Rev. Physiol. Biochem. Pharmacol., Vol. 121

Leukotrienes: Biosynthesis, Transport, Inactivation, and Analysis

DIETRICH KEPPLER

Contents

Division of Tumor Biochemistry, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, W-6900 Heidelberg 1, FRG

1 Introduction

The leukotrienes comprise a group of biologically highly potent mediators synthesized from 20-carbon polyunsaturated fatty acids, predominantly from arachidonate (Samuelsson et al. 1979; Murphy et al. 1979; Samuelsson 1983; Hammarström 1983). They include the cysteinyl leukotrienes LTC_4, LTD_4, LTE_4, and *N*-acetyl-LTE_4, as well as dihydroxyeicosatetraenoate leukotriene B_4 (LTB_4). Leukotrienes act at nanomolar concentrations in host defense, intercellular communication, and in signal transduction. The cysteinyl leukotrienes induce smooth muscle conctraction and increase vascular permeability (Dahlén et al. 1981; Lewis and Austen 1984; Piper 1984); LTB_4 elicits leukocyte sticking to vascular endothelia and inflammatory infiltration, and contributes in vivo to vascular permeability changes, immunoregulation, and pain responses (Ford-Hutchinson 1990, 1991b). Leukotrienes have been implicated as mediators in the pathogenesis of inflammatory, allergic, and other diseases, including bronchial asthma, arthritis, inflammatory bowel disease, anaphylaxis, shock, hepatorenal syndrome, pancreatitis, psorisasis, and tissue trauma (Piper 1984; Lewis and Austen 1984; Denzlinger et al. 1985; A. Keppler et al. 1987; Samuelsson et al. 1987; Keppler 1988, Huber et al. 1989; Huber and Keppler 1990; Ford-Hutchinson 1990). Only a limited number of cell types are capable of synthesizing LTC_4, LTB_4, or both. Predominant producer cells are macrophages, monocytes, neutrophils, eosinophils, mast cells, and basophils (Lewis and Austen 1984; Verhagen et al. 1984; Lewis et al. 1990). In addition, transcellular synthesis from the 5,6-epoxide LTA_4 released from some cells represents a pathway for synthesis of LTB_4 and LTC_4 in endothelial cells, platelets, mast cells, lymphocytes, and even erythrocytes (Odlander et al. 1988; Dahinden and Wirthmueller 1990; Feinmark 1990; Jones and Fitzpatrick 1990).

Recent progress in leukotriene research has led to a more detailed understanding of the enzymes and proteins mediating the biosynthesis of leukotrienes and to the development of potent inhibitors of biosynthesis as well as receptor antagonists interfering with signal transduction (for reviews see Rokach 1989; Piper and Krell 1991). Moreover, the mechanisms of leukotriene transport during release from biosynthetic cells (Lam et al. 1989, 1990; Schaub et al. 1991) and during hepatobiliary elimination (Ishikawa et al. 1990; Keppler et al. 1992) have been recognized, and pathways and compartmentation of leukotriene inactivation were further elucidated (Soberman et al. 1988; Stene and Murphy 1988; Keppler et al. 1989; Shirley and Murphy 1990; Sala et al. 1990; Jedlitschky et al. 1991). In addition to receptor-mediated leukotriene actions on the cell surface (Saussy et al.

1989; Herron et al. 1992), intracellular leukotriene actions in growth factor signal transduction have been recognized (Peppelenbosch et al. 1992).

2 Leukotriene Biosynthesis

Leukotriene biosynthesis is triggered under pathophysiological and experimental conditions by a variety of immunological and nonimmunological stimuli, including Ca^{2+} ionophores. The key enzyme, arachidonate 5-lipoxygenase (EC 1.13.11.34) depends on the availability of arachidonate, which is released from membrane phospholipids by phospholipase A_2 (EC 3.1.1.4) or by the sequential action of phospholipase C (EC 3.1.4.3) and diaclyglycerol lipase (EC 3.1.1.34). Ca^{2+}-dependent activation of phospholipase A_2 with subsequent release of arachidonate is associated with phosphorylation and translocation of the cytosolic phospholipase A_2 to membrane vesicles (Lin et al. 1992). The concentration of free arachidonate is controlled, in addition, by its reincorporation into lysophospholipids (Ferber and Resch 1973; Irvine 1982). Arachidonate 5-lipoxygenase is a

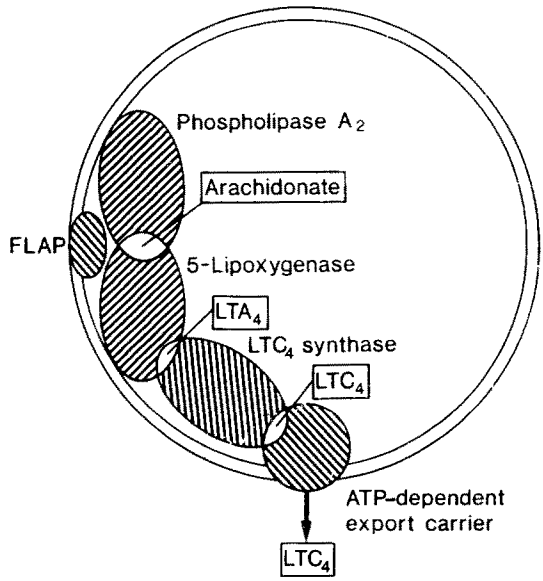

Fig. 1. Proposed scheme of the association and concerted action of enzymes and proteins involved in the synthesis of the parent cysteinyl leukotriene LTC_4. This association may allow for chanelling of the intermediates into the export carrier and may be localized in vesicles at the plasma membrane. FLAP designates the five-lipoxygenase-activating protein (Ford-Hutchinson 1991a). Release of LTC_4 from a leukotriene-synthesizing cell is mediated by an ATP-dependent export carrier (Schaub et al. 1991) which is distinct from the LTB_4 transporter

bifunctional enzyme and also catalyzes the synthesis of the 5,6-epoxide LTA_4. Depending on the differentiation of a leukotriene-generating cell, LTA_4 may be converted to LTB_4 by LTA_4 hydrolase (EC 3.3.2.6) or to LTC_4 by the membrane-bound enzyme LTC_4 synthase (EC 2.5.1.37). A protein termed *f*ive-*l*ipoxygenase-*a*ctivating *p*rotein (FLAP) is required, in addition, for the synthesis of LTC_4 and LTB_4 in intact cells (Dixon et al. 1990; Miller et al. 1990; Rouzer et al. 1990; Ford-Hutchinson 1991a). One may assume that the enzymes and proteins required for cellular synthesis of leukotrienes are closely associated and translocated to the cell membrane allowing for concerted catalysis and export from the cell (Fig. 1).

2.1 Biosynthetic Enzymes

Arachidonate 5-lipoxygenase catalyzes the first step in leukotriene synthesis by addition of oxygen to carbon 5 of arachidonate yielding (5*S*)-hydroperoxy-6,8,11,14-eicosatetraenoate. The latter is converted by the second catalytic activity of the 5-lipoxygenase protein, LTA_4 synthase, to 5,6-oxido-7,9,11,14-eicosatetraenoate (for review see Samuelsson and Funk 1989). 5-Lipoxygenase/LTA_4 synthase has been cloned and expressed in mammalian cells (Dixon et al. 1988; Matsumoto et al. 1988; Rouzer et al. 1988). This 78-kDa protein requires Ca^{2+} and ATP for maximal activity. Moreover, translocation of 5-lipoxygenase from the cytosol to the cell membrane, which is triggered by Ca^{2+}, is associated with activation of cellular leukotriene synthesis (Rouzer and Kargman 1988). Inhibition of this translocation by the indole derivative MK-886 inhibits leukotriene synthesis in intact cells (Rouzer et al. 1990). The target protein of MK-886 has been identified as the 18-kDa membrane protein FLAP, which is essential for leukotriene synthesis and must be coexpressed together with 5-lipoxygenase (Dixon et al. 1990; Miller et al. 1990; Reid et al. 1990; Ford-Hutchinson 1991a). FLAP may act to couple phospholipase A_2, membrane phospholipids, and 5-lipoxygenase. The presence of 5-lipoxygenase and FLAP is limited mostly to cells of the myeloid lineage and is related to cell differentiation (Habenicht et al. 1989).

The product of 5-lipoxygenase, LTA_4, is converted enzymatically either by LTA_4 hydrolase to LTB_4, by LTC_4 synthase to the glutathione conjugate LTC_4, by 15-lipoxygenation to 15-hydroxy-LTA_4, or by cytosolic epoxide hydrolase to 5(*S*),6(*R*)-dihydroxyeicosatetraenoate. *LTA_4 hydrolase* is a cytosolic monomeric protein of about 69 kDa which has been cloned and expressed in *Escherichia coli* (Samuelsson and Funk 1989). LTA_4 hydrolase has been detected in virtually all tissues as well as in blood plasma

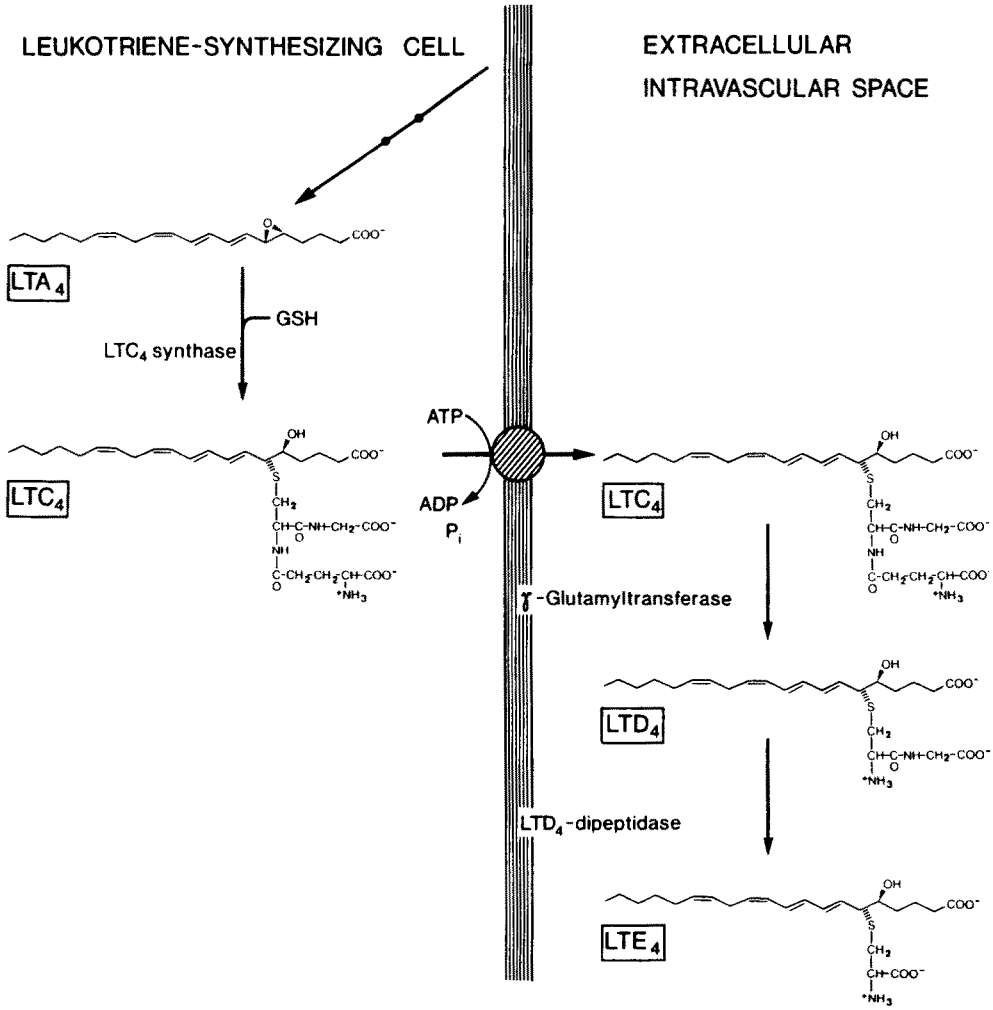

Fig. 2. Synthesis, export, and peptidolytic degradation of LTC_4. Synthesis of LTC_4 from LTA_4 and glutathione by microsomal LTC_4 synthase is followed by unidirectional ATP-dependent export from leukotriene-synthesizing cells, such as murine mastocytoma cells (Schaub et al. 1991). The ectoenzymes γ-glutamyltransferase and LTD_4 dipeptidase catalyze the biological activation and deactivation to LTD_4 and LTE_4, respectively (Hammarström et al. 1985)

from several species and in erythrocytes (McGee and Fitzpatrick 1985). Surprisingly, LTA_4 hydrolase has been identified as a Zn^{2+}-containing aminopeptidase with a sequence homologous to the active site of certain peptidases (Haeggström et al. 1990; Minami et al. 1990). Accordingly, the aminopeptidase inhibitor bestatin (Örning et al. 1991a) as well as the angiotensin-converting enzyme inhibitor captopril (Örning et al. 1991b) were

found to act as inhibitors of LTB_4 synthesis from LTA_4 in the micromolar concentration range.

