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MOLECULAR PHARMACOLOGY, 39:314-323

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Transformation of 5-Hydroperoxyeicosatetraenoic Acid Into Dihydroxy- and Cysteinyl-Leukotrienes by Rat Hepatocytes: Effects of Glutathione

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Received May 10, 1990; Accepted October 3, 1990

SUMMARY

In the presence of glutathione (GSH 400 μM), rat hepatocyte homogenates converted 5-hydroperoxyeicosatetraenoic acid (5-HPETE), via the intermediate leukotriene A_4, into leukotriene C_4 (LTC_4) and leukotriene B_4 (LTB_4); 5-hydroxyeicosatetraenoic acid (5-HETE) was also a prominent product. During a 5-min incubation with 100 μM (13.4 μg) 5-HPETE, 0.24 ng of LTC_4, 15.4 ng of all-*trans*-LTB_4, 4.3 ng of LTB_4, and 12.4 μg of 5-HETE were formed/mg of protein. In incubations devoid of GSH, 38.6 ng of all-*trans*-LTB_4, 8.8 ng of LTB_4, and 2.2 μg of 5-HETE were formed/mg of protein, and 3.3 μg of intact 5-HPETE could be recovered. The presence of GSH induced a time-dependent rapid depletion of 5-HPETE, paralleled by large increases in the formation of 5-HETE; formation of LTC_4 was detected in the presence but not in the absence of GSH. Addition of thiomalic acid (0.1 mM) or penicillamine (0.2 mM), both inhibitors of selenium-dependent GSH peroxidases, increased formation rates of LTC_4 by factors of 3 and 2, respectively, whereas the suppressive effects of GSH on the formation of LTB_4 were partially reversed. These results suggest that hepatocytes are capable of the simultaneous synthesis of cysteinyl- and dihydroxy-leukotrienes as well as 5-HETE; the availability of the precursor 5-HPETE and the profile of leukotrienes formed are dependent on the GSH concentration and the extent of GSH peroxidase activity.

Leukotrienes are a group of endogenous mediators active in hypersensitivity reactions and inflammation. In competent cells (i.e., macrophages, neutrophils, mast cells, and basophils), arachidonic acid is converted by the key enzyme of the arachidonic acid cascade, 5-lipoxygenase, which exhibits dual enzymic activities (1, 2), to 5-HPETE and subsequently to LTA_4. The hydrolysis of LTA_4 by LTA_4 hydrolases yields the dihydroxy-leukotriene LTB_4, which, at nanomolar concentrations, exerts a variety of distinct biological effects, among them chemotaxis of neutrophils and regulation of immune responses (3, 4). In the presence of glutathione, GSH-*S*-transferases convert LTA_4 into LTC_4, which gives rise to LTD_4 and LTE_4 upon sequential peptidolysis (5). These cysteinyl-leukotrienes are vasoactive and are thought to play a role in the bidirectional communication of the immune system and the central nervous system (6).

Leukotrienes have been implicated in the pathophysiology of hepatotoxicity. *In vivo* animal models of drug-induced hepatotoxicity (7) have revealed elevated levels of cysteinyl-leukotrienes and their metabolites in blood and bile, respectively. In an *in vitro* model of alcohol-induced hepatotoxicity, cultured human and rat hepatocytes released a chemotactic factor believed to be LTB_4 in response to challenges with ethanol (8), whereas Kupffer cells in culture responded mainly with LTC_4 release to an insult with frog virus 3 or calcium ionophore A23187 (9). In the latter experiments, trace amounts of LTB_4 were also formed. In previous studies, we have shown that LTA_4 can be converted to biologically fully active LTB_4 by human and rat liver microsomal preparations (10). In addition, homogenates of rat hepatocytes transformed exogenous 5-HPETE into active LTB_4 via the epoxide intermediate LTA_4; Kupffer cells showed a far lower capacity for this conversion (11).

Those studies did not take into account the abundance of glutathione in hepatocytes. GSH serves, together with LTA_4, as a co-substrate for GSH-*S*-transferases in the biosynthesis of cysteinyl-leukotrienes (12). In addition, GSH is a substrate for Se-dependent and Se-independent GSH peroxidases in the

This work was supported by the Swiss National Science Foundation (Grant 3-109.0.88) and the very generous help of the Roche Research Foundation. J.G. is the recipient of a START Research Career Development Award (3-018.0.87) from the Swiss National Science Foundation.

ABBREVIATIONS: 5-HPETE, 5-(*S*)-hydroperoxy-6-trans-8,11,14-*cis*-eicosatetraenoic acid; 5-HETE, 5-(*S*)-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid; LTA_4, 5-(*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTB_4, 5-(*S*)-12-(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; TMA, thiomalic acid; RP-HPLC, reversed phase high performance liquid chromatography; SP-HPLC, straight phase high performance liquid chromatography; RP-TLC, reversed phase thin layer chromatography; RIA, radioimmunoassay.

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reduction of organic peroxides to their corresponding alcohols (13). Thus, in the presence of GSH, 5-HPETE, which serves as a substrate for LTB_4 biosynthesis in hepatocytes devoid of GSH (11), might become reduced to its corresponding alcohol, 5-HETE, by means of GSH peroxidase activity (14), which is abundant in hepatocytes. Alternatively, the intermediate LTA_4, formed during the enzymatic transformation of 5-HPETE into LTB_4 (11), might become a preferential substrate for GSH-*S*-transferases, thereby affecting the amounts of LTC_4 and LTB_4 formed. So far, two studies have investigated the influence of cellular GSH supply on the profile of leukotrienes synthesized. Rouzer *et al.* (15) reported on diminished LTC_4 synthesis by GSH-deficient macrophages in response to challenges with phagocytic stimuli, whereas Pace-Asciak *et al.* (16) described catabolism of LTA_4 into LTB_4, LTC_4, and LTD_4 by rat liver homogenates; this latter study did not discriminate the capacity of distinct liver cell types (i.e., hepatocytes, Kupffer cells, and liver endothelial cells) for leukotriene synthesis.

This report provides the first quantitative investigation of the profile of concomitant biosynthesis of LTC_4, LTA_4, and LTB_4 from 5-HPETE by hepatocytes in the presence of GSH and explores the profound effects of GSH on that profile. Via the activity of GSH peroxidases, GSH limited the availability of the precursor 5-HPETE in a time-dependent fashion; a decrease in LTB_4 synthesis was compensated for by an increase in LTC_4 synthesis concomitant to the formation of large amounts of 5-HETE. Inhibition of the Se-dependent GSH peroxidase activity by specific inhibitors partially reversed the GSH-dependent constraint on LTB_4 synthesis, whereas the synthesis of LTC_4 was stimulated.

