Biochemical Pharmacology, Vol. 32, No. 3, pp. 381–387, 1983.
Printed in Great Britain.

0006-2952/83/030381-07 $03.00/0

COMMENTARY

NEW CONCEPTS IN THE MODULATION OF LEUKOTRIENE SYNTHESIS

PIERRE BORGEAT*†, BERNARD FRUTEAU DE LACLOS* and JACQUES MACLOUF‡
*Laboratoire d'Endocrinologie Moléculaire, Centre Hospitalier de L'Université Laval, Québec, Canada G1V 4G2, and ‡U150, INSERM, LA 334 CNRS, Hôpital Lariboisière, 75475, Paris Cedex 10, France

The metabolism of arachidonic acid is highly complex and leads to a variety of biologically active compounds, including the prostaglandins (PGs), the thromboxanes (TXs) and hydroxy- or hydroperoxy-eicosatetraenoic acids (HETEs or HPETES) [1]. The latest addition to the series of bioactive metabolites of arachidonic acid are the leukotrienes (LTs). LTs were initially discovered as the major products of the oxidative metabolism of arachidonic acid in rabbit peritoneal polymorphonuclear leukocytes (PMNL) [2, 3] and human blood PMNL [4]. Figure 1 shows the structures and the mechanism of biosynthesis of LTs with recognized biological activities. Another family of LTs derived from the C-15 lipoxygenase has been described recently [5, 6].

The biosynthesis of LTs is initiated in a lipoxygenase-type reaction leading to the 5S-hydroperoxy-6,8,11,14-(E,Z,Z,Z)-eicosatetraenoic acid (5S-HPETE), which is, in turn, transformed into leukotriene A_4 (LTA_4), an unstable allylic epoxide, the key compound in the LT pathway. LTA_4 is the precursor of leukotriene B_4 (LTB_4) and of leukotriene C_4 (LTC_4). Peptidases transform LTC_4 into leukotrienes D_4 and E_4 (LTD_4 and LTE_4) (see Ref. 7 for a review).

† Author to whom correspondence should be sent.

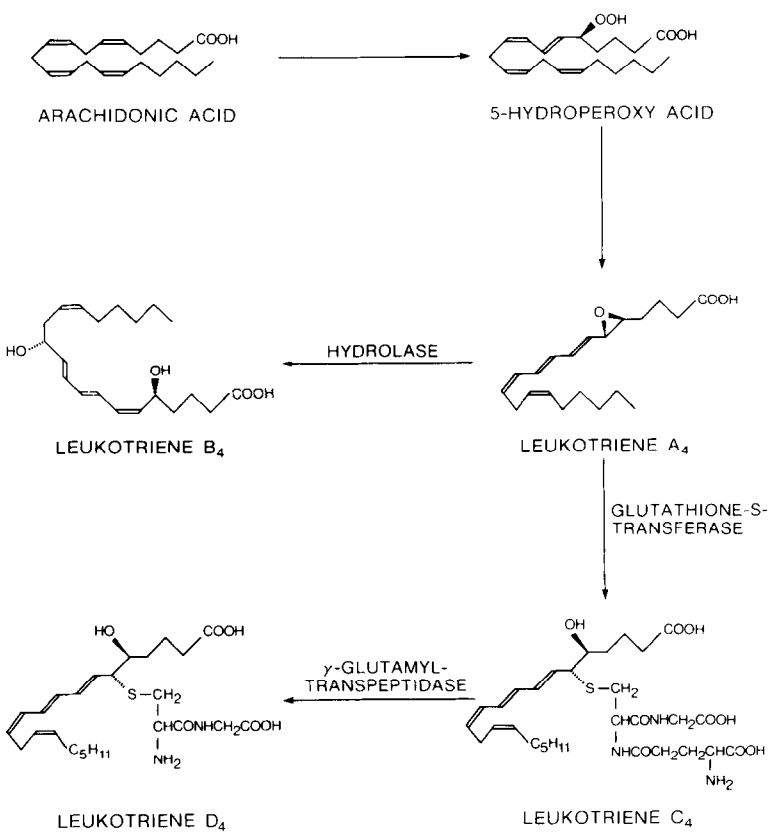

Fig. 1. Structures and biosynthesis of LTs. The chemical synthesis of the compounds depicted above has been performed; this allowed the determination of the detailed stereochemistry of the LTs and confirmed the sequence of biochemical events as presented in this scheme.

