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The Metabolism of Arachidonic and Eicosapentaenoic Acids in Human Neutrophils Stimulated by A23187 and FMLP

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A23187 stimulates the metabolism of endogenous as well as exogenous arachidonic acid (AA) and eicosapentaenoic acid (EPA) to their corresponding leukotrienes in human neutrophils. In contrast, conflicting results have been obtained concerning the effect of FMLP on the metabolism of these fatty acids. In the present study we compared the effect of A23187 and FMLP on the release and metabolism of these fatty acids in neutrophils. Stimulation of neutrophils with A23187, but not with FMLP, resulted in detectable levels of AA in the presence or absence of BW755C (a dual inhibitor of cyclooxygenase and lipoxygenase). The absolute amount of nonesterified AA in the extracts of neutrophils exposed to the agonist A23187 in the presence of BW755C was 20% higher than that obtained in the absence of BW755C, indicating that only a small fraction of the released AA was converted to lipoxygenase products. Furthermore, significant quantities of AA and EPA metabolites were detected only after treatment of neutrophils with A23187, but not with FMLP. Both A23187 and FMLP stimulated the conversion of exogenous EPA to 5-lipoxygenase products, with A23187 being somewhat more effective. In addition, significant differences were noted on the effect of EPA and DHA on the conversion of AA to its metabolites in A23187-stimulated neutrophils. Our results provide strong evidence that the amounts of eicosanoid precursors mobilized in response to FMLP are extremely small, if any, and this appears to be the likely explanation for the lack of eicosanoid detection by HPLC in FMLP-stimulated neutrophils.

Key words: arachidonic acid, neutrophils, eicosapentaenoic acid

It is becoming increasingly apparent that arachidonic acid and its metabolites play a predominant role in several inflammatory processes [1–3]. For example, leukotriene B_4 (LTB_4) is known to stimulate neutrophil chemotaxis and other functions [4–7]. Recently, a great deal of interest has arisen concerning the potential dampening effects of

Abbreviations used: BW755C, 3-amino-1-(3-trifluoromethylphenyl-2-pyrazoline) hydrochloride (a dual inhibitor of cyclooxygenase and lipoxygenases); FMLP, fmet-Leu-Phe.

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the metabolites derived from another 20C fatty acid, eicosapentaenoic acid (EPA), commonly present in fish oil-enriched diets [8–10]. Leukotriene B_5 (LTB_5), which is synthesized from EPA, has been shown to be less chemotactic than LTB_4 for neutrophils [11–13].

Arachidonic acid that is mobilized from membrane phospholipids as well as exogenous arachidonic acid are readily metabolized to their corresponding leukotrienes via the 5-lipoxygenase in neutrophils when stimulated with A23187 [14–17]. It is known that almost all of the tissue arachidonic acid, and possibly EPA as well, which are the precursors of 4- and 5-series leukotrienes, remain exclusively associated with membrane phosphoglycerides [18]. These eicosanoid precursor fatty acids must be mobilized enzymatically from membrane lipid stores before they can be metabolized to their corresponding metabolites. Thus, mobilization of these precursors from membrane lipids is frequently considered as the rate-limiting step in the biosynthesis of biologically active substances such as leukotrienes [19].

Two biochemical pathways have been recognized as important for mobilization of arachidonic acid and/or EPA from membrane phospholipids [20–25]. One pathway involves the release of arachidonic acid and/or EPA from phosphatidylinositol (PI) by the sequential action of phospholipase C and diacylglycerol lipase [20–22]. The other involves arachidonic acid and/or EPA release from phosphatidylcholine (PC), phosphatidylethanolamine (PE), and PI by the action of phospholipase A_2 [23–25]. However, determining the source of arachidonic acid/EPA in different cell types has proven extremely difficult, reflecting the complexity of various enzyme systems and precursor pools involved in the biosynthesis of these metabolites. Earlier studies with labeled human neutrophils showed that PC and PI were the principal sources of eicosanoids [26,27]. In addition, a recent study in neutrophils stimulated with A23187, in which the actual mass changes of phosphoglycerides were monitored, suggests also the involvement of alkenyl species of PE [28].

