaCl_2$ and 120 mM NaCl; the concentration of the substrate phosphatidylcholine (10 μM) far exceeds the critical micellar concentration. The reaction was carried out for 60 min at 37° and stopped by adding 25 μL 2 N HCl. The released arachidonic acid and remaining phosphatidylcholine were extracted three times with 2 vol. of ethyl acetate. Ethyl acetate was evaporated under a stream of nitrogen at 45°. The product (arachidonate) and the substrate (phosphotidylcholine) were separated by thin-layer chromatography with the solvent system of chloroform-methanol-ammonium hydroxide-water (90:30:0.5:4). The locations of arachidonic acid and phosphatidylcholine migration were determined with a radioactivity scanner (Bioscan), and spots were quantified by liquid scintillation counting. PLA_2 activity denotes the ratio of the radioactivity of arachidonic acid recovered to that of the total phosphatidylcholine plus arachidonic acid recovered [14]. The PLA_2 activities were normalized to protein content.

Southern Blot Analysis

The present PLA_2 nomenclature is somewhat confusing [15]. Groups I, II and III are extracellular (secreted) PLA_2 enzymes of approximately 14, 14 and 17 kDa, respectively, have been isolated from poisonous snakes and bees as well as rat pancreas [16], and require millimolar levels of Ca^{2+}.

Groups IV A and IV B comprise intracellular PLA_2 enzymes of approximately 85 and 40 kDa, respectively (for the purpose of simplicity, we have chosen to name "Group IV" in the Dennis review [15] as "IV B"). Group IV A was first isolated from the human monocytic U937 cell line, is specific for arachidonic acid, and requires submicromolar levels of Ca^{2+} [17]. Group IV B has been isolated from rat vascular smooth muscle cells [18] and canine myocardium [19], prefers arachidonyl-containing phospholipids, and is Ca^{2+}-independent.

We received as generous gifts the rat pancreatic PLA_2 I [16] and the rat vascular smooth muscle cell PLA_2 II [18] cDNA clones from Hiroshi Teraoka, and the human PLA_2 IV A [17] from John Knopf. The plasmids containing each of the three PLA_2 cDNAs were digested by restriction endonucleases and resolved via 2% nondenatured agarose gel electrophoresis. Southern hybridization was carried out as described [20]. The digested DNA was transferred to GeneScreen membranes, and the filter was blocked for 4 h in 0.5 M Na_2HPO_4 (pH 7.2), 7% SDS, 1% Fraction V BSA, 1 mM ethylenediaminetetraacetic acid, 2.5 μg/mL heat denatured salmon sperm, and 10% dextran sulfate at 65°; replicate filters were hybridized at 60° in the same solution with each of the ^{32}P-labeled PLA_2 cDNA probes. Following 40 hr of hybridization, the filters were washed twice with 1× SSC and 0.1% SDS at room temperature for 15 min, twice with the same solution at 37° for 15 min, and finally with 0.1× SSC and 1.0% SDS at 37° for 15 min. The filters were then exposed for 24–48 hr to Kodak XAR-5 film at −70° with intensifying screens.

RNA Extraction and Northern Blots

Total RNA from the *ch/ch* and *14CoS/14CoS* cells lines was extracted by the acid guanidinium thiocyanate method [21]. Total RNA (20 μg) was separated in 1% formaldehyde-agarose gels and transferred to GeneScreen membranes in 10× SSC. Replicate filters were blocked, hybridized to each of the three PLA_2 cDNA probes, and washed under conditions identical to those described above for the Southern blot analysis. The filters were exposed for 5 and 63 hr to Kodak XAR-5 film at −70° with intensifying screens. The densities of the 18S and 28S rRNA bands in each lane on the ethidium bromide-stained gels were used as RNA-loading controls per lane. The blots were then semi-quantitated by scanning densitometry of each of the three mRNAs, as a function of mRNA to [18S + 28S] rRNA ratio [22]. This hybridization analysis was repeated two additional times. Statistical analysis of the data was performed by Student's two-tailed *t* test.

