Leukotrienes and Lipoxins: Structures, Biosynthesis, and Biological Effects

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Arachidonic acid is released from membrane phospholipids upon cell stimulation (for example, by immune complexes and calcium ionophores) and converted to leukotrienes by a 5-lipoxygenase that also has leukotriene A_4 synthetase activity. Leukotriene A_4, an unstable epoxide, is hydrolyzed to leukotriene B_4 or conjugated with glutathione to yield leukotriene C_4 and its metabolites, leukotriene D_4 and leukotriene E_4. The leukotrienes participate in host defense reactions and pathophysiological conditions such as immediate hypersensitivity and inflammation. Recent studies also suggest a neuroendocrine role for leukotriene C_4 in luteinizing hormone secretion. Lipoxins are formed by the action of 5- and 15-lipoxygenases on arachidonic acid. Lipoxin A causes contraction of guinea pig lung strips and dilation of the microvasculature. Both lipoxin A and B inhibit natural killer cell cytotoxicity. Thus, the multiple interaction of lipoxygenases generates compounds that can regulate specific cellular responses of importance in inflammation and immunity.

STUDIES CARRIED OUT DURING THE PAST DECADE HAVE demonstrated the biological importance of products formed via various lipoxygenase-catalyzed reactions (1). The leukotrienes are formed by oxygenation of arachidonic acid at C-5 and further transformation into an unstable epoxide intermediate, leukotriene (LT) A_4. This derivative can be converted enzymatically by hydration to LTB_4 and by addition of gluathione to LTC_4 (Fig. 1). LTC_4 is metabolized to LTD_4 and LTE_4 by successive elimination of a γ-glutamyl residue and glycine. Slow reacting substance of anaphylaxis consists of a mixture of LTC_4, LTD_4, and LTE_4 (Fig. 2).

The cysteinyl-containing leukotrienes are potent bronchoconstrictors, increase permeability in postcapillary venules, and stimulate mucus secretion. The dihydroxy derivative, LTB_4, which is a calcium ionophore, causes adhesion and chemotactic movement of leukocytes and stimulates aggregation, enzyme release, and generation of superoxide in neutrophils. LTC_4, LTD_4, and LTE_4, which are released from lung tissues of asthmatic subjects on exposure to specific allergens, seem to play a pathophysiological role in immediate hypersensitivity reactions. Both these leukotrienes and LTB_4 have proinflammatory effects.

Leukotrienes are also formed in the central nervous system. The highest production of LTC_4 is found in hypothalamus and the median eminence. Immunohistochemical studies revealed the coexistence of LTC_4 and luteinizing hormone–releasing hormone (LHRH) in neurons in the median eminence. Furthermore, LTC_4 caused release of luteinizing hormone (LH) from rat anterior pituitary cells at $10^{-13}M$ suggesting a neuroendocrine role of LTC_4.

A new group of arachidonic acid–derived products was recently discovered. These compounds (lipoxins), formed by the actions of 5- and 15-lipoxygenases and additional reactions, contain a conjugated tetraene structure and three alcohol groups. Lipoxin A has effects on microvasculature and contracts guinea pig lung strips. Both lipoxin A and B inhibit natural killer cell cytotoxicity. These studies indicate that the multiple interaction of lipoxygenases generates compounds that can be involved in regulating specific cellular responses of importance in inflammation.

Formation of Leukotrienes and Lipoxins

The enzyme 5-lipoxygenase plays a central role in the biosynthesis of the leukotrienes and lipoxins. Within the past several years, considerable progress has been made in the purification of this enzyme from human, rat, porcine, and murine sources (*2–6*). The 5-lipoxygenase is composed of a single polypeptide chain of 75,000 to 80,000 daltons that is anionic at neutral *p*H. The human and murine enzymes are stimulated by a microsomal membrane preparation (*2, 5, 7*), whereas the rat enzyme is stabilized and stimulated by phosphatidylcholine (*3, 6*). Maximal activity of the 5-lipoxygenases from all sources required Ca^{2+} and adenosine triphosphate (ATP) (*2–6, 8*). Finally, as was first discovered for an enzyme from plants (*9*), the enzymes from all sources were shown to catalyze not only the formation of 5-hydroperoxytetraenoic acid (5-HPETE) from arachidonic acid, but also the subsequent conversion of 5-HPETE to leukotriene A_4. Thus 5-lipoxygenase has dual enzymatic activities and catalyzes the first two steps of leukotriene biosynthesis (*4–6, 8*) (Fig. 1).