In general, it has been difficult to evaluate the effect of inflammatory ligands such as FMLP and opsonized zymosan since they are relatively weaker than A23187 in their effects on AA mobilization and this has led to conflicting results on their ability to stimulate the mobilization of arachidonic acid and, further, to stimulate the metabolism of this fatty acid to leukotrienes in neutrophils [15,17,29,30]. The chemotactic peptide, FMLP, has, however, been shown to stimulate the turnover and remodeling of phosphoglycerides in human neutrophils [31–33]. On the other hand, the metabolism of EPA in neutrophils stimulated with A23187 has been extensively studied [34–37]. However, there is a paucity of information concerning the ability of inflammatory ligands such as FMLP and opsonized zymosan to stimulate the formation of leukotrienes from this fatty acid.

Although several ex vivo and in vitro studies have been conducted thus far with stimulated neutrophils, the significance of various phospholipases in stimulus-induced arachidonic acid/EPA mobilization from neutrophil membrane lipids has remained obscure. In human neutrophils, phospholipase A_2 has been found to be associated with the plasmalemma [38] and granular membranes [39]. Furthermore, conflicting results have been reported on the effects of agents such as FMLP, C5a, and LTB_4 to stimulate phospholipase A_2 in rabbit neutrophil preparations [40,41]. Recently it has been reported that differences exist in the activity of phospholipase A_2 between postnuclear supernatants of resting and A23187-activated neutrophils [42].

The present study was designed to compare the release and metabolism of EPA with arachidonic acid in human neutrophils stimulated with FMLP, a chemotactic peptide. Our results demonstrate that FMLP alone failed to stimulate the formation of metabolites of both endogenous EPA and arachidonic acid. However, FMLP stimulated the conversion of exogenous EPA to lipoxygenase products. These results indicate that the failure of this ligand to stimulate the metabolism of arachidonic acid or EPA may be due primarily to poor activation of phospholipases.

MATERIALS AND METHODS

Materials

Pure fatty acids, A23187, and FMLP were obtained from Sigma Chemical Co. (St. Louis, MO). Precoated thin-layer silica gel H60 plates were purchased from E. Merck (Darmstadt, FRG). Hank's modified buffers were obtained from Flow Laboratories (Ontario, Canada). Ficoll-Paque was obtained from Pharmacia (Quebec, Canada). All solvents and chemicals used were of analytical or HPLC grade.

Isolation of Neutrophils

Human neutrophils were prepared as previously described with some slight modifications [33,43]. Whole blood was mixed with equal volumes of 3% dextran T-500 in 0.9% NaCl solution, then the red blood cells were allowed to settle at room temperature for 45 min. The supernatant was aspirated and then centrifuged at 200g for 10 min at 4°C. The resulting pellet was resuspended in Hank's balanced salt solution (HBSS), layered on Ficoll-Paque followed by centrifugation at 400g for 40 min at 19°C. Contaminating red blood cells were removed by osmotic lysis and isotonicity was restored by the addition of 1.8% NaCl. The suspension was again centrifuged at 200g for 10 min at 4°C. The pellet was finally suspended in appropriate volumes of HBSS buffer containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$.

Incubation of Neutrophil Suspensions and Analysis of Fatty Acids

Neutrophil suspensions in HBSS that contained 0.1% albumin (1.6×10^7 cells/ml) were routinely incubated for 3 min with and without A23187 or FMLP in the presence and absence of BW755C (a dual inhibitor of cyclooxygenase and lipoxygenases). Lipids were immediately extracted by the method of Bligh and Dyer [44]. The lower chloroform phases were dried under oxygen-free nitrogen and redissolved in small volumes of chloroform:methanol (2:1, v/v). Nonesterified fatty acids present in these extracts were separated on precoated silica gel H plates being developed in heptane: isopropylether:acetic acid (60:40:3, v/v/v). The free fatty acid band was immediately scraped into a tube containing an internal standard, monopentadecanoate, and methylated in the presence of 6% methanolic sulfuric acid for 2 hr. Fatty acid methyl esters extracted with 2 ml petroleum ether were dried under nitrogen and analyzed on fused silica megabore columns (J.W. Scientific, California) at 210°C using the Hewlett-Packard gas chromatograph (5890). Helium was used as a carrier gas. The fatty acid peaks were identified by comparing their retention times with those of standard fatty acid methyl esters and quantitated on the basis of an internal standard [22]. To assess the formation of EPA metabolites, neutrophils were enriched with EPA by providing MaxEPA capsules to human volunteers for 4 weeks. Analysis of total cellular fatty acids

