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Stimulation of leukotriene biosynthesis in human blood leukocytes by platelet-derived 12-hydroperoxy-icosatetraenoic acid

(arachidonic acid/lipoxygenase/prostaglandins/inflammation)

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ABSTRACT **Addition of arachidonic acid to suspensions of human blood leukocytes induces the synthesis of small amounts only of the C-5 lipoxygenase products as demonstrated by HPLC. However, the coincubation of blood platelets with the leukocytes always resulted in an activation of the C-5 lipoxygenase and formation of (5*S*)-5-hydroxy-6,8,11,14-icosatetraenoic acid, (5*S*,12*S*)-5,12-dihydroxy-6,8,10,14-icosatetraenoic acid, and leukotriene B_4 from exogenous arachidonic acid. It was found that the activation of arachidonic acid metabolism in leukocytes was caused by a labile compound because the synthesis of the C-5 lipoxygenase products did not occur when platelets were preincubated for 1 min or more with the substrate prior to the addition of the leukocytes. The use of cyclooxygenase inhibitors did not suppress the activation of the leukocytes by the platelets. However, the addition of 5,8,11,14-icosatetraynoic acid, an inhibitor of cyclooxygenase and C-12 and C-15 lipoxygenases, completely suppressed the formation of leukotrienes, although this substance is not an inhibitor of the C-5 lipoxygenase in human leukocytes. This indicated that a product of the C-12 lipoxygenase was likely the mediator of the stimulatory effect of platelets on leukocyte arachidonic acid metabolism. The finding that the direct addition of (12*S*)-12-hydroperoxy-5,8,10,14-icosatetraenoic acid, but not of the corresponding hydroxy derivative, could activate the leukocyte's C-5 lipoxygenase confirmed this hypothesis. These data demonstrate that an interaction between C-12 and C-5 lipoxygenases can promote the formation of leukotrienes and support the possibility of a cooperation between platelets and leukocytes in inflammation and hypersensitivity reactions. Furthermore, the finding provides a new interest for the platelet C-12 lipoxygenase.**

The existence of a mammalian lipoxygenase was first demonstrated in human platelets, where arachidonic acid is converted to (12*S*)-12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE) and subsequently to the corresponding (12*S*)-12-hydroxy acid (12-HETE) (1). More recently, the discovery of leukotrienes (LTs) (2–4), a new family of bioactive metabolites formed in a lipoxygenase-type reaction (involving the C-5 of arachidonic acid), and the detection of a C-15 lipoxygenase in leukocytes (5, 6) have indicated the existence of several lipoxygenases in blood cells. In addition to differences in positional specificities, the heterogeneity of these enzymes is further emphasized by different reactivities. For instance, it has been shown that, in contrast to platelet suspensions which efficiently transform exogenous arachidonic acid into 12-HETE (and cyclooxygenase products), the addition of the fatty acid to preparations of human polymorphonuclear leukocytes (PMNL) was followed by only minor transformation of the substrate (5); however, the metabolism of arachidonic acid was strongly enhanced when leukocytes were incubated in the presence of arachidonic acid and of the divalent cation ionophore A23187, indicating clearly that the human PMNL C-5 lipoxygenase required activation for 5-HETE and leukotriene synthesis. Recent studies suggest the involvement of LTs in hypersensitivity reactions and inflammation (7, 8), pointing out the importance of a better understanding of the biochemical process involved in the control of the biosynthesis of these bioactive substances

We have isolated a new metabolite of arachidonic acid from leukocytes—namely, the (5*S*,12*S*)-5,12-dihydroxy-(*E*,*Z*,*E*,*Z*)-6,8,10,14-icosatetraenoic acid (5*S*,12*S*-diHETE), a stereoisomer of leukotriene B_4 (LTB_4) (9, 10). We have shown that this compound is formed upon addition of 12-HETE to leukocytes stimulated with the ionophore A23187, suggesting the occurrence of biochemical interactions between platelets and leukocytes.

We report here additional results on the interaction between platelet and leukocyte lipoxygenases and present evidence that the platelet-derived 12-HPETE stimulates the synthesis of LTs in human blood leukocytes.