The elucidation of the LT pathway and the concomitant identification of the bioactive components of the slow-reacting substance of anaphylaxis (SRS-A) and other SRS as mixtures of LTC_4 and/or LTD_4 and LTE_4 appear of major interest in the biochemistry and pharmacology of allergy and inflammation (see Ref. 8 for a review). Several years ago, SRS-A was proposed as an important mediator of the bronchospasm associated with anaphylactic reactions [9]. Recent studies with synthetic LTs support this hypothesis. Indeed, it has been shown that LTs are potent myotropic substances and cause the contraction of human and guinea pig respiratory tract smooth muscles. For instance, LTD_4 was found to be several orders of magnitude more potent (on a molar basis) than histamine or $PGF_{2\alpha}$ on the guinea pig lung parenchymal strip. LTs show some tissue selectivity, being more active on the peripheral airways [10, 11]. Interestingly, the LTs have little activity on rat and rabbit smooth muscles preparations [12]. Other studies have indicated vascular effects of LTs [13]. LTC_4, D_4 and E_4 are also active *in vivo* and cause bronchoconstrictions and cardiovascular effects [14]. In addition, LTB_4 shows several proinflammatory properties; indeed, the compound stimulates leukocyte aggregation and adhesion to vascular endothelium [15], is a potent chemotactic and chemokinetic agent towards PMNL, monocytes and macrophages [16, 17], alters Ca^{2+} homeostasis in PMNL [18], releases lysosomal enzymes [19], and increases vascular permeability [20].

The data already accumulated on the biological properties of LTs, together with the finding that LTs are released by lungs and leukocytes by allergic and inflammatory stimuli [21], clearly support the hypothesis that these compounds may be involved in immediate hypersensitivity reactions, for instance asthma, as mediators of the bronchospasm, mucosal oedema and leukocyte accumulation, as well as in non-immunological inflammatory reactions. Thus, the discovery of LTs and the elucidation of their mechanism of formation brought some hope for major progress in the pharmacology of allergy and inflammation. However, the rational development of new therapeutic approaches based on these recent findings requires a better understanding of the mechanisms involved in the modulation of LT biosynthesis in health and disease.

THE BIOSYNTHESIS OF LEUKOTRIENES

The successive findings in the past few years of several metabolic pathways of arachidonic acid in mammalian tissues have raised questions about the control of the synthesis of the various metabolites. The problem is particularly important in view of the potent biological properties of the various compounds formed and because of the presence of up to three different pathways in a single cell type (the human PMNL contains cyclooxygenase and C-5 and C-15 lipoxygenases; see Refs. 2, 22 and 23).

The concept that the formation of the various arachidonic acid metabolites is dependent on substrate availability is well accepted [24], and the addition of arachidonic acid to cells and tissues *in vitro* usually results in the rapid synthesis of PGs, TXs and hydroxy acids. The concentration of free arachidonic acid in cells under normal conditions is low as compared to the total amount of the fatty acid present in the form of esters. The formation of cyclooxygenase and lipoxygenase products is thus initially controlled by the activity of the various lipases which make arachidonic acid available to metabolizing enzymes [24]. The effects of the divalent cation ionophore A23187, which unspecifically stimulates the release of arachidonic acid (likely through stimulation of Ca^{2+}-dependent phospholipase A_2) and the synthesis of cyclooxygenase and lipoxygenase products in a variety of systems, support this concept [25, 26].

Concerning more specifically the C-5 lipoxygenase, this scheme must be somewhat modified. The synthesis of 5-HETE and LTs also depends on substrate availability; addition of arachidonic acid to a suspension of rabbit peritoneal PMNL (glycogen-induced) results in the formation of 5-HETE and LTB_4 [2, 3]. However, in other systems, such as in human blood PMNL, the C-5 lipoxygenase will not readily, or only to a small extent, transform exogenous arachidonic acid, whereas incubation of these cells in the presence of the ionophore A23187 leads to synthesis of substantial amounts of 5-HETE and LTB_4 from endogenous arachidonic acid [4]. These data indicated that the ionophore not only caused the release of arachidonic acid but also activated the C-5 lipoxygenase involved in the further transformation of the fatty acid, suggesting that this enzyme was Ca^{2+} dependent [4]. This stimulatory effect of the ionophore A23187 on the C-5 lipoxygenase was indirectly reported in previous studies on SRS-A synthesis [27]. Since then the Ca^{2+} requirement for the activation of LT synthesis has been supported in studies involving a cell-free system from rat basophilic leukemia cells (RBL-1) [28].

These considerations leave open the question of the control of the transformation of arachidonic acid through the multiple pathways in a system such as PMNL, since a single stimulus that alters Ca^{2+} homeostasis might unspecifically trigger the release and the metabolism of the fatty acid. Recent studies, however, suggested that given stimuli could induce Ca^{2+} influx (or delocalisation) in discrete areas of the cells causing the formation of selected metabolites of arachidonic acid. For instance, it has been observed that zymosan and the Ca^{2+} ionophore stimulate the formation of both cyclooxygenase and C-5 lipoxygenase products in mouse peritoneal macrophages, whereas phorbolmyristate acetate and lipopolysaccharides specifically induce PGE_2 synthesis, suggesting the presence of independent sources of substrates in these cells [29].