indicated that 1.6×10^7 cells contained 16 nmoles of arachidonic acid, 7 nmoles of EPA, and 4 nmoles of docosahexaenoic acid (DHA). The EPA-enriched neutrophils were then incubated for 5 min with A23187 or FMLP in the presence or absence of arachidonic acid (10 μM) or EPA (10 μM). The incubations were terminated with 0.5 ml chilled methanol and stored at −80°C until analyzed for arachidonic acid and EPA metabolites by RP-HPLC.

Analysis of Arachidonic Acid and EPA Metabolites

Arachidonic acid and EPA metabolites were analyzed by precolumn extraction-HPLC as previously described [45]. Two Millipore-Waters Model 510 pumps coupled to a Model 680 gradient controller were used to deliver the mobile phases. Products were detected at 235 and 280 nm by use of a Millipore-Waters Model 490 UV detector and a Raytest data system. The stationary phase was a Millipore-Waters Novapak C_{18} column (4 μM particle size; 3.9 × 150 mm). The mobile phase consisted of a linear gradient between water:acetonitrile:acetic acid (80:20:0.02) and water:acetonitrile:methanol: acetic acid (28:33:39:0.02) over 60 min. The flow rate was 2 ml/min. The sample was loaded onto a Millipore-Waters μBondapak C_{18} Guard-PAK precolumn insert attached to a Millipore-Waters WAVS automated switching valve [45]. The sample was pumped onto the precolumn by the use of a Milton Roy minipump in line with a Millipore-Waters WISP automatic injector. 19-HydroxyPGB_2 was used as an internal standard to correct for recovery [46].

RESULTS

Effect of A23187 and FMLP on Arachidonic Acid Release

To compare the effects of FMLP (1 μM) and A23187 (10 μM) on the mobilization of arachidonic acid from membrane phospholipids, neutrophils suspended in albumin-containing buffer were stimulated with FMLP (1 μM) or A23187 for 3 min in the presence or absence of BW755C (a dual inhibitor of cyclooxygenase and lipoxygenases). Albumin (0.1%) was used in the medium to reduce the degree of potential reacylation and to trap the released nonesterified arachidonic acid. Our preliminary results showed that BW755C (a dual inhibitor of cyclooxygenase and lipoxygenase) at 80–100 μM selectively inhibited the oxygenase pathways without affecting the activity of phospholipases. For example, phosphatidic acid formed in response to FMLP remained unaffected in the presence of BW755C. Furthermore, the amount of arachidonic acid released from endogenous phospholipids in response to A23187 was also not affected. These results are in general agreement with its effects on platelets [22,25]. As shown in Figure 1 and Table I, no nonesterified arachidonic acid was detectable in the lipid extracts obtained from either unstimulated or FMLP-stimulated human neutrophils while significant levels were detected in the extracts of neutrophils stimulated with A23187. As expected, a greater quantity of free arachidonic acid was detected following stimulation with A23187 in the presence of BW755C (600 ng as opposed to 503 ng in the absence of BW755C). These results indicate that only 16% of the arachidonic acid released following stimulation with A23187 was converted to eicosanoids, assuming that complete inhibition of cyclooxygense and lipoxygenase enzymes occurred in the presence of BW755C.