The synthesis of LTC_4 is catalyzed by membrane-bound *LTC_4 synthase* (Fig. 2), which is distinct from cytosolic and microsomal glutathione *S*-transferases (Söderström et al. 1988; Yoshimoto et al. 1988). The enzyme is highly specific for its substrate LTA_4 and has an isoelectric point of about 6, whereas other members of the glutathione *S*-transferase family are basic, with isoelectric points at or above 8.5, and catalyze the synthesis of a wide range of xenobiotic and endogenous glutathione *S*-conjugates (Söderström et al. 1988). LTC_4 synthase also reacts with LTA_3 and LTA_5. LTA_3 is a potent competitive inhibitor of LTC_4 synthesis from glutathione and LTA_4 (Yoshimoto et al. 1988). Further properties of LTC_4 synthase will be elucidated when this protein has been purified to homogeneity, cloned, and expressed. LTC_4 synthase is present not only in cells of the myeloid lineage, including mast cells and eosinophils, but also in several tissues and in endothelial cells (Feinmark 1990).

A *γ-glutamyltransferase* catalyzes the conversion of LTC_4 to LTD_4. Since LTD_4 is biologically much more potent than LTC_4 (Lewis and Austen 1984; Piper 1984), the partial degradation of the glutathione moiety to the cysteinylglycine derivative LTD_4 (Fig. 2) may be considered a biosynthetic reaction generating the ligand for the LTD_4/LTE_4 receptor. γ-Glutamyltransferase is a glycoprotein enzyme widely distributed on cell surfaces. It has not been established whether a specific γ-glutamyltransferase isoenzyme is responsible for LTD_4 generation. This reaction depends on catalysis in the low nanomolar concentration range by a high-affinity ectoenzyme (Weckbecker and Keppler 1986; Huber and Keppler 1987).

2.2 Transcellular Leukotriene Synthesis

Interaction between different cell types allows for enzymatic cooperation in leukotriene synthesis, also termed transcellular synthesis (Dahinden et al. 1985; McGee and Fitzpatrick 1986; Odlander et al. 1988; Dahinden and Wirthmueller 1990; Feinmark 1990; Jones and Fitzpatrick 1990). In neutrophils, LTA_4 formed in excess of the capacity for intracellular LTB_4 synthesis is released into the extracellular fluid where it can be stabilized by albumin. Neutrophil-derived LTA_4 is a precursor for leukotriene synthesis particularly in cell types deficient in 5-lipoxygenase, such as erythrocytes, platelets, and vascular endothelial cells. As an example, LTA_4 hydrolase in erythrocytes generates LTB_4 from neutrophil-derived LTA_4 (McGee and Fitzpatrick 1986). Moreover, LTA_4, released from neutrophils and bound to albumin, serves in the synthesis of LTC_4 by mast cells

(Dahinden et al. 1985; Dahinden and Wirthmueller 1990). Thereby, the capacity of mast cells for LTC_4 generation is augmented. Leukotriene production under conditions where cell-cell cooperation occurs differs quantitatively and qualitatively from the sum of the separate cellular biosynthetic capacities. In disease processes different cell combinations may exist as compared to the normal condition. Transcellular leukotriene synthesis not only contributes to systemic leukotriene production but also influences the efficacy of inhibitors of leukotriene biosynthesis, which differ in their action on different cell types.

2.3 Inhibition of Leukotriene Biosynthesis and Action

Selective inhibition of leukotriene biosynthesis or selective blockade of the receptors for LTD_4 and LTE_4 or for LTB_4 is not only of therapeutic interest. These approaches furthermore serve to define the role of the leukotrienes under pathophysiological conditions. The recent development of biosynthesis inhibitors and of receptor antagonists has resulted in a considerable increase in selectivity and in compounds which are effective in the low nanomolar concentration range (Fitzsimmons and Rokach 1989; Ford-Hutchinson 1991a, b; Aharony and Krell 1991).

Direct inhibitors of 5-lipoxygenase have been described and many of them are "redox" inhibitors, presumably reducing the iron at the active site of the enzyme (for review see Fitzsimmons and Rokach 1989). Natural lipoxygenase inhibitors include the flavonoid compounds and hydroxylated cinnamic acids with cirsiliol and caffeic acid, respectively, as potent representatives in both groups. Nordihydroguaiaretic acid (NDGA) is a commercial antioxidant which inhibits lipoxygenase enzymes. NDGA is a widely used antioxidant inhibitor which lacks sufficient selectivity for 5-lipoxygenase and efficacy in the living mammalian organism. Among the quinone inhibitors Takeda's AA-861 represents a prototype compound with limitations comparable to NDGA. These compounds may be valuable in studies with cells in culture, but, in addition to a number of side effects, they do not sufficiently suppress systemic leukotriene production in the intact organism. Direct 5-lipoxygenase inhibitors that are effective in vivo and exhibit sufficient selectivity include compounds with hydroxamate or *N*-hydroxyurea functionalities. One of these drugs- A-64077 or zileuton [*N*-(1-benzo-thien-2-ylethyl)-*N*-hydroxyurea] is an effective inhibitor of leukotriene biosynthesis in man (Bell et al. 1992). As indicated in Fig. 3, this leads to an inhibition of both LTB_4 and systemic LTC_4 synthesis.

As an alternative approach to direct enzyme inhibition, *interference of 5-lipoxygenase translocation* to the plasma membrane by compounds

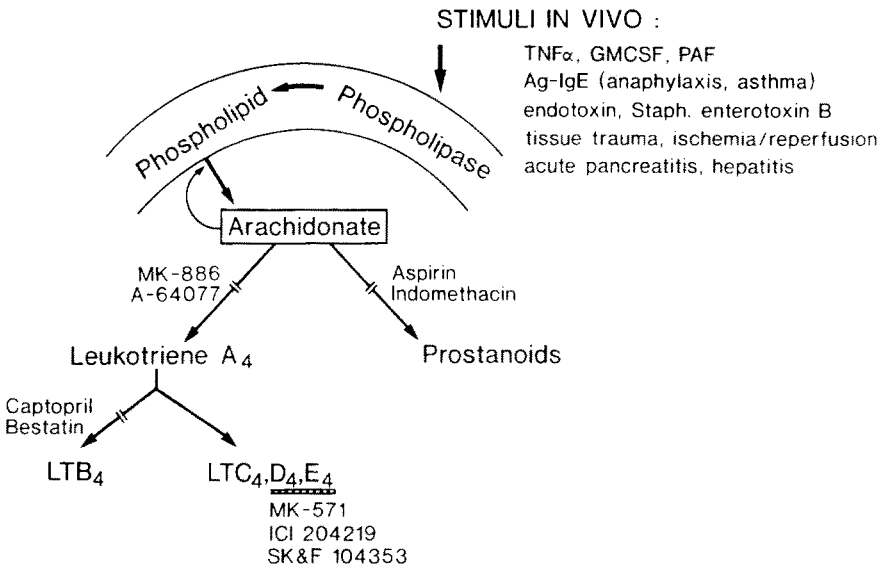

Fig. 3. The arachidonate cascade with several of its stimuli in the intact mammalian organism and with the sites of action of biosynthetic inhibitors and examples for LTD_4/LTE_4 receptor antagonists. Synthesis of leukotrienes may be elicited in vivo by a number of pathophysiological stimuli (for review see Keppler 1988) as well as physiological primers and elicitors such as the granulocyte-macrophage conoly-stimulating factor (*GMCSF*; Denzlinger et al. 1990). Inhibitors of leukotriene biosynthesis from the first generation of drug development include MK-886 (Gillard et al. 1989; Ford-Hutchinson 1991a) and A-64077 (Bell et al. 1992), as well as captopril and bestatin with their additional potential to inhibit LTA_4 hydrolase (Örning 1991a, b). More potent, second generation inhibitors of LTA_4 synthesis include MK-591, A-78773, and ICI's D-2138. Some selective and potent antagonists of LTD_4/LTE_4 receptors, MK-571, ICI 204219, and SK&F 104353, are indicated (Snyder and Fleisch 1989; Piper and Krell 1991; Lewis et al. 1991)

which bind to FLAP induces potent and selective suppression of the synthesis of leukotrienes (Gillard et al. 1989; Ford-Hutchinson 1991a; Evans et al. 1991). The indole derivative MK-886 (Gillard et al. 1989), which binds with high affinity to FLAP (Rouzer et al. 1990), does not significantly affect 5-lipoxygenase itself but blocks leukotriene synthesis in intact cells and in vivo. Systemic leukotriene production, measured by an index metabolite in bile during guinea pig anaphylaxis, is completely suppressed by MK-886 (Guhlmann et al. 1989). MK-886 also suppresses cysteinyl leukotriene excretion into human urine to a large extent (Ford-Hutchinson 1991a). FLAP, as a novel drug target for inhibiting the biosynthesis of leukotrienes, also binds a group of quinoline derivatives which inhibit leukotriene synthesis in intact cells with a similar mechanism of action and at lower concentrations than MK-886 (Evans et al. 1991).

Selective inhibition of LTB_4 biosynthesis, without inhibition of LTC_4 synthesis, has become feasible as a consequence of the discovery that bestatin and captopril inhibit LTA_4 hydrolase with IC_{50} concentrations of 4 and 11 μM, respectively (Örning et al. 1991a, b). The functional resemblance of LTA_4 hydrolase to metallohydrolase enzymes (Haeggström et al. 1990) will necessitate chemical modification of the drugs for successful and selective inhibition of LTB_4 synthesis in vivo, without or with little inhibition of peptidases and angiotensin-converting enzyme.

Inhibition of LTB_4 action has been achieved by the development of LTB_4 receptor antagonists. Among these, the hydroxyacetophenone derivative LY 255283 has 50% inhibitory potency in the binding assay at a concentration of 87 nM (Herron et al. 1992).