Other interesting aspects in the control of arachidonic acid metabolism concern interactions between the various pathways. It is now well documented that 15-HPETE is a potent inhibitor of PGI_2 synthesis [30]; more recently, 15-HETE was shown to inhibit the platelet C-12 lipoxygenase [31] as well as the leukocyte C-5 lipoxygenase [32]. In addition, LTs stimulate the synthesis of cyclooxygenase products in leukocytes [33] and lungs [34, 35]. These data clearly point out the occurrence of inhibitory and stimulatory interactions between the various dioxy-

genases. In the next paragraphs, we should like to summarize and discuss some recent data on lipoxygenase interactions which might be of major importance for the modulation of LT biosynthesis.

Double dioxygenation of arachidonic acid by lipoxygenases

The discovery of metabolites of arachidonic acid formed by successive dioxygenation brought direct evidence of interactions between lipoxygenases and lipoxygenase products. We have recently reported the synthesis of a stereoisomer of LTB_4 in porcine blood leukocytes and human blood leukocytes [36]. The compound was isolated and identified by gas chromatography–mass spectrometry (GC–MS), ultraviolet spectrometry, and steric analysis as the 5S,12S-dihydroxy-6,8,10,14(E,Z,E,Z)-eicosatetraenoic acid (5S,12S-DiHETE). Studies on its mechanism of biosynthesis, involving $^{18}O_2$ labeling experiments, indicated that the 5S,12S-DiHETE was the product of a double dioxygenation of arachidonic acid, as opposed to LTB_4 and its Δ^6-*trans* isomers which are derived from hydrolysis of LTA_4 [37, 38]. The mechanism of biosynthesis was confirmed in experiments where leukocytes incubated with 12-HETE, and platelets incubated with 5-HETE, released the 5S,12S-DiHETE. We also showed that direct addition of LTA_4 to leukocyte suspensions did not lead to detectable formation of 5S,12S-DiHETE, whereas LTB_4 and its Δ^6-*trans* isomers were produced in substantial amounts. The acetylenic acid inhibitor of cyclooxygenase, C-12 and C-15 lipoxygenases, 5,8,11,14-eicosatetraynoic acid (ETYA), inhibited the formation of the 5S,12S-DiHETE (but not of other C-5 lipoxygenase products), in agreement with a role for the C-12 lipoxygenase in the formation of this compound [37]. In human leukocyte suspensions, the synthesis of the 5S,12S-DiHETE was dependent on the presence of contaminating platelets as the source of C-12 lipoxygenase activity. In porcine leukocytes, the 5S-12S-DiHETE was produced by the actions of endogenous C-5- and C-12 lipoxygenases on arachidonic acid (porcine platelets have very low C-12 lipoxygenase activity). Rabbit peritoneal PMNL, as well as human blood PMNL, contain C-5 and C-15 lipoxygenase activities [3, 4, 23]; the 5-HETE and 15-HETE both fulfill the structural requirements for reaction with lipoxygenases, suggesting the possible occurrence of a 5,15-DiHETE in leukocytes. Careful analysis of the products formed upon incubation of porcine blood leukocytes with arachidonic acid and the ionophore A23187 led to the detection of an unknown metabolite which was identified as a 5,15-DiHETE by GC–MS and ultraviolet spectrometry analysis. An identical compound [as judged by GC–MS, ultraviolet spectrometry and high performance liquid chromatography (HPLC) analysis] was obtained upon incubation of human blood leukocytes with 15-HETE or 15-HPETE, suggesting that the compound could be formed by successive dioxygenations by C-15- and C-5 lipoxygenases, and was probably the 5S,15S-dihydroxy-6,8,11,13(E,Z,Z,E)-eicosatetraenoic acid (5S,15S-DiHETE, unpublished data; the 5S,15S-DiHETE was also reported by other workers, see Ref. 6). These data constituted the first observations of the transformations of HETEs into DiHETEs and raised the question of the biological significance of these lipoxygenase interactions. Figure 2 shows the products of the double dioxygenation of arachidonic acid in leukocytes.