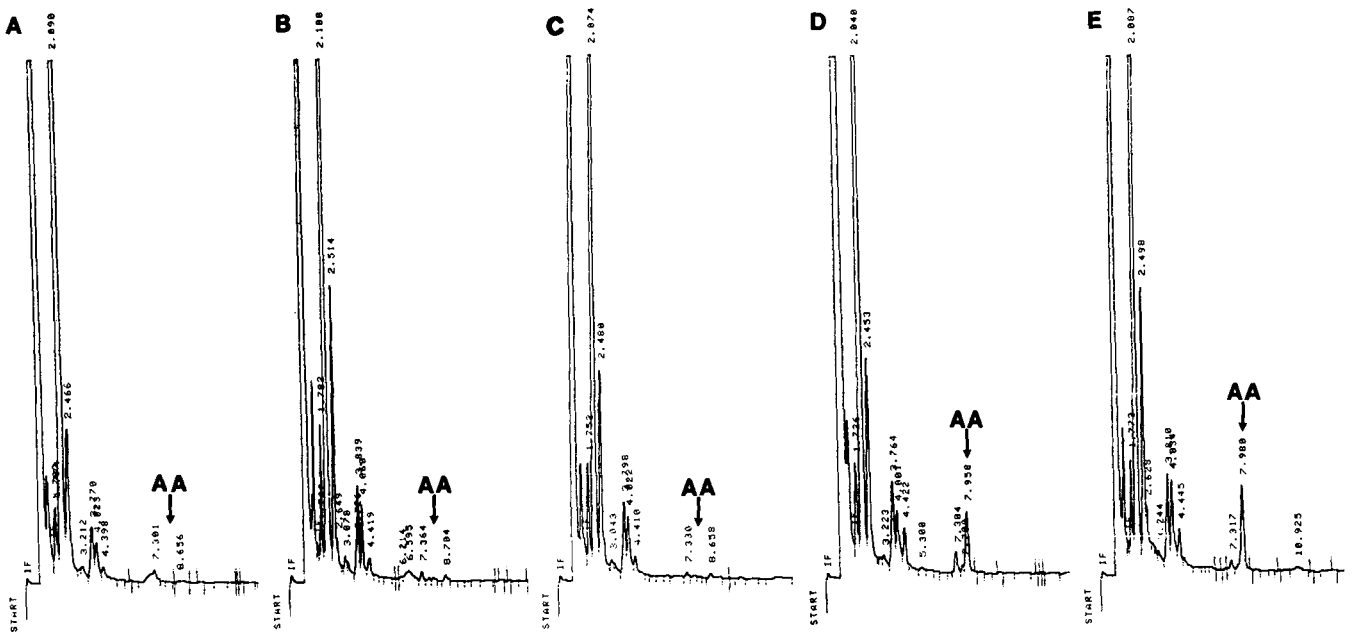

Fig. 1. Gas chromatograms of free fatty acids obtained from unstimulated and stimulated human neutrophils. Free fatty acids were extracted, separated, and quantitated by combined thin-layer and gas chromatography as described in Materials and Methods. **A:** Unstimulated. **B:** Neutrophils plus FMLP. **C:** Neutrophils, BW755C plus FMLP. **D:** Neutrophils plus A23187. **E:** Neutrophils, BW755C plus A23187. **AA:** Arachidonic acid.

TABLE I. Effect of A23187 and FMLP on Free Arachidonic Acid Levels in Human Neutrophils*

Treatment	Nanograms of free arachidonic acid (1.4×10^7 cells/ml)	
	Minus BW755C	Plus BW755C
Unstimulated	ND	ND
Plus FMLP	ND	ND
Plus A23187	503 ± 27	600 ± 14

*Human neutrophils suspended in albumin-containing buffer were stimulated with A23187 (10 μM) or FMLP (1 μM) for 3 min in the presence and absence of BW755C (80 μM), a dual inhibitor of cyclo- and lipoxygenase. At 80–100 μM, BW755C does not affect the activation of phospholipases in human neutrophils. Free arachidonic acid was extracted, separated, and quantitated by combined thin-layer and gas chromatography as described in Materials and Methods. Results represent the mean ± SE of triplicate determinations from one of the two similar experiments with two different donors. ND, none detected.

