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# MINI-REVIEW

# Lipoxins: Eicosanoids Carrying Intra- and Intercellular Messages

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## Abstract

The lipoxins are a recent addition to the family of biologically active products derived from arachidonic acid. Compounds of this series contain a conjugated tetraene structure and can be generated by the actions of the major lipoxygenases of human tissues (5-, 12-, and 15-LO's). Biosynthesis of the lipoxins from cellular sources of unesterified arachidonic acid is triggered by the initial actions of either the 15-LO or 5-LO followed by additional reactions. Recent results indicate that lipoxins are also generated by receptor-mediated events during cell–cell interactions with the transcellular metabolism of key intermediates. Lipoxin $A_4$ and lipoxin $B_4$ each possess a unique spectrum of biological activities unlike those of other eicosanoids in both *in vivo* and *in vitro* systems. Lipoxin $A_4$ stimulates changes in the microvasculature and can block some of the proinflammatory effects of leukotrienes (*in vivo*). Lipoxin $A_4$ and lipoxin $B_4$ both inhibit natural killer cells (*in vitro*), and lipoxin $B_4$ displays selective actions on hematopoietic cells. The finding that lipoxin $A_4$ activates isolated protein kinase C suggests that it may also serve an intracellular role in its cell of origin before it is released to the extracellular milieu. Thus, cell–cell interactions, along with multiple oxygenations by lipoxygenases, generate compounds that can regulate cellular responses by serving as both intra- and intercellular messages.

**Key Words:** Arachidonic acid; lipoxygenases; blood cells; signal transduction.

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## Introduction

Signal transduction in a variety of cell types is associated with the release and oxygenation of arachidonic acid by lipoxygenases (LO's).[2] LO's, which are widely distributed in human tissues, transform nonesterified arachidonic acid to biologically active products that may play roles in both normal and pathophysiologic processes (Samuelsson, 1983). In humans, the three major LO's (5-, 15-, and 12-LO) are compartmentalized within different cell types of peripheral blood. For example, upon stimulation, human platelets activate an arachidonate 12-LO, while human neutrophils (PMN) possess both 5- and 15-LO's (Borgeat *et al.*, 1983). Initial oxygenation of arachidonic acid via 5-LO is a key event in the biosynthesis of leukotrienes, which are held to play

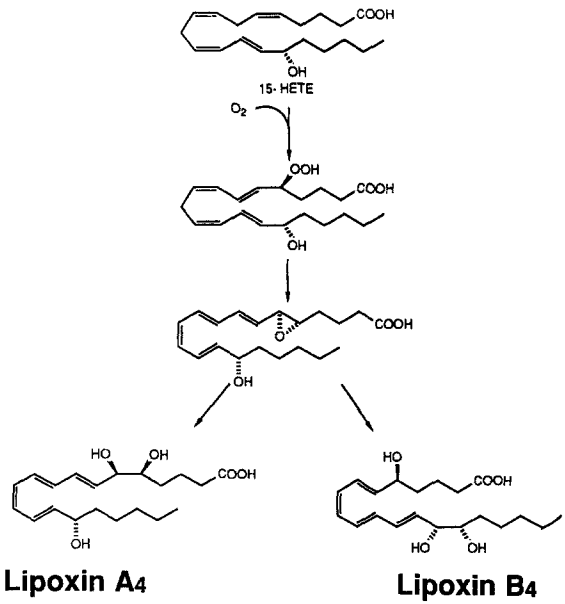


**Fig. 1.** Biosynthesis of lipoxins by activated human leukocytes (transformation of 15-HETE; see text for details).

[2]Abbreviations used: RP-HPLC, reverse-phase HPLC; GC, gas–liquid chromatography; MS, mass spectrometry; $Me_3Si$, trimethylsilyl; lipoxin $A_4$, ($LXA_4$), 5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid; lipoxin $B_4$, ($LXB_4$), 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid; 7-*cis*-11-*trans*-$LXA_4$, 5*S*,6*R*,15*S*-trihydroxy-9,11,13-*trans*-7-*cis*-eicosatetraenoic acid; $LTB_4$, leukotriene $B_4$, 5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-dihydroxy-eicosatetraenoic acid; $LTA_4$, leukotriene $A_4$, 5*S*-trans-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; 12-HETE, 12*S*-hydroxy-5,8,14-*cis*-10-*trans*-eicosatetraenoic acid; 15-HETE, 15*S*-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LO, lipoxygenase; FMLP, formyl-methionyl-leucyl-phenylalanine.

important roles in both inflammation and allergic reactions (reviewed in Samuelsson and Serhan, 1987). In view of the potential importance of LO products, studies were initiated to examine the consequences of initial lipoxygenation at carbon-15 of arachidonic acid as well as to investigate interactions between the major LO pathways (Serhan *et al.*, 1984a,b), since the roles of these other LO's in human tissues are not well understood. These studies led to the identification of a new series of biologically active eicosanoids termed lipoxins (Fig. 1). The interactions observed between peripheral blood cells may serve as models for metabolic events in human tissues. This mini-review summarizes the isolation and biosynthesis of the lipoxins as well as reviews recent results from several laboratories concerning their formation and biological activities.