The 5-lipoxygenase purified from human peripheral blood leukocytes is an 80,000-dalton protein (*2*). It requires a number of other cellular components for maximal activity. Although the 5-lipoxygenase is a soluble protein, significant activity is lost from the supernatant upon centrifugation at 100,000*g*. The 100,000*g* pellet does not have intrinsic 5-lipoxygenase activity, but addition of this fraction to the 100,000*g* supernatant effects a concentration-dependent restoration of the supernatant activity (*7*).

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A second 5-lipoxygenase stimulatory component was revealed during ammonium sulfate fractionation studies. The enzyme itself precipitates at 30 to 60% saturation of ammonium sulfate. However, addition of the protein precipitating at 60 to 90% saturation of ammonium sulfate (60 to 90% ppt) results in a two- to fourfold stimulation of 5-lipoxygenase activity and an increase in the apparent recovery of about 70% (*2*).

The existence of a third stimulatory component for 5-lipoxygenase became apparent during initial purification attempts with the anion exchange resin Mono Q (HR10/10, Pharmacia). Once again, the recovery of the enzyme from the column is unexpectedly low, but if the nonadherent protein (pass-through fraction, designated MQ-PTF) from the column is added back to the sample, enzymatic activity is increased 1.5- to 2-fold (*2*). Maximal activity of the purified enzyme requires the inclusion of all three components. The LTA_4 synthase activity of the enzyme has the same requirements for all three factors as does the 5-lipoxygenase activity (*8*).

These results suggested that the human leukocyte 5-lipoxygenase is regulated by a complex, multicomponent system involving Ca^{2+}, ATP, and at least three nondialyzable factors—two cytosolic and one membrane-associated. To test the possibility that the enzyme might interact with membranes in the presence of Ca^{2+}, leukocytes were suspended in homogenization buffer containing 2 m*M* EDTA and various concentrations of added Ca^{2+}. After sonication, 1000*g*, 10,000*g*, and 100,000*g* supernatants and a pellet were prepared from the homogenates and assayed for 5-lipoxygenase activity. The data indicated that, in the absence of added Ca^{2+}, 5-lipoxygenase was a cytosolic protein. At 3 m*M* added Ca^{2+} (in the presence of 2 m*M* EDTA), there was greater activity in the 100,000*g* pellet than in the corresponding supernatant (*10*).

It is important to note that detection of 5-lipoxygenase activity in the membrane fractions required the inclusion of the 60 to 90% ppt factor, which caused a four- to eightfold increase in the activity of these samples. However, addition of the MQ-PTF caused no further increase in activity. When the membranes were washed by resuspension in Ca^{2+}-free buffer and centrifugation, 60 to 75% of the 5-lipoxygenase activity was released into the pellet wash supernatant. The enzyme activity in this supernatant was not itself stimulated by the MQ-PTF. However, when the supernatant was subjected to chromatography on Mono Q, an enzyme sample was obtained that was stimulated by the MQ-PTF. Furthermore, the nonadherent protein from the Mono Q chromatography of the pellet wash supernatant possessed 5-lipoxygenase stimulatory activity characteristic of the MQ-PTF.