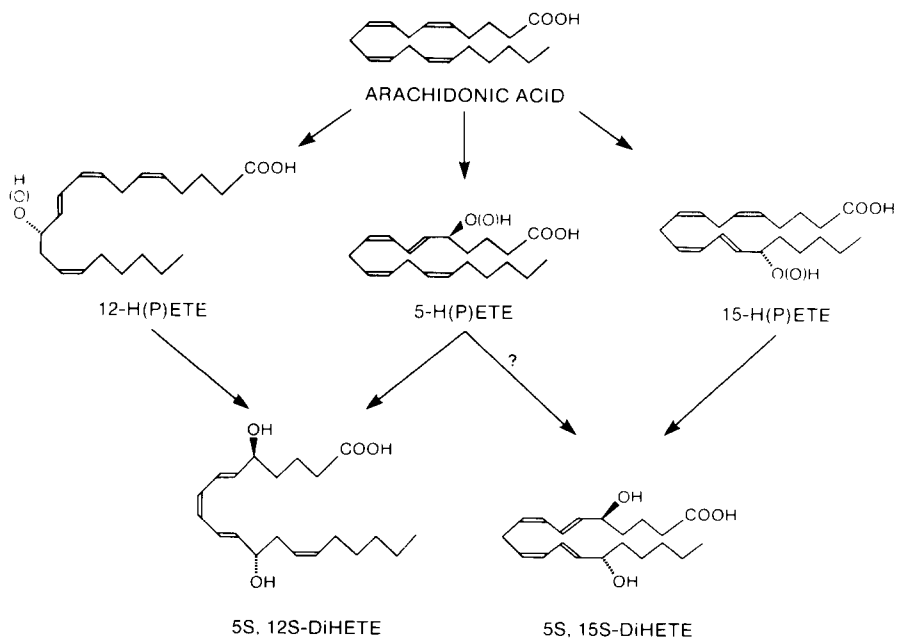

Fig. 2. Pathways of the double dioxygenation of arachidonic acid in leukocytes. C-5 and C-15 lipoxygenase activities are present in human leukocytes; however, C-12 lipoxygenase has not been reported in these cells. Therefore, the synthesis of 5S,12S-DiHETE most likely involves leukocytes and platelets.

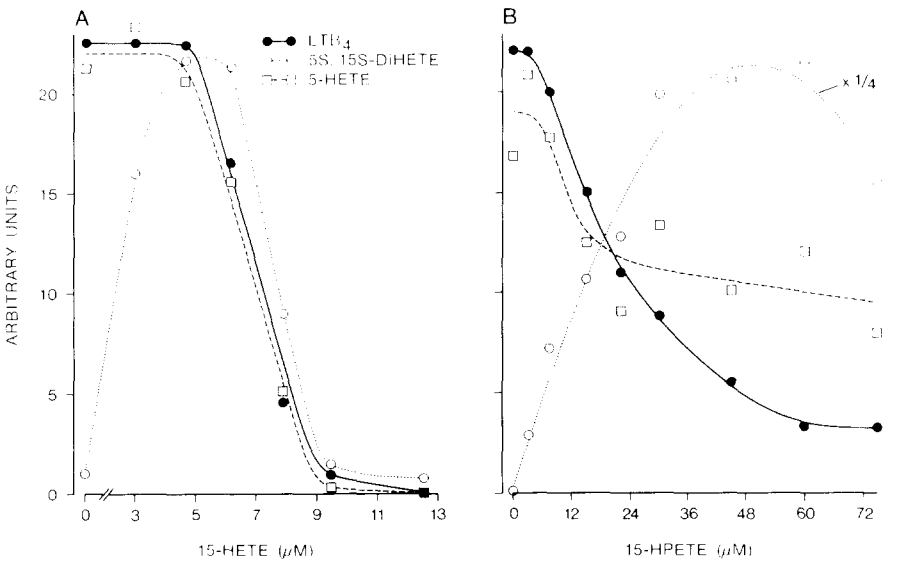

Fig. 3. Effect of increasing concentrations of 15-HETE (A) and 15-HPETE (B) on the synthesis of C-5 lipoxygenase products by human leukocytes. The cells were obtained from EDTA-treated blood by dextran sedimentation and ammonium chloride lysis for elimination of red cells. The mononuclear cells and PMNL were not separated. The leukocyte suspensions (60×10^6 cells/2 ml of Dulbecco's phosphate-buffered saline) were incubated for 5 min at 37° in the presence of the ionophore A23187 (2 μM) and of increasing concentrations of 15-HETE or 15-HPETE, which were added 15–30 sec before the ionophore. The samples were analyzed by adsorption HPLC and ultraviolet spectrophotometry using an internal standard. Results are expressed in arbitrary units; in the sample incubated with 7.5 μM 15-HPETE, the amounts (approximate) of metabolites formed, as measured by comparison of peak areas (corrected for differences in attenuation settings and absorption coefficients) were as follows: LTB_4, 0.9 μg; Δ^6-*trans* LTB_4 and Δ^6-*trans*-12-epi-LTB_4 (not shown), 0.1 μg each; 5,15-DiHETE, 4 μg; and 5-HETE, 2.5 μg. As indicated, the magnitude of the 5S,15S-DiHETE curve in part B has been reduced 4-fold.