Experimental Procedures

Materials. L-α-Dilauroyl-phosphatidylcholine, Lubrol PX, prostaglandin B_1, acivicin, TMA (mercaptosuccinic acid), DL-penicillamine, γ-glutamyltranspeptidase, L-γ-glutamyl-*p*-nitroanilide, glutathione (reduced form; GSH), glutathione (oxidized form; GSSG), *N*-ethylmaleimide, cysteine, and glutathione reductase were purchased from Sigma Chemical Co. (St. Louis, MO). Collagenase/dispase was from Boehringer (Mannheim, FRG). Synthetic LTA_4 methyl ester, LTB_4, LTC_4, and LTD_4 were generous gifts from Dr. J. Rokach (Merck Frosst, Canada). Hydrolysis of synthetic LTA_4 methyl ester was done in 9:1 (v/v) methanol/50% (w/v) sodium hydroxide at 4°C for 30 min; aliquots of this solution were used in subsequent experiments (10). [14,15-^{3}H] LTC_4 as well as the RIA kits for LTC_4 and LTB_4 were obtained from Amersham, UK. Arachidonic acid was from Calbiochem (San Diego, CA). 5-(*S*)-HPETE was synthesized enzymatically from arachidonic acid using potato tuber 5-lipoxygenase (17) and purified by preparative SP-HPLC, using a LiChrosorb Si-60 column (8 × 500 mm, 7 μm; Merck, Darmstadt, FRG) in 980:20:1 (v/v/v) hexane/isopropanol/glacial acetic acid, at a flow rate of 4 ml/min. Further purification of 5-HPETE was achieved by RP-HPLC on Nucleosil 100 C_{18} (4.6 × 250 mm, 5 μm; Marchery-Nagel, Düren, FRG), using 80:20:0.1 (v/v/v) methanol/water/glacial acetic acid containing 50 μM EDTA, at a flow rate of 0.9 ml/min. 5-HETE was prepared from 5-HPETE by reduction with triphenylphospine (Sigma) in wet ether (18), followed by purification on RP-HPLC, using a Nucleosil 100 C_{18} column (4.6 × 250 mm, 5 μm; Marchery-Nagel) in 80:20:0.1 (v/v/v) methanol/water/glacial acetic acid containing 50 μM EDTA, at a flow rate of 0.9 ml/min. 5-HPETE and 5-HETE were further analyzed by UV spectrometry and separation on RP-TLC (C-18; Merck), using 90:10 (v/v) methanol/water as a solvent. Identification of 5-HPETE was by reaction with a $FeSO_4$-NH_4SCN spray. All solvents were HPLC grade (LiChrosolv) and obtained from Merck. HPLC was done with a system comprising a Perkin Elmer series 410 quaternary solvent pump connected to a Perkin Elmer series LC 235 photo diode array detector and a Perkin Elmer LCI 100 computing integrator. Liquid scintillation spectrometry was carried out in a Tri-Carb 2200 CA counter, using Insta-Gel (Packard, IL) liquid scintillation cocktail.

Preparation of rat hepatocytes and Kupffer cell homogenates. Single-cell suspensions of hepatocytes were prepared from male Sprague-Dawley rats (200 g) by *in situ* perfusion of collagenase/dispase, as described (11). Separation of hepatocytes from nonparenchymal liver cells was performed according to the method of Smedsrod and Pertoft (19) with slight modifications. Briefly, cell suspensions were filtered through nylon mesh and then centrifuged for 4 min at 50 × *g*, yielding a pellet enriched in hepatocytes and a supernatant fraction enriched in nonparenchymal cells (i.e., Kupffer cells and liver endothelial cells). The pellet, containing mostly hepatocytes, was washed another four times in Hanks' buffered salt solution (GIBCO), pH 7.4; diluted aliquots of resuspended hepatocytes (25 ml) were immediately layered on top of a Percoll cushion (25 ml) with a density of 1.070 g/ml and centrifuged at 130 × *g* for 10 min. Viable hepatocytes, which only penetrated the Percoll layer, were collected and washed once more with Hanks' buffered salt solution. Hepatocytes prepared by passage through a Percoll layer of a density of 1.070 g/ml typically contained less than 2% Kupffer cells [as tested by peroxidase staining (20)]. Finally, the pellet obtained was resuspended in a buffer containing 10 mM potassium phosphate, 20% (v/v) glycerol, 150 mM NaCl, 0.1 mM EDTA, and 50 μg/ml L-α-dilauroyl-phosphatidylcholine. Hepatocytes were disrupted by four strokes in a Potter Elvehjem homogenizer at 4°C, followed by sonication for 10 min in a bath-type sonifier (Branson) in an ice-water bath. The homogenates were stored in aliquots at −80° and typically thawed only once, just before the experiment.

Kupffer cells were obtained essentially as described (19), by means of Percoll gradient enrichment and selective adherence to plastic. Briefly, the fraction of nonparenchymal cells obtained after the low speed sedimentation of hepatocytes (see above) was applied in aliquots (10 ml) onto a preformed step gradient of Percoll comprising a lower and upper cushion (10 ml each) with densities of 1.066 and 1.037 g/ml, respectively. Gradients were centrifuged at 800 × *g* for 20 min. Kupffer cells, which were spread throughout the 1.066 g/ml cushion and quantitatively separated from erythrocytes, liver endothelial cells, and nonviable cell debris, were freed of Percoll, transferred into RPMI 1640 culture medium (GIBCO), and plated on 10 × 35-mm plastic culture dishes (Costar) at a density of 5 × 10^6 cells/plate. Cells were allowed to adhere for 20 min at 37°C in a humidified atmosphere of air/CO_2 (95%/5%); after a 20-min incubation, nonadherent cells were removed through vigorous rinsing (twice) of the culture dishes with medium (see above). Viability of adherent cells (assessed by Trypan blue exclusion) was >95%; cells were identified as Kupffer cells by peroxidase staining and ingestion of latex particles (0.8 μm). At that point, >99% of cells were Kupffer cells; they were harvested and homogenized as described for hepatocytes.

Incubations of cell homogenates with precursors of leukotriene synthesis. All incubations were carried out in sealed Reactivials, under an argon atmosphere, at room temperature for the indicated length of time. Cell homogenates were incubated (for protein concentrations see figure legends) in 400 μl of 10 mM potassium phosphate, pH 7.4, 150 mM NaCl, 50 μM EDTA, subsequently termed minimal incubation buffer, with 5-HPETE (100 μM) and the amounts of GSH indicated. In a separate set of experiments, hepatocyte homogenates were incubated (0.75 mg of protein in 400 μl of 50 mM Tris-HCl, pH 6.0, 150 mM NaCl, 50 μM EDTA, 5 mg/ml bovine serum albumin) with LTA_4 (20 μM) in the presence of the indicated amounts of GSH. In order to study the stability of LTC_4 in the presence of 5-HPETE, incubations of hepatocyte homogenates with 5-HPETE (100 μM) were spiked with 500 ng (800 pmol) of synthetic LTC_4 and a trace amount (10000 cpm) of [14,15-^{3}H]LTC_4. Reaction products were extracted and analyzed by RP-HPLC, as described below. The eluting material was collected in intervals of 30 sec and transferred to glass scintillation

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vials. Fractions containing radioactive LTC_4 and/or reaction products of LTC_4 were determined by liquid scintillation spectrometry.

Extraction, separation, and quantitation of reaction products. Extractions and RP-HPLC-based separation of products were carried out as described (21), at room temperature, using silanized glassware throughout in order to minimize nonspecific binding of lipids. Incubations (400 μl, here referred to as aqueous phase) were terminated by addition of a 3-fold volume of isopropanol containing sufficient formic acid (5 N) to give a final pH of 3.0. To that mixture, a 3-fold volume of methylene chloride was added under vortexing to give a final composition of the extraction mixture of 400:1200:4800 (v/v/v) aqueous phase/isopropanol/methylene chloride. Phase separation was achieved with centrifugation at 10,000 rpm for 10 min (Sigma 202 labtop centrifuge). The aqueous top layer and the interphase material were discarded. To the organic phase, 100 μl of double-distilled water were added under vortexing; the sample was centrifuged at 10,000 rpm for 2 min, and the organic phase was removed under a stream of nitrogen at 50°C until a residual volume of 50 μl was achieved. This material was taken up into a 100-μl Hamilton syringe. The reaction tube was thoroughly rinsed with 50 μl of starting eluent used in the HPLC separation (see below); this material was combined with the former material into the same 100-μl Hamilton syringe and injected into the HPLC, using a 200-μl injection loop connected to a Rheodyne 7125 valve. This split tube extraction procedure yielded maximal recoveries of both dihydroxy- and cysteinyl-leukotrienes, as well as 5-HPETE and 5-HETE. RP-HPLC separation of products was achieved on a Nucleosil 100 C_{18} column (5 μm, 4.6 × 250 mm) operated with a guard column, at a flow rate of 0.9 ml/min, using a gradient program comprising 72:28:0.02 (v/v/v) methanol/water/glacial acetic acid from 0 to 15 min, followed by a convex gradient (shape #7, according to the Perkin Elmer series 410 LC pump operator's manual) over 20 min to 76:24:0.02 (v/v/v) methanol/water/glacial acetic acid, followed by a 10-min hold at this methanol concentration. At the end of each run, the column was flushed for 10 min with 80:20:0.02 (v/v/v) methanol/acetonitrile/glacial acetic acid and reequilibrated at the initial conditions. The eluents used were adjusted to pH 6.0 with triethylamine, contained 50 μM EDTA, and were sparked with helium throughout.