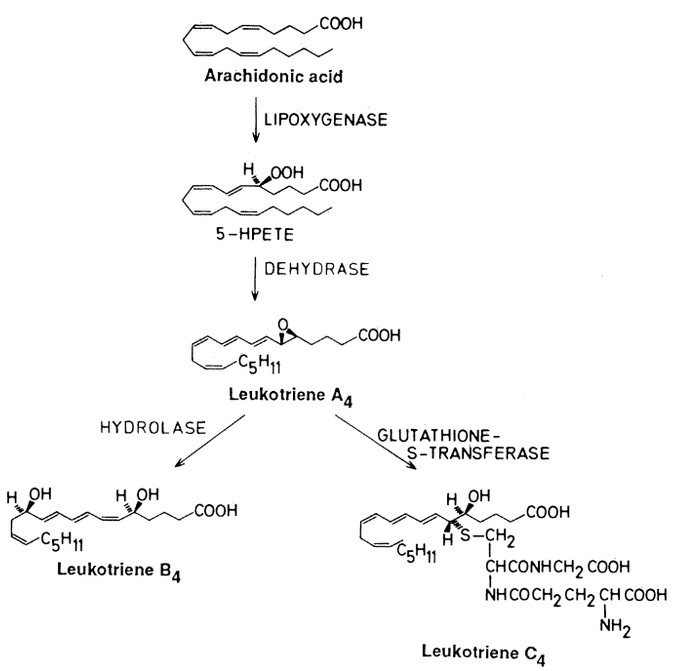

Fig. 1. Formation of leukotrienes from arachidonic acid.

These data indicate that both the 5-lipoxygenase and the stimulatory component in the MQ-PTF exhibit a reversible, Ca^{2+}-dependent association with cellular membranes. This finding suggests the possibility that neutrophil activation, resulting in an increase in intracellular Ca^{2+} might lead to the formation of an activated complex at the membrane surface. However, caution must be exercised in drawing such a conclusion, because the Ca^{2+} concentrations required to obtain high levels of membrane association (1 m*M* in excess of EDTA) are probably never achieved, even in an activated cell. On the other hand, the degree of membrane association was probably underestimated in these studies because the interaction was easily reversible and because of the poor stability of the membrane-associated 5-lipoxygenase activity (*10*). Obviously, a Ca^{2+}-directed translocation of the 5-lipoxygenase to a membrane site would be a very plausible activation mechanism considering the hydrophobicity of the enzyme and the nature of its substrate.

In conclusion, the human leukocyte 5-lipoxygenase is in many ways similar to the other mammalian 5-lipoxygenases that have been purified. However, it appears to be unique in its requirement for multiple cellular stimulatory components. Obviously, much remains to be learned concerning the mechanism of regulation of this enzyme and the relevance of the various stimulatory components for the activation of the 5-lipoxygenase in the intact neutrophil.

Biological Effects

The leukotrienes have potent biological actions in a number of areas (Fig. 3). Leukotriene B_4 has the leukocyte as its prime target, whereas the cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) primarily affect smooth muscle and other cells with contractile capacity.

The bronchospastic action of LTC_4 and its congeners has been studied extensively in animal preparations and also documented in human bronchi (*11, 12*). In general LTC_4, LTD_4, and LTE_4 have very similar activities on airway smooth muscle and are on a molar basis 100 to 1000 times as potent as histamine. Indeed, inhalation studies in healthy volunteers and asthmatic subjects have corroborated that LTC_4 and LTD_4 are potent inducers of airway obstruction [for example, (*13*)]. In addition, asthmatics are hyperreactive to inhaled leukotrienes [for example, (*14*)]. The cysteinyl leukotrienes predominantly activate airway smooth muscle, as well as several other relevant effectors, by direct mechanisms (*15*). The structure-activity requirements for the bronchospastic action has been outlined (*16*) and putative leukotriene receptors are being examined by binding studies (*17*); however, the correlation between radioligand binding and functional receptors is not evident (*18*).

Moreover, the cysteinyl leukotrienes increase microvascular permeability in the airways (*19*) and elsewhere, by an apparently direct action specifically on the endothelial lining of the postcapillary venule (*20*). The leakage of plasma induced by LTC_4 and its metabolites is generally preceded by an arteriolar constriction.

When given systemically, the vascular response to the cysteinyl leukotrienes is more complex and varies with factors such as species and the degree of anesthesia. There is often a long-lasting hypotension after injection of LTC_4 or LTD_4 (*19, 21–23*). One cause of the hypotension appears to be generalized leakage of plasma in the microvasculature (*19*), as evidenced by a marked hemoconcentration after LTC_4 (*19, 22*). In addition LTC_4, LTD_4, and LTE_4 may also

decrease blood pressure by an action on the heart, because cysteinyl leukotrienes reduce myocardial contractility and coronary blood flow (*24*). The hypotensive phase is usually preceded by an initial pressor effect (*21, 22*), which is primarily a consequence of generalized arteriolar constriction.