Inhibitory effect of HETEs on the C-5 lipoxygenase

In the course of our initial studies on the mechanism of formation of the 5S,12S-DiHETE, we observed that the synthesis of the C-5 lipoxygenase products in human blood leukocytes was progressively inhibited by increasing concentrations of 12-HETE (50% inhibition at about 30 μM), whereas in the same experiments the formation of the 5S,12S-DiHETE increased up to concentrations of 12-HETE of 30–45 μM and decreased at higher concentrations [37]. These data led us to speculate on the role of the platelet C-12 lipoxygenase in the control of LTs synthesis [39]. Other workers reported that 15-HETE inhibited the activity of the C-5 lipoxygenase in rabbit peritoneal PMNL (50% inhibition at 6 μM) and that addition of arachidonic acid to these cells led to the formation of inhibitory concentrations of the 15-HETE, in agreement with a regulatory role of 15-HETE on the activity of the C-5 lipoxygenase [32]. Figure 3A shows the effect of 15-HETE on human blood leukocytes activated with the ionophore A23187. The 15-HETE caused a rapid inhibition of the formation of 5-HETE and LTB_4 (50% inhibition at 7 μM), whereas the synthesis of the 5S,15S-DiHETE increased up to concentrations of 15-HETE of 4–6 μM and decreased rapidly at higher concentrations. The observations that two hydroxy acids (12-HETE and 15-HETE) with inhibitory effects on the C-5 lipoxygenase were also substrates for the enzyme were suggestive that the metabolism of the HETEs was related to their inhibitory action. However, as seen in Fig. 3A, the maximal formation of the 5S,15S-DiHETE occurred at a 15-HETE concentration that did not inhibit the synthesis of 5-HETE or LTB_4. In other experiments, we have found that 15S-hydroxy-8,11,13(Z,Z,E)-eicosatrienoic acid was as good an inhibitor of the C-5 lipoxygenase as 15-HETE although it did not react with the enzyme, indicating that there is no clear relationship between the inhibitory action of HETEs and the formation of corresponding DiHETEs. A comparison of Fig. 3A and 3B indicates that the effects of 15-HETE and 15-HPETE on the C-5 lipoxygenase differ considerably. The amount of 5S,15S-DiHETE formed from 15-HPETE was four times that obtained from 15-HETE. The difference between the inhibitory effects of 15-HETE and 15-HPETE (see the concentration scales in Fig. 3A and 3B) is striking in view of the expected rapid reduction of the 15-HPETE into 15-HETE in leukocytes; these results clearly suggest that the inhibition of the C-5 lipoxygenase by the 15-HETE generated upon addition of the 15-HPETE is reduced significantly by an opposing stimulatory effect of the hydroperoxide.

Stimulatory effect of platelets on the C-5 lipoxygenase

We consistently observed that human leukocytes do not efficiently metabolize exogenous arachidonic acid, the formation of 5-HETE and LTB_4 being less

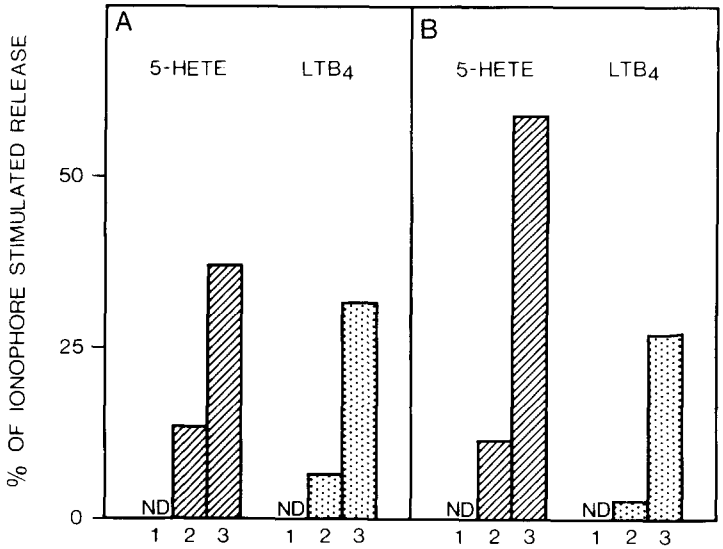

Fig. 4. Effect of platelet–leukocyte coincubation on the synthesis of 5-HETE and LTB_4. Human blood leukocytes (mixed cells) were prepared as described in the legend to Fig. 3. Platelets were obtained by centrifugation (650 g × 15 min) of the platelet rich-plasma from the same blood sample. The cells were incubated for 5 min at 37° in Dulbecco's phosphate-buffered saline. The products were measured by HPLC as described in the legend to Fig. 3. A and B refer to experiments performed using cells from two different donors. Key: (1) incubation of platelets (250 × 10^6/ml) with arachidonic acid (60 μM): the C-5 lipoxygenase products were not detectable (ND); (2) incubation of leukocytes (10 × 10^6/ml) with arachidonic acid, the platelet–leukocyte ratio being about 2:1; and (3) coincubation of leukocytes (10 × 10^6/ml) with platelets, the platelet–leukocyte ratio being about 25:1. Results are expressed as the percentage of the ionophore A23187 (2 μM) stimulated release.

than 15% of that obtained from cells stimulated with the ionophore A23187 (no arachidonic acid added). We also observed that platelets alone did not produce 5-HETE or LTB_4 upon addition of arachidonic acid. However, the mixed suspensions of leukocytes and platelets did produce substantial amounts of the C-5 lipoxygenase metabolites [40]. Figure 4 shows the results obtained with leukocytes and platelets from two human subjects. The coincubation of leukocytes and platelets with arachidonic acid caused, respectively, 3- to 5-fold and 4- to 8-fold increases in 5-HETE and LTB_4 formation compared with the amounts of these compounds obtained upon incubation of leukocytes alone with the fatty acid. The use of inhibitors (indomethacin and ETYA) indicated that a metabolite of the oxidative metabolism of arachidonic acid in platelets, most likely a product of the C-12 lipoxygenase pathway, was the mediator of this effect on 5-HETE and LTB_4 synthesis. Time–course studies showed that, in a 5-min preincubation period of the platelets with arachidonic acid, the stimulatory effect on leukocyte oxidative metabolism was lost, suggesting the involvement of the unstable 12-HPETE. The direct addition of the 12-HPETE to leukocyte suspensions confirmed this hypothesis. Indeed, 12-HPETE was found to stimulate (maximal effect at 5 μM) the synthesis of 5-HETE, LTB_4 and 5S,12S-DiHETE to levels equal to, or higher than, those obtained upon stimulation of the cells with the ionophore A23187; the corresponding 12-HETE had no effect under similar conditions [40].