MATERIALS AND METHODS

The ionophore A23187 was purchased from Calbiochem. Arachidonic acid (>99%, Sigma) was purified by silicic acid chromatography before use. The various standards used for HPLC analysis—i.e., 5-HETE, LTB_4, 5*S*,12*S*-diHETE, Δ^6-*trans*-LTB_4, and Δ^6-*trans*-12-epi-LTB_4—have been prepared by incubation of porcine blood leukocytes with arachidonic acid and the ionophore A23187 (9, 11) and purified by HPLC; their identity was confirmed by gas chromatography/mass spectrometry. The 5-hydroxy-12-(2-hydroxy)ethoxy-6,8,10,14-icosatetraenoic acid was prepared by treatment of (5*S*,6*S*)-5,6-oxido-7,9,11,14-icosatetraenoic acid (LTA_4) with ethylene glycol in acetonitrile.

The 12-HPETE was prepared from human platelet lysates incubated with 200 μM indomethacin and 260 μM arachidonic acid at 37°C; after 5 min, the incubation mixture was acidified to pH 3 and extracted with diethyl ether; this extract was fractionated by silicic acid chromatography as described (5). The 12-HPETE was separated from the corresponding 12-HETE by adsorption HPLC (12) and further purified by reversed-phase (C-18) HPLC with the system described previously for the analysis of HETEs (5). The identity of the compound was confirmed

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Abbreviations: 12-HPETE, (12*S*)-12-hydroperoxy-5,8,10,14-icosatetraenoic acid; 12-HETE, (12*S*)-12-hydroxy-5,8,10,14-icosatetraenoic acid; 5-HETE, (5*S*)-5-hydroxy-6,8,11,14-icosatetraenoic acid; 5*S*, 12*S*-diHETE, (5*S*,12*S*)-5,12-dihydroxy-(*E*,*Z*,*E*,*Z*)-6,8,10,14,-icosatetraenoic acid; LT(s), leukotriene(s); LTB_4, leukotriene B_4, (5*S*,12*R*)-5,12-dihydroxy-(*Z*,*E*,*E*,*Z*)-6,8,10,14-icosatetraenoic acid; HHT, (12*S*)-12-hydroxy-5,8,10-heptadecatrienoic acid; ETY, (5,8,11,14)-icosatetraynoic acid; PMNL, polymorphonuclear leukocytes; (5*S*,15*S*)-diHETE, (5*S*,15*S*)-5,15,-dihydroxy-6,8,11,13-icosatetraenoic acid.

by reductive conversion to 12-HETE with stannous chloride in methanol (1) and subsequent gas chromatographic analysis; the reduction was ascertained by TLC as described (1). Quantitation was done by using both isotopic dilution and UV absorption. The purified material was stored under nitrogen at −80°C. The radioimmunoassay of thromboxane B_2 was performed by the radioiodinated tracer method (13).

Suspensions of Human Leukocytes and Platelets. Blood from normal donors was collected in 0.077 M sodium EDTA at a blood/EDTA ratio of 9:1 and was centrifuged at 120 × *g* for 20 min; the platelet-rich plasma was collected. Human blood leukocytes were prepared as described (5) with the exception that the final step (centrifugation over Ficoll/sodium diatrizoate) was omitted. At each step, the resulting suspension was centrifuged at 100–120 g for 15 min to remove most of the platelets. The final pellet containing lymphocytes, monocytes, and PMNL was resuspended in Dulbecco's phosphate-buffered saline (pH 7.4) without Ca^{2+} and Mg^{2+} (14). Contamination by platelets expressed as a ratio of platelets to leukocytes, was always found to be 1:3–1:1. Blood platelets were obtained by centrifugation of the platelet-rich plasma of the same donor at 1,000 × *g* for 15 min. After careful removal of plasma, the platelets were resuspended in Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}).

Incubation and Extraction. The cells were incubated with 50–100 μM arachidonic acid (see figure legends) or 2 μM ionophore A23187 added as ethanolic solutions (the final concentration of ethanol in the buffer did not exceed 0.5%) and in the presence of 1 mM Ca^{2+}. The incubations were performed at 37°C for 5 min and terminated by the addition of 1.5 vol of ice-cold methanol containing 250 ng of prostaglandin B_2 added as an internal standard. Extractions were performed as described (5). Each type of experiment was carried out three times or more.