## Isolation and Biosynthesis of the Lipoxins

While studying the metabolism of [1-$^{14}$C]arachidonate (*in vitro*) with suspensions of mixed leukocytes (i.e., neutrophils, eosinophils, basophils, etc.) obtained from peripheral blood of human donors, we found that labeled C20 : 4 was transformed and associated with polar compounds that had not been previously described. Since human leukocytes rapidly oxygenate exogenous C20 : 4 (without addition of other stimuli, i.e., A23187) at the C-15 position (Borgeat *et al.*, 1983; Serhan *et al.*, 1984c), we studied the products associated with 15-lipoxygenation. Results from several laboratories suggest that initial lipoxygenation at carbon-15 can lead to the formation of eicosanoids of biological interest (Hamberg *et al.*, 1980; Murray *et al.*, 1986; and reviewed in Needleman *et al.*, 1986). 15*S*-Hydroxy-5,8,11,-*cis*-13-*trans*-eicosatetraenoic acid (15-HETE), for example, had been identified as a major product of C20 : 4 in both normal and asthmatic human lung tissue (Hamberg *et al.*, 1980). 15-HETE is also found in microgram quantities in bronchoalveolar lavage fluids obtained from patients with chronic stable asthma following antigenic challenge (Murray *et al.*, 1986). Although 15-lipoxygenation of C20 : 4 is a major route of its transformation in a wide range of mammalian tissues (reviewed in Needleman *et al.*, 1986), receptor-mediated activation of this enzyme system and general physiological role of its products has remained an area of interest.

To mimic cellular events that could have given rise to the novel polar compounds, as well as to prepare large amounts of these materials for further studies, 15-HPETE and 15-HETE were prepared and the products formed upon their exposure to human leukocytes were studied (Serhan *et al.*, 1984a,b, 1985, 1986a,b). This approach revealed a new series of oxygenated products of C20 : 4 that contain a unique conjugated tetraene. Since both of these

compounds can arise via the interaction(s) between LO pathways (Fig. 1) and proved to display novel biological properties, the trivial name lipoxins (lipoxygenase interaction products) was introduced (Serhan *et al.*, 1984b, 1987a).

Activation of human leukocytes with an ionophore of divalent cations (A23187) led to increased formation of these tetraene-containing eicosanoids, which facilitated their isolation (Serhan *et al.*, 1984a, 1986a,b). A fraction containing several of the compounds was obtained from incubations with leukocytes following silicic acid chromatography and thin-layer chromatography of material, which had been treated with diazomethane. The basic structures of the first two members of this series were determined by physical methods that included HPLC, UV spectroscopy, gas chromatography-mass spectrometry (GC-MS) and oxidative ozonolysis. One compound was identified as 5,6,15*L*-trihydroxy-7,9,11,13-eicosatetraenoic acid (Serhan *et al.*, 1984a), and the other proved to be its positional isomer 5*D*,14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid (Serhan *et al.*, 1984b). When these biologically derived products were isolated following HPLC and added to either human neutrophils, natural killer (NK) cells, or guinea pig lung strips, they elicited bioactions different from those previously observed with other eicosanoids. Therefore the new bioactive compounds were termed lipoxin $A_4$ ($LXA_4$) and lipoxin $B_4$ ($LXB_4$), respectively (Ramstedt *et al.*, 1985; Serhan *et al.*, 1984b, 1985).

We next determined the complete stereochemistry and identified a route of biosynthesis for the biologically active $LXA_4$ and $LXB_4$, as well as identified the structures and actions of their naturally occurring isomers. Results from isotopic oxygen studies guided these experiments, since they revealed that each of the tetraene-containing compounds generated by intact leukocytes carried an $^{18}O$ atom at C-5 and that the oxygen atoms at either C-6 of $LXA_4$ or C-14 of $LXB_4$, as well as their isomers, were not exclusively derived from molecular oxygen (Serhan *et al.*, 1985). These findings and results of alcohol trapping studies suggested that the formation of lipoxins in human leukocytes proceeds via an epoxide-containing intermediate (Puustinen *et al.*, 1986; Serhan *et al.*, 1986a,b).

At this stage, a synthetic approach was undertaken to establish the stereochemistry of $LXA_4$ and $LXB_4$ (i.e., geometry of the double bonds and the chirality of the carbon-6 and carbon-14 position alcohol groups; cf. Serhan *et al.*, 1984a,b). Several criteria and matching studies were employed with materials prepared by total synthesis. Synthetic and biologically derived materials were subject to analysis by UV spectroscopy, HPLC (co-chromatography in several systems), and GC-MS of several derivatives, and, most critically, they were assessed for biological activities in experiments performed in parallel. The complete structure of $LXB_4$ proved to be 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid (Serhan *et al.*, 1986a),

and $LXA_4$ is 5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (Serhan *et al.*, 1986b). A 6*S* isomer of $LXA_4$ (6*S*-$LXA_4$) and two *all-trans* epimers (6*S*-11-*trans*-$LXA_4$ and 11-*trans*-$LXA_4$) were also identified. However, these compounds have proven to be substantially less active than native $LXA_4$ in several bioassay systems (see Tables III–V). Synthetic approaches coupled with matching analyses performed with HPLC alone were attempted by others (Adams *et al.*, 1985; Fitzsimmons *et al.*, 1985).