LTD_4/LTE_4 receptor antagonists are, at present, most promising in drug development for antiasthma therapy (Piper and Krell 1991). Reasons to develop LTD_4/LTE_4 receptor antagonists have included the lack of evidence for signal transduction via LTC_4 receptors in man, the higher biological potency of LTD_4 relative to LTC_4, and the rapid formation of LTD_4 and LTE_4 from LTC_4 on cell surfaces and in the blood circulation in vivo. At least three new structural classes of high-affinity LTD_4/LTE_4 receptor antagonists have been developed and tested in man. These are SK&F 104353, ICI 204,219, and MK-571 (for reviews see Snyder and Fleisch 1989; Piper and Krell 1991; Lewis et al. 1991). These third-generation LTD_4/LTE_4 receptor antagonists are several orders of magnitude more potent and display a several hundred-fold improvement in their selectivity for LTD_4/LTE_4 receptors than the first antagonist, FPL 55712, developed in 1973 against slow-reacting substance of anaphylaxis (Augstein et al. 1973). Affinities of these antagonists were determined in the low nanomolar concentration range for the LTD_4/LTE_4 receptor in human airways and guinea pig trachea (Aharony and Krell 1991). Doses that are 50% effective in vivo after intravenous administration in the guinea pig are 46 nmol/kg for the indole-based ICI 204,219, 2 nmol/kg for the quinoline-based analog MK-571, and 550 nmol/kg for the LTD_4/LTE_4 analog SK&F 104353 (Aharony and Krell 1991). These compounds act as competitive antagonists, are highly effective in man, and contribute to a definition of LTD_4-mediated pathophysiological processes.

3 Transport of Leukotrienes During Biosynthesis and Excretion

Transport controls not only the release of LTC_4 (Lam et al. 1989; Schaub et al. 1991) and LTB_4 (Lam et al. 1990) from leukotriene-generating cells but also the removal of these mediators from the blood circulation in vivo. The liver is the most active organ for uptake, metabolic inactivation, and biliary excretion of leukotrienes (Appelgren and Hammarström 1982; Keppler et al. 1985; Hagmann et al. 1989; Wettstein et al. 1989). In addition, transport during renal excretion and during the limited intestinal reabsorption of cysteinyl leukotrienes contributes to the control of leukotriene concentrations in body fluids.

3.1 The Export Carrier Releasing LTC_4 After Its Biosynthesis

The release of LTC_4 has been studied in cultured human eosinophils incubated with exogenous LTA_4 (Lam et al. 1989). This transport is saturable, temperature-dependent, and inhibited by intracellular LTC_5, suggesting a carrier mediated process. The mechanism underlying the export of LTC_4 has been elucidated in plasma membrane vesicles prepared from murine mastocytoma cells and characterized as a primary-active, ATP-dependent process with apparent K_M values of 48 μM for ATP and 110 nM for LTC_4 (Schaub et al. 1991). Among the cysteinyl leukotrienes, LTC_4 is the best substrate for this ATP-dependent export carrier (Fig. 2). The relative transport rates at a concentration of 10 nM are 1.00, 0.31, 0.12, and 0.08 for LTC_4, LTD_4, LTE_4, and *N*-acetyl-LTE_4, respectively (Schaub et al. 1991). LTC_4 transport is competitively inhibited by the glutathione *S*-conjugate *S*-(2,4-dinitrophenyl)glutathione, and by several other amphiphilic anions including LTD_4/LTE_4 receptor antagonists (Schaub et al. 1991). Primary-active ATP-dependent transport is insignificant with LTB_4 as a substrate. Therefore, inhibition of the LTC_4 export carrier in leukotriene-synthesizing cells by structural analogs and LTD_4/LTE_4 receptor antagonists may serve as a novel pharmacological approach to interfere selectively with LTC_4 production without influencing LTB_4 generation. Isolation and molecular characterization of the LTC_4 export carrier from leukotriene-generating cells, such as mast cells, eosinophils, and monocytes, will answer the question whether this carrier belongs to the family of the ATP-dependent glutathione conjugate export carrier originally described in the erythrocyte plasma membrane (Kondo et al. 1980).

3.2 Leukotriene Uptake into Hepatocytes

Leukotrienes released into the blood circulation, with or without prior or subsequent interaction with leukotriene receptors, undergo rapid elimination from blood predominantly due to uptake by the liver (Appelgren and Hammarström 1982; Hagmann et al. 1984; Denzlinger et al. 1985; Huber and Keppler 1990; Hagmann and Korte 1990). Albumin serves as transport protein in the blood circulation (Falk et al. 1989). Uptake by hepatocytes has been demonstrated both for cysteinyl leukotrienes (Ormstad et al. 1982; Uehara et al. 1983; Weckbecker and Keppler 1986; Leier et al. 1992) and for LTB_4 (Hagmann and Korte 1990; Leier et al. 1992). Uptake of LTC_4, LTD_4, LTE_4, and *N*-acetyl-LTE_4 across the sinusoidal (basolateral) membrane into hepatocytes is independent of a Na^+-gradient and a K^+-diffusion potential (Leier et al. 1992). The uptake may be driven by high-affinity binding to intracellular proteins and by the unidirectional, ATP-dependent transport across the canalicular (apical) membrane into bile (Fig. 4; Ishikawa et al. 1990). At a concentration of 10 n*M*, the relative uptake rates into rat hepatocytes for LTC_4, LTD_4, LTE_4, and LTB_4 are 1.0, 1.3, 1.6 and 1.6, respectively. The K_M values for the leukotrienes range between 100 and 200 n*M* (Leier et al. 1992). Leukotriene-binding proteins possibly involved in hepatocellular transport were identified by the method of direct photoaffinity labeling in the deep-frozen state using the ^3H-labeled leukotriene itself as the photolabile ligand (Falk et al. 1989; Müller et al. 1991b, Leier et al. 1992). Liver membrane subfractions enriched with sinusoidal plasma membranes contain a 48-kDa polypeptide labeled both with [^3H]LTE_4 and [^3H]LTB_4. This polypeptide is not labeled by cysteinyl leukotrienes in hepatoma cells which are deficient in cysteinyl leukotriene uptake (Müller et al. 1991b; Leier et al. 1992). There is no convincing

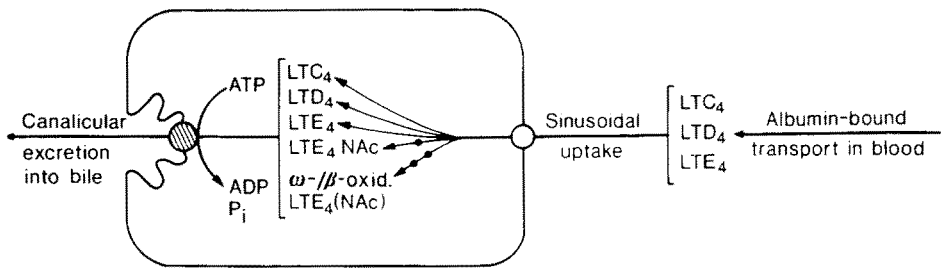

Fig. 4. Transport of cysteinyl leukotrienes through hepatocytes. Uptake across the sinusoidal membrane may be followed by intracellular degradation (Keppler et al. 1989; Jedlitschky et al. 1991) and ATP-dependent export across the canalicular membrane into bile (Ishikawa et al. 1990). The latter process may be rate-limiting in overall hepatobiliary cysteinyl leukotriene elimination

evidence, however, that his 48-kDa polypeptide represents the transporter responsible for leukotriene uptake. It may rather be an intracellular, membrane-associated polypeptide binding the leukotrienes (Leier et al. 1992) as well as related amphiphilic substances (Kurz et al. 1989). The dihydroxy fatty acid LTB_4 differs from the cysteinyl leukotrienes by its entry into hepatoma cells, possibly as a result of its facilitated diffusion (Leier et al. 1992). Kinetic studies in hepatocytes employing inhibitors indicate the existence of distinct uptake systems for the cysteinyl leukotrienes and LTB_4 in the sinusoidal membrane. The substrate specificity of the transporters involved in leukotriene uptake across the sinusoidal hepatocyte membrane will be defined more precisely after reconstitution of the purified transporter in liposomes. The interaction of both cysteinyl leukotrienes and LTB_4 with hepatocytes does not lead to detectable receptor-mediated signal transduction if the mediators are added in the physiological nanomolar concentration range. This indicates that the hepatocyte uptake systems are transporters and not receptors for the leukotrienes.

3.3 The Cysteinyl Leukotriene Export Carrier in the Hepatocyte Canalicular Membrane

During the vectorial transport across the hepatocyte some of the leukotriene metabolites retain their structure and some undergo oxidative degradation from the ω-end (Figs. 4, 5). Products of ω- and β-oxidation of LTE_4, *N*-acetyl-LTE_4, and LTB_4, as well as unmodified LTC_4, LTD_4, LTE_4, and *N*-acetyl-LTE_4, are substrates for the leukotriene export carrier in the canalicular (apical) membrane of hepatocytes (Ishikawa et al. 1990). The mechanisms of this transport has been analyzed by use of plasma membrane vesicles enriched in canalicular membranes. The inside-out vesicles incubated in the presence of labeled cysteinyl leukotrienes and ATP showed primary-active, ATP-dependent uptake, corresponding to ATP-dependent export across the canalicular membrane into bile (Ishikawa et al. 1990). Primary-active, ATP-dependent transport seems to be domain-specific with a location in the canalicular but not in the sinusoidal hepatocyte membrane (Fig. 4). This is indicated by transport studies in vesicle preparations from different membrane domains (Ishikawa et al. 1990) and by photoaffinity labeling with the ^{35}S-labeled ATP analog ATP-γ-S of canalicular and sinusoidal membranes (Müller et al. 1991a). Among the cysteinyl leukotrienes, LTC_4 is the best substrate for the canalicular export carrier. Apparent K_M values are 0.25, 1.5, and 5.2 μ*M* for LTC_4, LTD_4, and *N*-acetyl-LTE_4, respectively, whereas the K_M value for the cysteine *S*-conjugate, LTE_4, is more than 10 μ*M* (Ishikawa et al. 1990). In addition, ω-carboxy-LTB_4, but not LTB_4 itself, is a substrate for ATP-dependent trans

port across the canalicular membrane. Mutual competition among the cysteinyl leukotrienes and between leukotrienes and several glutathione *S*-conjugates and glucuronate conjugates suggests a common export carrier (Ishikawa et al. 1990; Akerboom et al. 1991). The term leukotriene export carrier is preferred since LTC_4 is the endogenous substrate with the highest known affinity for this carrier. As indicated by the transport of LTD_4 and *N*-acetyl-LTE_4 via this ATP-dependent carrier, the glutathione moiety is not a structural determinant of the substrate properties, although it may be a property providing higher affinity for the active site of the carrier. ATP-dependent glutathione *S*-conjugate transport has been originally described in erythrocyte inside-out membrane vesicles (Kondo et al. 1980) and subsequently observed in other tissues (Kobayashi et al. 1988, 1990; Ishikawa et al. 1989; Akerboom et al. 1991). The carriers expressed in different tissues may be similar in substrate specificity but are distinct as evidenced by the hereditary deficiency of the leukotriene export carrier in liver (Huber et al. 1987) and its simultaneous presence in erythrocytes (Board et al. 1992).

The ATP-dependent leukotriene export carrier in the canalicular membrane is apparently absent or inactive in a mutant strain of rats in which cysteinyl leukotriene excretion into bile is reduced to less than 2% of normal (Huber et al. 1987; Ishikawa et al. 1990). These mutant rats are partially deficient in the hepatobiliary excretion of several other non-bile salt amphiphilic organic anions, such as bilirubin glucuronide and dibromosulfophthalein (Jansen et al. 1985). The defect in this TR^- mutant rat strain is considered analogous to the one in Dubin-Johnson syndrome in man and in Corriedale sheep (Jansen et al. 1985; Kitamura et al. 1992). Deficiency of the leukotriene export carrier in the canalicular membrane is compensated by metabolic inactivation and degradation of the leukotrienes in the hepatocyte resulting in an increased renal excretion of leukotriene catabolites (Huber et al. 1987; Keppler et al. 1991).