RESULTS

Arachidonic Acid Release

Figure 1 shows that, without any stimulation, the basal levels of arachidonic acid release from the *ch/ch* and *14CoS/14CoS* cell lines were comparable. When stimulated by melittin, the *14CoS/14CoS* cells exhibited a 3-fold higher release of arachidonic acid and its metabolites, as compared with *ch/ch* cells. The arachidonic acid release was 2-fold higher in *14CoS/14CoS* than in *ch/ch* cells, when stimulated by either fetal calf serum or the calcium ionophore A23187. We found that other well-established arachidonic acid-releasing factors were incapable of stimulating arachidonic acid release in these two cell lines (data not shown): bradykinin, gastric release peptide, vasopressin, bombesin, epidermal growth factor, and 12-*O*-tetradecanoylphorbol 13-acetate (TPA). Because melittin, fetal calf serum, and A23178 are stimuli known to increase intracellular calcium [23], these data (Fig. 1) would suggest that the increase in arachidonic acid release from *14CoS/14CoS*, as compared with that from the *ch/ch* wild-type cell line, might be associated with an enhanced calcium influx in response to the endogenous ROM-mediated oxidative stress.

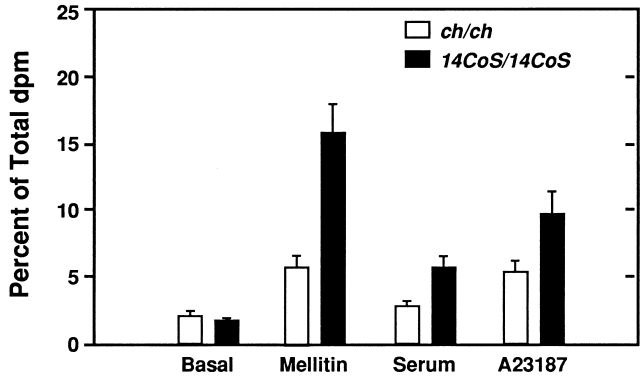

FIG. 1. Arachidonic acid release in *ch/ch* and *14CoS/14CoS* liver cell lines. Cultures (in logarithmic growth and about 75% confluent) were serum-deprived and prelabeled with [^{3}H]arachidonic acid for 6.0 h—prior to removal of the radiolabeled medium and no stimulation (basal), melittin (2.5 μg/ml), 10% fetal bovine serum, or A23187 (2.5 μg/ml). Values represent means ± SD of the percentage of cell-associated counts released during the 5-min assay period ($n = 8$).

HPLC Analysis of Arachidonic Acid Metabolites

To identify which predominant arachidonic acid metabolites might contribute to most of the increased arachidonic acid release from *14CoS/14CoS* cells, we qualitatively analyzed the cell supernatant fractions by radiochromatographic HPLC separation (Fig. 2). The major peak, with a retention time of 35 min (Fig. 2 insets, *top* and *bottom*), co-migrated with authentic arachidonic acid. Hence, the material released is either arachidonic acid or a closely related metabolite of arachidonic acid. This major peak obtained from *14CoS/14CoS* was 3- to 4-fold greater than that from *ch/ch* cells—consistent with the magnitude of increase in arachidonic acid release shown in Fig. 1.

Three additional peaks (having retention times of 7, 9, and 10.5 min) were observed from *14CoS/14CoS* but not from *ch/ch* cells (Fig. 2). Although these peaks might represent more than single metabolites, these eluates co-migrated at the positions of the arachidonic acid metabo-