Although it is clear that multiple biosynthetic routes as well as cell–cell interactions can lead to formation of tetraene-containing eicosanoids, the finding that 15-HETE is rapidly transformed by activated leukocytes provided a model for studying one pathway in their formation (Serhan *et al.*, 1986a,b). In this route, schematically summarized in Fig. 1, 15-HETE is converted to 5(*S*)-hydroperoxy-15(*S*)-hydroxy-6,13-*trans*-8,11-*cis*-eicosatetraenoic acid by activated human leukocytes and further transformed to a 5(6)-epoxytetraene. This epoxide or its equivalent could be enzymatically transformed to either $LXA_4$ (by the action of an epoxide hydrolase to yield a 5*S*,6*R* diol) or $LXB_4$ (by attack of the C-14 position with the generation of an 8-*cis* double bond). Further evidence for a 5(6)-epoxytetraene intermediate in the biosynthesis was obtained with 15*S*-hydroxy-5(6)-oxido-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid prepared by total chemical synthesis. This epoxide was rapidly and quantitatively converted to $LXA_4$ (*in vitro*) by a purified cytosolic epoxide hydrolase (Puustinen *et al.*, 1986). It remains to be determined whether a similar enzyme is solely responsible for the formation of $LXA_4$ by human leukocytes. Others have also postulated the role of epoxytetraenes in the formation of $LXA_4$, $LXB_4$ and their isomers (Adams *et al.*, 1985; Kühn *et al.*, 1984) in model systems. In particular, Kühn *et al.* (1984), Fitzsimmons *et al.* (1985), and Adams *et al.* (1985) considered several possible biosynthetic schemes to generate lipoxins, which relied heavily upon theoretical knowledge of the action of LO's available at that time. One scheme involved an epoxide intermediate and another involved three consecutive oxygenations catalyzed by LO's. It is now clear from these studies as well as results obtained from our biosynthetic studies with intact human leukocytes that the generation of a 5(6)-epoxytetraene from 15-HETE is an enzymatic event (Serhan *et al.*, 1985, 1986a,b). Fitzsimmons *et al.* (1985) proposed that, once formed, the 5(6)-epoxytetraene could undergo nonenzymatic hydrolysis to yield a mixture of products including 14*S*-8-*trans*-$LXB_4$, 8-*trans*-$LXB_4$, and a racemic mixture of both $LXA_4$ and its 6(*S*) isomer (6*S*-$LXA_4$), but not $LXB_4$. This suggestion was based upon the results of an experiment in which synthetic 5(6)-epoxytetraene was added to leukocytes in suspension. However, it is not clear from these data whether the 5(6)-epoxytetraene entered the cells or if it underwent hydrolysis in the extracellular medium before gaining access to cellular compartments. Moreover,

the results of time course studies are not consistent with this proposal, namely, equal amounts of $LXA_4$ and 6*S*-$LXA_4$ would be expected, rather than the quantitatively higher amounts of $LXA_4$ that are observed with cells that have not undergone lysis (Serhan *et al.*, 1984a, 1986b, 1989).

## Cell-Free Systems and Isolated Enzymes Which Generate Lipoxins and Related Compounds

The proposal that tetraene-containing eicosanoids, in particular $LXA_4$ and $LXB_4$, could be generated by several distinct biosynthetic routes (Serhan *et al.*, 1984a,b) has now been explored in several model systems using both purified and partially purified enzymes (Table I). Initial studies by Kühn *et al.* (1984) in this area indicated that a purified LO obtained from reticulocytes could convert 5*S*,15*S*-DiHETE to trihydroxytetraenes. Since the generation of these compounds was blocked by inhibitors, heat denaturing of the enzyme, and by anaerobic conditions, it was proposed that the products were enzymatically generated by LO-type reactions. As stated above, two independent LO mechanisms could account for the formation of these products by the isolated lipoxygenase. One involves a 5(6)-epoxide, while the other implicates the formation of an intermediate that is generated by the action of a LO on either 5,15-DHPETE or its reduced form 5,15-DHETE (Kühn *et al.*, 1984). This second route is considered a sequential triple lipoxygenation where a product of dioxygenation becomes a substrate for the enzymatic insertion of molecular oxygen as the third step. More recently, these investigators (Kühn *et al.*, 1986, 1987) demonstrated that the isolated reticulocyte enzyme can convert 5,15-DHETE to $LXB_4$ by incorporation of molecular oxygen, thus illustrating the potential of this triple LO route with purified enzyme.