Absolute recovery and amount of leukotrienes formed (via external standardization) were determined by comparison (in percentages) of peak heights of the internal standard prostaglandin B_1, LTC_4, Δ^6-*trans*-LTB_4, Δ^6-*trans*-12-epi-LTB_4, LTB_4, 5-HPETE, and 5-HETE with those of the directly injected corresponding synthetic standards. An area-height percentage method, using baseline projection from the last baseline reference point after the previous peak to the next point satisfying baseline criteria (Perkin-Elmer LCI 100 computing integrator software, code 2), was used to calculate peak heights.

Other methods. The concentrations of leukotrienes, 5-HPETE, and 5-HETE were estimated with UV spectrophotometry. Extinction coefficients were $\epsilon_{270\ nm}$ = 50,000 M^{-1} cm^{-1} for LTB_4, ϵ_{280} = 40,000 M^{-1} cm^{-1} for LTC_4 and LTD_4, and ϵ_{235} = 28,000 M^{-1} cm^{-1} for 5-HPETE and 5-HETE. The measurements were carried out in ethanol. During RP-HPLC, on-line spectra of leukotrienes were obtained and, for identification, compared with those in a library obtained from synthetic standards. The [^{3}H]LTB_4 and [14,15-^{3}H]LTC_4 RIAs were performed, according to manufacturer's guidelines, on material collected during elution from RP-HPLC; the material was immediately brought to pH 7.4 by addition of an aliquot of 100 mM sodium carbonate, pH 9.6, and was stored at −80°C until assayed. The cross-reactivity (at 50% B/B_0 displacement) of the LTC_4 antiserum used was 64%, 64%, <0.001%, and <0.001% towards LTD_4, LTE_4, LTB_4, and GSH, respectively. GSH and GSSG concentrations were determined according to the method of Hissin and Hilf (22). GSH peroxidase activity was measured in a coupled assay as described (23). In brief, hepatocyte homogenates (0.35 mg/ml) were incubated in 10 mM potassium phosphate, pH 7.4, containing 150 mM sodium chloride, 0.01% Lubrol PX (v/v), 0.2 mM NADPH, 1 mM sodium azide, and the amounts of GSH indicated, in a 1-ml final volume, at 25°C for 10 min before addition of 5-HPETE in ethanol (kept constant at 2% v/v). Exogenous GSH reductase was omitted. Measurements were carried out using an Aminco DW-2 spectrophotometer in the dual wavelength mode, with sample and reference wavelengths at 340 and 410 nm, respectively. γ-Glutamyltranspeptidase activity was determined as described (24), using L-γ-glutamyl-*p*-nitroanilide as a substrate. Enzymatic conversion by γ-glutamyltranspeptidase activity of LTC_4 to LTD_4 was carried out as described (25). Protein concentrations were estimated by the Bio-Rad assay procedure, using bovine serum albumin as a standard. Statistical analysis and presentation of data were done with StatView 512+ software on a Macintosh IIx computer.

Results

We have recently shown that both 5-HPETE and LTA_4 are converted to biologically fully active LTB_4 by rat hepatocyte homogenates (10, 11). Because GSH, which is abundant in hepatocytes, is a co-substrate for GSH-*S*-transferase in the biosynthesis of cysteinyl-leukotrienes [i.e., LTC_4, LTD_4, etc. (1, 5, 12)], we have extended these studies and probed the profile and quantity of leukotrienes synthesized from 5-HPETE by hepatocytes in the presence of GSH.

Acidified methylene chloride extracts of rat hepatocyte incubations with 5-HPETE were analyzed on RP-HPLC, and five prominent UV-absorbing peaks were reproducibly detected (Fig. 1). Peaks I through V co-chromatographed with and exhibited retention times identical to those of synthetic standards of Δ^6-*trans*-LTB_4, Δ^6-*trans*-12-epi-LTB_4, LTB_4, 5-HPETE, and 5-HETE, respectively. Δ^6-*trans*-LTB_4 and Δ^6-*trans*-12-epi-LTB_4 corresponded to products formed in model systems upon nonenzymatic mild acid hydrolysis of the epoxide intermediate LTA_4 (26); their presence in the chromatograms of these methylene chloride extracts suggests that LTA_4 was an intermediate in the transformation of 5-HPETE to LTB_4.

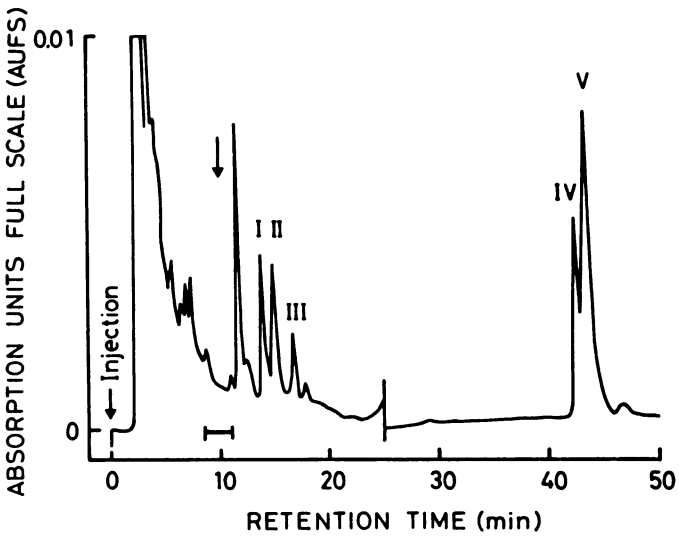

Fig. 1. HPLC profile of dichloromethane extracts of 5-HPETE incubations with hepatocyte homogenates. Hepatocyte homogenates (0.375 mg of protein/400 μl of minimal incubation buffer) were incubated with 5-HPETE (100 μM) for 30 min. Extraction of reaction products and separation on HPLC was done as described in Experimental Procedures. Up to 25 min, detection was at 270 nm and 0.01 AUFS; after 25 min, detection was at 235 nm and 0.2 AUFS. Peaks I through V eluted with the same retention times as synthetic standards of Δ^6-*trans*-LTB_4, Δ^6-*trans*-12-epi-LTB_4, LTB_4, 5-HPETE, and 5-HETE, respectively. The peak eluting at 11.8 min is the internal standard prostaglandin B_1. *Arrow*, elution position of synthetic LTC_4; *bar*, position where the column eluates were collected and tested for LTC_4 content in a RIA.

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The identity of peak III with biologically active LTB_4 has previously been established (11) by UV spectrophotometry, mass spectral analysis (27), RIA, and LTB_4 receptor replacement, whereas peaks IV and V were compared with and found to be identical to synthetic standards of 5-HPETE and 5-HETE, respectively, as assayed by UV spectrometry, RP-TLC analysis, and both RP- and SP-HPLC.

Influence of GSH on the synthesis of LTB_4. When incubations (30 min) of hepatocyte homogenates with 5-HPETE (100 μM) were supplemented with exogenous GSH, a GSH concentration-dependent 3- to 4-fold decrease in the formation of both all-*trans*-LTB_4[1] and LTB_4 was observed (Fig. 2A)[2] at rather low GSH concentrations (160 μM); no significant further decrease was seen at 400 μM GSH. Increasing GSH concentrations up to 1 mM or 10 mM resulted in a complete loss of formation of both all-*trans*-LTB_4 and LTB_4 [within the detection limit of our chromatographic system, i.e., 2 ng of LTB_4 (data not shown)]. Prior control experiments had revealed that the incubations of hepatocyte homogenates contained less than 20 μM GSH. These findings are compatible with at least two possibilities. First, GSH, as a co-substrate for Se-dependent and Se-independent GSH peroxidases (28), might induce a rapid reduction of 5-HPETE to its corresponding alcohol, 5-HETE, thereby limiting the availability of 5-HPETE for biosynthesis of LTB_4 (and possibly other leukotrienes). Second, GSH, as a co-substrate for GSH-*S*-transferases active in LTC_4 biosynthesis (12), might induce the synthesis of LTC_4; this pathway may compete for the branchpoint substrate LTA_4, which in hepatocyte homogenates is an intermediate in the transformation of 5-HPETE into LTB_4 by an as yet uncharacterized enzyme(s) (29).