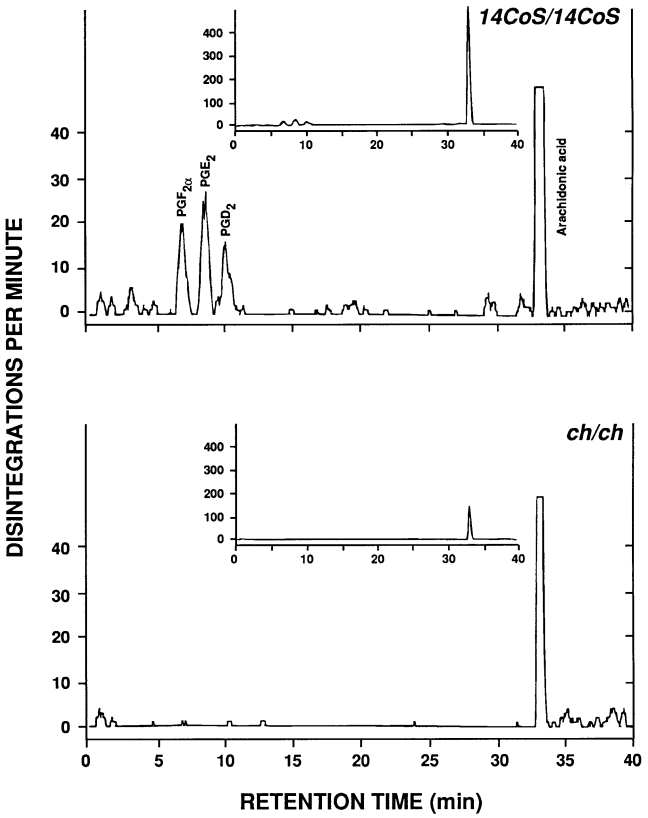

FIG. 2. HPLC radiochromatographic analysis of released [^{3}H]arachidonic acid metabolites. *14CoS/14CoS* and *ch/ch* cultures were prelabeled, as described in the Fig. 1 legend, and then stimulated with melittin (2.5 μg/ml) for 5 min. *Inset*: total radioactivity, showing the size of the peak representing nonmetabolized arachidonic acid (retention time = 33 min).

lites $PGF_{2\alpha}$, PGE_2, and PGD_2, respectively. All three of these eicosanoids are derivatives of prostaglandin H_2. The presence of these increased metabolites would suggest that the cyclooxygenase pathway [23, 24] is more active in the oxidatively stressed *14CoS/14CoS* cell line, when compared with that in the *ch/ch* line.

PLA_2 Enzyme Activity

Arachidonic acid, the precursor of eicosanoids, is the product of phosphatidylcholine cleavage by PLA_2 [15, 25]. We therefore measured PLA_2 enzymic activity in the two cell lines.

Figure 3 illustrates that PLA_2 activity in oxidatively stressed *14CoS/14CoS* cells was three times higher than that in the *ch/ch* cells. This magnitude of the difference in PLA_2 activity between the two cell lines is comparable to that of the mellitin-stimulated arachidonic acid release from the two cell lines. These findings suggest an association among the increases in PLA_2 enzyme activity, the increased arachidonic acid release, and the increased oxidative stress in *14CoS/14CoS* cells, as compared with that from the *ch/ch* wild-type cells. Consistent with these data is a report [26] showing that type B ultraviolet light (UVB)-induced oxidative stress causes a stimulation of PLA_2 synthesis.

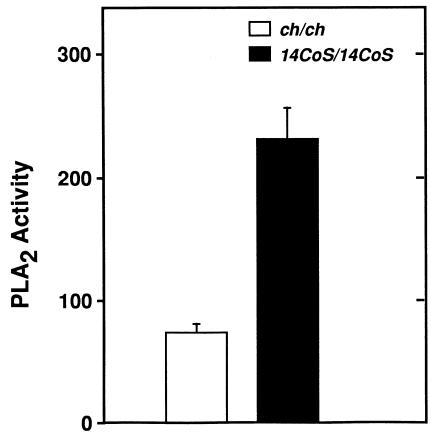

FIG. 3. PLA_2 activity in untreated *ch/ch* and *14CoS/14CoS* liver cell lines. The PLA_2 activity is presented as the percent of arachidonic acid released from the total amount of phosphatidylcholine added, per microgram of protein. The values represents the means ± SD (n = 3 experiments).

PLA_2 mRNA Levels in the 14CoS/14CoS *and* ch/ch *Cell Lines*

Measurement of increased PLA_2 enzymic activity in *14CoS/14CoS* cells does not distinguish as to which of the six or more PLA_2 enzymes has been induced [15]. We therefore chose to compare mRNA levels of PLA_2 I [16], PLA_2 IV A [17] and PLA_2 II [18] in the untreated *14CoS/14CoS* and *ch/ch* cell lines.

Because these three PLA_2 genes exhibit >40% identity [27], we first examined whether these three types of PLA_2 cDNA probes might cross-hybridize to one another under the experimental conditions that were chosen for Northern blot analysis. Figure 4 shows one band in PLA_2 II and PLA_2 IV A that cross-hybridized to the PLA_2 I cDNA probe, no cross-hybridization between PLA_2 I or PLA_2 IV A when probed with the PLA_2 II cDNA, and no cross-hybridization with PLA_2 I plus very slight hybridization with PLA_2 II when probed with the PLA_2 IV A cDNA. The arrows denote each of the specific bands that were gel-purified for use in Northern hybridizations. The Fig. 4 data show that these three cDNA probes should allow us to examine specifically the level of mRNA expression of three distinctly different groups of PLA_2.