Ueda *et al.* (1987a) have shown that a 5-LO purified from porcine leukocytes can enzymatically generate a 5(6)-epoxytetraene with either 15-HPETE or 15-HETE. In contrast, studies using a purified 12-LO from porcine leukocytes indicate that $LXB_4$ can be generated by both the 14*R*-oxygenase activity as well as the $LTA_4$ synthase activity of this enzyme with 5,15-DiHPETE (Ueda *et al.*, 1987b). Thus, this enzyme can generate $LXB_4$ by both triple oxygenation and by the formation of a putative 14(15)-epoxytetraene. It should be noted, however, that, unlike the porcine leukocyte, human leukocytes do not possess a prominent 12-LO, and when 5,15-DiHPETE was added to intact human platelets, which contain active 12-LO, $LXB_4$ was not generated (Serhan *et al.*, 1986a). Nevertheless, results obtained with purified 12-LO from porcine leukocytes have offered another enzymatic route for $LXB_4$ production with intriguing mechanistic implications.

**Table I.** Generation of Lipoxins and Related Compounds by Cell-Free and Isolated Enzyme Systems

| Enzyme | Source | Substrate | Major enzymatic product | Reference |
|---|---|---|---|---|
| 15-/12-Lipoxygenase | Rabbit reticulocytes | 5,15-DiHETE | Trihydroxytetraenes | Kühn *et al.*, 1984 |
| 15-Lipoxygenase | Soybean | 5,15-DiHETE | Trihydroxytetraenes | Kühn *et al.*, 1984 |
| 15-/12-Lipoxygenase | Reticulocyte | 15-HETE, arachidonic acid | Lipoxin $B_4$ | Kühn *et al.*, 1986<br>Kühn *et al.*, 1987 |
| 5-Lipoxygenase | Porcine leukocytes | 15-HPETE, 5,15-DiHPETE | 5(6)-Epoxytetraene | Ueda *et al.*, 1987a |
| 12-Lipoxygenase | Porcine leukocytes | 15-HPETE, 5,15-DiHPETE | Lipoxin $B_4$ | Ueda *et al.*, 1987b |
| 5-Lipoxygenase | Potato tubers | 15-HPETE/C20:4 | Lipoxin $A_4$/lipoxin $B_4$ | Ho and Wong, 1989<br>Reddy *et al.*, 1989 |
| 15-Lipoxygenase | Soybean | Leukotriene $A_4$ | 5(6)-Epoxytetraene | Serhan *et al.*, 1990 |
| 12-Lipoxygenase | Human platelets (100,000 *g* supernates) | Leukotriene $A_4$ | Lipoxin $A_4$ | Serhan *et al.*, 1990 |
| Epoxide hydrolase (cytosolic) | Human liver | 5(6)-Epoxytetraene | Lipoxin $A_4$ | Puustinen *et al.*, 1986 |

The 5-LO of potato tubers has also been shown to generate $LXB_4$, as well as a novel 6,7-dihydrolipoxin $B_4$, when incubated with 15-HPETE (Ho and Wong, 1989; Reddy *et al.*, 1989). In addition to utilizing hydroxy- and hydroperoxy-containing compounds as substrates for dioxygenation-type reactions, recent results indicate that LO's can also utilize epoxide-containing compounds as substrates to lipoxin precursors. Along these lines, the soybean 15-LO added to $LTA_4$ rapidly transforms this epoxide to a 5(6)-epoxytetraene (Serhan *et al.*, 1990), suggesting that 15-lipoxygenation of $LTA_4$ may serve as an additional route to lipoxin formation in various cells.

The role of initial oxygenation of C20:4 by 5-LO, followed by interactions with either the 12-LO or 15-LO, has been explored in studies with intact human platelets (Edenius *et al.*, 1988; Serhan *et al.*, 1990), rat mesangial cells (Garrick *et al.*, 1989), and neutrophils (Serhan and Sheppard, 1990). Recent results with human platelet-derived 100,000 *g* supernatants, which display 12-LO activity, show that the platelet 12-LO can transform $LTA_4$ to lipoxins (Serhan and Sheppard, 1990; Serhan *et al.*, 1990). This action of the human 12-LO with $LTA_4$ exemplifies its $\omega$-6 oxygenase activity and provides an unexpected role for this enzyme in lipoxin biosynthesis. Once formed, the 5(6)-epoxytetraene could be a substrate for lipoxin production by several distinct mechanisms (Serhan *et al.*, 1986a,b). It is clear, at least *in vitro*, that synthetic epoxytetraenes [e.g., 5(6)-epoxytetraene and 14(15)-epoxytetraene] can undergo nonenzymatic hydrolysis to generate both $LXA_4$ and $LXB_4$, respectively, as well as some but not all of their isomers (Fitzsimmons *et al.*,