Inhibition of ATP-dependent transport of LTC_4 in liver is observed in the presence of various glutathione *S*-conjugates in the micromolar concentration range (Ishikawa et al. 1989). Moreover, structural analogs of LTD_4 and LTE_4, developed as LTD_4/LTE_4 receptor antagonists and devoid of a glutathione moiety and a fatty acid side chain, are not only potent inhibitors of LTC_4 transport in mastocytoma cells (Schaub et al. 1991) but also of the export carrier in the canalicular membrane. Cyclosporin A interferes with the hepatobiliary excretion of cysteinyl leukotrienes (Hagmann et al. 1989). Recent studies demonstrate 50% inhibition of ATP-dependent LTC_4 transport across the rat liver canalicular membrane at a cyclosporin A concentration of 4.5 μM. This inhibition by cyclosporin A is analogous to the inhibition of the ATP-dependent multidrug export carrier

(p-glycoprotein) by this immunosuppressant (Foxwell et al. 1989; Speeg et al. 1992).

3.4 Elimination and Transport In Vivo

The pathways of elimination of leukotrienes in the intact organism were originally studied by autoradiographic (Appelgren and Hammarström 1982) and invasive techniques, mostly by use of ^{3}H-labeled leukotrienes (Hagmann et al. 1984; Denzlinger et al. 1985, 1986; Hammarström et al. 1985; Huber et al. 1990; Maltby et al. 1990). Few studies addressed the elimination and in vivo degradation of LTB_4 (Serafin et al. 1984; Hagmann and Korte 1990). More extensive investigations dealt with the elimination of different cysteinyl leukotrienes (Hagmann et al. 1984, 1986; Örning et al. 1985, 1986; Sala et al. 1990; Keppler et al. 1991, 1992). Once released into the blood circulation, the leukotrienes are selectively bound to albumin (Falk et al. 1989) and eliminated predominantly by hepatobiliary excretion. Using *N*-acetyl-LTE_4 as a representative tracer, half-lives in blood during the initial elimination period were 38 s in the rat and 4 min in man (Keppler et al. 1992). The advantage of using *N*-acetyl-LTE_4, radioactively labeled in the *N*-acetylcysteine moiety, is the metabolic stability of the label as opposed to the extensive loss of tritium from leukotrienes labeled in the arachidonate-derived fatty acid moiety during β-oxidation from the ω end (Keppler et al. 1989; Jedlitschky et al. 1991). *N*-Acetyl-LTE_4 is also an endogenous metabolite of LTC_4 in human urine (Huber et al. 1989; Maltby et al. 1990) and in rodent bile (Hagmann et al. 1986). Moreover, *N*-acetyl-LTE_4 is eliminated and transported on the same routes and at comparable rates as the other cysteinyl leukotrienes, LTC_4, LTD_4, and LTE_4. For administration of the labeled compound in vivo it is advantageous that the biological activity of *N*-acetyl-LTE_4 is low when compared to LTD_4 and LTC_4 (Lewis et al. 1981; Samhoun et al. 1989). Within 1 h, 80% of intravenously administered *N*-acetyl-LTE_4 is excreted in the rat with bile, either intact or after partial oxidative degradation from the ω end of the fatty acid chain (Jedlitschky et al. 1991). At the same time, renal excretion in the rat amounts to about 2%. In man and in the monkey cysteinyl leukotriene excretion into urine represents a much higher proportion than in rodents and amounts to about 50% of the hepatobiliary excretion (Denzlinger et al. 1986; Maltby et al. 1990; Keppler et al. 1992).

Positron emission tomography using carbon-11 labeled, positron-emitting *N*-[^{11}C]acetyl-LTE_4 enables noninvasive analyses of elimination kinetics, organ distribution, and transport of this cysteinyl leukotriene (Keppler et al. 1991). In the rat, the initial distribution phase was characterized by a

rapid disappearence of ^{11}C radioactivity from the blood circulation. This was accompanied by an increase in the leukotriene concentration in liver reaching its maximum 4 min after intravenous injection. In the *cynomolgus* monkey this maximum was reached after 12 min. As a consequence of hepatobiliary excretion, increasing amounts of *N*-[^{11}C]acetyl-LTE_4 and its ω-/β-oxidized metabolites were detected in the intestines. Only negligible amounts of the leukotrienes were monitored in the urinary bladder of the rat within 50 min. Renal excretion was significant, however, in the monkey, which is in accordance with previous invasive tracer studies in this species (Denzlinger et al. 1986). Kinetic analyses indicated a mean transit time of the cysteinyl leukotriene through the liver of 17 min in the rat and of 34 min in the monkey (Keppler et al. 1991). In a mutant rat strain with a hereditary defect of the hepatobiliary transport of cysteinyl leukotrienes across the hepatocyte canalicular membrane (Huber et al. 1987; Ishikawa et al. 1990) elimination of leukotriene radioactivity from the blood circulation was retarded, the mean transit time or storage period in the liver was extended to 54 min, and leukotriene excretion into the intestines was below detectability. This impaired hepatobiliary elimination was compensated by transport of ω-/β-oxidized metabolites from the liver back into blood with subsequent renal excretion. This was monitored by the sharp rise in ^{11}C radioactivity in the urinary bladder of mutant rats. A similar shift from hepatobiliary to renal cysteinyl leukotriene elimination was observed in rats with extrahepatic cholestasis due to surgical ligation of the bile duct. Leukotrienes labeled with a short-lived, positron-emitting radioisotope thus provide quantitative insight into the pathways of their elimination and transport in vivo and into the relative contribution of liver and kidney to these processes under normal and under pathopysiological conditions.

4 Metabolic Deactivation and Inactivation of Leukotrienes

Enzyme-catalyzed chemical modification of the leukotrienes determines their biological activity. Removal of the γ-glutamyl moiety from LTC_4 yields the biologically most potent cysteinyl leukotriene, LTD_4 (Fig. 2). On the other hand, modification of the cysteinylglycine moiety of LTD_4 and ω-oxidation followed by β-oxidation of LTE_4, *N*-acetyl-LTE_4, and LTB_4 result in deactivation and inactivation of these leukotrienes (Fig. 5). Inactivation of potent mediators is equally important as their biosynthesis since the relative rates of synthesis and inactivation determine the concentration of the biologically active leukotrienes at the receptor.

4.1 Deactivation of LTD_4 in the Mercapturate Pathway

The rank order of molar potencies of the cysteinyl leukotrienes in most assay systems is LTD_4>LTC_4>LTE_4>*N*-acetyl-LTE_4 (Lewis et al. 1981; Samhoun et al. 1989). LTD_4 is irreversibly hydrolyzed to LTE_4 and glycine (Fig. 2). The reaction is catalyzed by a dipeptidase (Bernström and Hammarström 1981), which has been purified from microvillus membranes (Kozak and Tate 1982) and characterized as an ectoenzyme (Huber and Keppler 1987). The dipeptidase is associated in the membrane with other enzymes of the mercapturate pathway (Hughey et al. 1978). Removal of the glycine moiety from LTD_4 leads to a considerable loss of biological activity by about two orders of magnitude (Samhoun et al. 1989). Degradation of the glutathione conjugate LTC_4 to LTD_4, LTE_4, and *N*-acetyl-LTE_4 follows the mercapturate pathway, originally known as a route of detoxification of xenobiotics (Hagmann et al. 1986; Huber and Keppler 1988). In this pathway, the cysteinyl leukotrienes are endogenous substrates in the nanomolar concentration range (Denzlinger et al. 1985). Inhibition of the deactivation of LTD_4 to LTE_4, both on cultured cells and in the rat, is induced by L-penicillamine (Huber and Keppler 1987). This interference also prevents the generation of the mercapturate, *N*-acetyl-LTE_4, and of ω-oxidized polar metabolites of LTE_4 and *N*-acetyl-LTE_4.

N-Acetyl-LTE_4 is formed by intracellular *N*-acetylation of LTE_4 with acetyl-coenzyme A (CoA). The enzyme catalyzing this reaction is present in liver, kidney, spleen, skin, and lung of the rat (Bernström and Hammarström 1986). Endogenous *N*-acetyl-LTE_4 was originally identified in rat bile (Hagmann et al. 1985, 1986) and feces (Örning et al. 1986) as the predominant LTC_4 catabolite. In human urine, but not in the bile, this mercapturate is present as a minor metabolite amounting to about 10% of LTE_4 (Huber et al. 1989, 1990; Sala et al. 1990; Maltby et al. 1990). *N*-acetyl-LTE_4 retains at least 30% of activity relative to LTE_4 (Lewis et al. 1981) and may be equipotent as LTE_4 in some assays (Samhoun et al. 1989). Therefore, catabolism of LTC_4 in the mercapturate pathway is associated with biological activation to LTD_4, followed by partial deactivation to LTE_4 and *N*-acetyl-LTE_4. Complete inactivation of the cysteinyl leukotrienes is only achieved by oxidation at the ω end of the fatty acid moiety (Samhoun et al. 1989).

4.2 Oxidative Inactivation of Leukotrienes

ω-Oxidation of LTB_4 to ω-hydroxy-LTB_4, ω-aldehyde-LTB_4, and ω-carboxy-LTB_4, which is associated with a reduction of biological activity, has

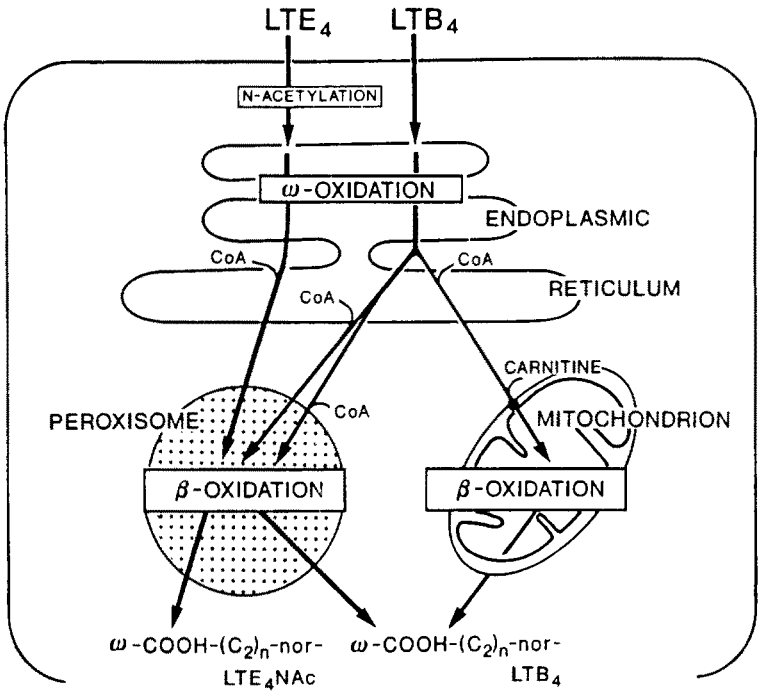

Fig. 5. Compartmentation of inactivation and degradation of LTE_4 and LTB_4 in the hepatocyte. Products of peroxisomol β-oxidation may be dinor, tetranor, and hexanor metabolites of the LTE_4, *N*-acetyl-LTE_4, or LTB_4 [ω-COOH-$(C_2)_n$-nor-leukotriene]. (Reproduced with permission from Jedlitschky et al. 1991)

been observed in leukocytes (Hansson et al. 1981; Powell 1984; Soberman et al. 1988; Lewis et al. 1990) as well as in hepatocytes (Harper et al. 1986; Baumert et al. 1989; Shirley and Murphy 1990; Sumimoto et al. 1990; Jedlitschky et al. 1990; Shirley et al. 1992). By identification of ω-carboxy-dinor-LTB_4 and ω-carboxy-tetranor-LTB_3 in hepatocyte suspensions, these cells were shown to β-oxidize ω-carboxy-LTB_4 from the ω end (Harper et al. 1986; Jedlitschky et al. 1991). Ethanol at moderate concentrations interferes with the further catabolism of ω-hydroxy-LTB_4 (Baumert et al. 1989). As a result, not only LTB_4 and ω-hydroxy-LTB_4 (Baumert et al. 1989) but also 3-hydroxy-LTB_4 increases in hepatocytes (Shirley et al. 1992). The latter are potent calcium-mobilizing and chemotactic metabolites (Shirley et al. 1992).