Using the same hybridization protocol for Northern blot analysis as we did in the Southern blots, we examined the levels of PLA_2 mRNA expression in the untreated *14CoS/14CoS* and *ch/ch* cell lines (Fig. 5). The strongest signal was found for the PLA_2 II mRNA, and the oxidatively stressed *14CoS/14CoS* cell line displayed about five times more of this extracellular Ca^{2+}-dependent PLA_2 mRNA than the *ch/ch* wild-type line. With a 65-hr exposure, the PLA_2 I mRNA (extracellular, secreted) can be visualized but is approximately the same in both cell lines. Also with the

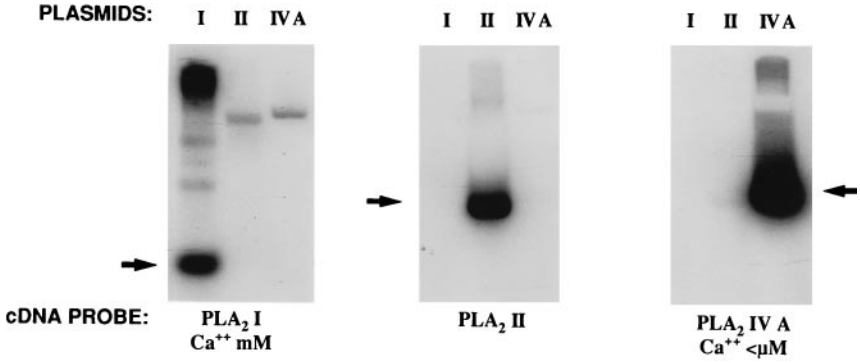

FIG. 4. Southern hybridization analysis of the three PLA_2 cDNAs. Plasmids containing each of the three PLA_2 cDNAs were digested by the appropriate restriction endonucleases. Each PLA_2 cDNA was then separated from the vector in a 2% nondenatured agarose gel, transferred to GeneScreen membranes, and probed with the PLA_2 cDNA probes. *Arrows* denote the fragment that subsequently was gel-purified and used for cDNA probing of the Northern blots.

65-hr exposure, the PLA_2 IV A mRNA can be seen faintly, with slightly more in the *14CoS/14CoS* than the *ch/ch* cells; this faint pattern and slight difference would be consistent with the amount of cross-hybridization between the PLA_2 II and PLA_2 IV A cDNA probes seen in Fig. 4. This fold difference in PLA_2 II mRNA expression between the *14CoS/14CoS* and *ch/ch* cell lines (Fig. 5) is remarkably parallel to the fold differences in PLA_2 enzyme activity and mellitin-stimulated arachidonic acid release between the two cell lines described above. These results suggest that enhanced expression of the Ca^{2+}-dependent extracellular PLA_2 II is correlated with the increased arachidonic acid release from untreated *14CoS/14CoS* cells.

DISCUSSION

In the present study we have demonstrated that, compared with the *ch/ch* wild-type, the untreated *14CoS/14CoS* liver cell line—undergoing endogenous ROM-mediated oxidative stress because of the deleted *Fah* gene on Chr 7—exhibits 2- to 5-fold increases in: 1) PLA_2 enzyme activity, 2) a Group II secreted PLA_2 mRNA, 3) arachidonic acid release, and 4) formation of prostaglandins D_2, E_2, and $F_{2\alpha}$. Included are illustrations of the phospholipid signaling pathways (Fig. 6) and the arachidonic acid cascade (Fig. 7) pertinent to the data of the present report.

Figure 6 shows that PLA_2 is but one of at least six classes of phospholipid-cleaving enzymes, and the only class of which a subset gives rise to arachidonic acid formation. PLA_2 catalyzes the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids to liberate free fatty acids and lysophospholipids. The PLA_2 proteins represent a very diverse class of enzymes with regard to function, localization, regulation, mechanism, structure and role of divalent metal ions [25]. The functions of PLA_2 proteins include such cellular processes as signal transduction pathways, host defense, and phospholipid digestion and metabolism [15]. Figure 6 also shows that arachidonic acid is one of at least eight second messengers, which activate many more than eight target proteins involved in signal transduction pathways. Furthermore, arachidonic acid is the starting material for generating all eicosanoids, and is liberated by the action of membrane-bound PLA_2 upon various types of stimulation—including oxidative stress [26]. PLA_2 also up-regulates certain protein kinases C by way of the arachidonic acid cascade (Fig. 6). As determined by Western immunoblot analysis, we found no differences in protein kinase C levels between the untreated *14CoS/14CoS* and *ch/ch* cell lines (M. Yu and G. A. Jamieson, Jr, data not shown).