TABLE II. Effect of Exogenous EPA and DHA on 5-Lipoxygenase Metabolites Formed From AA (Endogenous and Exogenous) in Response to A23187*

Treatment	Picomoles of AA metabolites (1.6×10^7 cells/ml)		
	5-HETE	LTB_4	20h-LTB_4
A23187	304 ± 102	217 ± 65	373 ± 92
EPA + A23187	427 ± 93	125 ± 15	169 ± 28
DHA + A23187	601 ± 105	115 ± 4	262 ± 24
AA + A23187	3,302 ± 1,251	915 ± 27	727 ± 150
AA + EPA + A23187	1,395 ± 128	650 ± 19	608 ± 60
AA + DHA + A23187	1,565 ± 203	1,115 ± 30	855 ± 56

*Human neutrophils were stimulated with A23187 (10 μM) in the presence and absence of AA (10 μM), EPA (10 μM), DHA (10 μM), AA and EPA (10 μM each), and AA and DHA (10 μM each). AA metabolites were immediately extracted with 0.5 ml chilled methanol and analyzed by RP-HPLC as described in Materials and Methods. Values represent the mean ± SE (n = 4) from two independent experiments with different subjects. HETE: Hydroxyeicosatetraenoic acid.

Effects of A23187 and FMLP on the Generation of Leukotrienes from Arachidonic Acid and Eicosapentaenoic Acid

There are conflicting reports in the literature concerning the ability of certain inflammatory agents such as FMLP to induce the synthesis of arachidonic metabolites in human neutrophils. In addition, it is not known whether FMLP stimulates the metabolism of endogenous and/or exogenous EPA.

In this study we compared the effect of A23187 (10 μM) and FMLP (1 μM) on the relative conversion of endogenous and exogenous arachidonic acid and EPA to their lipoxygenase metabolites in intact human neutrophils. A23187 (10 μM) stimulated the conversion of both endogenous and exogenous arachidonic acid to lipoxygenase metabolites (Table II). In our experiments, the mean values for LTB_4 and its ω-oxidation products (ω-carboxy-LTB_4 and 20h-LTB_4) formed in response to A23187 were 915 and 727 pmoles with, and 217 and 373 pmoles without, added arachidonic acid, respectively (Table II). Stimulation of neutrophils with FMLP alone, however, failed to produce detectable quantities of any lipoxygenase metabolites of arachidonic acid, but small amounts of LTB_4 and its ω-oxidation products were formed when FMLP (1 mM) and arachidonic acid (10 μM) were added simultaneously (Table III). However, addition of

TABLE III. Effect of Free AA, EPA, and FMLP on 5-Lipoxygenase Metabolites Formed From Arachidonic Acid*

Treatment	Picomoles of AA metabolites (1.6×10^7 cells/ml)		
	5-HETE	LTB_4	20h-LTB_4
FMLP	0	0	0
AA	4,187	102	37
AA + FMLP	3,353	78	178
EPA	0	10	37
EPA + FMLP	0	0	0

*Human neutrophils treated with cytochalasin B (5 μg/ml) were stimulated with FMLP (1 μM), free AA (10 μM), AA (10 μM) and FMLP (1 μM), EPA (10 μM), EPA (10 μM) and FMLP (1 μM) for 5 min at 37°C. AA metabolites were immediately extracted with 0.5 ml chilled methanol and analyzed by RP-HPLC as described in Materials and Methods. Values represent the average of duplicate determinations from one experiment.