Indeed, 5-HPETE was a substrate for GSH peroxidase At 20 μM 5-HPETE, GSH concentrations of >200 μM were sufficient for apparent maximal GSH peroxidase activity. Varying 5-HPETE concentrations from 2.5 to 100 μM revealed similar apparent saturation effects above 50 μM 5-HPETE at an apparently saturating GSH concentration (i.e., 1 mM, data not shown). Here, one should note that the endogenous GSH reductase present in the incubations of hepatocyte homogenates was not limiting in the coupled assay, in that a linear increase in NADPH oxidation ($\Delta A_{340\ nm}$) upon addition of GSSG (up to 1 mM) was observed (data not shown). TMA (mercaptosuccinate, 100 μM), a potent reversible inhibitor (30) of Se-dependent GSH-peroxidase, completely abolished the action of GSH-peroxidase on 5-HPETE at GSH concentrations < 400 μM; this effect was partially reversed by increasing GSH concentrations up to 2 mM. Correspondingly, in the presence of 100 μM TMA in incubations of hepatocyte homogenates with 100 μM 5-HPETE, GSH concentrations of >1 mM were required to decrease formation rates of all-*trans*-LTB_4 and LTB_4 to those observed at 400 μM GSH in the absence of TMA (Fig. 2B). In all these experiments, a GSH concentration-dependent increase in the amount of 5-HETE recovered was noted; no 5-HPETE, however, could be detected in these incubations (30 min).

GSH-dependent depletion of 5-HPETE. These data in-

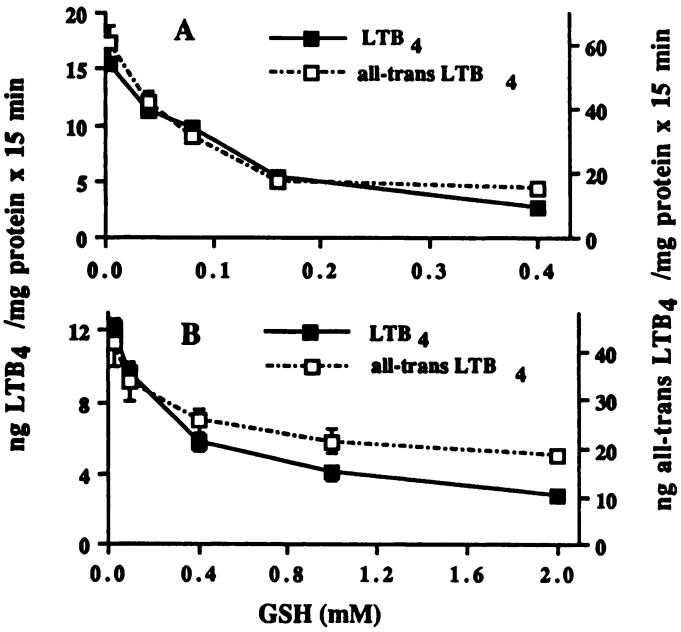

Fig. 2. Effect of GSH on the formation of LTB_4 from 5-HPETE by rat hepatocyte homogenates. Hepatocyte homogenates (0.375 mg of protein/400 μl of minimal incubation buffer) were incubated with 5-HPETE (100 μM) for 30 min in the presence of amounts of GSH as indicated. A, Incubations in the absence of TMA; B, incubations in the presence of 100 μM TMA. Note here the extended range of GSH concentrations. Values are means ± standard deviations of two separate experiments. Where not visible, *bars* reflecting standard deviation are smaller than the *symbol* reflecting the data point.

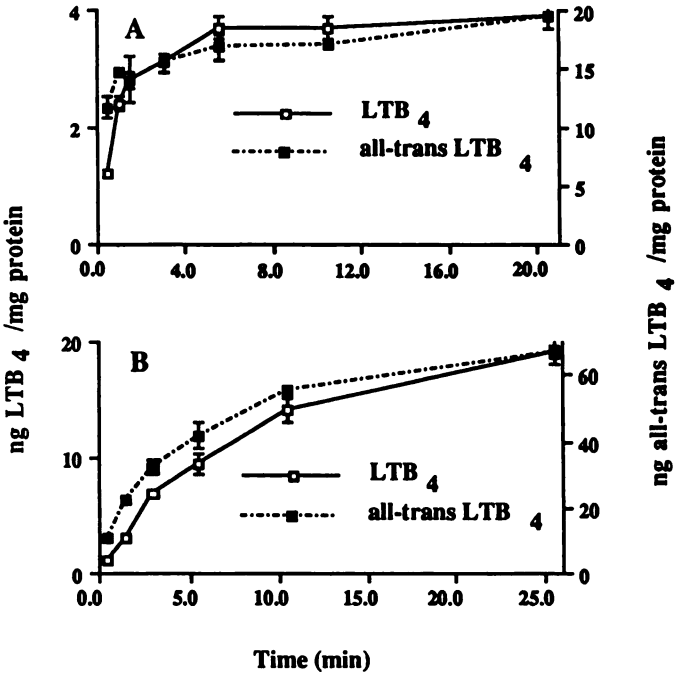

Fig. 3. Time-dependent effects of GSH on the formation of LTB_4 from 5-HPETE by rat hepatocyte homogenates. Hepatocyte homogenates (0.75 mg of protein/400 μl of minimal incubation buffer) were incubated with 5-HPETE (100 μM) and acivicin (250 μM) for the indicated times. A, Incubations in the presence of 400 μM GSH; B, incubations in the absence of GSH. Values are means ± standard deviations of two separate experiments. Where not visible, *bars* reflecting standard deviation are smaller than the *symbol* reflecting the data point.

[1] The collective term all-*trans*-LTB_4 is used to denote the combined acid hydrolysis products of LTA_4 (i.e., Δ^6-*trans*-LTB_4 and Δ^6-*trans*-12-epi-LTB_4).

[2] Due to inter-individual variations, hepatocyte homogenates obtained from individual rats varied in their basic capacity for LTB_4 synthesis in absence of GSH. Typically, at 100 μM 5-HPETE, a value of 11.6 ± 1.03 ng LTB_4/mg protein × 15 min (mean ± standard error, n = 7; range = 8.0 – 15.5 ng LTB_4/mg protein × 15 min) was obtained.

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dicate that, in the presence of GSH, substrate depletion via GSH peroxidase activity might limit LTB_4 synthesis from 5-HPETE by hepatocyte homogenates. To explore this possibility, we examined the time dependency of both LTB_4 production and 5-HPETE disappearance in the presence and absence of GSH. We also monitored the formation of 5-HETE, which would be expected to be the major product of GSH peroxidase-dependent 5-HPETE depletion. As shown in Fig. 3A, the initial increase in formation of all-*trans*-LTB_4 and LTB_4 ceased rapidly (within 2.5 min) in incubations of hepatocyte homogenates with 5-HPETE (100 μM) and GSH (400 μM). The maximal amounts of all-*trans*-LTB_4 and LTB_4 formed in these incubations were about 17.0 ± 1.1 and 3.7 ± 0.2 ng/mg of protein, respectively, and remained constant up to 20 min. A similar observation was made concerning the formation of 5-HETE in these incubations (Fig. 4A). A very rapid increase (within 1 min) in the amount of 5-HETE recovered was followed by almost constant amounts recovered over the next 20 min, with maximal values of approximately 15 μg of 5-HETE/mg of protein being detected. No 5-HPETE could be detected in these incubations, not even at very short (30 sec) incubation times. In sharp contrast were the findings when GSH was omitted from these incubations. A gradual increase, ceasing at about 25 min, in the formation of all-*trans*-LTB_4 and LTB_4 was observed (Fig. 3B), with maximal amounts of 66.8 ± 1.8 and 19.1 ± 1.1 ng/mg of protein of all-*trans*-LTB_4 and LTB_4, respectively, being formed. The amount of 5-HETE recovered reached a low level plateau at 2.2 ± 0.6 μg/mg of protein (Fig. 4B) within 5 min. Residual 5-HPETE could be detected up to 5 min, and thereafter no significant amounts of 5-HPETE were detectable. In contrast to incubations with GSH, the quantity of all-trans LTB_4, LTB_4, and 5-HETE formed by hepatocyte homogenates as well as that of 5-HPETE recovered does not match the amount of 5-HPETE used as a substrate in incubations without GSH. In absence of GSH, other routes of hydroperoxide decay, possibly yielding dihydroxy-, epoxyhydroxy-, and trihydroxy-fatty acid derivatives similar to those described by Dix and Marnett (31) for hematin-catalyzed transformation of linoleic acid hydroperoxide (i.e., 13-OOH-18:2), may contribute to 5-HPETE depletion. Furthermore, Haeggström *et al* (32) reported on the presence in mouse liver of an epoxide hydrolase acting on LTA_4, giving rise to (5S,6R)-5,6-dihydroxy-7,9-trans-11,14-cis-eicosatetraenoic acid as a major metabolite. Such compounds have not yet been included into the current extraction and quantitation scheme.