The specificity and mechanism of the effect of the 12-HPETE are presently unknown; experiments such as those shown in Fig. 3A and 3B, however, indicate that hydroperoxy fatty acids other than 12-HPETE (including PGG_2) show some stimulatory activity on the C-5 lipoxygenase.

DISCUSSION AND COMMENTS

It has become obvious that interactions between the various dioxygenases involved in the metabolism of arachidonic acid occur and may have important biological significance. Thus, in addition to the inhibitory effect of 15-HPETE on PGI_2 synthesis [30], recent studies suggest that generation of hydroperoxy and hydroxy acids may constitute important biochemical mechanisms in the physiological control of the synthesis of LTs. From the data available, it seems most likely that C-15 and C-12 lipoxygenases exert, respectively, inhibitory and stimulatory influences on the synthesis of LTs in leukocytes. Indeed, 15-HETE, which is formed in leukocytes, is a more potent inhibitor of the C-5 lipoxygenase than 12-HETE (50% inhibition at 6 and 30 μM respectively), whereas platelet-derived 12-HPETE is an efficient activator of the enzyme. Vanderhoek and coworkers first suggested a role for the C-15 lipoxygenase in the regulation of the LT pathway [32]; interestingly, the same group also reported that the 15-HETE was even more potent as an inhibitor of the C-12 lipoxygenase (50% inhibition at 0.34 μM) [31]. Thus, our finding of the stimulatory effect of 12-HPETE on the activity of the C-5 lipoxygenase brings additional support to the hypothetical role of 15-HETE in the regulation of the synthesis of LTs. The biological significance of the 5S,12S-DiHETE remains unclear; so far it might be regarded as a product reflecting the stimulatory interaction of 12-HPETE with the

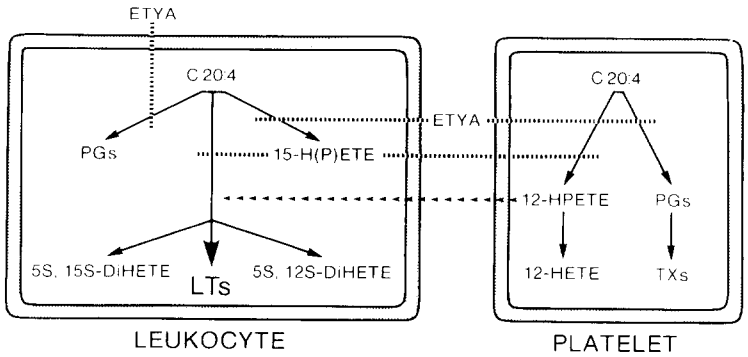

Fig. 5. Hypothetical scheme of the interactions between C-5, C-12 and C-15 lipoxygenases in platelets and leukocytes, in relation to the control of LT synthesis. Key: (▪▪▪▪▪▪▪) inhibitory effect; and (◂◂◂◂◂◂) stimulatory effect.

C-5 lipoxygenase. Figure 5 illustrates the various interactions that may take place between lipoxygenases and directly affect the synthesis of LTs.

It is noteworthy that these findings also suggest an attractive hypothesis for the long elusive biological significance of the platelet lipoxygenase.

Several other hypotheses emerge from the data summarized above. The stimulatory effect of platelet oxidative metabolism on the synthesis of LTs provides a biochemical basis for the role of platelets in the initiation or potentiation of inflammatory and allergic reactions and supports the concept of the possible involvement of platelet–leukocyte interactions in some pathophysiological states. In this regard, one may speculate that, in aspirin-sensitive asthmatics, the administration of the drug causes an increased release of 12-HPETE either by altering the balance between the transformations of arachidonic through the cyclooxygenase and the lipoxygenase (in favor of the last one) as shown *in vitro* [41] or by partial inhibition of the fatty acid hydroperoxide peroxidase associated with the platelet cytosolic C-12 lipoxygenase [42]; the 12-HPETE released might, in turn, activate the C-5 lipoxygenase of circulating leukocytes or lung macrophages with subsequent release of bronchoconstricting LTs.