TABLE IV. The Formation of 5-Lipoxygenase Metabolites From EPA in EPA-Enriched Human Neutrophils Stimulated With A23187 and FMLP*

Treatment	Picomoles of EPA metabolites (1.6×10^7 cells/ml)		
	5-HEPE	LTB_5	20h-LTB_5
A23187	8 ± 4	20 ± 4	44 ± 8
EPA + A23187	2,084 ± 484	364 ± 9	567 ± 7
FMLP	0	0	0
EPA + FMLP	1,515 ± 453	362 ± 98	185 ± 57

*Human neutrophils enriched in EPA (by dietary means) were stimulated with A23187 or FMLP in the presence and absence of EPA (10 μM). EPA-enriched neutrophils had contained 16, 7, and 4 nmoles of AA, EPA, and DHA, respectively, in total phospholipids (PC, PE, PI, and PS). EPA metabolites were then extracted with 0.5 ml chilled methanol and analyzed by RP-HPLC as described in Materials and Methods. Values represent the mean ± SE (n = 3). HEPE: Hydroxyeicosapentaenoic acid.

arachidonic acid (10 μM) alone to neutrophils resulted in the formation of similar amounts of 5-lipoxygenase products (Table III).

Stimulation of EPA-enriched neutrophils with A23187 resulted in the formation of EPA-derived lipoxygenase metabolites (Table IV). However, LTB_4 and its ω-oxidation metabolites were still the major products detected in A23187-stimulated EPA-enriched cells (110 pmoles of LTB_4, 373 pmoles of ω-oxidation products). In contrast, no lipoxygenase metabolites derived either from arachidonic acid or EPA were detected in neutrophils stimulated with FMLP (Table IV, V). Exogenous EPA (10 μM) was converted to lipoxygenase metabolites by both EPA-enriched neutrophils and neutrophils, which had not been enriched with EPA (less than 0.5 mole % EPA) when exposed to the agonist A23187 (Tables IV, V). Arachidonic acid was a much better substrate than EPA for neutrophil 5-lipoxygenase. Only 168 pmoles of LTB_5 and 256 pmoles of 20h-LTB_5 were detected following stimulation of non-EPA-enriched neutrophils with A23187 in the presence of exogenous EPA (10 μM) (Fig. 2, Table V). Much greater quantities of leukotrienes (915 and 727 pmoles of LTB_4 and its ω-oxidation products, respectively) were detected after treatment of neutrophils with A23187 in the presence of arachidonic acid (10 μM) (Table II). In contrast to what was observed with free arachidonic acid (10 μM) (Table III), EPA (10 μM) alone was converted to only very

TABLE V. Effect of A23187 and FMLP on 5-Lipoxygenase Metabolites Formed From Eicosapentaenoic Acid*

Treatment	Picomoles of EPA metabolites (1.6×10^7 cells/ml)		
	5-HEPE	LTB_5	20h-LTB_5
EPA	214	15	11
EPA + A23187	1,848	168	256
FMLP	0	10	13
EPA + FMLP	741	84	288

*Human neutrophils were stimulated with EPA (10 μM), EPA (10 μM) and A23187 (10 μM), FMLP (1 μM), EPA (10 μM) and FMLP (1 μM) for 5 min at 37°C. EPA-derived metabolites were immediately extracted with 0.5 ml chilled methanol and analyzed by RP-HPLC as described in Materials and Methods. Values represent the average of duplicate samples from one experiment.

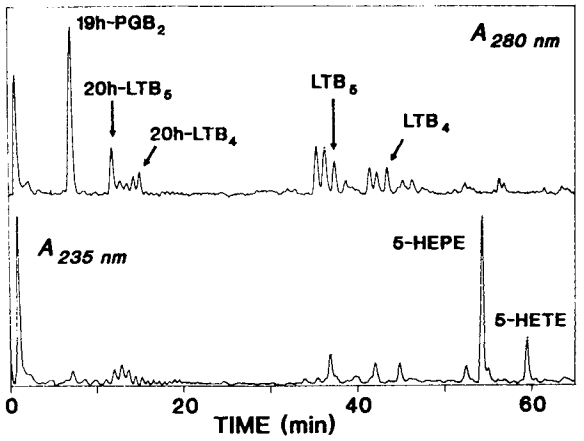

Fig. 2. High pressure liquid chromatogram of the products formed after incubation of human neutrophils (1 ml) with EPA (10 μM) and A23187 (10 μM) for 5 min at 37°C. 19-Hydroxy PGB_2 (19h-PGB_2) was used as an internal standard. **Bottom:** Absorbance at 235 nm. **Top:** Absorbance at 280 nm.

small amounts of 5-lipoxygenase products (Table V). To test the ability of FMLP to stimulate the conversion of exogenous EPA to its corresponding leukotrienes, neutrophil suspensions were incubated with 10 μM EPA in the presence of FMLP. Analysis of the products by RP-HPLC indicated that moderate quantities of 5-lipoxygenase products were formed (Tables IV, V). These results indicate that stimulation of neutrophils with FMLP results in the conversion of exogenous EPA to its corresponding metabolites.