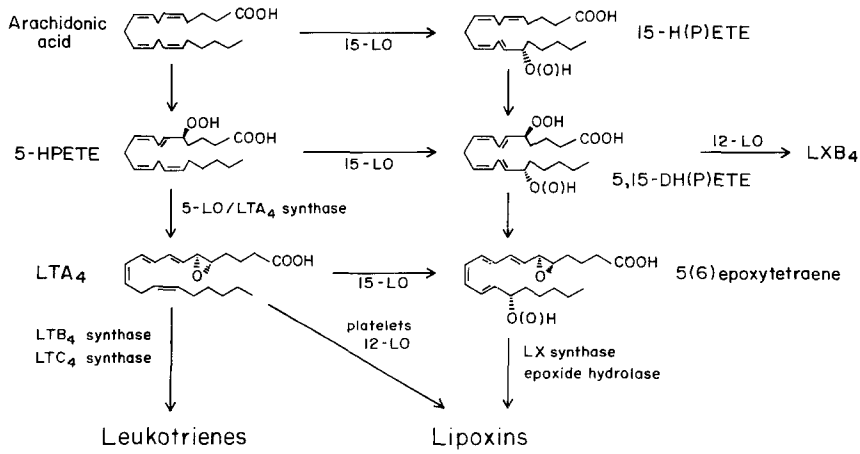


**Fig. 2.** Key switch points between leukotriene and lipoxin production. Arrows indicate reactions documented by investigations cited in Tables I and II. Together, they may represent potential sites or routes for formation of lipoxins by cell–cell interactions or within a given cell type, which possesses all the necessary enzymes.

1985; Leblanc *et al.*, 1987; Nicolaou *et al.*, 1989). However, further transformations of the epoxytetraenes by various cell types have not been examined in detail. Taken together, results obtained with isolated enzymes and cell-free systems (Table I) have illustrated the existence of multi-potential routes for lipoxin generation, some of which may prove to be utilized by specific cellular systems. These routes and their relationship to leukotriene biosynthesis are illustrated in Fig. 2. The existence of multiple pathways makes it essential to characterize the pathway of LX biosynthesis utilized in the particular cell systems under investigation.

## Cellular Origins of Lipoxins: Role of Cell–Cell Interactions

Cell types from several species have now been shown to generate lipoxins from either endogenous and/or exogenous sources of C20 : 4 (Table II). The first reports of lipoxin formation from endogenous sources of either C20 : 4 or eicosapentaenoic acid were presented by Wong *et al.* (1986). Lam *et al.* (1987), utilizing an isolated $PLA_2$ isoenzyme added to porcine leukocytes, demonstrated the formation of $LXB_4$ and its isomers, while eosinophil-rich granulocyte suspensions from humans generate $LXA_4$, which correlated with the number of eosinophils present (Serhan *et al.*, 1987b). The biosynthetic route for $LXA_4$ formation from endogenous sources could not be concluded from the results in this study because of the limited amounts of cells; that is, was $LXA_4$ produced exclusively from C20 : 4 released and processed within a single cell? Upon activation of eosinophil-enriched cell suspensions, suitable substrates may have been mobilized by one cell type and transformed to $LXA_4$ by another (Serhan *et al.*, 1987b). The 5(6)-epoxytetraene, $LTA_4$, or 15-HETE are each potential candidates and hence may shuttle between individual cells for further processing. Along these lines, we have previously documented that interactions between human platelets and neutrophils result in the transcellular metabolism of both lipoxygenase products and native platelet-derived arachidonic acid (Marcus *et al.*, 1982). During the past several years, the role of cell–cell interactions in the biosynthesis of eicosanoids and other putative inflammatory mediators has captured considerable interest. Edenius *et al.* (1988) have recently reported that mixed platelet–granulocyte suspensions stimulated with the ionophore A23187 generate $LXA_4$, as well as four other tetraene-containing compounds, from endogenous sources. They also showed that $LTA_4$ is converted to $LXA_4$ by platelets and suggested that the reported 15-LO activity of platelets may be involved in this enzymatic conversion. Garrick *et al.* (1989) found that mesangial cells from rat kidney can also utilize $LTA_4$ to form lipoxins, providing additional

**Table II.** Cells That Generate Lipoxins

| Cell type | Substrate | Stimulus | References |
|---|---|---|---|
| Human leukocytes | 15-HPETE | ± Ionophore A23187 | Serhan *et al.*, 1984a,b |
| Human leukocytes | 15-HPETE | Ionophore A23187 | Fitzsimmons *et al.*, 1985 |
| Human leukocytes | 15-HETE | FMLP; ionophore A23187 | Serhan *et al.*, 1986a |
| Human eosinophil-rich granulocyte suspension | C20:4, endogenous C20:4 | Ionophore A23187 | Serhan *et al.*, 1987b |
| Porcine leukocytes | Endogenous C20:4 | $PLA_2$ isoenzyme | Lam *et al.*, 1987 |
| Bovine leukocytes | C20:4, 15-HPETE, 15-HETE, 5-HETE, 5-HPETE | Ionophore A23187 | Walstra *et al.*, 1988 |
| Rat alveolar macrophages | 15-HPETE | Ionophore A23187 | Kim, 1988 |
| Rat basophilic leukemia cells (RBL-1) | 15-HPETE | PMA, FMLP, ionophore A23187 | Ng *et al.*, 1989 |
| Canine mastocytoma cells | C20:4 | Ionophore A23187 | Lazarus and Zocca, 1988 |
| Human neutrophils | Endogenous C20:4 | Ionophore A23187 | Serhan, 1989 |
| Human platelets | $LTA_4$ | ± Ionophore A23187, stimulation | Edenius *et al.*, 1988 |
| Human platelets | $LTA_4$ | ± Ionophore A23187, thrombin | Serhan *et al.*, 1990 |
| Rat kidney mesangial cells | $LTA_4$ | None required | Garrick *et al.*, 1989 |
| Human eosinophils | 15-HETE | Ionophore A23187 | Steinhilber and Roth, 1989 |
| Porcine leukocytes | 15-HPEPE | ± Ionophore A23187 | Wong *et al.*, 1986<br>Lam *et al.*, 1987 |
| *Cell–cell interactions* | | | |
| Human granulocyte–eosinophil | Endogenous C20:4 | Ionophore A23187 | Serhan *et al.*, 1987b |
| Human platelet–granulocyte | Endogenous C20:4 | Ionophore A23187 | Edenius *et al.*, 1988 |
| Human neutrophil–platelet | Endogenous C20:4 | FMLP, thrombin | Serhan and Sheppard, 1990 |