The liver converts LTE_4 and *N*-acetyl-LTE_4 to the respective ω-hydroxy and ω-carboxy metabolites (Örning 1987; Ball and Keppler 1987; Stene and Murphy 1988). Further degradation by β-oxidation from the ω end yields ω-carboxy-dinor, -tetranor, and -hexanor derivates of LTE_4

and *N*-acetyl-LTE_4 (Stene and Murphy 1988; Sala et al. 1990; Huber et al. 1990). All these ω-carboxy derivates of LTE_4 and *N*-acetyl-LTE_4 are biologically inactive (Samhoun et al. 1989).

Additional pathways for the catabolism of cysteinyl leukotrienes have been described on the basis of in vitro experiments. These include the degradation of LTC_4, LTD_4, and LTE_4 to 6-*trans*-LTB_4 diastereoisomers and the subclass-specific *S*-diastereoisomeric sulfoxides by myeloperoxidase from activated human polymorphonuclear leukocytes and monocytes (Lee et al. 1983). Additionally, cysteinyl leukotrienes may be inactivated by hydroxyl radicals yielding 6-*trans*-isomers of LTB_4 (Henderson et al. 1982). These pathways for inactivation of cysteinyl leukotrienes have been outlined repeatedly (Lewis et al. 1990), however, there is no evidence for their significance in the intact organism where the metabolites recovered from injected LTC_4, LTD_4, LTE_4, or *N*-acetyl-LTE_4 in bile and urine account for most of the administered leukotrienes and exclude a detectable contribution from myeloperoxidase-catalyzed degradation in vivo (Huber et al. 1987; Jedlitschky et al. 1991).

4.3 Peroxisomal Degradation by β-Oxidation from the ω End

The peroxisomal β-oxidation pathway for very long-chain fatty acids involves acyl-CoA oxidase (EC 1.3.99.3), the bifunctional or rather trifunctional protein displaying enoyl-CoA hydratase (EC 4.2.1.17), 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), and Δ^3, Δ^2-enoyl-CoA isomerase (EC 5.3.3.8) activity, and the peroxisomal 3-ketoacyl-CoA thiolase (EC 2.3.1.16), as well as auxiliary enzymes such as 2,4-dienoyl-CoA reductase (EC 1.3.1.34; Osmundsen et al. 1991). The increased degradation of leukotrienes in the β-oxidation pathway after treatment of rats with clofibrate, an inducer of peroxisome proliferation, led to the suggestion that β-oxidation of leukotrienes may be localized in peroxisomes (Keppler et al. 1989). Both the long-chain structure of the leukotrienes and the structures of their degradation products by β-oxidation in rat hepatocytes (Shirley and Murphy 1990) and in human urine (Sala et al. 1990; Huber et al. 1990) are in line with peroxisomal leukotriene breakdown. It is of interest that the leukotrienes, in contrast to the prostaglandins, are not degraded from the carbon-1-carboxyl group but from the ω end by β-oxidation of the ω-carboxy metabolites derived from LTB_4, LTE_4, and *N*-acetyl-LTE_4. Direct evidence for an exclusive degradation of cysteinyl leukotrienes in peroxisomes has been obtained by use of isolated liver peroxisomes and direct photoaffinity labeling of the peroxisomal enzymes of β-oxidation with ω-carboxy-*N*-[^{3}H]acetyl-LTE_4 (Jedlitschky et al. 1991). In addition,

isolated peroxisomes catalzye the β-oxidation from the ω end of ω-carboxy-LTB_4 yielding the dinor and the tetranor catabolites (Jedlitschky et al. 1991). In vitro experiments indicate that the degradation of LTB_4 can also proceed in liver mitochondria, as indicated in Fig. 5. It is unlikely, however, that the mitochondrial β-oxidation of ω-carboxy-LTB_4 plays a major role in the intact organism since LTB_4 degradation is severly impaired in patients with Zellweger syndrome, a disorder of peroxisomal biogenesis (Mayatepek et al. 1992). In this inherited disease the defect of peroxisomal leukotriene degradation results in increased levels of the biologically active, proinflammatory mediators LTE_4 and LTB_4. In addition, the concentrations in urine of ω-carboxy-LTE_4 and ω-carboxy-LTB_4, which are the immediate substrates for peroxisomal β-oxidation, are manifold increased (Mayatepek et al. 1992). These findings in humans with peroxisome deficiency underline the essential role of peroxisomes in the catabolism of leukotrienes.

5 Analysis of Cysteinyl Leukotrienes and LTB_4

Quantitative determinations of the leukotrienes can be accomplished by radioimmunoassays, high-performance liquid chromatography (HPLC), mass spectrometry after gas chromatography, bioassays, or combinations of these techniques. Separation of leukotriene metabolites by HPLC (Borgeat et al. 1990) often serves as initial step prior to detection with high sensitivity. For unequivocal identification gas chromatography/mass spectrometry is the method of choice (Murphy 1984; Mathews 1990; Murphy and Sala 1990).

5.1 Methods for Determination in Biological Fluids

Difficulties in leukotriene analysis include (a) the short half-life of these mediators in vivo and in most biological fluids, (b) their presence in low nanomolar or picomolar concentration, (c) their susceptibility to oxidative degradation during sample preparation, and (d) the artificial generation of leukotrienes from cells during sampling particularly the leukotriene release from blood leukocytes during attempts to measure blood plasma leukotrienes (Denzlinger et al. 1986).

Most measurements of LTB_4 in biological fluids have employed sensitive radioimmunoassays after verification of the identity of substance by HPLC or mass spectrometry (Tateson et al. 1988; Lehr et al. 1991; Mayatepek et al. 1992).

The endogenous cysteinyl leukotrienes have been analyzed in fluids into which these substances are excreted and present at sufficient concentrations, particularly in bile (Hagmann et al. 1984, 1985; Denzlinger et al. 1985, 1986; Keppler 1988) and urine (Denzlinger et al. 1986; Keppler et al. 1988; Huber et al. 1989; Tagari et al. 1989; Taylor et al. 1989; Nicoll-Griffith et al. 1990; Denzlinger et al. 1990; Fauler et al. 1991). These determinations have been based on tracer studies which have defined species-characterictic index metabolites for systemic cysteinyl leukotriene production (Keppler et al. 1988). In humans, the measurement of urinary LTE_4 reflects about 5% of the systemic LTC_4 production (Maltby et al. 1990); in the rat, *N*-acetyl-LTE_4 represents the index metabolite of choice to be analyzed in bile and corresponding to about 13% of systemic LTC_4 generation (Huber and Keppler 1987) and in the guinea pig biliary LTD_4 amounts to 20%–50% of LTC_4 administered into the systemic blood circulation (A. Keppler et al. 1987; Guhlmann et al. 1989). In each case, HPLC separation of the respective index metabolite in urine or bile should precede the quantitative analysis by immunoassay or mass spectrometry, and the results should be corrected for the recovery of internal standards. The percentages of cysteinyl leukotrienes eliminated into bile and urine are influenced by the relative transport capacities of these organs as well as by the enzyme activities degrading the cysteinyl leukotrienes in the vascular bed, hepatocytes, and kidney. Nevertheless, these determinations provide useful information on the role of leukotrienes under pathophysiological conditions and on the action of inhibitors of their synthesis, whereas analyses in blood are less meaningful because of the short half-life of these mediators in the systemic circulation and the risk of their artificial ex vivo synthesis and release from blood cells (Denzlinger et al. 1986; Heavy et al. 1987; Keppler 1988).

5.2 Generation of Cysteinyl Leukotrienes In Vivo Under Pathophysiological Conditions

Pathophysiological conditions associated with enhanced systemic generation of cysteinyl leukotrienes have been described in experimental animals and in humans. In most instances, local release of the mediators leads to elimination with the blood circulation followed by biliary and renal excretion of detectable quantities. Under a few experimental conditions, such as in the anaphylactic shock in the guinea pig (A. Keppler et al. 1987; Guhlmann et al. 1989), a causal relationship has been established between the quantitiy of leukotriene release and the clinical symptoms.

In humans, biliary LTE_4 is increased in acute pancreatitis (Keppler 1988). Enhanced urinary LTE_4 excretion is associated with fulminant hepatitis, liver cirrhosis, and hepatorenal syndrome (Huber et al. 1989; Moore et al. 1990), antigen challenge in asthma patiens (Taylor et al. 1989; Tagari et al. 1989, 1990; Christie et al. 1991), adult respiratory distress syndrome and burns (Fauler et al. 1991; Westcott et al. 1991), systemic lupus erythematosus (Hackshaw et al. 1992), and treatment with certain cytokines, such as granulocyte-macrophage colony-stimulating factor (Denzlinger et al. 1990), and tumor necrosis factor-α. In addition to urinary LTE_4, both LTE_4 and LTB_4 have been determined in significant quantities in sputum from patients with cystic fibrosis and asthma (Piper et al. 1991). In the monkey, intoxication with staphylococcal enterotoxin B (Denzlinger et al. 1986) and endotoxin from *Salmonella abortus equi* elicited increased biliary and urinary LTE_4 excretion.

Systemic anaphylaxis leads to an immediate release of relatively large amounts of cysteinyl leukotrienes detected as LTD_4 in guinea pig bile (A. Keppler et al. 1987; Guhlmann et al. 1989), or as *N*-acetyl-LTE_4 in rat bile (Foster et al. 1988), or as LTE_4 in sheep lymph during cyclooxygenase blockade (Robinson et al. 1986). Biliary cysteinyl leukotrienes also increase after immunological challenge of the isolated rat or guinea pig liver (Hagmann et al. 1991).

In the rat, where 85%–90% of the systemic LTC_4 production is reflected by the biliary excretion of metabolites, various pathophysiological conditions have been studied by analysis of *N*-acetyl-LTE_4 in bile. These disease states include endotoxin shock (Hagmann et al. 1984, 1985, 1986; D. Keppler et al. 1987), different types of tissue trauma such as surgical trauma, bone fracture, burn injury (Denzlinger et al. 1985), shock induced by platelet-activating factor (Huber and Keppler 1987) and by tumor necrosis factor-α (Huber et al. 1988), and fulminant experimental hepatitis (Hagmann et al. 1987).