In a recent report [32], 2,3,7,8 - tetrachlorod - ibenzo-*p*-dioxin (dioxin, TCDD) was shown in mouse hepatoma Hepa-1c1c7 wild-type cells to evoke AHR-mediated *Cox2* gene activation and high concentrations of 12-HHT formation, but shown to have no measurable

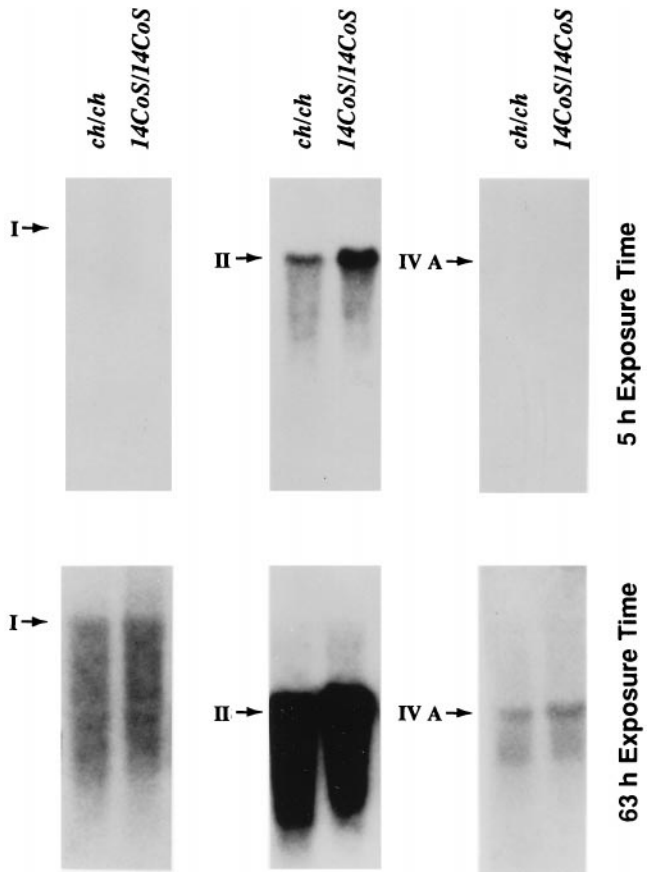

FIG. 5. Northern hybridization analysis of PLA_2 mRNA expression in the untreated *ch/ch* and *14CoS/14CoS* liver cell lines. Ten μg of total RNA from the *ch/ch* and *14CoS/14CoS* cells were separated on a 1% formaldehyde-agarose gel, transferred to GeneScreen membranes, and probed with the three different PLA_2 cDNA probes. Two exposure times are shown.