We also investigated the effect of exogenous EPA (10 μM) and DHA (10 μM) on the synthesis of leukotrienes from endogenous and/or exogenous arachidonic acid in neutrophils stimulated with A23187. In the presence of either 10 μM EPA or 10 μM DHA, a marked reduction occurred in the quantities of LTB_4 and its ω-oxidation products formed from endogenous arachidonic acid when compared with the levels obtained with A23187 alone. The mean values of LTB_4 and 20h-LTB_4 were reduced by 42–55% and 30–47% in the presence of 10 μM EPA and 10 μM DHA, respectively. Similarly, the absolute amount of LTB_4 formed by neutrophils stimulated with 10 μM arachidonic acid and A23187 were significantly reduced in the presence of exogenous

EPA (10 μM) (Table II). In contrast, the amount of LTB_4 released from neutrophils stimulated with 10 μM arachidonic acid, 10 μM DHA, and A23187 were slightly higher than those obtained from neutrophils stimulated only with 10 μM arachidonic acid and A23187 (Table II).

DISCUSSION

Calcium ionophore, A23187, is employed extensively as an agonist for investigating the metabolism of arachidonic acid and EPA in neutrophils [14–17,34–37]. In addition, other agents such as FMLP, serum-treated zymosan, heat-aggregated IgG, C5a, and phorbol myristate have been used in similar studies to assess the release of esterified arachidonic acid and its further metabolism to eicosanoids [15,17,26,27,29,30]. In this study we have compared the effects of A23187 and FMLP on the metabolism of arachidonic acid and EPA in human neutrophils. Our results on the actual mass of free (nonesterified) arachidonic acid released by stimulated neutrophils in the presence or absence of BW755C (a dual inhibitor of cyclooxygenase and lipoxygenases) demonstrate that no significant mobilization of arachidonic acid from membrane phospholipids occurred in response to FMLP. These results confirm the findings of Clancy et al. [15], but are in contrast to another recent study utilizing radioactively labeled arachidonic acid [29]. If a significant amount of arachidonic acid had been released in response to FMLP it would have been detected in our studies, since BW755C would have inhibited the metabolism of arachidonic acid without affecting the activation of phospholipases, and albumin would have trapped the released arachidonic acid. It is evident from the results obtained in A23187-stimulated neutrophils that arachidonic acid indeed accumulates in the presence of BW755C and albumin. Our findings on the observed difference in the absolute mass of arachidonic acid mobilized from phospholipid sources following stimulation with A23187, with and without BW755C, are in very close agreement with those of Chilton and O'Connell [28]. They showed recently that only a small fraction (10–35%) of the arachidonic acid released from all phospholipid sources following exposure of neutrophils to A23187 was converted to eicosanoids. Our results show that arachidonic acid accumulates only in A23187-stimulated neutrophils. On the contrary, it has been shown before that no lysophospholipids are detectable in neutrophils stimulated with A23187 [27]. The inability of being able to detect lysophospholipids in A23187-stimulated neutrophils therefore may be due to a rapid degradation of lysophospholipids rather than the rapid reacylation of released arachidonic acid. Therefore, the differences observed in our study between the effects of A23187 and FMLP on arachidonic acid release cannot be explained by simple rapid reacylation reactions. However, a small degree of reacylation of the released arachidonic acid in the presence of albumin (0.1%) cannot be ruled out [47,48].