6 Leukotriene-Mediated Disease Processes and Their Prevention

The biological actions of LTC_4, LTD_4 LTE_4, and LTB_4, as well as actions of some of the metabolites, such as *N*-acetyl-LTE_4 and ω-hydroxy-LTB_4, have been well defined (Dahlén et al. 1981; Lewis and Austen 1984; Piper 1984; Feuerstein 1985; Drazen and Austen 1987; Samuelsson et al. 1987; Guhlmann et al. 1989; Rola-Pleszczynski 1989; Ford-Hutchinson 1990; Lehr et al. 1991; Shaw and Krell 1991; Shirley et al. 1992). Moreover, analysis of leukotriene concentrations in biological fluids and tissues have established that the concentrations and amounts of these mediators under

some conditions are sufficient to elicit pathophysiological responses in humans and experimental animals (A. Keppler et al. 1987; Keppler et al. 1988; Guhlmann et al. 1989; Taylor et al. 1989). Of particular importance are the recent results from clinical studies with receptor antagonists and inhibitors of leukotriene biosynthesis. The development of both types of compounds has reached a high degree of selectivity in their actions and sufficient bioavailability (Gillard et al. 1989; Aharony and Krell 1991; Ford-Hutchinson 1991b; Jones et al. 1991; Lewis et al. 1991; Piper and Krell 1991; Herron et al. 1992). Thus, the criteria to define and the means to treat leukotriene-mediated disease processes are available. Anaphylactic shock in the sensitized guinea pig may serve as an example where selective inhibition of leukotriene biosynthesis in vivo (by MK-886) prevents the generation of otherwise lethal amounts of endogenous LTC_4, and where the above mentioned criteria have been fulfilled (Guhlmann et al. 1989).

Asthma is the human disease in which the most convincing evidence has been presented to implicate the cysteinyl leukotrienes as key mediators (Piper and Krell 1991; Lewis et al. 1991). This conclusion is based on the bronchoconstrictor activity of inhaled LTC_4, LTD_4, and LTE_4 (Drazen and Austen 1987), on the generation of cysteinyl leukotrienes during the asthmatic attack (Taylor et al. 1989), and on the results from clinical studies with third-generation LTD_4/LTE_4 receptor antagonists (Lewis et al. 1991). In other inflammatory diseases, local or systemic leukotriene production has been measured and suggests a role in pathogenesis; however, the importance remains to be proven by successful clinical intervention or prevention by use of leukotriene biosynthesis inhibitors, LTD_4/LTE_4 receptor antagonists, and/or LTB_4 receptor antagonists. The leukotrienes may act within a network of mediators involving cytokines and other arachidonate metabolites. Diseases in which inhibition of leukotriene synthesis or action may prove to be beneficial include, in addition to asthma and anaphylaxis, psoriasis, adult respiratory distress syndrome, neonatal pulmonary hypertension, allergic rhinitis, gout, rheumatoid arthritis, inflammatory bowel disease, acute and fulminant hepatitis, hepatorenal syndrome, glomerulonephritis, and possibly sepsis. The use of selective leukotriene synthesis inhibitors and receptor antagonists may result not only in therapeutic progress but also in a deeper and more detailed understanding of the role of leukotrienes under normal and pathophysiological conditions.

References

Aharony D, Krell RD (1991) Pharmacology of peptide leukotriene receptor antanonists. Ann NY Acad Sci 629:125–132

Akerboom TPM, Narayanaswami V, Kunst M, Sies H (1991) ATP-dependent *S*-(2,4-dinitrophenyl)glutathione transport in canalicular plasma membrane vesicles from rat liver. J Biol Chem 266:13147–13152

Appelgren LE, Hammarström S (1982) Distribution and metabolism of [^{3}H]-labeled leukotriene C_3 in the mouse. J Biol Chem 257:531–535

Augstein J, Farmer JB, Lee TB, Sheard P, Tattersall ML (1973) Selective inhibition of slow reacting substance of anaphylaxis. Nature New Biol 245:214–216

Ball HA, Keppler D (1987) ω-oxidation products of leukotriene E_4 in bile and urine of the monkey. Biochem Biophys Res Commun 148:664–670

Baumert T, Huber M, Mayer D, Keppler D (1989) Ethanol-induced inhibition of leukotriene degradation by ω-oxidation. Eur J Biochem 182:223–229

Bell RL, Young PR, Albert D, Lanni C, Summers JB, Brooks DW, Rubin P, Carter GW (1992) The discovery and development of zileuton – an orally active 5-lipoxygenase inhibitor. Int J Immunopharmacol 14:505–510

Bernström K, Kammarström S (1981) Metabolism of leukotriene D by porcine kidney. J Biol Chem 256:9579–9582

Bernström K, Kammarström S (1986) Metabolism of leukotriene E_4 by rat tissues: formation of *N*-acetyl leukotriene E_4. Arch Biochem Biophys 244:486–491

Board P, Nishida T, Gatmaitan Z, Che M, Arias IM (1992) Erythrocyte membrane transport of glutathione conjugates and oxidized glutathione in the Dubin-Johnson syndrome and in rats with hereditary hyperbilirubinemia. Hepatology 15:722–725

Borgeat P, Picard S, Vallerand P, Bourgoin S, Odeimat A, Sirois P, Poubelle PE (1990) Automated on-line extraction and profiling of lipoxygenase products of arachidonic acid by high-performance liquid chromatography. Meth Enzymol 187:98–116

Christie PE, Tagari P, Ford-Hutchinson AW, Charleson S, Chee P, Arm JP, Lee TH (1991) Urinary leukotriene-E_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Resp Dis 143:1025–1029

Dahinden CA, Wirthmueller U (1990) Release and metabolism of leukotriene A_4 in neutrophil-mast cell interactions. Methods Enzymol 187:567–577

Dahinden CA, Clancy RM, Gross M, Chiller JM, Hugli TE (1985) Leukotriene C_4 production by murine mast cells: evidence of a role for extracellular leukotriene A_4. Proc Natl Acad Sci USA 82:6632–6636

Dahlén SE, Björck T, Hedqvist P, Arfors KE, Hammarström S, Lindgren JÅ, Samuelsson B (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 80:3887–3891

Denzlinger C, Rapp S, Hagmann W, Keppler D (1985) Leukotrienes as mediators in tissue trauma. Science 230:330–332

Denzlinger C, Guhlmann A, Scheuber PJ, Wilker D, Hammer DK, Keppler D (1986) Metabolism and analysis of cysteinyl leukotrienes in the monkey. J Biol Chem 261: 15601–15606

Denzlinger C, Kapp A, Grimberg M, Gerhartz HH, Wilmanns W (1990) Enhanced endogenous leukotriene biosynthesis in patients treated with granulocyte-macrophage colony-stimulating factor. Blood 76:1765–1770

Dixon RAF, Jones RE, Diehl RE, Bennett CD, Kargman S, Rouzer CA (1988) Cloning of the cDNA for human 5-lipoxygenase. Proc Natl Acad Sci USA 85:416–420

Dixon RAF, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW, Miller DK (1990) Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. Nature 343:282–284

Drazen JM, Austen KF(1987) Leukotrienes and airway responses. Am Rev Respir Dis 136:985–998

Evans JF, Léveillé C, Mancini JA, Prasit P, Thérien M, Zamboni R, Gauthier JY, Fortin R, Charleson P, MacIntyre DE, Luell S, Bach TJ, Meurer R, Guay J, Vickers PJ, Rouzer CA, Gillard JW, Miller DK (1991) 5-Lipoxygenase-activating protein is the target of a quinoline class of leukotriene synthesis inhibitors. Molec Pharmacol 40:22–27

Falk E, Müller M, Huber M, Keppler D, Kurz G (1989) Direct photoaffinity labeling of leukotriene binding sites. Eur J Biochem 186:741–747

Fauler J, Tsikas D, Holch M, Seekamp A, Nerlich ML, Sturm J, Frölich JC (1991) Enhanced urinary excretion of leukotriene E_4 by patients with multiple trauma with or without adult respiratory distress syndrome. Clin Sci 80:497–504

Feinmark SJ (1990) Leukotriene C_4 biosynthesis during polymorphonuclear leukocyte-vascular cell interactions. Methods Enzymol 187:559–567

Ferber E, Resch K (1973) Phospholipid metabolism of stimulated lymphocytes: activation of acyl-CoA: lysolecithin acyltransferases in microsomal membranes. Biochim Biophys Acta 296:335–349

Feuerstein G (1985) Autonomic pharmacology of leukotrienes. J Auton Pharmac 5: 149–168

Fitzsimmons BJ, Rockach J (1989) Enzyme inhibitors and leukotriene receptor antagonists. In: Rockach J (ed) Leukotrienes and lipoxygenases. Chemical, biological and clinical aspects. Elsevier, Amsterdam, pp 427–502

Ford-Hutchinson AW (1990) Leukotriene B_4 in inflammation. Crit Rev Immunol 10:1–12

Ford-Hutchinson AW (1991a) FLAP: a novel drug target for inhibiting the synthesis of leukotrienes. TIPS 121:68–70

Ford-Hutchinson AW (1991b) Leukotrienes and chemotaxis – 5-lipoxygenase activation and control. In: Sies H, Flohé L, Zimmer G (eds) Molecular aspects of inflammation. Springer, Berlin Heidelberg New York, pp 33–39

Foster A, Letts G, Charleson S, Fitzsimmons B, Blacklock B, Rokach J (1988) The in vivo production of peptide leukotrienes after pulmonary anaphylaxis in the rat. J Immunol 141:3544–3550

Foxwell BM, Mackie A, Ling V, Ryffel B (1989) Identification of the multidrug resistance-related P-glycoprotein as a cyclosporine binding protein. Mol Pharmacol 36:543–546

Gillard J, Ford-Hutchinson AW, Chan C, Charleson S, Denis D, Foster A, Fortin R, Leger S, McFarlane CS, Morton H, Piechuta H, Riendeau D, Rouzer CA, Rokach J, Young R, MacIntyre DE, Peterson L, Bach T, Eiermann G, Hopple S, Humes J, Hupe L, Luell S, Metzger J, Meurer R, Miller DK, Opas E, Pacholok S (1989) L-663,536 (MK-886) (3-[1-(4-chlorbenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel orally active leukotriene biosynthesis inhibitor. Can J Physiol Pharmacol 67:456–464

Guhlmann A, Keppler A, Kästner S, Krieter H, Brückner UB, Messmer K, Keppler D (1989) Prevention of endogenous leukotriene production during anaphylaxis in the guinea pig by an inhibitor of leukotriene biosynthesis (MK-886) but not by dexamethasone. J Exp Med 170:1905–1918

Habenicht AJR, Goerig M, Rothe DER, Specht E, Ziegler R, Glomset JA, Graf T (1989) Early reversible induction of leukotriene synthesis in chicken myelomonocytic cells transformed by a temperature sensitive mutant of avian leukemia virus E26. Proc Natl Acad Sci USA 86:921–924

Hackshaw KV, Voelkel NF, Thomas RB, Westcott JY (1992) Urine leukotriene E_4 levels are elevated in patients with active systemic lupus erythematosus. J Rheumatol 19: 252–258

Haeggström JR, Wetterholm A, Vallee BL, Samuelsson B (1990) Leukotriene A_4 hydrolase: an epoxide hydrolase with peptidase activity. Biochem Biophys Res Commun 173: 431–437

Hagmann W, Korte M (1990) Hepatic uptake and metabolic disposition of leukotriene B_4 in rats. Biochem J 267:467–470

Hagmann W, Denzlinger C, Keppler D (1984) Role of peptide leukotrienes and their hepatobiliary elimination in endotoxin action. Circ Shock 14:223–235

Hagmann W, Denzlinger C, Keppler D (1985) Production of peptide leukotrienes in endotoxin shock, FEBS Lett 180:309–313

Hagmann W, Denzlinger C, Rapp S, Weckbecker G, Keppler D (1986) Identification of the major endogenous leukotriene metabolite in the bile of rats as *N*-acetyl leukotriene E_4. Prostaglandins 31:239–251