Column Chromatography. Silicic acid column chromatography was performed with a glass column (internal diameter, 0.5 cm) packed with 0.5 g of silicic acid (SilicAR CC-4, Mallinckrodt). The first solvent mixture (15 ml of diethyl ether/hexane, 10:90, vol/vol) eluted unreacted arachidonic acid and other unpolar lipids and was discarded; the second solvent mixture (15 ml of diethyl ether/methanol, 95:5, vol/vol) eluted mono- and dihydroxy acids and was collected.

HPLC Analysis. The ether/methanol fractions were evaporated to dryness and treated with diazomethane to form methyl esters. The samples were dissolved in diethyl ether and analyzed on silica gel columns. The solvent systems used were nonlinear gradients as described in the legend to Fig. 1. Quantitation was done by relating peak areas of the various compounds to the peak area of the internal standard after correction for attenuation settings. The results have been expressed in arbitrary units.

RESULTS

Effect of Platelets on the Metabolism of Arachidonic Acid in Human Leukocytes. Peripheral leukocytes incubated with arachidonic acid produced small amounts of lipoxygenase metabolites as shown by HPLC analysis (Fig. 1*A*). However, when leukocytes were incubated in the presence of platelets, a strong stimulation (4- to 8-fold) of the transformation of the fatty acid occurred (Fig. 1*B*), and substantial amounts of 5-HETE, Δ^6-*trans*-LTB_4, Δ^6-*trans*-12-epi-LTB_4, 5*S*,12*S*-diHETE, and LTB_4 were detected. Platelets incubated under similar conditions produced large amounts of 12-HETE and (12*S*)-12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), but the C-5 lipoxygenase products were not detectable (data not shown). The HPLC profile obtained from incubation of leukocytes and platelets resembled that obtained from incubation of leukocytes stimulated with the ionophore A23187 (Fig. 1*C*).

The stimulatory effect of platelets on arachidonic acid metabolism in leukocytes was studied in more detail. The progressive addition of platelets to the leukocytes increased the production of the C-5 lipoxygenase products (Fig. 2). The 5-HETE and LTB_4 production was already stimulated at low platelet concentrations and culminated at a platelet/leukocyte ratio of about 25:1 in this experiment; the production of 5-HETE and LTB_4 decreased when platelets were added in excess. In contrast, the 5*S*,12*S*-diHETE increased with platelet concentrations up to the highest platelet/leukocyte ratio tested. The formation of HHT and 12-HETE reflected the increasing concentration of platelets in the medium.

Time-Course Incubations of Platelets with Arachidonic Acid Before Addition of Leukocytes. In a first attempt to define the nature of the factor responsible for the effect of platelets on leukocyte C-5 lipoxygenase activity, kinetic studies were performed. The preincubation of platelets with arachidonic acid prior to the addition of leukocytes (as opposed to the direct addition of the fatty acid to platelet/leukocyte mixtures) resulted in a decreased formation of the C-5 lipoxygenase metabolites; after only 1 min of preincubation, the stimulation of the leukocyte metabolism was lowered by 75% (Fig. 3), indicating the lability of the stimulatory agent generated by the platelets upon addition of arachidonic acid. This suggested the involvement of either the unstable platelet lipoxygenase product 12-HPETE or of the cyclooxygenase metabolites thromboxane A2 or prostaglandin endoperoxides PGG_2 and PGH_2. Experiments with inhibitors were undertaken to clarify this question.

Effect of Inhibitors of the Oxidative Metabolism of Arachidonic Acid in Platelet/Leukocyte Mixtures. The total blockade of the cyclooxygenase pathway (as measured by the complete inhibition of HHT formation) by 10 μM indomethacin or 10 μM flurbiprofen decreased the C-5 lipoxygenase metabolism by 25–50%, depending on the measured compound (Fig. 4; the effect of flurbiprofen was similar to that of indomethacin). In contrast, the use of the acetylenic analog of arachidonic acid, (5,8,11,14)-icosatetraynoic acid (ETY), an inhibitor of both cyclooxygenase and C-12 lipoxygenase, totally suppressed the leukocyte lipoxygenase activity (Fig. 4), although the inhibitor did not significantly alter the formation of 5-HETE and LTB_4 in ionophore-stimulated cells (data not shown). The inhibition of cyclooxygenase activity also was monitored by measurements of thromboxane B_2 by radioimmunoassay; the results (not shown) were in agreement with those mentioned above. These data suggested a major role for the platelet lipoxygenase in the activation of the leukocyte C-5 lipoxygenase.