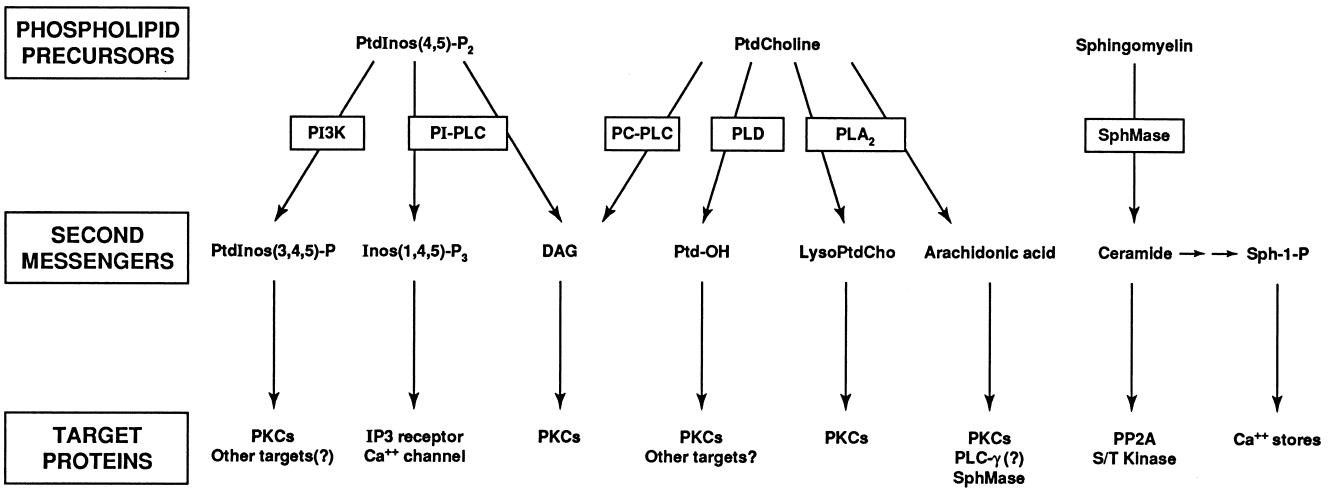

FIG. 6. Phospholipid signaling pathways, and some of their putative second-messengers and target proteins. Ptd, phosphatidyl; Inos, inositol; PI3K, phosphatidylinositol(1,4,5)triphosphate kinase; PI-PLC, phosphatidylinositol phospholipase C; PC-PLC, phosphatidylcholine phospholipase C; PLD, phospholipase D; SphMase, sphingomyelinase; DAG, diacylglycerol; Lyso, lysolecithin; Sph-1-P, sphingomyelin; PKCs, protein kinases C; PP2A, phosphoprotein-2A; S/T kinase, serine-threonine kinase. [derived from refs. 28–30].

effect on the levels of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGD_2, PGE_2, 5-HETE, 12-HETE or 15-HETE; TCDD also had no detectable effect on lipid peroxidation, GSH levels, or cysteine or protein thiol levels [32]. In contrast, in the present study we found that the oxidatively stressed untreated *14CoS/14CoS* cell line exhibits increased levels of prostaglandins D_2, E_2, and $F_{2\alpha}$. The results of these two studies suggest that there might be key differences between the TCDD-induced response in Hepa-1 cells and the electrophile-induced oxidative stress response in *14CoS/14CoS* cells.

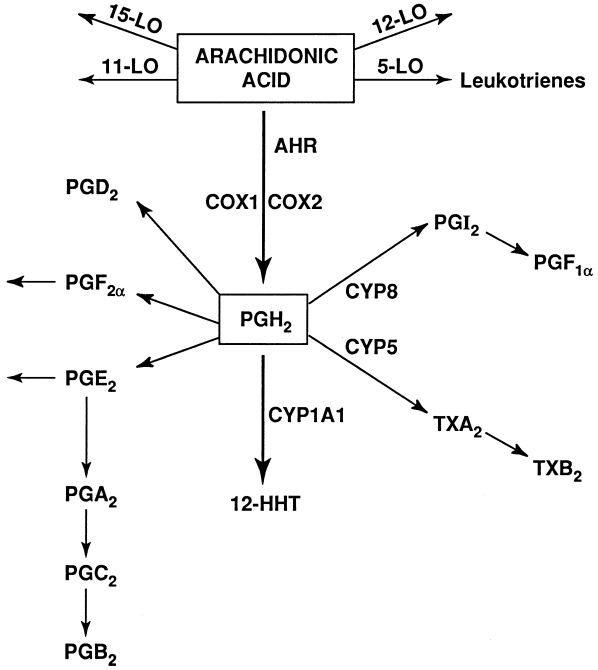

FIG. 7. Simplified diagram of the arachidonic acid cascade [derived from ref. 31]. The different forms of lipoxygenase (LO) can act in the 15-, 12-, 11- and -5 positions. Dioxin induction of COX2 requires the AHR [32, 33], but COX2 can also be induced by the tumor-promoting 4β-12-O-tetradecanoylphorbol-13-acetate (TPA) [34, 35] and peroxisomal proliferators [35]. PGI_2, prostacyclin, formed by prostacyclin synthase, a P450 (CYP8); TXA_2, thromboxane A_2, formed by thromboxane synthesis, a P450 (CYP5).