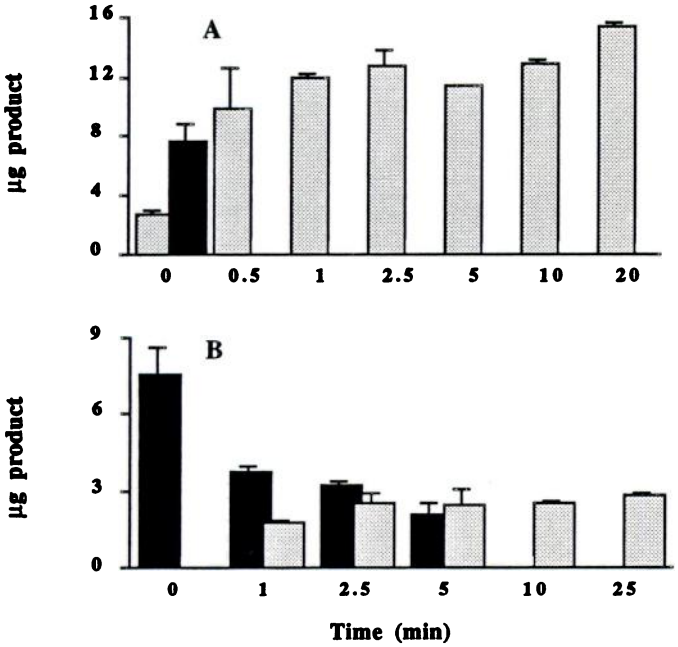

Fig. 4. Time-dependent effects of GSH on the formation of 5-HETE from 5-HPETE by rat hepatocyte homogenates. Hepatocyte homogenates (0.75 mg protein/400 μl minimal incubation buffer) were incubated with 5-HPETE (100 μM) and acivicin (250 μM) for the indicated times. A, Incubations in presence of 400 μM GSH. B, incubations in absence of GSH. Figures are means ± standard deviation of two separate experiments. Total 5-HPETE recovered in μg (■) or 5-HETE formed in μg/mg protein (■) are given.

5-HETE itself was not an inhibitor of LTB_4 synthesis in hepatocyte homogenates, because, in a separate set of experiments, we incubated hepatocyte homogenates (0.375 mg of protein/400 μl of minimal incubation buffer) with 100 μM 5-HPETE in the presence and absence of 5-HETE (100 μM) and found no effect of 5-HETE on formation rates of all-*trans*-LTB_4 (41.5 ± 3.6 versus 41.1 ± 1.1 ng/mg of protein × 15 min for the control, three experiments) or LTB_4 (13.8 ± 3.1 versus 12.8 ± 0.4 ng/mg of protein × 15 min for the control, three experiments). We also ruled out the possibility that, in the presence of GSH, mechanism-based inactivation of the enzyme(s) responsible for LTB_4 formation in hepatocytes might occur. We incubated hepatocyte homogenates (0.75 mg of protein/400 μl) with 5-HPETE (100 μM) in the presence of GSH (400 μM) for an initial 5-min period. Under these conditions, all substrate (i.e., 5-HPETE) was used and maximal amounts of 5-HETE were formed (Fig. 4A and Table 1). We then added another 100 μM 5-HPETE and measured the total amounts of products formed after an additional 5-min incubation period. Comparison of the amounts (ng of product/mg of protein) of all-*trans*-LTB_4, LTB_4, and 5-HETE formed in the original 5-min incubations with those in the substrate-supplemented 10-min incubations revealed a clear doubling of those values (Table 1). These data indicate that none of the enzymic activities involved in transformation of 5-HPETE into the products followed here is inactivated during this process.

GSH-dependent synthesis of LTC_4. We wondered whether, in spite of the observed rapid substrate depletion, the presence of GSH still could lead to a channeling of the intermediate LTA_4 into pathways other than LTB_4 synthesis. Although GSH was present in the previous experiments, no LTC_4 was detected chromatographically, possibly for two reasons. First, the concentration of the intermediate LTA_4 reached in incubations of hepatocyte homogenates with 100 μM 5-HPETE might not be sufficient to allow the synthesis of amounts of LTC_4 detectable with the current RP-HPLC method (the lower detection limit for LTC_4 is ~6–10 ng, with a signal to noise ratio of >10:1). Second, the LTC_4 formed might undergo rapid sequential peptidolysis to LTD_4 and LTE_4 through γ-glutamyltranspeptidase and dipeptidase activities, respectively. Therefore, in the experiments presented in Figs. 3, 5 and Table 1, as well as in all subsequent experiments, we blocked presumable peptidolysis of LTC_4 by addition of acivicin (see below), collected the column eluates that corresponded in retention time to that of synthetic LTC_4 (Fig. 1), and subjected this material to analysis by a RIA specific for LTC_4 (the detection limit of this assay is about 10 pg of LTC_4). As shown in Fig. 5, a time-dependent formation of LTC_4, which paralleled LTB_4

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TABLE 1

Incubation of 5-HPETE with rat hepatocyte homogenate in the presence of GSH

Hepatocyte homogenates (0.75 mg of protein/400 μl of minimal incubation buffer) were incubated for 5 min (experiment A) with 5-HPETE (100 μM), the co-substrate GSH (400 μM), and 250 μM acivicin. After 5 min, incubations were supplemented with another 100 μM 5-HPETE (experiment B). Reaction products were extracted and separated on RP-HPLC, as described in Experimental Procedures. Values are means ± standard deviations of two separate experiments.

Experiment	Incubation time	Product				
		LTC_4[a]	All-*trans*-LTB_4	LTB_4	5-HETE	5-HPETE
	min	*ng of product/mg of protein*				
A	5	0.24 ± 0.08[b]	15.4 ± 0.4[b]	4.3 ± 1.0[b]	12.4 ± 1.5[b,c]	ND[d]
B	5 + 5	0.66 ± 0.11[e]	31.3 ± 1.8[e]	9.1 ± 0.0[e]	22.3 ± 0.5[c,e]	ND

[a] LTC_4 determined by RIA.
[b] Product formation/5 min.
[c] μg of product.
[d] ND, not detectable.
[e] Product formation/10 min.