In addition, it seems possible that the effect of 12-HPETE on LT synthesis is relevant to the postulated role of oxygen-centred radicals in inflammation [43]. Indeed, although the mechanism of action of 12-HPETE is presently unclear, the data available leave no doubt of the importance of the hydroperoxy group (12-HETE is completely inactive). It is thus conceivable that oxygen-centered radicals generated upon reduction of 12-HPETE by leukocyte (or platelet) peroxidases may account for the activation of the C-5 lipoxygenase and increased formation of LTs. This would constitute one out of many explanations for the anti-inflammatory properties of superoxide dismutase [44] and of radical scavengers [45] in some animal models.

Finally, the data summarized in this paper may also bring some light on the controversy concerning the effect of ETYA on the C-5 lipoxygenase. It was reported that ETYA does not block but rather increases the formation of 5-HETE and LTB_4 in rabbit peritoneal PMNL incubated with arachidonic acid [2, 32], and similar results have been obtained using human or porcine blood leukocytes stimulated with the ionophore A23187 [37]; thus, in systems where the C-5 lipoxygenase is active (rabbit peritoneal PMNL), or has been activated (ionophore-treated human blood PMNL), ETYA does not inhibit the formation of C-5 lipoxygenase products. However, ETYA completely inhibits the production of C-5 lipoxygenase metabolites generated upon addition of arachidonic acid to suspensions of human blood leukocytes contaminated with platelets (data not shown); in this case it seems likely that the inhibition of the synthesis of the C-5 lipoxygenase products by ETYA does not reflect a direct inhibition of the enzyme but rather the blockade of the synthesis of the stimulatory 12-HPETE by the platelets. The numerous reports that SRS-A (LTC_4, D_4, E_4) release was inhibited by ETYA [27, 46, 47] could probably be explained by the inhibition of the glutathione transferase involved in the transformation of LTA_4 into LTC_4 [48] rather than by a direct effect on the C-5 lipoxygenase. This newly reported effect of polyacetylenic acids on LTA_4 metabolism, as well as the inhibitory effect of ETYA on the synthesis of 15-HETE [23], may account for the stimulatory effect of ETYA on 5-HETE and LTB_4 formation observed in some systems [2, 32, 37]. It is also noteworthy that LTB_4 and 5S,12S-DiHETE comigrate in some chromatographic systems [36], which might have led to erroneous conclusions concerning the action of ETYA or LTB_4 formation (the synthesis of the 5S,12S-DiHETE is inhibited by ETYA).

These new observations of lipoxygenase interactions certainly suggest explanations to controversial data and raise new hypotheses on the biochemical mechanisms involved in allergy and inflammation. Further studies are required to establish the biological importance of the inhibitory and stimulatory effects of hydroxy and hydroperoxy fatty acids in LT synthesis.

Acknowledgements—The original work mentioned in this paper was supported by grants from the National Cancer Institute of Canada and the Canadian Lung Association to P. B. and by a Fellowship of the Association Claude Bernard to J. M.