Effect of 12-HPETE on the Activity of the Leukocyte C-5 Lipoxygenase. Experiments were performed to evaluate the effect of direct additions of the unstable platelet lipoxygenase product, 12-HPETE, on the metabolism of arachidonic acid by human blood leukocytes.

Leukocytes were pretreated with 10 μM ETY to inhibit the C-12 and C-15 lipoxygenase and the cyclooxygenase activities of the cells. Addition of 12-HPETE (final concentration, 1.5 μM) initiated the oxidative metabolism of exogenous arachidonic acid into 5-HETE, LTB_4, and 5*S*,12*S*-diHETE. At 3–4 μM 12-HPETE, the formation of 5-HETE and LTB_4 reached a plateau. In contrast, the synthesis of the 5*S*,12*S*-diHETE continued to increase with higher concentrations of 12-HPETE (Fig. 5). No stimulation was observed when the corresponding 12-hydroxy derivative was used at the same concentrations. These experiments supported a role for the 12-HPETE in the platelet-mediated activation of the C-5 lipoxygenase.

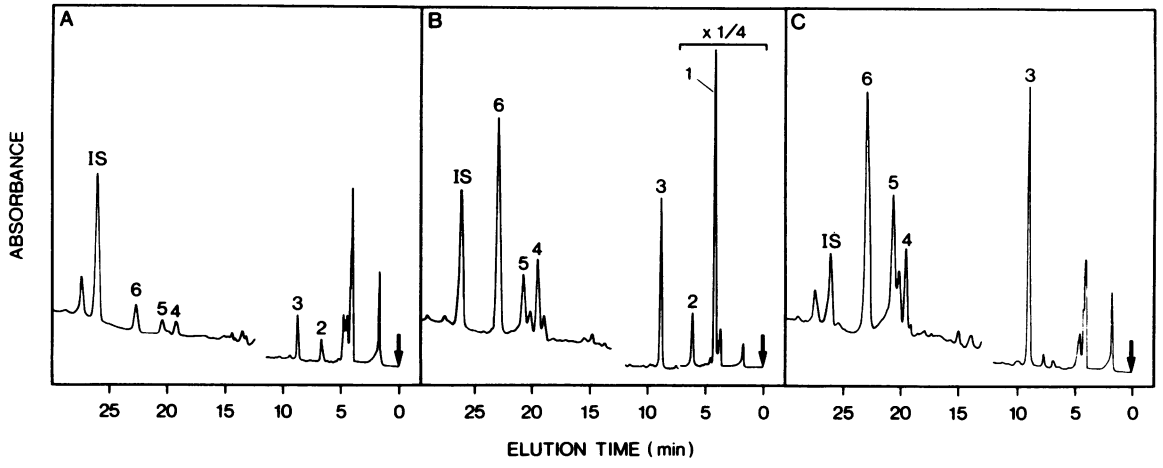

FIG. 1. HPLC chromatograms of the products obtained from incubations of leukocytes (5 ml; 8×10^3 cells per μl) with 60 μM arachidonic acid (*A*), of leukocytes and platelets (5 ml; 8×10^3 and 200×10^3 cells per μl, respectively) with 60 μM arachidonic acid (*B*), and of leukocytes (5 ml; 8×10^3 cells per μl) with 3 μM ionophore A23187 (*C*) for 5 min at 37°C. The incubations were stopped by the addition of 7.5 ml of methanol (0°C) containing 350 ng of (5*S*)-5-hydroxy-12-(2-hydroxy)ethoxy-6,8,10,14-icosatetraenoic acid as internal standard (IS). Eluting compounds were monitored by UV photometry at 235 nm (0–12 min) and at 280 nm (12–30 min). Signal attenuation was 10 times higher at 235 than at 280 nm; in the initial part of *B*, an additional four-fold attenuation was used, as indicated. Peak identifications: 1, 12-HETE; 2, HHT; 3, 5-HETE; 4, Δ^6-*trans*-LTB_4; 5, Δ^6-*trans*-12-epi-LTB_4; and 6, LTB_4. The 5*S*,12*S*-diHETE was eluted immediately before peak 4 and was present only in *B*. The amount of each metabolite formed upon stimulation of leukocytes with the ionophore in *C* were as follows: ≈14 μg (peak 3); ≈0.3 μg (peak 4); ≈0.35 μg (peak 5); and ≈1.7 μg (peak 6). Chromatographic conditions: column, 250 × 4.6 mm packed with 5-μm particle silica gel (Nucleosil 50-5, Macherey-Nagel, Düren, Germany); solvent, gradients of isopropanol in hexane [0–1 min, 1% (isocratic); 1–7 min, 1–3.8%; 7–8 min, 3.8–4.5%; 8–19 min, 4.5–5.6%; 19–22 min, 5.6–8.7%; 22–30 min, 8.7% (isocratic)].