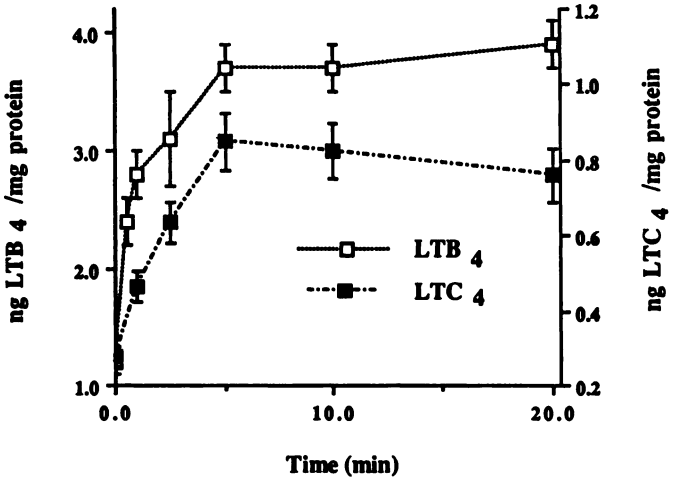

Fig. 5. Formation of LTB_4 and LTC_4 from 5-HPETE by rat hepatocyte homogenates in the presence of GSH. Hepatocyte homogenates (0.75 mg of protein/400 μl of minimal incubation buffer) were incubated with 5-HPETE (100 μM) in the presence of GSH (400 μM) and acivicin (250 μM), for the indicated times. Extraction of reaction products and separation on HPLC were done as described in Experimental Procedures. The column eluates corresponding to the elution position of synthetic LTC_4 (see Fig. 1) were collected and tested for LTC_4 content in the RIA. Values are means ± standard deviations of two separate experiments. Where not visible, *bars* reflecting standard deviation are smaller than the *symbol* reflecting the data point.

formation, was found. The maximal amounts formed were about 0.8 ng of LTC_4/mg of protein over 5 min, as compared with about 17.0 and 3.7 ng/mg of protein of all-*trans*-LTB_4 and LTB_4 formed, respectively. Here again, the initial increase in LTC_4 formation levelled off after about 5 min. Heat-denatured hepatocyte homogenates (80°C, 30 min) did not form any LTB_4 and LTC_4. The formation of LTC_4 was dependent on the availability of both 5-HPETE and GSH; supplementation of hepatocyte homogenate (0.75 mg of protein/400 μl, 400 μM GSH) at the end of a 5-min incubation with another 100 μM 5-HPETE resulted in a duplication of the amount of LTC_4 formed/mg of protein (Table 1). In addition, the absence of GSH virtually abolished the formation of LTC_4, and cysteine (0.4 mM) or GSSG (0.4 mM) could not substitute for GSH (Table 2). Control experiments had revealed inherent γ-glutamyltranspeptidase activity (i.e., 14.2 units/mg) of the hepatocyte homogenates towards the model substrate L-γ-glutamyl-*p*-nitroanilide, as well as towards LTC_4. Thus, in order to block peptidolysis of the formed LTC_4 in our incubations, the potent γ-glutamyltranspeptidase inhibitor acivicin [250 μM (33)] was included in the above experiments; this concentration was sufficient to block the formation of LTD_4 from synthetic LTC_4. Here, one should also note that the LTC_4 formed might undergo oxidative modification, similar to that observed with activated polymorphonuclear leukocytes or myeloperoxidase (34), in the presence of the substrate 5-HPETE. In fact, in a separate set of experiments, we have found that, in incubations (30 min) of hepatocyte homogenates (0.375 mg of protein/400 μl of minimal incubation buffer) with 5-HPETE (100 μM) fortified with 800 pmol of synthetic LTC_4 and a trace amount of [14,15-^{3}H]LTC_4, about 30% of the labeled [14,15-^{3}H]LTC_4 was found to be associated with more polar products derived from LTC_4, with the remainder co-chromatographing with the authentic LTC_4. No such degradation effects have been observed with LTB_4.

In separate experiments, we verified the capacity of hepatocyte homogenates for LTC_4 synthesis from very low concentrations of exogenously added LTA_4. Concentrations of exogenously added LTA_4 were varied from ~20 μM to 10 nM, a range covering reported K_m values of GSH-*S*-transferases acting on LTA_4 methyl esters (12) and the estimated intermediate LTA_4 concentrations reached in the transformation of 5-HPETE to LTB_4 by hepatocyte homogenates, respectively (Fig. 3A). The formation of LTC_4 detected was dependent on the concentration of both LTA_4 and GSH (Table 3). At 400 μM and 10 mM GSH, 100 nM LTA_4 was sufficient to yield 0.06 ± 0.0013 and 0.23 ± 0.11 ng of LTC_4/mg of protein × min, respectively. Increasing LTA_4 concentrations resulted in increased formation rates of LTC_4 (Table 3). These studies suggest that very low (i.e., ~100 nM) transient LTA_4 concentrations, can lead, in addition to that of all-*trans*-LTB_4 and LTB_4, to production of LTC_4 in a manner that is dependent on the GSH concentration. In an attempt to establish the identity with synthetic LTC_4 of the material collected from column eluates (Fig. 1), we rechromatographed this material, recorded the corresponding UV spectrum on-line, re-collected the material, and subjected it to digestion with γ-glutamyltranspeptidase (25). Analysis of the products on RP-HPLC revealed a complete transformation of LTC_4 to material coeluting with and exhibiting a retention time identical to that of synthetic LTD_4; this transformation was inhibited by acivicin (250 μM). The UV spectra recorded were compared and found to be identical to those of synthetic LTC_4 and LTD_4, respectively. These experiments indicate that hepatocyte homogenates indeed form LTC_4 from either 5-HPETE or LTA_4 in the presence of GSH.

Influence of GSH peroxidase activity on leukotriene biosynthesis. The formation of LTC_4 from 5-HPETE is

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TABLE 2

Incubation of 5-HPETE with rat hepatocyte homogenate in the presence of effectors

Hepatocyte homogenates (0.75 mg of protein/400 μl of minimal incubation buffer) were incubated for 5 min with 5-HPETE (100 μM), the co-substrate GSH, and the effectors indicated. All incubations were done in the presence of 250 μM acivicin. Reaction products were extracted and separated on RP-HPLC, as described in Experimental Procedures. Except where noted, values represent means ± standard deviations of two separate experiments. The baseline signal obtained in the RIA with aliquots of column eluates devoid of LTC_4 was 0.16 ± 0.015 (mean ± standard deviation, six experiments) and was subtracted from the signal obtained in the presence of LTC_4.

GSH	Effector	Product				
		LTC_4[a]	All-*trans*-LTB_4[b]	LTB_4	5-HETE	5-HPETE
mM		ng of product/mg of protein × 5 min				
0	None	0.03[c]	38.6 ± 2.2	8.8 ± 0.1	2.2 ± 1.2[d]	3.3 ± 1.4[e]
0	Cysteine (0.4 mM)	ND[f]	45.1 ± 2.6	11.6 ± 1.8	3.1 ± 0.2[d]	1.4 ± 0.2[e]
0	GSSG (0.4 mM)	0.01 ± 0.009	45.3 ± 4.5	10.9 ± 0.6	2.8 ± 0.2[d]	2.9 ± 0.5[e]
0.4	None	0.24 ± 0.08	15.4 ± 0.4	4.3 ± 1.0	12.45 ± 1.5[d]	ND
0.4	TMA (0.1 mM)	0.82 ± 0.08	24.0 ± 2.0	5.3 ± 0.3	10.0 ± 0.2[d]	ND
0.4	Penicillamine (0.2 mM)	0.44 ± 0.02	31.3 ± 1.8	7.2 ± 1.6	10.9 ± 2.0[d]	ND

[a] LTC_4 determined by RIA.
[b] Δ^6-*trans*-LTB_4 and Δ^6-*trans*-12-epi-LTB_4 combined.
[c] Single determination.
[d] μg of product/mg of protein × 5 min.
[e] μg recovered.
[f] ND, not detectable.

TABLE 3

Incubation of LTA_4 with rat hepatocyte homogenate in the presence of GSH

Hepatocyte homogenates (0.75 mg of protein/400 μl of minimal incubation buffer) were incubated with the indicated amounts of LTA_4 and the co-substrate GSH and 250 μM acivicin for 1 min. Reaction products were extracted and separated on RP-HPLC, as described in Experimental Procedures, except that an isocratic eluent system (i.e., 72:28:0.02 (v/v/v) methanol/water/glacial acetic acid, pH 6.0 adjusted with triethylamine, containing 50 μM EDTA) was used. Values represent means ± standard deviations of two separate experiments.