Hagmann W, Steffan A-M, Kirn A, Keppler D (1987) Leukotrienes as mediators in frog virus 3-induced hepatitis in rats. Hepatology 7:732–736

Hagmann W, Parthé S, Kaiser I (1989) Uptake, production and metabolism of cysteinyl leukotrienes in the isolated perfused rat liver. Inhibition of leukotriene uptake by cyclosporine. Biochem J 261:611–616

Hagmann W, Kaiser I, Jakschik BA (1991) The sensitized liver represents a rich source of endogenous leukotrienes. Hepatology 13:482–488

Hammarström S (1983) Leukotrienes. Ann Rev Biochem 52:355–377

Hammarström S, Örning L, Bernström K (1985) Metabolism of leukotrienes. Mol Cell Biochem 69:7–16

Hansson G, Lindgren J.-Å, Dahlén SE, Hedqvist P, Samuelsson B (1981) Identification and biological activity of novel omega-oxidized metabolites of leukotriene B_4 from human leukocytes. FEBS Lett 130:107–112

Harper TW, Garrity MJ, Murphy RC (1986) Metabolism of leukotriene B_4 in isolated rat hepatocytes. Identification of a novel 18-carboxy-19,20-dinor leukotriene B_4 metabolite. J Biol Chem 261:5414–5418

Heavey DJ, Soberman RJ, Lewis RA, Spur B, Austen KF (1987) Critical considerations in the development of an assay for sulfidopeptide leukotrienes in plasma. Prostaglandins 33:693–708

Henderson WR, Jörg A, Klebanoff SJ (1982) Eosinophil peroxidase-mediated inactivation of leukotrienes B_4, C_4 and D_4. J Immunol 128:2609–2613

Herron DK, Goodson T, Bollinger NG, Swansonbean D, Wright IG, Staten GS, Thompson AR, Froelich LL, Jackson WT (1992) Leukotriene-B_4 receptor antagonists – the LY255283-series of hydroxyacetophenones. J Med Chem 35:1818–1828

Huber M, Keppler D (1987) Inhibition of leukotriene D_4 catabolism by D-penicillamine. Eur J Biochem 167:73–79

Huber M, Keppler D (1988) Leukotrienes and the mercapturate pathway. In: Sies H, Ketterer B (eds) Glutathione conjugation. Mechanisms and biological significance. Academic, London, pp 449–470

Huber M, Keppler D (1990) Eicosanoids an the liver. In: Popper H, Schaffner F (eds) Progress in liver diseases, vol 9. Saunders, Philadelphia, pp 117–141

Huber M, Guhlmann A, Jansen PLM, Keppler D (1987) Hereditary defect of hepatobiliary cysteinyl leukotriene elimination in mutant rats with defective hepatic anion excretion. Hepatology 7:224–228

Huber M, Beutler B, Keppler D (1988) Tumor necrosis factor α stimulates leukotriene production in vivo. Eur J Immunol 18:2085–2088

Huber M, Kästner S, Schölmerich J, Gerok W, Keppler D (1989) Cysteinyl leukotriene analysis in human urine: enhanced excretion in patients with liver cirrhosis and hepatorenal syndrome. Eur J Clin Invest 19:53–60

Huber M, Müller J, Leier I, Jedlitschky G, Ball HA, Moore KP, Taylor GW, Williams R, Keppler D (1990) Metabolism of cysteinyl leukotrienes in monkey and man. Eur J Biochem 194:309–315

Hughey RP, Rankin BB, Elce JS, Curthoys NP (1978) Specificity of a particulate rat renal peptidase and its localization along with other enzymes of mercapturic acid synthesis. Arch Biochem Biophys 186:211–217

Irvine RF (1982) How is the level of free arachidonic acid controlled in mammalian cells? Biochem J 204:3–16

Ishikawa T, Kobayashi K, Sogame Y, Hayashi K (1989) Evidence for leukotriene C_4 transport mediated by an ATP-dependent glutathione *S*-conjugate carrier in rat heart and liver plasma membranes. FEBS Lett 259:95–98

Ishikawa T, Müller M, Klünemann C, Schaub T, Keppler T (1990) ATP-dependent primary active transport of cysteinyl leukotrienes across liver canalicular membrane. Role of the ATP-dependent transport system for glutathione *S*-conjugates. J Biol Chem 265: 19279–19286

Jansen PLM, Peters WH, Lamers WH (1985) Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. Hepatology 5:573–579

Jedlitschky G, Leier I, Huber M, Mayer D, Keppler D (1990) Inhibition of leukotriene ω-oxidation by ω-trifluoro analogs of leukotrienes. Arch Biochem Biophys 282:333–339

Jedlitschky G, Huber M, Völkl A, Müller M, Leier I, Müller J, Lehmann W-D, Fahimi HD, Keppler D (1991) Peroxisomal degradation of leukotrienes by β-oxidation from the ω-end. J Biol Chem 266:24763–24772

Jones DA, Fitzpatrick FA (1990) Leukotriene B_4 biosynthesis by erythrocyte-neutrophil interactions. Methods Enzymol 187:553–559

Jones TR, Zamboni R, Belley M, Champion E, Charette L, Ford-Hutchinson AW, Gauthier J-Y, Leger S, Lord A, Masson P, McFarlane CS, Metters KM, Pickett C, Piechuta H, Young RN (1991) Pharmacology of the leukotriene antagonist verlukast: the (*R*)-enantiomer of MK-571. Can J Physiol Pharmacol 69:1847–1854

Keppler A, Örning L, Bernström K, Hammarström S (1987) Endogenous leukotriene D_4 formation during anaphylactic shock in the guinea pig. Proc Natl Acad Sci USA 84: 5903–5907

Keppler D (1988) Stoffwechsel und Analyse von Leukotrienen in Vivo. Klin Wochenschr. 66:997–1004

Keppler D, Hagmann W, Rapp S, Denzlinger C, Koch HK (1985) The relation of leukotrienes to liver injury. Hepatology 5:883–891

Keppler D, Hagmann W, Rapp S (1987) Role of leukotrienes in endotoxin action in vivo. Rev Infect Diseases 9:580–584

Keppler D, Huber M, Hagmann W, Ball HA, Guhlmann A, Kästner S (1988) Metabolism and analysis of endogenous cysteinyl leukotrienes. Ann NY Acad Sciences 524:68–74

Keppler D, Huber M, Baumert T, Guhlmann A (1989) Metabolic inactivation of leukotrienes. Adv Enzyme Regul 28:307–319

Keppler D, Guhlmann A, Oberdorfer F, Krauss K, Müller J, Ostertag H, Huber M (1991) Generation and metabolism of cysteinyl leukotrienes in vivo. Ann NY Acad Sciences 629:100–104

Keppler D, Müller M, Klünemann C, Guhlmann A, Krauss K, Müller J, Berger U, Leier I, Mayatepek E (1992) Transport and in vivo elimination of cysteinyl leukotrienes. Adv Enzyme Regul 32:107–116

Kitamura T, Alroy J, Gatmaitan Z, Inoue M, Mikami T, Jansen P, Arias IM (1992) Defective biliary excretion of epinephrine metabolites in mutant (TR^-) rats: relation to the pathogenesis of black liver in the Dubin-Johnson syndrome and Corriedale sheep with an analogous excretory defect. Hepatology 15:1154–1159

Kobayashi K, Sogame Y, Hayashi K, Nicotera P, Orrenius S (1988) ATP stimulates the uptake of *S*-dinitrophenylglutathione by rat liver plasma membrane vesicles. FEBS Lett 240:55–58

Kobayashi K, Sogame Y, Hara H, Hayashi K (1990) Mechanism of glutathione *S*-conjugate transport in canalicular and basolateral rat liver plasma membranes. J Biol Chem 265:7737–7741

Kondo T, Dale GL, Beutler E (1980) Glutathione transport by inside-out vesicles from human erythrocytes. Proc Natl Acad Sci USA 77:6359–6362

Kozak EM, Tate SS (1982) Glutathione-degrading enzymes of microvillus membranes. J Biol Chem 257:6322–6327

Kurz G, Müller M. Schramm U, Gerok W (1989) Identification and function of bile salt binding polypeptides of hepatocyte membrane. In: Petzinger E, Kinne RK-H, Sies H (eds) Hepatic transport of organic substances. Springer, Berlin Heidelberg New York, pp 267–278

Lam BK, Owen WF Jr, Austen KF, Soberman RJ (1989) The identification of a distinct export step following the biosynthesis of leukotriene C_4 by human eosinophils. J Biol Chem 264:12885–12889

Lam BK, Gagnon L, Austen KF, Soberman RJ (1990) The mechanism of leukotriene B_4 export from human polymorphonuclear leukocytes. J Biol Chem 265:13438–13441

Lee CW, Lewis RA, Tauber AI, Mehrotra M, Corey EJ, Austen KF (1983) The myeloperoxidase-dependent metabolism of leukotrienes C_4 D_4 and E_4 to 6-*trans*-leukotriene B_4 diastereoisomers and the subclass-specific *S*-diastereoisomeric sulfoxides. J Biol Chem 258:15004–15010

Lehr HA, Guhlmann A, Nolte D, Keppler D, Messmer K (1991) Leukotrienes as mediators in ischemia-reperfusion injury in a microcirculation model in the hamster. J Clin Invest 87:2036–2041

Leier I, Müller M. Jedlitschky G, Keppler D (1992) Leukotriene uptake by hepatocytes and hepatoma cells. Eur J Biochem 209: in press

Lewis MA, Krell RD, Jones TR (1991) Third-generation peptidoleukotriene receptor antagonists. In: Crooke ST, Wong A (eds) Lipoxygenases and their products. Academic, San Diego, pp 207–234

Lewis RA, Austen KF (1984) The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. J Clin Invest 73:889–897

Lewis RA, Drazen JM, Austen KF, Toda M, Brion F, Marfat A, Corey EJ (1981) Contractile activities of structural anlogs of leukotrienes C and D: role of the polar substituents. Proc Natl Acad Sci USA 78:4579–4583

Lewis RA, Austen KF, Soberman RJ (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. New Engl J Med 323:645–655

Lin L-L, Lin AY, Knopf JL (1992) Cytosolic phospholipase A_2 is coupled to hormonally regulated release of arachidonic acid. Proc Natl Acad Sci USA 89:6147–6151

Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT (1990) Leukotriene C_4 elimination and metabolism in man. J Allergy Clin Immunol 85:3–9

Mathews R (1990) Quantitative gas chromatography-mass spectrometry analysis of leukotriene B_4. Meth Enzymol 187:76–81

Matsumoto T, Funk CD, Radmak O, Höög JO, Jörnvall H, Samuelsson B (1988) Molecular cloning and amino acid sequence of human 5-lipoxygenase. Proc Natl Acad Sci USA 85: 26–30

Mayatepek E, Lehmann W-D, Fauler J, Tsikas D, Frölich JC, Schutgens RBH, Wanders RJA, Keppler D (1992) Impaired degradation of leukotrienes in patients with peroxisome deficiency disorders. J Clin Invest, in press

McGee JE, Fitzpatrick F (1985) Enzymatic hydration of leukotriene A_4: purification and characterization of a novel epoxide hydrolase from human erythrocytes. J Biol Chem 260:12832–12837

McGee JE, Fitzpatrick FA (1986) Erythrocyte-neutrophil interactions: formation of leukotriene B_4 by transcellular biosynthesis. Proc Natl Acad Sci USA 83:1349–1353

Miller DK, Gillard JW, Vickers PJ, Sadowski S, Léveillé C, Mancini JA, Charleson P, Dixon RAF, Ford-Hutchinson AW, Fortin R, Gauthier JY, Rodkey J, Rosen R, Rouzer CA, Sigal IS, Strader CD, Evans JF (1990) Identification and isolation of a membrane protein necessary for leukotriene production. Nature 343:278–281