DISCUSSION

In this paper, we show that the 12-HPETE derived from platelet arachidonic acid oxidative metabolism activates the C-5 lipoxygenase of human blood leukocytes *in vitro*. Indeed, addition of platelets to suspensions of leukocytes enhanced the metabolism of exogenous arachidonic acid by the leukocytes (Fig. 1). The platelet/leukocyte ratio appeared to be critical; although the optimal ratio varied in different experiments, possibly because of variations in platelet lipoxygenase activity, we consistently observed the same pattern—i.e., stimulation of 5-HETE and LTB_4 synthesis, followed by inhibition above a critical ratio (Fig. 2). It is possible that in addition to the platelet/leukocyte ratio, the cell concentration in the incubation medium also is an important parameter, which has been observed in studies of platelet-derived endoperoxides as substrates for endothelial cell prostacyclin synthetase (15). The observation that platelets lost their stimulatory effect, upon short preincubation with arachidonic acid prior to the addition of leukocytes, favored the involvement of a short-lived factor as the activator of the leukocyte C-5 lipoxygenase. The use of pharmaco-

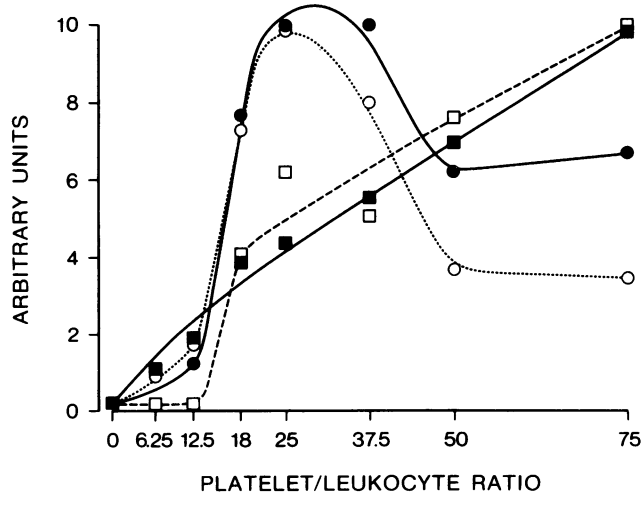

FIG. 2. Effect of platelet concentration on the metabolism of arachidonic acid in human leukocytes. ○, 5-HETE; ●, LTB_4; □, 5*S*,12*S*-diHETE; ■, HHT + 12-HETE. Leukocyte suspensions (2 ml; 8×10^3 cells per μl) were incubated 5 min at 37°C with 60 μM arachidonic acid in the presence of increasing concentrations of human platelets (from the same donor). Incubations were stopped by addition of 3 ml of ice-cold methanol containing 250 ng of prostaglandin B_2 as an internal standard. The compounds formed were analyzed by HPLC. Mass values of arbitrary units: 5-HETE, 575 ng; LTB_4, 65 ng; 5*S*,12*S*-diHETE, 15 ng.