evidence to support the hypothesis that cell–cell interactions play a pivotal role in lipoxin biosynthesis.

Although ionophore-stimulated cells can generate lipoxins without addition of exogenous substrate (Edenius *et al.*, 1988; Serhan, 1989; Serhan *et al.*, 1987b), it remained to be determined if receptor occupancy could lead to their formation. Recent results from this laboratory indicate that simultaneous activation of human neutrophils and platelets by receptor-mediated agonists (FMLP and thrombin) leads to formation of both $LXA_4$ and $LXB_4$ (Serhan and Sheppard, 1990). In these studies, $LTA_4$ was transformed to $LXA_4$ as well as the recently identified 7-*cis*-11-*trans*-$LXA_4$ (Nicolaou *et al.*, 1989) by cell-free 100,000 *g* supernatants of human platelets, which displayed 12-LO activity. Taken together, these results indicate that lipoxins can also be formed by initial oxygenation by the 5-LO followed by transcellular metabolism of $LTA_4$. This new route of lipoxin formation is of particular interest, since results with human neutrophils indicate that, when these cells utilize 15-HETE to generate lipoxins, an inverse relationship is observed between leukotriene and lipoxin generation (Serhan, 1989).

Canine mastocytoma cells in culture can generate lipoxins, suggesting that mast cells are also a source of lipoxins (Lazarus and Zocca, 1988). In nonhuman cell types, preliminary evidence has been obtained for triple lipoxygenation biosynthesis to generate lipoxins with intact bovine leukocytes (Walstra *et al.*, 1988). Unlike human neutrophils, which possess both 5- and 15-LO activities, bovine leukocytes also display 12-LO activity. As a result, bovine leukocytes can generate lipoxins by utilizing either C20:4, 15-HETE, 5,15-DHETE, 5-HPETE, or 5-HETE. Steinhilber and Roth (1989) isolated peptido-containing lipoxins from human eosinophils. These new additions to the lipoxin series appear to be generated via conjugation of a 5(6)-epoxytetraene intermediate. The biological role and significance of these new lipoxins is currently being explored.

## Intra- and Intercellular Actions of Lipoxins

Since the lipoxins were found to be LO products, initial studies evaluating their biological actions focused on their comparative responses with respect to other LO products including leukotrienes. Results from these studies quickly revealed that the actions of lipoxin $A_4$ and lipoxin $B_4$ are distinct from those of other eicosanoids (Tables III–V). In each system, including results from both *in vivo* and *in vitro* evaluations, the responses evoked by either $LXA_4$ or $LXB_4$ have proven to be stereospecific, and in some systems responses are observed in the nanomolar concentration range. In general, the magnitude and extent of the responses induced by the lipoxins, like other

**Table III.** Biological Activities of Lipoxins: *in vivo* Models

| Bioassay system | Compounds | Response | Reference |
|---|---|---|---|
| Microcirculation: hamster cheek pouch | $LXA_4$ | Arteriolar dilation without vascular permeability or leukocyte adherence | Dahlén *et al.*, 1987 |
| Systemic and renal hemodynamics in rats | $LXA_4$ | Selective fall in afferent arteriolar resistance, glomerular hyperperfusion, hypertension and hyperfiltration (arteriolar dilation) | Badr *et al.*, 1987 |
| Renal hemodynamics | $LXA_4$ | Antagonizes leukotriene $D_4$-induced fall in glomerular filtration rate but not renal plasma flow (counterregulatory actions) | Badr *et al.*, 1989 |
| Cerebral arterioles of newborn pigs | $LXA_4$ and $LXB_4$ | Stimulate dilation of cerebral arterioles not prostanoid mediated | Busija *et al.*, 1989 |
| Mesenteric circulation in conscious rats | $LXA_4$ and $LXB_4$ | I.V. administration constricts mesenteric vessels | Feuerstein and Siren, 1988 |
| Hamster cheek pouch | $LXA_4$ | Inhibits leukotriene $B_4$-induced inflammation; $LXA_4$ blocks plasma leakage and leukocyte migration | Hedqvist *et al.*, 1989 |
| Mouse hematopoietic stem cells | $LXB_4$, but not $LXA_4$ | Radioprotective actions without diarrhea or ataxia | Walden, 1988 |