Minami M, Ohishi N, Mutoh H, Izumi T, Bito H, Wada H, Seyama Y, Toh H, Shimizu T (1990) Leukotriene A_4 hydrolase is a zinc-containing aminopeptidase. Biochem Biophys Res Commun 173:620–626

Moore KP, Taylor GW, Maltby NH, Siegers D, Fuller RW, Dollery CT, Williams R (1990) Increased production of cysteinyl leukotrienes in hepatorenal syndrome. J Hepatol 11:263–271

Müller M, Ishikawa T, Berger U, Lucka L, Klünemann C, Schreyer A, Kannicht C, Reutter W, Kurz G, Keppler D (1991a) ATP-dependent transport of taurocholate across the hepatocyte canalicular membrane mediated by a 110 kDa glycoprotein binding ATP and bile salt. J Biol Chem 266:18920–18926

Müller M, Falk E, Sandbrink R, Berger U, Leier I, Jedlitschky G, Huber M. Kurz G, Keppler D (1991b) Photoaffinity labeling of leukotriene binding sites in hepatocytes and hepatoma cells. Advan Prostaglandin, Thromboxane, Leukotriene Res 21A:395–398

Murphy RC (1984) Mass spectrometric quantitation and analysis of leukotrienes and other 5-lipoxygenase metabolites. Prostaglandins 28:597–601

Murphy RC, Sala A (1990) Quantitation of sulfidopeptide leukotrienes in biological fluids by gas chromatography-mass spectrometry. Meth Enzymol 187:90–98

Murphy RC, Hammarström S, Samuelsson B (1979) Leukotriene C: a slow-reacting substance from murine mastocytoma cells. Proc Natl Acad Sci USA 76:4275–4279

Nicoll-Griffith D, Zamboni R (1990) BIO-fully automated sample treatment high-performance liquid chromatography and radioimmunoassay for leukotriene E_4 in human urine from asthmatics. J Chromatography 526:341–354

Odlander B, Jakobsson PJ, Rosén A, Claesson H-E (1988) Human B and T lymphocytes convert leukotriene A_4 into leukotriene B_4. Biochem Biophys Res Commun 153: 203–208

Örning L (1987) ω-Oxidation of cysteine-containing leukotrienes by rat liver microsomes. Isolation and characterization of ω-hydroxy and ω-carboxy metabolites of leukotriene E_4 and *N*-acetyl-leukotriene E_4. Eur J Biochem 170:77–85

Örning L, Kaijser RL, Hammarström S (1985) In vivo metabolism of leukotriene C_4 in man: Urinary excretion of leukotriene E_4. Biochem Biophys Res Commun 130:214–220

Örning L, Norin E, Gustafsson B, Hammarström S (1986) In vivo metabolism of leukotriene C_4 in germ-free and conventional rats. Fecal excretion of *N*-acetyl-leukotriene E_4. J Biol Chem 261:766–771

Örning L, Krivi G, Bild G, Gierse J, Aykent S, Fitzpatrick FA (1991a) Inhibition of leukotriene A_4 hydrolase/aminopeptidase by captopril. J Biol Chem 266:16507–16511

Örning L, Krivi G, Fitzpatrick FA (1991b) Leukotriene A_4 hydrolase. Inhibition by bestatin and intrinsic aminopeptidase activity establish its functional resemblance to metallohydrolase enzymes. J Biol Chem 266:1375–1378

Ormstad K, Uehara N, Orrenius S, Örning L, Hammarström S (1982) Uptake and metabolism of leukotriene C_4 by isolated rat organs and cells. Biochem Biophys Res Commun 104:1434–1440

Osmundsen H, Bremer J, Pedersen JI (1991) Metabolic aspects of peroxisomal β-oxidation. Biochim Biophys Acta 1085:141–158

Peppelenbosch MP, Tertoolen LGJ, Den Hertog J, De Laat SW (1992) Epidermal growth factor activates calcium channels by phospholipase A_2/5-lipoxygenase-mediated leukotriene C_4 production. Cell 69:295–303

Piper PJ (1984) Formation and actions of leukotrienes. Physiol Rev 64:744–761

Piper PJ, Krell RD (eds) (1991) Advances in the understanding and treatment of asthma. Ann NY Acad Sciences 629:89–167

Piper PJ, Conroy DM, Costello JF, Evans JM, Green CP, Price JF, Sampson AP, Spencer DA (1991) Leukotrienes and inflammatory lung disease. Ann NY Acad Sciences 629: 112–119

Powell WS (1984) Properties of leukotriene B_4 hydrolase from polymorphonuclear leukocytes. J Biol Chem 259:3082–3089

Reid GK, Kargman S, Vickers PJ, Mancini JA, Léveillé C, Ethier D, Miller DK, Gillard JW, Dixon RAW, Evans JF (1990) Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. J Biol Chem 265: 19818–19823

Robinson DR, Skoskiewicz M, Bloch JK, Castorena G, Hayes E, Lowenstein E, Melvin C, Michelassi F, Zapol WM (1986) Cyclooxygenase blockade elevates leukotriene E_4 production during acute anaphylaxis in sheep. J Exp Med 163:1509–1517

Rokach J (ed) (1989) Leukotrienes and lipoxygenases. Chemical, biological and clinical aspects. Elsevier, Amsterdam

Rola-Pleszczynski M (1989) Leukotrienes and the immune system. J Lipid Mediators 1: 149–159

Rouzer CA, Kargman S (1988) Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. J Biol Chem 263:10980–10988

Rouzer CA, Rands E, Kargman S, Jones RE, Register RB, Dixon RAF (1988) Characterization of cloned human leukocyte 5-lipoxygenase expressed in mammalian cells. J Biol Chem 263:10135–10140

Rouzer CA, Ford-Hutchinson AW, Morton HE, Gillard JW (1990) MK886, a potent and specific leukotriene biosynthesis inhibitor blocks and reverses the membrane association of 5-lipoxygenase in ionophore-challenged leukocytes. J Biol Chem 265:1436–1442

Samhoun MN, Conroy RM, Piper PJ (1989) Pharmacological profile of leukotriene E_4, *N*-acetyl E_4 and four of their novel ω- and β-oxidative metabolites in airways of guinea-pig and man in vitro. Br J Pharmacol 98:1406–1412

Samuelsson B (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220:568–575

Samuelsson B, Funk CD (1989) Enzymes involved in the biosynthesis of leukotriene B_4. J Biol Chem 264:19469–19472

Samuelsson B, Borgeat P, Hammarström S, Murphy RC (1979) Introduction of a nomenclature: leukotrienes. Prostaglandins 17:785–787

Samuelsson B, Dahlén S-E, Lindgren JÅ, Rouzer CA, Serhan CN (1987) Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science 237:1171–1176

Saussy DL Jr, Sarau HM, Mong S, Crooke ST (1989) Mechanisms of leukotriene E_4 partial agonist activity at leukotriene D_4 receptors in differentiated U-937 cells. J Biol Chem 264:19845–19855

Schaub T, Ishikawa T, Keppler D (1991) ATP-dependent leukotriene export from mastocytoma cells. FEBS Lett 279:83–86

Serafin WE, Oates JA, Hubbard WC (1984) Metabolism of leukotriene B_4 in the monkey. Identification of the principal nonvolatile metabolite in the urine. Prostaglandins 27: 899–911

Shaw A, Krell RD (1991) Peptide leukotrienes: current status of research. J Medicinal Chem 34:1235–1242

Shirley MA, Murphy RC (1990) Metabolism of leukotriene B_4 in isolated rat hepatocytes. Involvement of 2,4-dienoyl-coenzyme A reductase in leukotriene B_4 metabolism. J Biol Chem 265:16288–16295

Shirley MA, Reidhead CT, Murphy RC (1992) Chemotactic LTB_4 metabolites produced by hepatocytes in the presence of ethanol. Biochem Biophys Res Commun 185:604–610

Snyder DW, Fleisch JK (1989) Leukotriene receptor antagonists as potential therapeutic agents. Annu Rev Pharmacol Toxicol 29:123–143

Soberman RJ, Sutyak JP, Okita RT, Wendelborn DF, Roberts II LJ, Austen KF (1988) The identification and formation of 20-aldehyde leukotriene B_4. J Biol Chem 263:7996–8002

Söderström M, Hammarström S, Mannervik B (1988) Leukotriene C synthase in murine mastocytoma cells: a novel enzyme distinct from cystolic and microsomal glutathione transferase. Biochem J 250:713–718

Sola A, Voelkel N, Maclouf J, Murphy RC (1990) Leukotriene E_4 elimination and metabolism in normal human subjects. J Biol Chem 265:21771–21778

Speeg KV, Maldonado AL, Liaci J, Muirhead D (1992) Effect of cyclosporine on colchicine secretion by a liver canalicular transporter studied in vivo. Hepatology 15:899–903

Stene DO, Murphy RC (1988) Metabolism of leukotriene E_4 in isolated rat hepatocytes. Identification of β-oxidation products of sulfidopeptide leukotrienes. J Biol Chem 263: 2773–2778

Sumimoto H, Kusunose E, Gotoh Y, Kusunose M, Minakami S (1990) Leukotriene B_4 ω-hydroxylase in rat liver microsomes: identification as a cytochrome P-450 that catalyzes prostaglandin A_1 ω-hydroxylation, and participation of cytochrome b_5. J Biochem 108:215–221

Tagari P, Ethier D, Carry M, Korley V, Charleson S, Girard Y, Zamboni R (1989) Measurement of urinary leukotrienes by reversed-phase liquid chromatography and radioimmunoassay. Clin Chem 35:388–391

Tagari P, Rasmussen JB, Delorme D, Girard Y, Eriksson L-O, Charleson S, Ford-Hutchinson AW (1990) Comparison of urinary leukotriene E_4 and 16-carboxytetranordihydro leukotriene E_4 excretion in allergic asthmatics after inhaled antigen. Eicosanoids 3:75–80

Tateson JE, Randall RW, Reynolds CH, Jackson WP, Bhattacherjee P, Salmon JA, Garland LG (1988) Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: biochemical assessment in vitro and ex vivo. Br J Pharmacol 94:528–539

Taylor GW, Taylor I, Black P, Maltby NH, Fuller RW, Dollery CT (1989) Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. Lancet i:584–589

Uehara N, Ormstad K, Örning L, Hammarström S (1983) Characteristics of the uptake of cysteine-containing leukotrienes by isolated hepatocytes. Biochim Biophys Acta 732:69–74

Verhagen J, Bruynzeel PLB, Koedam JA, Wassink GA, de Boer M, Terpstra GK, Kreukniet J, Veldink GA, Vliegenthart JFG (1984) Specific leukotriene formation by purified human eosinophils and neutrophils. FEBS Lett 168:23–28

Weckbecker G, Keppler D (1986) Leukotriene C_4 metabolism by hepatoma cells deficient in the uptake of cysteinyl leukotrienes. Eur J Biochem 154:559–562

Westcott JY, Thomas RB, Voelkel NF (1991) Elevated urinary leukotriene E_4 in patients with ARDS and severe burns. Prostaglandins Leukotrienes and Essential Fatty Acids 43:151–158

Wettstein M, Gerok W, Häussinger D (1989) Metabolism of cysteinyl leukotrienes in nonrecirculating rat liver perfusion. Eur J Biochem 181:115–124

Yoshimoto T, Soberman RJ, Spur B, Austen KF (1988) Properties of highly purified leukotriene C_4 synthase of guinea pig lung. J Clin Invest 81:866–871