Prostaglandins D_2, E_2, and $F_{2\alpha}$ are products of the constitutive (COX1) and inducible (COX2) cyclooxygenase pathway via PGH_2 (Fig. 7). Aromatic hydrocarbon response elements (AhREs) have been found upstream of the *Cox2* gene [33]. Because TCDD in Hepa-1 cells induces AHR-mediated CYP1A1 and COX2 and increased 12-HHT formation—but not increases in prostaglandins D_2, E_2, and $F_{2\alpha}$—we suggest that CYP1A1 induction (as found in the untreated *14CoS/14CoS* cell line) might be associated with the lack of PGD_2, PGE_2, and $PGF_{2\alpha}$ formation. These findings are consistent with the possibility that CYP1A1 might play a role in the formation of 12-HHT from PGH_2 (Fig. 7).

The reaction of PGH_2 to 12-HHT represents either a removal of the endoperoxide or a metal-mediated breakdown of the endoperoxide followed by enzymic removal of the resulting hydroxyl group and loss of a 3-carbon fragment. Selenium-dependent and -independent glutathione peroxidases are known to catalyze GSH-dependent 2-electron reductions of a variety of fatty acid hydroperoxides to the corresponding alcohols [36]. Capdevila and coworkers [36] have provided several lines of indirect evidence consistent with a unique selenium-dependent cytosolic GSH peroxidase that specifically inhibits prostanoid and 12-HHT formation by COX1. Curiously, however, GSH concentrations are 3-fold higher in *14CoS/14CoS* than in *ch/ch* cells [12], and 12-HHT formation is not detectable in the *14CoS/14CoS* hepatocyte line or Hepa-1 hepatoma cells—unless treated with TCDD.

It is clear that the electrophile-induced COX2 pathway in *14CoS/14CoS* cells and the TCDD-induced AHR-mediated COX2 pathway in Hepa-1 cells result in the accumulation of different arachidonic acid metabolite profiles. This difference might be caused by excessive amounts of GSH in the former, CYP1A1 induction in the latter, or another as-yet-unappreciated chemical or enzyme. For example, might TCDD induce a specific glutathione peroxidase? Thromboxane synthase (CYP5) is a cytochrome P450 that carries out an oxygen-oxygen bond reductive cleavage and GSH supports a presumably enzymatic carbon-carbon bond scission [37, 38], similar to that seen in the formation of 12-HHT [36]. Whereas the role of GSH and the mechanism of 12-HHT formation from PGH_2 remain to be determined [36], the present study suggests that CYP1A1 might participate in this reaction.

Some of the PLA_2 enzymes appear to play a role in the "acute phase response," in which proinflammatory effects via COX2 induction are produced in activated neutrophils, mast cells, platelets, chondrocytes, vascular endothelial cells and macrophage-like cells [25, 39, 40]. The Group II PLA_2 may provide the critical link between the Ca^{2+}-mobilizing ligands that are regulated by G proteins and the stimulation of arachidonic acid release [17]. PGE_2 participates in differentiation of T cells, cell migration in response to inflammation, cell division, and activation of IL-2 and interferon-γ [23]. PGA_2, formed from PGE_2 (Fig. 7), participates in a Ca^{2+}-mediated stress gene response leading to growth arrest [41]. It is very likely that many of the effects of potent electrophiles that cause oxidative stress involve the accumulation of specific—and sometimes different—eicosanoids, as described in the present study. The mechanism(s) of oxidative stress in the presence of elevated GSH levels or in the absence of CYP1A1 induction, and the possible role of CYP1A1 in the arachidonic acid cascade will require further study.

NOTE ADDED IN PROOF: Two reviewers of this manuscript insisted that our Group II PLA_2 clone that we had obtained as a generous gift from Shionogi and Company, Ltd (Osaka) [18] must be a Group IV PLA_2, because the secretory Group II PLA_2 proteins are generally not found in liver cells. We therefore sequenced a portion of this clone (S. F. Reuter & D. W. Nebert, unpublished) and, as predicted, found it to be identical to the rat vascular smooth muscle cell Group II PLA_2 cDNA. Moreover, the Group II clone [18] expressed in yeast gave a protein of 14,000 MW (Jun Ishizaki, personal communication). Human Group II PLA_2 expression and secretion has been shown to be induced in hepatoma HepG2 cells treated with interleukin-6, tumor necrosis factor, or interleukin-1 [39]. Accession numbers are D00036 (also N00036) for the Group I PLA_2 and M25148 for the Group II PLA_2 cDNAs from Shionogi and Company, Ltd. Hence, we conclude that it is the Group II Ca^{2+}-dependent secreted PLA_2 of 14-kDa size that is increased in oxidatively stressed *14CoS/14CoS* cell cultures.

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