eicosanoids, appear to be both species- and cell type-dependent (Tables III and IV). In several *in vivo* models (Table III), $LXA_4$ and $LXB_4$ each stimulate changes in microcirculation (Badr *et al.*, 1987, 1989; Busija *et al.*, 1989; Dahlén *et al.*, 1987; Feuerstein and Siren, 1988; Hedqvist *et al.*, 1989). At submicromolar concentrations, $LXA_4$ induces rapid arteriolar dilation in both the hamster cheek pouch and renal hemodynamics of the rat (Badr *et al.*, 1987; Dahlén *et al.*, 1987). $LXA_4$ has recently been found to antagonize $LTD_4$-induced vasoconstriction (*in vivo*) and block the binding of $LTD_4$ to its receptors on mesangial cells (in culture). These cells are responsible, in part, for regulating glomerular hemodynamics. Thus, these results suggest that $LXA_4$ may regulate the actions of vasoconstrictor leukotrienes *in vivo* (Badr *et al.*, 1989). $LXA_4$ has also been shown to block $LTB_4$-induced inflammation in the hamster cheek pouch (Hedqvist *et al.*, 1989). In this *in vivo* system, $LXA_4$ inhibited both $LTB_4$-induced plasma leakage and leukocyte migration, which are important components in inflammatory responses.

The actions of lipoxins have now been evaluated by several groups utilizing isolated organ bath systems (Table IV). Unlike the leukotrienes,

**Table IV.** Biological Activities of Lipoxins: Isolated Organ Paths

| Bioassay system | Compounds | Response | Reference |
|---|---|---|---|
| Guinea pig lung strips | Lipoxin $A_4$ | Contracts with slow onset | Serhan *et al.*, 1986b |
| Guinea pig lung strips | Lipoxin $A_4$ | Cyclooxygenase-independent contraction | Dahlén *et al.*, 1987 |
| Guinea pig lung strips | $LXA_4$ geometric isomers | Structure–function relationship with smooth muscle contraction | Dahlén *et al.*, 1989 |
| Guinea pig lung strips | Lipoxin $A_4$ but not $LXB_4$ | Contracts parenchymal strips, and not tracheal spirals; $LXA_4$ contractions were not blocked by a 5-LO inhibitor | Jacques *et al.*, 1988 |
| Guinea pig lung parenchyma and ileum smooth muscle | $LXA_4$ | Contracts lung strips but not ileum smooth muscles in cascade superfusion | Cristol and Sirois, 1988 |
| Rat tail artery | $LXA_4$ | Contracts | Ng *et al.*, 1989 |
| Guinea pig, rabbits, and rat lung strips | $LXA_4$ and $LXB_4$ | Bronchoconstrictors and exert endothelium-dependent vasorelaxation | Lefer *et al.*, 1988 |
| Guinea pig lung | $LXA_4$ | Stimulates thromboxane production | Wikstrom *et al.*, 1989 |

$LXA_4$ contracts guinea pig lung strips but not ileum smooth muscle preparations, which suggests a degree of organ specificity at the receptor level as well (Cristol and Sirois, 1988; Dahlén *et al.*, 1987, 1989; Jacques *et al.*, 1988; Serhan *et al.*, 1986b). Contractions induced by $LXA_4$ are characteristically slow in onset and are not blocked by either cyclooxygenase or LO inhibitors with lung strips (Dahlén *et al.*, 1987), although $LXA_4$ can stimulate thromboxane production in these preparations (Wikström *et al.*, 1989). In addition to bronchoconstrictor activities, both $LXA_4$ and $LXB_4$ can stimulate endothelium-dependent vasorelaxation (Lefer *et al.*, 1988), which may be mediated in part or related to their ability to rapidly stimulate prostacyclin formation and release from cultured human endothelial cells (Brezinski *et al.*, 1989).

Lipoxins also display unique actions on specific cell types *in vitro* (Table V). For example, $LXA_4$ elicits a selective profile of action on human neutrophils which is unlike that of leukotriene $B_4$ (Lee *et al.*, 1989a,b; Luscinskas *et al.*, 1989; Nigam *et al.*, 1990; Palmblad *et al.*, 1987; Serhan and Reardon, 1989; Serhan *et al.*, 1984b). In addition to inducing a modest chemotactic response (Palmblad *et al.*, 1987), $LXA_4$ is also reported to block both $LTB_4$- and FMLP-induced responses (i.e., $IP_3$ generation and $Ca^{2+}$