On the other hand, LTB_4 has no direct effects on blood flow but specifically stimulates a number of leukocyte functions (Fig. 3), including the emigration of leukocytes from the bloodstream (*20*). The initial response to LTB_4 is an increase of leukocytes adhering to the endothelium in venules; thereafter leukocytes start to infiltrate the tissue. All types of leukocytes may be affected, but with a brief exposure to LTB_4, polymorphonuclear leukocytes are primarily affected (*25*). Furthermore, LTB_4 also increases microvascular permeability (*26*). In contrast to the effect of the cysteinyl leukotrienes, the plasma leakage induced by LTB_4 is slow in onset and a consequence of leukocyte activation (*27*).

Bronchial asthma remains the area where the strongest experimental evidence supports a mediator role for leukotrienes. In addition to their effects on bronchi and microvessels, LTC_4 and LTD_4 stimulate mucus secretion in airways (*28*). Thus, the biological activity of the cysteinyl leukotrienes is more than sufficient to provoke the symptoms of airway obstruction in man.

Furthermore, it is well documented that lung tissue as a whole (*12, 29*), as well as relevant cells such as mast cells, basophils, eosinophils, and alveolar macrophages, share with neutrophils the capacity to synthesize leukotrienes (*1*). In particular, allergen to which the patient is sensitive proved to be a potent stimulus for the release of cysteinyl leukotrienes from lungs of asthmatic patients (*12*).

Finally, ablation of leukotriene mechanisms abrogates the bronchial response to allergen challenge in human bronchi. Thus, inhibition of leukotriene formation (determined biochemically) blocked the antihistamine-resistant contraction evoked by allergen in bronchi of atopic asthmatics (*12*). Likewise, an antagonist for SRS-A attenuated the allergen response in asthmatic bronchi, and it was recently observed that a leukotriene-receptor antagonist blocked the contraction induced by antibody to immunoglobulin E in human bronchi (*30*). Thus, the available evidence indicates that the cysteinyl leukotrienes are major mediators of the acute bronchial contraction evoked by immunoglobulin E–dependent reactions in man.

It is likely that we will soon be able to evaluate the contribution of this leukotriene-dependent bronchial contraction to the overall airway obstruction evoked by allergen or other stimuli, as well as the role of LTB_4 in inflammation.

Role of Leukotrienes in the Central Nervous System

Recent findings demonstrate formation of leukotrienes in the central nervous system. We and other groups demonstrated the production of LTC_4, LTD_4, and LTE_4 in rat and gerbil brain tissue (*31, 32*).

After stimulation of sliced rat brain tissue with ionophore A23187 and arachidonic acid, LTC_4, LTD_4, and LTE_4 were detected (*31*). In addition, LTB_4 and various monohydroxyeicosatetraenoic acids (5-HETE, 9-HETE, 11-HETE, 12-HETE, and 15-HETE) were isolated and identified from incubations of chopped rat brain tissue with ionophore A23187 (*33*). The formation of 11-HETE was inhibited by indomethacin, indicating that this compound was formed as a by-product via the cyclooxygenase pathway (*34*). The isolation of 9-HETE suggested the presence of a novel lipoxygenase activity in the brain, since formation of this compound

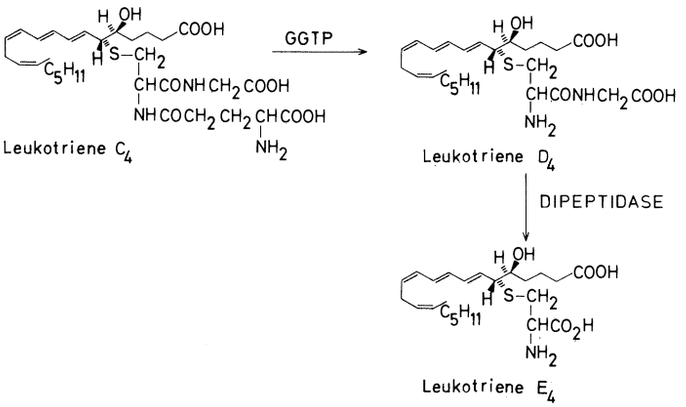

Fig. 2. Metabolism of leukotriene C_4.

had not been reported to occur in mammalian tissue. Ionophore A23187 caused a dose-dependent stimulation of LTB_4 and LTC_4 synthesis (*35*). Regional distribution studies indicated the highest production of LTC_4 in the hypothalamus and median eminence, whereas the formation in cerebellum and brain cortex was low (*31, 36*).