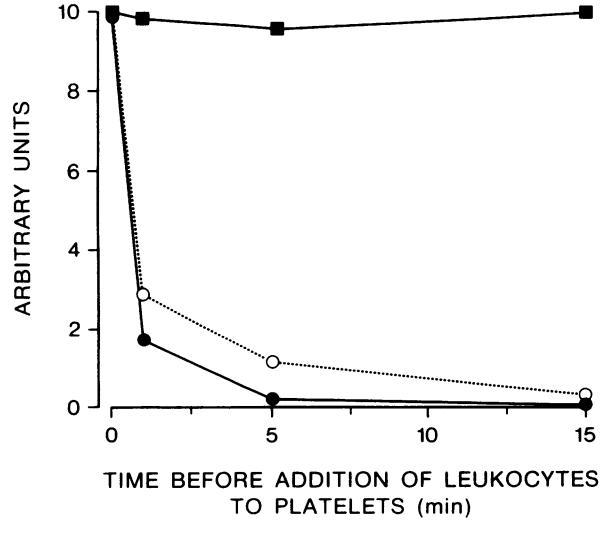

FIG. 3. Effect of the preincubation of human platelets with arachidonic acid on the subsequent metabolism of the fatty acid by added human leukocytes. ■, HHT + 12-HETE; ○, 5-HETE; ●, LTB_4. Platelets were preincubated with 50 μM arachidonic acid during the indicated time; leukocytes were then added for a further 5-min incubation period (with a supplement of 7.5 μg of arachidonic acid to raise the final concentration to 75 μM). The samples were treated as described in the legend to Fig. 2. Mass values of arbitrary units: 5-HETE, 80 ng; LTB_4, 25 ng.

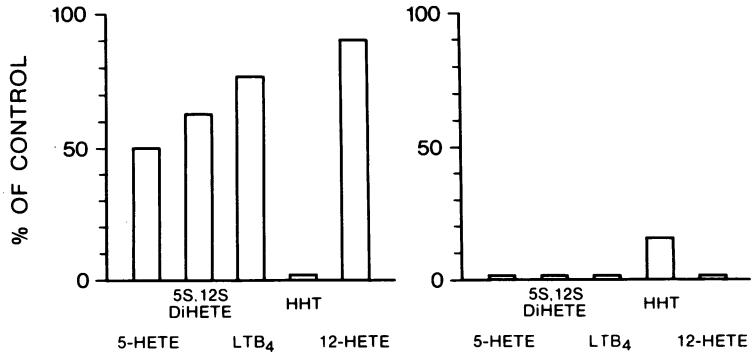

FIG. 4. Inhibitory effects of 10 μM indomethacin (*Left*) and 7 μM ETY (*Right*) on platelet and leukocyte arachidonic acid oxidative metabolism. Mixed suspensions (2 ml) of human leukocytes (22×10^3 cells per μl) and human platelets (31×10^4 cells per μl) were preincubated 30 min at room temperature with the drugs prior to incubation with 100 μM arachidonic acid for 5 min. The samples were treated as described in the legend to Fig. 2. Results are expressed as the percentage of untreated controls.

logical agents suggested that a platelet lipoxygenase product—i.e., 12-HPETE—was mainly responsible for the activation of the C-5 lipoxygenase.

Indeed, 7 μM ETY totally suppressed the C-12 lipoxygenase activity and the activation of the C-5 lipoxygenase, although at a concentration of 10 μM ETY, the C-5 lipoxygenase activity was not inhibited directly (data not shown). The lack of inhibitory effect of ETY on the C-5 lipoxygenase has been observed in rabbit peritoneal PMNL (16, 17) and in porcine leukocytes (10). However, the partial inhibition by nonsteroidal antiinflammatory drugs (cyclooxygenase inhibitors) of the synthesis of C-5 lipoxygenase metabolites (Fig. 4) leaves the possibility that prostaglandin G_2, which possesses a hydroperoxy group at C-15, might also contribute to the platelet-induced stimulation of arachidonic acid metabolism in leukocytes.

Conclusive evidence for a role of 12-HPETE in the platelet activation of the leukocyte C-5 lipoxygenase was obtained by direct addition of the compound to leukocytes incubated with arachidonic acid. Micromolar concentrations of 12-HPETE stimulated 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 was completely inactive (see Fig. 5). It is noteworthy that the low concentrations of platelets required to induce the leukocyte C-5 lipoxygenase activation are compatible with the small amounts of 12-hydroperoxide necessary to achieve the same effect. The transient accumulation (30–60 sec) of 12-HPETE observed in incubations of whole platelets with arachidonic acid (18) is also in good agreement with our data (Fig. 3). The reasons for the decrease of 5-HETE and LTB_4 formation and for the further increase in 5S,12S-diHETE synthesis (Fig. 2) at high platelet/leukocyte ratio are presently unclear; however, in the light of the present and other studies, it appears likely that the 12-HPETE and the 12-HETE are both involved in the expression of the effect of platelets on C-5 lipoxygenase activity. Indeed, in a separate study, we have observed that 12-HETE progressively inhibited the synthesis of 5-HETE and LTB_4, whereas it was increasingly metabolized into the 5S,12S-diHETE (in the same dose range) in ionophore-stimulated leukocytes (10). This opposing effect of the 12-HETE and 12-HPETE suggests that their relative concentrations are critical factors for the activity of the C-5 lipoxygenase; these data also point out that fatty acid hydroperoxide reductases constitute important components of the regulatory mechanism of leukotriene synthesis.