**Table V.** Biological Activities of Lipoxins: *in vitro* Systems

| Bioassay system | Compounds | Response | Reference |
|---|---|---|---|
| *Isolated cell systems* | | | |
| Human neutrophils | $LXA_4 > LXB_4$ | Chemotaxis without aggregation | Palmblad *et al.*, 1987; Serhan *et al.*, 1984b |
| Human neutrophils | $LXA_4$ | Inhibits leukotriene and FMLP-induced chemotactic responses, $Ca^{2+}$ mobilization and $IP_3$ generation | Lee, 1989; Lee *et al.*, 1989a,b |
| Human neutrophils | $LXA_4$ and $LXB_4$ | Do not inhibit FMLP- or A23187-induced superoxide anion generation | Serhan and Reardon, 1989 |
| Human neutrophils | $LXA_4$ and $LXB_4$ | Stimulate lipid remodeling and release of C20 : 4 without LO activation, or blocking $LTB_4$ binding | Nigam *et al.*, 1990 |
| Human neutrophils | 5(6)-Epoxytetraene | Stimulates mobilization of intracellular $Ca^{2+}$ | Luscinskas *et al.*, 1989 |
| Human endothelial cells | $LXA_4$ and $LXB_4$ | Stimulates prostacyclin generation and release | Brezinski *et al.*, 1989 |
| Rat mesangial cells | $LXA_4$ | Inhibits $LTD_4$-induced $IP_3$ generation and $LTD_4$ binding | Badr *et al.*, 1989 |
| Human mononuclear cells | $LXB_4$ | Stimulates colony formation in diffusion chambers | Popov *et al.*, 1989 |
| Human natural killer cells | $LXA_4$ and $LXB_4$ | Blocks cytoxicity without altering target-cell binding | Ramdstedt *et al.*, 1985, 1987 |
| *Isolated enzymes* | | | |
| Protein kinase C (PKC) human placental-derived | $LXA_4$ | Activates PKC in the presence of $Ca^{2+}$ and selects substrate specificity | Hansson *et al.*, 1986 |
| PKC $\gamma$-subspecies bovine cerebellum | $LXA_4$ | Selective activator | Shearman *et al.*, 1989 |

mobilization) with these cells (Lee *et al.*, 1989a,b). Neither compound, however, can inhibit FMLP- or ionophore-stimulated superoxide anion generation (Serhan and Reardon, 1989). We have recently found that both $LXA_4$ and $LXB_4$ selectively stimulate lipid remodeling with neutrophils and release C20 : 4 without initiating LO activity, extensive $Ca^{2+}$ mobilization, or blocking $LTB_4$ binding to these cells (Nigam *et al.*, 1990). These results

suggest that the lipoxins activate a unique pathway in the stimulus–response coupling sequence in human PMN. Although neither compound elicits $Ca^{2+}$ mobilization alone with neutrophils, one of their precursors, 5(6)-epoxytetraene, can mobilize intracellular $Ca^{2+}$ (Luscinskas *et al.*, 1989), which may play a role in transcellular metabolism of epoxides. $LXA_4$ and $LXB_4$ also display selective actions with NK cells and block cytotoxicity (Ramstedt *et al.*, 1985, 1987). Results indicate that lipoxins also induce some long-term responses. $LXB_4$, for example, stimulate colony formation with human mononuclear cells (Popov *et al.*, 1989) and this lipoxin, but not $LXA_4$, possesses a selective radioprotective effect with hematopoietic stem cells (Walden, 1988).

In addition to its actions on various tissues and hence potential extracellular roles, $LXA_4$ may also have an intracellular role within its cell type of origin before its release to the extracellular milieu (Table V). Since arachidonic acid is released and oxygenated by a wide range of cell types during stimulus–response coupling, we have been interested in determining whether eicosanoids themselves can serve as intracellular messengers (i.e., modulate the actions of enzyme systems within their cells of origin prior to their membrane transport). We therefore examined the action of several classes of LO products and native fatty acids on the activities of isolated protein kinase C as a model system, given the pivotal role of this enzyme and its isoforms in cell activation (Hansson *et al.*, 1986). $LXA_4$ activated the kinase and proved to be more potent than both diacylglyceride (a proposed intracellular signal in the activation of protein kinase C) and native arachidonic acid. Other oxygenated derivatives of arachidonic acid, including leukotriene $B_4$ ($LTB_4$), were without a direct effect in this enzyme. In this isolated enzyme system, $LXB_4$ was found to be approximately 10 times less potent than $LXA_4$ while both 8-*trans*-$LXB_4$ and 14*S*-$LXB_4$ were essentially inactive. Results of these experiments also showed that the substrate specificity of this kinase can be modulated, depending upon the stereochemistry of the activator. These results suggest that lipoxygenase products, in particular $LXA_4$, may serve an intracellular role. Recent evidence from Shearman *et al.* (1989) has extended these observations by providing evidence indicating that the action of $LXA_4$ is specific for the $\gamma$-subspecies of PKC, which is abundant in both brain and spinal cord.

Since $LXA_4$ is found in the bronchoalveolar lavage of patients with pulmonary diseases (Lee *et al.*, 1990), the bioactions of this compound in model systems (reviewed here) may provide some insights into the role it may play during various stages of pathogenesis in pulmonary tissues.

To conclude, lipoxins are generated by several biosynthetic routes, which can include lipoxygenase as well as cell–cell interactions. $LXA_4$ and $LXB_4$ each carry selective biological actions demonstrated in both *in vivo* and *in vitro* systems. Results from several laboratories now provide

a body of evidence to support the notion that lipoxins may be involved in regulating cellular responses of interest in host defense, vascular tone, and inflammation.

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