Immunohistochemical analysis, with the indirect immunofluorescence technique, demonstrated LTC_4-immunoreactive fibers in the median eminence (*31*). The fibers terminated close to the portal vessels. In addition, single fibers with the same appearance were observed in other brain regions, including many areas in the hypothalamus. Weakly fluorescent cell bodies were also observed in the medial preoptic area. The immunoreactivity could be abolished after absorption of the antiserum to LTC_4 with LTC_4–bovine serum albumin conjugate. The distribution pattern of LTC_4-positive fibers in the median eminence was similar to the pattern of LHRH-immunoreactive fibers (*37*). Furthermore, elution-restaining experiments showed that a large portion of LTC_4- and LHRH-positive neurofibers were identical. This indicated that the two compounds were present in the same neurons.

The immunohistochemical data, together with the finding of the prominent LTC_4 synthesis in the hypothalamus, suggested a possible neuroendocrine role for LTC_4. Further evidence for such a role was obtained by the finding that LTC_4 in extremely low concentrations (maximal effects at 10^{-12} to $10^{-13}M$) stimulated LH release from dispersed rat anterior pituitary cells after incubation for 30 minutes (*37*). Using the maximal effective concentration of LTC_4, we observed a stimulation after 5 minutes of incubation (*38*). In contrast to the fast action of LTC_4, a stimulatory effect of LHRH on LH release was not obvious after 30 minutes, but could be observed after 3 hours (*37*). This indicated that LTC_4 and LHRH stimulated LH release at least partly through different mechanisms. In addition, it was recently reported that LTC_4 also stimulated the release of

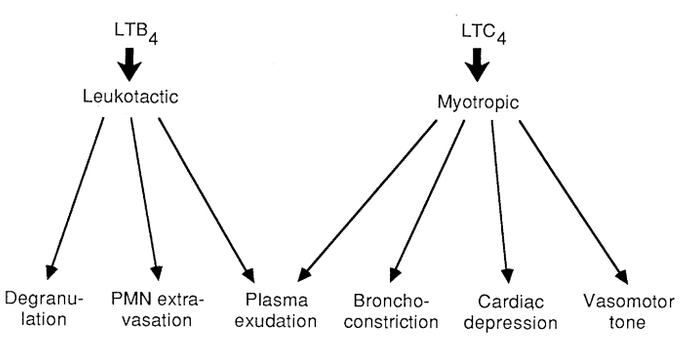

Fig. 3. Some biological effects of leukotrienes.

LHRH from the rat median eminence (*39*). Taken together, these findings demonstrate a complex relation between LTC_4 and LHRH and gives further evidence for a physiological role of LTC_4 in the regulation of LH secretion.

A putative role of leukotrienes as neuromodulators was suggested by the finding that LTC_4 and LTD_4 caused excitation of cerebellar Purkinje neurons (*40*). Further evidence for such a role was obtained by the findings of LTC_4 binding sites in the rat and guinea pig brain (*41*). The highest binding capacity was observed in the brainstem and hippocampus, whereas the binding in the hypothalamus was low. Very weak or no binding was observed for LTB_4, LTD_4, or LTE_4, indicating that LTC_4 may be the active metabolite in the brain.

Lipoxins

Results from several studies have suggested that initial lipoxygenation at C-15 can lead to the formation of compounds of biological interest (*1, 42*). To mimic cellular events, we prepared both 15-HETE and 15-HPETE and examined the products formed upon incubation with human leukocytes.