LTA_4[a]	GSH	Product		
		LTC_4[b]	All-*trans*-LTB_4[c]	LTB_4
μM	mM	ng of product/mg of protein × min		
0.01	0.4	0.04 ± 0.003	ND[d]	ND
0.1	0.4	0.06 ± 0.013	ND	ND
1.0	0.4	0.27 ± 0.003	28.8 ± 0.6	ND
21.4	0.4	7.20 ± 0.16	604.1 ± 62.0	14.3 ± 0.4
0.01	10.0	0.04 ± 0.009	ND	ND
0.1	10.0	0.23 ± 0.11	4.4 ± 0.1	ND
1.0	10.0	3.90 ± 1.10	66.3 ± 4.5	1.1 ± 0.0
22.0	10.0	105.10 ± 4.00	984.1 ± 57.6	19.4 ± 0.8

[a] The concentration of LTA_4 was determined spectrophotometrically just before the experiment.
[b] ng of LTC_4/mg of protein × min; LTC_4 determined by RIA.
[c] Δ^6-*trans*-LTB_4 and Δ^6-*trans*-12-epi-LTB_4 combined.
[d] ND, not detectable within the limits of the chromatographic method (i.e., ~2.0 ng of LTB_4).

strongly dependent on the availability of that substrate. The presence of an inhibitor of the Se-dependent GSH peroxidases, TMA, has profound effects on the profile of products formed from 5-HPETE (100 μM) by hepatocyte homogenates (0.375 mg of protein/400 μl) in the presence of GSH (400 μM). Under these conditions, an approximately 3-fold drop in formation rates of LTB_4 and all-*trans*-LTB_4 was observed, when compared with those of controls lacking GSH (Fig. 2A), whereas the formation of 5-HETE increased dramatically (Fig. 4A). TMA, in a concentration-dependent manner, partially reverses these effects. Elevated levels of LTB_4 (Fig. 6) and all-*trans*-LTB_4 (not shown) have been observed; constant values (which corresponded to about two thirds of those obtained with controls devoid of GSH) were reached above 100 μM TMA. In contrast, the formation of 5-HETE was reduced from 25 to ~20 μg/mg of protein × 30 min (Fig. 6). Results obtained for the formation of LTC_4 were different; addition of TMA (100 μM) or penicillamine (200 μM) to incubations containing GSH enhanced the formation of LTC_4 by factors of 3 and 2, respectively (Table 2). In control experiments, no influence of TMA was detected on the synthesis of LTB_4 and all-*trans*-LTB_4 from 5-HPETE in the absence of GSH (data not shown). These experiments suggest that the activity of GSH peroxidase, which limits the availability of 5-HPETE, consequently also determines the extent of production of both LTC_4 and LTB_4.

Cellular and enzymic origin of hepatic LTB_4-generating activity. The liver comprises several functionally different cell types; hepatocytes constitute about 65% of the total cell number, whereas nonparenchymal sinusoidal cells, including Kupffer cells, endothelial cells, and fat-storing cells, constitute about 35%. Kupffer cells have been shown to be very active in prostaglandin and cysteinyl-leukotriene production upon appropriate stimulation. Therefore, in accordance with previous control experiments (11), we have ruled out a significant contribution of contaminating Kupffer cells (<2%) to the hepatocyte homogenate-dependent formation of LTB_4 from exogenous 5-HPETE. Thus, isolated Kupffer cells (1×10^6 cells/200-μl incubation volume) were incubated in the presence of 100 μM 5-HPETE, and the formation of LTB_4 was compared with that in identical incubations of 7.2×10^5 hepatocytes, obtained after passage through Percoll (1.070 g/ml). These hepatocyte preparations contained less than 2% nonhepato-

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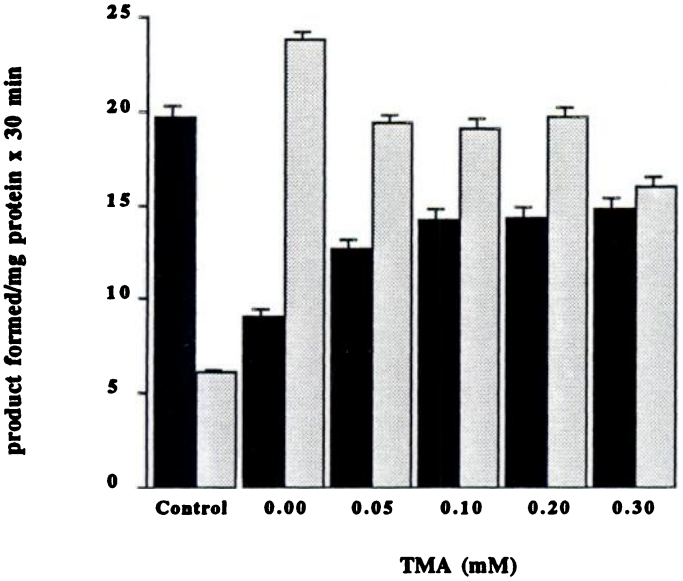

Fig. 6. Effect of the GSH-peroxidase inhibitor TMA on the formation of LTB_4 from 5-HPETE by rat hepatocyte homogenates. Hepatocyte homogenates (0.375 mg protein/400 μl of minimal incubation buffer) were incubated with 5-HPETE (100 μM), acivicin (250 μM), and GSH (400 μM) for 30 min. The concentration of TMA was varied as indicated. The bars labelled as *Control* reflect data obtained in absence of GSH and TMA. The formation of LTB_4 (■), ng/mg protein) and 5-HETE (□), μg/mg protein) have been measured. Figures are means ± standard deviation of two separate experiments.

cytes, mostly Kupffer cells (i.e., at most 1.4×10^4 Kupffer cells). Using a RIA specific for LTB_4, it was found that 7.2×10^5 hepatocytes produced 885 ± 141 pg of LTB_4/15 min (two experiments). In contrast, 1×10^6 Kupffer cells produced only 137 ± 53 pg of LTB_4/15 min (two experiments), indicating only a marginal contribution (i.e., <0.2%) of Kupffer cells to the formation by hepatocyte homogenates of LTB_4 from exogenously added 5-HPETE in this *in vitro* assay system. These findings do not exclude mutually stimulatory or inhibitory interactions by distinct hepatic cell types in leukotriene biosynthesis in the intact liver or when the 5-HPETE precursor is generated endogenously.

The enzymic origin of LTB_4 formation in hepatocytes was tested through trypsin treatment (36.4 units, from bovine pancreas; 15 min at 37°C) or heat inactivation (80°C, 30 min) of hepatocyte homogenates. In incubations of such material (0.75 mg of protein/400 μl of minimal buffer, 100 μM 5-HPETE), LTB_4 formation ceased completely, whereas, in keeping with earlier reports (11, 29), residual formation of LTA_4 (20–40%, three experiments) was found (data not shown). This is reflected by the detection of relatively large amounts of all-*trans*-LTB_4 (i.e., ~10 ng) at time 0 in experiments in which the time dependency of LTB_4 formation from 5-HPETE was investigated (Fig. 3), both in the presence and in the absence of GSH. These baseline signals for all-*trans*-LTB_4 did not stem from a contamination of the substrate 5-HPETE, in that incubation and extraction of 100 μM 5-HPETE in hepatocyte homogenate-free buffer resulted in no detectable signal for all-*trans*-LTB_4 on RP-HPLC (detection limit, ~2 ng of LTB_4). These findings point to a partial contribution of nonenzymatic processes to the transformation of 5-HPETE to all-*trans*-LTB_4 in hepatocyte homogenates, facilitated by complexed ferric iron species [possibly heme centers (29, 35)].

Discussion

In this report we show that, in the presence of GSH, rat hepatocyte homogenates convert exogenous 5-HPETE into LTC_4, all-*trans*-LTB_4, LTB_4, and 5-HETE. Several lines of experimental evidence support this conclusion. First, hepatocyte homogenates catalyzed a GSH-dependent formation of LTC_4 from 5-HPETE; all-*trans*-LTB_4, LTB_4, and 5-HETE were additional prominent reaction products of this reaction. No LTC_4 production could be detected in the absence of GSH or in experiments in which GSH was replaced by cysteine or GSSG. Second, a GSH concentration-dependent decrease in the formation of all-*trans*-LTB_4 and LTB_4 from 5-HPETE was observed in these incubations. Third, the presence of GSH induced a very rapid depletion of 5-HPETE, concomitant with a rapid increase in the formation of the corresponding alcohol, 5-HETE. Finally, inhibitors of GSH peroxidases partially reversed the effects exerted by GSH on production rates of all-*trans*-LTB_4, LTB_4, LTC_4, and 5-HETE. We have ruled out the possibility that GSH or 5-HETE might induce any kind of inactivation of LTB_4-forming activity in hepatocyte homogenates. These data suggest that hepatocytes contain enzymes that are capable of catalyzing the biosynthesis of LTB_4 and, in the presence of GSH, that of LTC_4 from peroxidized arachidonic acid precursors (i.e., 5-HPETE) via the common epoxide intermediate LTA_4. However, the activity of the GSH peroxidases limits the availability of the substrate 5-HPETE via its reduction to the corresponding alcohol, 5-HETE, thereby modulating the extent and profile of leukotrienes formed.