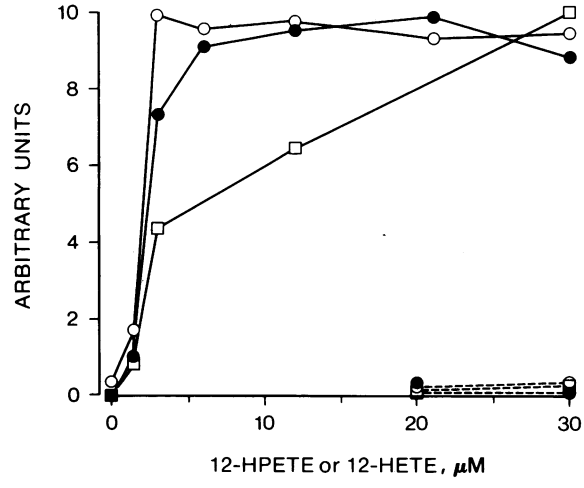

FIG. 5. Effect of 12-HPETE on the metabolism of arachidonic acid by human leukocytes. □, 5S,12S-diHETE; ○, 5-HETE; ●, LTB_4. The leukocyte suspensions (2 ml; 13×10^3 cells per μl) were pretreated with 10 μM ETY. The 12-HPETE (——) or 12-HETE (----) was added immediately before the addition of 100 μM arachidonic acid to the cells, which were incubated 5 min. The samples were treated as described in the legend to Fig. 2. Mass values of arbitrary units: 5-HETE, 785 ng; LTB_4, 65 ng; 5S,12S-diHETE, 15 ng.

The mechanism by which the 12-HPETE activates the C-5 lipoxygenase of leukocytes is presently unknown, although it seems that the hydroperoxy moiety is a prerequisite. It has been shown earlier that the kinetics of platelet and soya bean lipoxygenase reactions were altered by low concentrations of fatty acid hydroperoxides, which reduced the initial lag phase of these reactions. Parent compounds lacking the hydroperoxide group did not evoke such enhancing effect (19, 20), and isomeric specificity for this effect was also reported (20). However, the phenomenon reported in our studies is different in that the 12-HPETE rather activates the C-5 lipoxygenase, which otherwise shows only minor activity (Fig. 1); in this respect, the effect of the 12-HPETE on leukocytes resembles that of the ionophore A23187.

In connection with the present findings, we speculate that the spontaneous activity of the C-5 lipoxygenase (in the absence of the ionophore A23187) that we occasionally observed in some preparations of human blood leukocytes (5) may be due to the formation of fatty acid hydroperoxides by contaminating platelets or the leukocyte C-15 lipoxygenase or cyclooxygenase. Accordingly, we have observed that the systematic treatment of leukocytes with 5–10 μM ETY always suppressed the spontaneous activity of the C-5 lipoxygenase in spite of the lack of effect of the inhibitor on this enzyme. Our findings also may be relevant to previous reports by other workers that (*i*) combination

of platelets and leukocytes produced different profiles of arachidonic acid metabolites than either cell types alone (21), (*ii*) 12-HPETE stimulates some human neutrophil functions (22), and (*iii*) fatty acid hydroperoxides enhance anaphylactic mediator release from guinea pig lungs (23).

It has been suggested that LTs play some roles in immediate hypersensitivity reactions, in view of their potent broncho-constricting properties (8), and in inflammation, because they promote macromolecule leakage, leukocyte recruitment and adhesion, and lysosomal enzyme release (7). The interaction described herein between C-12 and C-5 lipoxygenases could signify that thrombocytes are implicated in the initiation or amplification of those reactions (even when platelet cyclooxygenase is inhibited by nonsteroidal antiinflammatory drugs). Fig. 6 shows a hypothetical scheme of the platelet–leukocyte interactions that lead to increased release of C-5 lipoxygenase products. As indicated in Fig. 6, we have found that the C-15 lipoxygenase also interacts with the C-5 lipoxygenase to produce a (5*S*,15*S*)-5,15-dihydroxy-6,8,11,13-icosatetraenoic acid (5*S*,15*S*-diHETE) (these data will be reported separately). The formation of the 5*S*,12*S*-diHETE and of the 5*S*,15*S*-diHETE reflects the interactions between lipoxygenases.