After column chromatography and thin-layer chromatography, a fraction containing tetraene-containing compounds was examined by reversed-phase high-pressure liquid chromatography (RP-HPLC). The basic structures of two main compounds of this series were elucidated by ultraviolet spectrometry, gas chromatography–mass spectrometry, and oxidative ozonolysis and shown to be 5,6,15L-trihydroxy-7,9,11,13-eicosatetraenoic acid and 5D,14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid. Addition of these materials to either human neutrophils or human natural killer (NK) cells evoked selective responses different from those observed with leukotrienes. Hence the bioactive compounds were termed lipoxins (lipoxygenase interaction products) A (LXA) and lipoxin B (LXB), respectively (*43, 44*).

Next, we determined both the complete stereochemistry of these compounds and their routes of biosynthesis. Each of the biologically derived compounds was subject to analysis with several RP-HPLC systems, followed by determination of their physical and biological characteristics. Studies with isotopic oxygen demonstrated that LXA, LXB, and their isomers each carried an ^{18}O atom at C-5 and that neither the C-6 of LXA nor C-14 of LXB was derived exclusively from molecular oxygen. These findings, along with results of alcohol trapping studies, provide evidence for involvement of an epoxide in the formation of these compounds (*45–48*). At this point, a synthetic approach was undertaken to establish the complete stereochemistry (that is, geometry of the double bonds and the chirality of the C-6 and C-14 position alcohol groups) of LXA and LXB (Fig. 4) (*45–47, 49, 50*). Biologically derived LXB proved to be 5*S*, 14*R*, 15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid, and its two naturally occurring isomers are 5*S*, 14*R*, 15*S*-trihydroxy-6,8,10,12-*trans*-eicosatetraenoic acid (8-*trans*-LXB) and 5*S*, 14*S*, 15*S*-trihydroxy-6,8,10,12-*trans*-eicosatetraenoic acid (14*S*-8-*trans*-LXB) (*45*). In collaboration with K. C. Nicolaou (University of Pennsylvania), we established that, by comparison with several synthetic materials, biologically derived LXA is 5*S*,6*R* 15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (*46*). 6*S*-LXA was also identified from leukocyte extracts as well as two all-*trans* isomers assigned as 6*S*-11-*trans*-LXA and 11-*trans*-LXA. Synthetic approaches and matching analyses have also been attempted by others (*51, 52*), and intriguing mechanisms of biosynthesis have been proposed (*53*).

Although it is clear that several biosynthetic routes can lead to the formation of tetraene-containing eicosanoids, the finding that 15-HETE is transformed by activated leukocytes to these compounds provided us with a model for studying one biosynthetic path in their formation (*46, 47*). In this route 15-HETE is transformed to 5(*S*)-hydroperoxy-15(*S*)-hydroxy-6,13-*trans*-8,11-*cis*-eicosatetraenoic acid, which can be transformed to a 5(6)-epoxide tetraene. This epoxide, or its equivalent, could be enzymatically transformed to either LXA (by the action of an epoxide hydrolase) or LXB (by attack at the C-14 position and generation of an 8-*cis* double bond) (Fig. 4). Other naturally occurring isomers can be generated by nonenzymatic hydrolysis of the 5(6)-epoxytetraene or by isomerizations of LXA or LXB during isolation or interaction with hemoprotein (*46, 47*). To gain further evidence for the role of a 5(6)-epoxytetraene intermediate in the biosynthesis, 15(*S*)-hydroxy-5(6)-oxido-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid was prepared by total