We have speculated (29) that the intermediate LTA_4 might not be freely accessible as a substrate to cytosolic or microsomal GSH-*S*-transferases (36) but remains, in a transition state leading from 5-HPETE to LTB_4, tightly bound to the catalytic center of the responsible enzyme(s). However, the fact that, in the presence of GSH, 5-HPETE is transformed into both LTB_4 and LTC_4 suggests that the endogenous formed intermediate LTA_4 is accessible to LTA_4 hydrolases and GSH-*S*-transferases present in hepatocyte homogenates. With 100 μM exogenous 5-HPETE and 400 μM GSH, the transient concentrations of LTA_4 observed in hepatocyte homogenates are very low (i.e.,

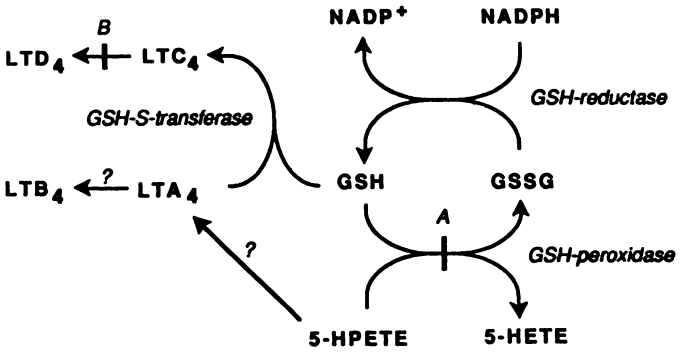

Fig. 7. Proposed scheme of GSH-dependent synthesis of LTC_4 and LTB_4 in hepatocytes. In hepatocytes, 5-HPETE is transformed to LTA_4 and subsequently to LTB_4 by as yet poorly understood enzymic steps (*?*). In the presence of GSH, LTA_4 serves as a branchpoint substrate for the GSH-*S*-transferase-dependent synthesis of LTC_4, whose peptidolysis to LTD_4 can be blocked by acivicin (*B*). At the expense of GSH, 5-HPETE can also be reduced to 5-HETE by GSH peroxidases; the activity of Se-dependent GSH-peroxidases can be reversibly inhibited by TMA or penicillamine (*A*). GSH in turn is regenerated from GSSG via the NADPH-dependent activity of GSH reductase.

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100 nM and less; see Fig. 3A). Although far below reported K_m values of GSH-*S*-transferases for LTA_4 methyl esters [i.e., 2–22 μM (12)], these concentrations are obviously still sufficient for LTC_4 synthesis from both exogenous (i.e., LTA_4 added as a substrate) and endogenous LTA_4 (i.e., 5-HPETE added as a substrate).

In competent cells, arachidonic acid is transformed into a variety of hydroperoxy intermediates by cyclooxygenases and lipoxygenases (1). The enzyme 5-lipoxygenase exhibits dual enzymic activity; it catalyzes the synthesis of 5-HPETE from arachidonic acid as well as the subsequent conversion of 5-HPETE to LTA_4 and is, therefore, the key enzyme in leukotriene synthesis (37, 38). In contrast, no reports have indicated the presence of 5-lipoxygenase activity in hepatocytes. In keeping with findings by Decker (39), experiments in our laboratory have shown that hepatocytes do not possess considerable 5-lipoxygenase activity (11); these results have been corroborated by studies by Balcarek *et al.* (40), showing a low abundance of 5-lipoxygenase-specific messages in whole rat liver when assayed for with a cDNA probe on Northern blots. Despite this apparent lack of 5-lipoxygenase activity, hepatocytes can convert 5-HPETE to LTA_4 and subsequently to all-*trans*-LTB_4 and LTB_4 (11), indicating the occurrence of alternative pathways (sensitive to both heat and trypsin treatment) in hepatocytes for 5-HPETE transformation into LTA_4. However, in hepatocytes, at least part of this conversion might occur non-enzymatically (see Fig. 3, Results in this report, and Ref. 29), whereas transformation of LTA_4 to both LTB_4 and LTC_4 is strictly enzyme catalyzed (11, 29).

The question arises, then, how hepatocytes would acquire 5-HPETE as a precursor for leukotriene biosynthesis. They might not produce it on their own but rather obtain it through intercellular transfer processes resembling those occurring in platelet-endothelial cell interactions that provide vascular prostacyclin synthetase with platelet-derived endoperoxides as substrates (41). Transcellular exchange of hydroperoxy- or hydroxy-acids was shown to lead to a variety of modulators in responsive target cells (42). Earlier reports suggested that neutrophil-derived exogenous LTA_4 could serve as a transcellularly generated substrate for leukotriene synthesis by mast cells (43), human platelets (44), and erythrocytes (45). We and others (16, 46) have shown that LTA_4 is a substrate for hepatocyte homogenates in the transformation into active leukotrienes. In fact, our experiments show that, over a wide range of LTA_4 concentrations, hepatocyte homogenates produce appreciable amounts of both LTC_4 and LTB_4 at any given GSH concentration (Table 3). Thus, the role of hepatocytes might not be restricted to transcellular degradation of leukotrienes (47); instead, they might be actively involved in intra- and/or transcellular synthesis of these mediators as well.

Besides transport from other cells, in hepatocytes, lipid peroxidation might be a contributing factor to the acquisition of arachidonic acid-derived hydroperoxides as precursors of leukotrienes. Under conditions of low O_2 concentrations, occurring physiologically within the liver lobule (48), drug-induced lipid peroxidation is markedly increased in hepatocyte suspensions and rat liver microsomes, as demonstrated for haloalkanes [i.e., halothane and carbon tetrachloride (49)] and redox-cycling drugs (50). In fact, both 5-HPETE and 15-HPETE were among the predominant peroxidation products of arachidonic acid in an *in vitro* model system of drug-induced peroxidation using 1-chloro-2,2,2-trifluoroethyl free radical, which arises as a major metabolite upon reductive metabolism of the anesthetic agent halothane, as a peroxidation-initiating species (18). It is expected that, under conditions of enhanced lipid peroxidation, 100 μM concentrations of 5-HPETE, as used in the *in vitro* studies herein, would rarely be achieved *in vivo*; local lipid hydroperoxide (i.e., 5-HPETE) concentrations in the liver in the nanomolar range could still be achieved and yield substantial amounts of leukotrienes and 5-HETE. It remains unknown whether such processes could lead to systemically enhanced leukotriene concentrations. However, under conditions of low but physiologically occurring O_2 tensions (48) that facilitate both synthesis of LTB_4 (10, 11) from LTA_4 or 5-HPETE and halocarbon-induced lipid peroxidation (49), cultured hepatocytes were sensitive to toxic effects of leukotrienes and hydroperoxides (51). In that hypoxia (i.e., <4% O_2) also increases the half-life of LTB_4 in cultured hepatocytes (52), a local derangement of leukotriene homeostasis within the liver under conditions of peroxidative stress is conceivable.

In this study, the relationship of GSH concentration and GSH peroxidase activity to the availability of 5-HPETE as a precursor for the formation of LTC_4, all-*trans*-LTB_4, LTB_4, and 5-HETE in hepatocyte homogenates has been investigated and quantitated. A derangement of either the cellular GSH concentration or the GSH peroxidase activity changes the capacity of hepatocytes for leukotriene biosynthesis *in vitro*; similar processes may disturb the delicate equilibrium of hepatic leukotriene homeostasis *in vivo*.

Acknowledgments

We are indebted to Dr. J. Rokach, Merck-Frosst Canada, for generously providing synthetic leukotrienes.

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