REFERENCES

1. B. Samuelsson, S. Hammarström and P. Borgeat, in *Advances in Inflammation Research* (Eds. G. Weissman, B. Samuelsson and R. Paoletti), Vol. 1, p. 405. Raven Press, New York (1979).
2. P. Borgeat, M. Hamberg and B. Samuelsson, *J. biol. Chem.* **251**, 7816 (1976) [Correction: *J. biol. Chem.* **252**, 8772 (1977)].
3. P. Borgeat and P. Samuelsson, *J. biol. Chem.* **254**, 2643 (1979).
4. P. Borgeat and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **76**, 2148 (1979).
5. W. Jubiz, O. Radmark, J. A. Lindgren, C. Malmsten and B. Samuelsson, *Biochem. biophys. Res. Commun.* **99**, 976 (1981).
6. R. L. Maas, A. R. Brash and J. A. Oates, *Proc. natn. Acad. Sci. U.S.A.* **78**, 5523 (1981).
7. P. Borgeat and P. Sirois, *J. med. Chem.* **24**, 121 (1981).
8. P. Sirois and P. Borgeat, *Int. J. Immunopharmac.* **2**, 281 (1980).
9. W. E. Brocklehurst, in *Progress in Allergy* (Eds. P. Kallos and B. H. Waksman), Vol. 6, p. 539. Karger, Basel (1962).
10. P. Hedqvist, S.-E. Dahlén, L. Gustafsson, S. Hammarström and B. Samuelsson, *Acta physiol. scand.* **110**, 331 (1980).
11. P. Sirois, S. Roy, J. P. Tétrault, P. Borgeat, S. Picard and E. J. Corey, *Prost. Med.* **7**, 327 (1981).
12. S.-E. Dahlén, P. Hedqvist, S. Hammarström and B. Samuelsson, *Nature, Lond.* **288**, 484 (1980).
13. J. M. Drazen, K. F. Austen, R. A. Lewis, D. A. Clark, G. Goto, A. Marfat and E. J. Corey, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4354 (1980).
14. P. Schiantarelli, S. Bongrani and G. Folco, *Eur. J. Pharmac.* **73**, 363 (1981).
15. M. A. Bray, A. W. Ford-Hutchinson and M. J. Smith, *Prostaglandins* **22**, 213 (1981).
16. A. W. Ford-Hutchinson, M. A. Bray, M. V. Doig, M. E. Shipley and M. J. H. Smith, *Nature, Lond.* **286**, 264 (1980).
17. P. Bhattacherjee, B. Hammond, J. A. Salmon, R. Stepney and K. E. Eakins, *Eur. J. Pharmac.* **73**, 21 (1981).
18. R. I. Sha'afi, P. H. Naccache, T. F. Molski, P. Borgeat and E. J. Goetzl, *J. cell. Physiol.* **108**, 401 (1981).
19. H. Showell, P. H. Naccache, P. Borgeat, S. Picard, P. Vallerand, E. Becker and R. Sha'afi, *J. Immun.* **128**, 811 (1982).
20. M. A. Bray, F. M. Cunningham, A. W. Ford-Hutchinson and M. J. H. Smith, *Br. J. Pharmac.* **72**, 483 (1981).
21. E. J. Goetzl, *Med. Clins N. Am.* **65**, 809 (1981).
22. R. B. Zurier and D. M. Sayadoff, *Inflammation* **1**, 93 (1975).
23. S. Narumiya, J. A. Salmon, F. H. Cottee, B. C. Weatherley and R. J. Flower, *J. biol. Chem.* **256**, 9583 (1981).
24. P. Needleman, *Biochem. Pharmac.* **27**, 1515 (1978).
25. B. Wentzell and R. M. Epand, *Fedn Eur. Biochem. Soc. Lett.* **86**, 255 (1978).
26. W. C. Pickett, R. L. Jesse and P. H. Cohen, *Biochem. biophys. Acta* **486**, 209 (1977).
27. B. A. Jakschik, A. Falkenhein and C. W. Parker, *Proc. natn. Acad. Sci. U.S.A.* **74**, 4577 (1977).
28. B. A. Jakschik, F. F. Sun, L. Lee-Hauh and M. M. Steinhoff, *Biochem. biophys. Res. Commun.* **95**, 103 (1980).
29. J. L. Humes, S. Sadowski, M. Galavage, M. Goldenberg, E. Subers, R. J. Bonney and F. A. Kuehl, Jr., *J. biol. Chem.* **257**, 1591 (1982).
30. G. J. Dusting, S. Moncada and J. R. Vane, *Prostaglandins* **13**, 3 (1977).
31. J. Y. Vanderhoek, R. W. Bryant and J. M. Bailey, *J. biol. Chem.* **255**, 5996 (1980).
32. J. Y. Vanderhoek, R. W. Bryant and J. M. Bailey, *J. biol. Chem.* **255**, 10064 (1980).
33. N. Feuerstein, M. Foegh and P. W. Ramwell, *Br. J. Pharmac.* **72**, 389 (1981).
34. P. Sirois, S. Roy, P. Borgeat, S. Picard and P. Vallerand, *Prost. Leukotr. Med.* **8**, 157 (1982).
35. G. Folco, G. Hansson and E. Granström, *Biochem. Pharmac.* **30**, 2491 (1981).
36. P. Borgeat, S. Picard, P. Vallerand and P. Sirois, *Prost. Med.* **6**, 557 (1981).
37. P. Borgeat, B. Fruteau de Laclos, S. Picard, J. Drapeau, P. Vallerand and E. J. Corey, *Prostaglandins* **23**, 713 (1982).
38. J. A. Lindgren, G. Hansson and B. Samuelsson, *Fedn Eur. Biochem. Soc. Lett.* **128**, 329 (1981).
39. P. Borgeat, B. Fruteau de Laclos, S. Picard, P. Vallerand and P. Sirois, in *Leukotrienes and Other Lipoxygenase Products* (Eds. B. Samuelsson and R. Paoletti), Vol. 9, p. 45. Raven Press, New York (1982).
40. J. Maclouf, B. Fruteau de Laclos and P. Borgeat, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6042 (1982).
41. M. Hamberg and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3400 (1974).
42. M. I. Siegel, R. T. McConnell, N. A. Porter and P. Cuatrecasas, *Proc. natn. Acad. Sci. U.S.A.* **77**, 308 (1980).
43. F. A. Kuehl, Jr. and R. W. Egan, *Science* **210**, 978 (1980).
44. I. Fridovich, *A. Rev. Biochem.* **44**, 147 (1975).
45. F. A. Kuehl, Jr., J. L. Humes, M. L. Torchiana, E. A. Ham and R. E. W. Egan, in *Advances in Inflammation Research* (Eds. G. Weissman, B. Samuelsson and R. Paoletti), Vol. 1, p. 419. Raven Press, New York (1979).
46. S. Watanabe-Kohno and C. W. Parker, *J. Immun.* **125**, 946 (1980).
47. N. A. M. Paterson, J. F. Burka and I. D. Craig, *J. Allergy clin. Immun.* **67**, 426 (1981).
48. B. A. Jakschik, D. M. DiSantis, S. K. Sankarappa and H. Sprecher, *Biochem. biophys. Res. Commun.* **102**, 624 (1981).