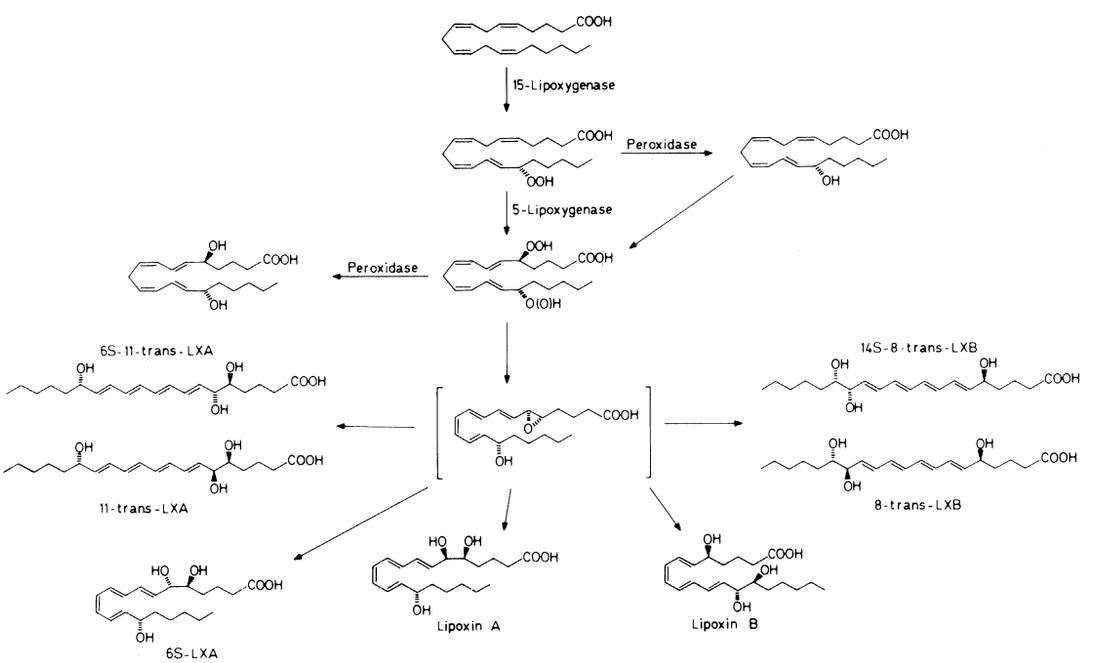

Fig. 4. Scheme of formation of lipoxins A and B and their isomers.

chemical synthesis and added to a purified cytosolic epoxide hydrolase (*48*). The epoxide was rapidly and quantitatively converted into LXA. This system provides a clear model for the enzymatic formation of LXA (*48*). It remains to be determined, however, if a similar enzyme and 5(6)-epoxide tetraene of this double-bond geometry are involved in the formation of LXA in human leukocytes. Other investigators have also postulated the role of epoxide tetraenes in the formation of lipoxins and related compounds (*51, 54*) and have described lipoxins of the 5 series derived from EPA (*55*).

We have recently found that eosinophil-rich granulocyte suspensions from peripheral blood of patients with hypereosinophilic syndrome generate LXA when exposed (in vitro) to ionophore A23187. The formation of LXA from endogenous sources of arachidonate was proportional to the percentage of eosinophils present (*56*).

Biological Effects of Lipoxins

The activities of lipoxins have been evaluated in several systems. In each, the responses evoked have proven to be stereospecific. When added to neutrophils, for example, LXA stimulates superoxide anion generation without provoking aggregation. Recently LXA, but not 6(*S*)-LXA, has been shown to cause chemotaxis (*57*). LXA also possesses spasmogenic activities (*58*). In submicromolar concentrations, LXA elicits long-lasting contractions of the guinea pig lung strip. The response to LXA was not due to release of either acetylcholine, histamine, norepinephrine, or cyclooxygenase products. Synthetic LXA causes responses that are indistinguishable from those obtained with leukocyte-derived material and the 6(*S*) isomer of LXA [6(*S*)-LXA] does not provoke contractions. Furthermore, with guinea pig ileum, LXA, unlike LTC_4, does not stimulate contraction.

Intravital microscopy of the hamster cheek pouch revealed that LXA induces arteriolar dilation but has no visible effects on microvascular permeability or leukocyte adherence to venular endothelium (*58*). When the systemic and renal hemodynamic responses to intrarenal arterial administration of LXA were examined in rats, LXA induced glomerular hyperperfusion, hypertension, and hyperfiltration. In these experiments, micropuncture measurements of glomerular dynamics revealed that LXA provokes a selective and dramatic fall in afferent, but not efferent, arteriolar resistance (*59*). Thus, LXA displays a pattern of activity in spasmogenic assays and in microvasculature that is distinct from those known for prostaglandins, thromboxanes, or leukotrienes.

LXA and LXB also block human NK cell activity againt K562 target cells (*44*). Here 15-HETE, LTB_4, and LTC_4 displayed no effects on NK cell cytotoxicity. LXA appears to stereospecifically block NK cell cytotoxicity by disrupting "signals" involved in the orientation of its Golgi complex, an event that appears to be of importance in cytotoxicity (*50*).