In summary, the results reported here (*i*) suggest a role for the platelet lipoxygenase, (*ii*) propose a mechanism of activation of LT biosynthesis that might have a pathophysiological importance, and (*iii*) support the concept of cellular interactions in the development of inflammatory and allergic reactions.

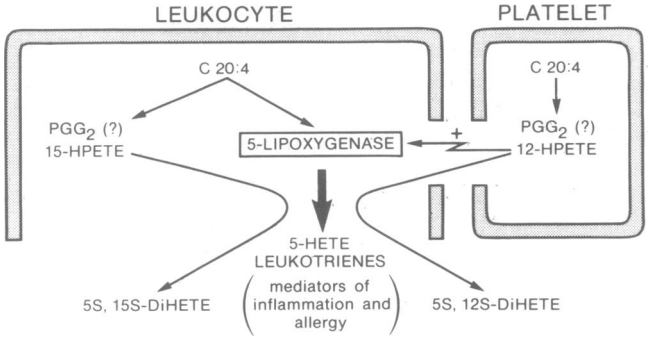

FIG. 6. Hypothetical scheme of platelet and leukocyte lipoxygenases interactions. +, activation.

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1. Hamberg, M. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3400–3404.
2. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3213–3217.
3. Murphy, R. C., Hammarström, S. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4275–4279.
4. Morris, H. R., Taylor, G. W., Piper, P. J. & Tippins, J. R. (1980) *Nature (London)* **285**, 104–106.
5. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2148–2152.
6. Narumiya, S., Salmon, J. A., Cottee, F. H., Weatherley, B. C. & Flower, R. J. (1981) *J. Biol. Chem.* **256**, 9583–9592.
7. Bray, M. A., Ford-Hutchinson, A. W. & Smith, M. J. H. (1981) *Prostaglandins* **22**, 213–222.
8. Dahlén, S. E., Bjork, J., Hedqvist, P., Arfors, K. E., Hammarström, S., Lindgren, J. A. & Samuelsson, B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3887–3891.
9. Borgeat, P., Picard, S., Vallerand, P. & Sirois, P. (1981) *Prostaglandins Med.* **6**, 557–570.
10. Borgeat, P., Fruteau de Laclos, B., Picard, S., Drapeau, J., Vallerand, P. & Corey, E. J. (1981) *Prostaglandins* **23**, 713–724.
11. Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254**, 7865–7869.
12. Porter, N. A., Logan, J. & Kontoyiannidou, V. (1979) *J. Org. Chem.* **44**, 3177–3181.
13. Maclouf, J., Pradel, M., Pradelles, P. & Dray, F. (1976) *Biochim. Biophys. Acta* **431**, 139–146.
14. Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182.
15. Marcus, A. J., Weksler, B., Jaffe, E. A. & Broerman, M. J. (1980) *J. Clin. Invest.* **66**, 979–986.
16. Borgeat, P., Hamberg, M. & Samuelsson, B. (1977) *J. Biol. Chem.* **251**, 7816–7820, and correction (1977) **252**, 8772.
17. Vanderhoek, J. Y., Bryant, R. W. & Bailey, J. M. (1980) *J. Biol. Chem.* **255**, 10064–10065.
18. Hamberg, M. & Hamberg, G. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1090–1097.
19. Funk, M. O., Kim, S.H.-S. & Alteneder, A. W. (1981) *Biochem. Biophys. Res. Commun.* **98**, 922–929.
20. Siegel, M. I., McConnell, R. T., Abrahams, S. L., Porter, N. A. & Cuatrecasas, P. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1273–1280.
21. Killackey, J. J., Killackey, B. A. & Philp, R. B. (1981) *Thromb. Haemostasis* **46**, 78 (abstr. 0833).
22. Goetzl, E. J., Hill, H. R. & Gorman, R. R. (1980) *Prostaglandins* **19**, 71–85.
23. Adcock, J. J., Garland, L. G., Moncada, S. & Salmon, J. A. (1978) *Prostaglandins* **16**, 163–177.