It became of interest to determine whether eicosanoids can serve intracellular roles (that is, modulate enzyme systems). As a model, we examined the action of lipoxygenase products on isolated protein kinase C (*60*). LXA activated the kinase and proved to be more potent than either diacylglyceride (a proposed intracellular signal in the activation of protein kinase C) or native arachidonic acid. Other oxygenated derivatives of arachidonic acid, including LTB_4, were without effect or less potent than LXA. Results from these studies also showed that substrate specificity of the kinase can be modulated depending upon the stereochemistry of the activator. Such findings suggest that lipoxygenase products, in particular LXA, may also serve in intracellular roles.

Whether lipoxins prove to be intracellular rather than extracellular signals remains to be determined. Nonetheless, results suggest a role for interactions among lipoxygenases in regulating specific cellular responses. Moreover, the lipoxins provide additional or alternative means by which the oxygenation of arachidonic acid, either within various cells, or by transcellular metabolism can exert an effect on the responses of interest in inflammation and host defense.

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Transgenic Plants as Tools to Study the Molecular Organization of Plant Genes

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Transgenic plants are generated in nature by *Agrobacterium tumefaciens*, a pathogen that produces disease through the transfer of some of its own DNA into susceptible plants. The genes are carried on a plasmid. Much has been learned about how the plasmid is transferred, how the plasmid-borne genes are organized, regulated, and expressed, and how the bacteria's pathogenic effects are produced. The *A. tumefaciens* plasmid has been manipulated for use as a general vector for the transfer of specific segments of foreign DNA of interest (from plants and other sources) into plants; the activities of various genes and their regulation by enhancer and silencer sequences have been assessed. Future uses of the vector (or others like it that have different host ranges) by the agriculture industry are expected to aid in moving into vulnerable plants specific genes that will protect them from such killers as nonselective herbicides, insects, and viruses.

THE SOIL PHYTOPATHOGEN *Agrobacterium tumefaciens* IS A sophisticated parasite that uses genetic engineering processes to force infected plant cells to divert some of their organic carbon and nitrogen supplies for the synthesis of nutrients (called opines), which the infecting bacteria can specifically catabolize (*1*). The genetically engineered plant cells are also stimulated to proliferate and thus form typical tumor tissues called crown galls.

A detailed genetic and molecular analysis of this phenomenon became possible when it was discovered (*2*) that large extrachromosomal plasmids in agrobacteria carry the genes responsible for both tumorous growth and opine synthesis of crown gall tissues as well as for opine catabolism by agrobacteria. Genetic evidence thus pointed to specific transfer of genes from the Ti plasmids in agrobacteria to the genome of plant cells.

Transposon mutagenesis of Ti plasmids (*3, 4*) revealed that two segments of these plasmids were involved in oncogenicity and therefore, presumably, in the transfer of DNA from bacteria to plant cells. One of these segments, later called the *vir* region, contains genes whose inactivation leads to a loss of tumor-inducing capacity by the mutant strains. Insertions into the other segment, called the T region, produced mutant strains that could still transfer DNA into plant cells, but the cells then either lacked the capacity to synthesize opines or the tissues had aberrant morphologies (shoot-forming teratomas or root-forming calli). A striking observation made with these insertions was that a transposon within the T region of a Ti plasmid was physically integrated into the genome of transformed plant cells (*5*). This finding confirmed that the T region of Ti plasmids was physically transferred to plant cells and showed that Ti plasmids could be used to introduce foreign DNA into plant cells.

When small DNA fragments from Ti plasmids were hybridized with DNA from transformed plant cells a well-defined segment called transfer DNA (T-DNA), derived from the T region of these plasmids, was covalently integrated into the plant nuclear genome (*6*). RNA-DNA hybridizations showed that the T-DNA in plant cells was transcribed into a number of well-defined polyadenylated transcripts. Some of these transcripts correlated with the T-DNA functions genetically identified by transposon mutagenesis of the T region of Ti plasmids (*7*).

T-DNA Oncogenes Code for Enzymes

Plant cells harboring a T-DNA segment in their genome do not require supplementation with cellular growth factors, such as auxins

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