In agreement with previous reports [14–17], significant quantities of leukotrienes and other lipoxygenase metabolites originating from both endogenous arachidonic acid and EPA were detected in the supernatants of neutrophils stimulated with A23187. In contrast, stimulation of neutrophils with FMLP alone failed to produce detectable amounts of arachidonic acid or EPA metabolites. This could be due to either insufficient mobilization of arachidonic acid or EPA from membrane phospholipid sources or insufficient activation of the 5-lipoxygenase pathway. The levels of nonesterified arachidonic acid detected in lipid extracts of FMLP-stimulated neutrophils supports the former concept that insignificant mobilization of eicosanoid precursor fatty acids occurs

in response to this ligand. Furthermore, the inability of FMLP to stimulate the production of EPA-derived metabolites from endogenous sources indicates that neither the arachidonic acid nor the EPA pools are affected by this agonist. Although FMLP has been known to affect other responses such as phosphoinositide turnover, intracellular calcium changes, and remodeling of phosphoglycerides, it does not seem to be an effective stimulus to induce the mobilization of arachidonic acid or EPA [31–33].

In general agreement with previous findings [15,16,30,34–37], exogenous arachidonic acid and EPA, at near pathophysiological concentrations (10 μM), were readily metabolized by neutrophils stimulated with A23187. The finding that the levels of arachidonic acid metabolites in neutrophils stimulated with arachidonic acid (10 μM) alone were similar to those of neutrophils stimulated with FMLP and arachidonic acid suggested that no appreciable increase in the activity of lipoxygenase under these conditions occurred as a result of the addition of FMLP. These results also indicate that exposure of neutrophils to free arachidonic acid at concentrations as low as 10 μM can result in the stimulation of 5-lipoxygenase. However, it has previously been reported that such stimulation occurs at much higher concentrations of arachidonic acid [15,30]. On the other hand, FMLP increased the conversion of exogenous EPA (10 μM) to leukotrienes. It is apparent that the 5-lipoxygenase pathway is affected in neutrophils when stimulated with FMLP in the presence of EPA. The differences observed in the activation of the 5-lipoxygenase pathway between neutrophils stimulated with arachidonic acid and FMLP, and EPA and FMLP, are likely due to the effects of free arachidonic acid and EPA. Although no clear biochemical explanation can be offered for these differences from our studies, it should be noted that EPA has been shown to be a poor activator of protein kinase C [49]. Recent results from our laboratory also indicate that EPA is less effective than arachidonic acid at stimulating the phosphoinositide response and release of superoxide anion in human neutrophils [unpublished observations].

As reported by Prescott [34] and Lee et al. [50], stimulation of human neutrophils with A23187 in the presence of EPA and DHA resulted in a marked inhibition of arachidonic acid metabolism. It is apparent from our results that EPA was less efficiently utilized than arachidonic acid by the 5-lipoxygenase pathway for the synthesis of LTB_5 and LTB_4, respectively, even though EPA has been shown, with a partially purified enzyme, to be a slightly better substrate for 5-lipoxygenase as compared to arachidonic acid [51,52]. Our results demonstrate that added EPA at levels as low as 10 μM inhibited the metabolism of endogenous and/or exogenous arachidonic acid following stimulation of neutrophils with A23187. Interestingly, the levels of leukotrienes produced in the presence of added arachidonic acid were unaffected by the addition of equimolar concentrations of DHA. Similar findings have previously been reported with higher concentrations of these fatty acids in human neutrophils [50]. It has been shown that 5,8,11-eicosatrienoic acid, which accumulates in essential fatty acid deficiency states, and EPA affect leukotriene production by inhibiting the activity of leukotriene A hydrolase [34,53]. It is known that both EPA and DHA are poor substrates for and competitive inhibitors of cyclooxygenase [9,54–56]. The effects of EPA and DHA, which attenuate the oxidative metabolism of arachidonic acid to proinflammatory products such as leukotrienes, appear to be distinctly different on the 5-lipoxygenase pathway.

In summary, our results indicate that FMLP may not be a potent ligand for the mobilization of arachidonic acid or EPA in human neutrophils. Furthermore, our results show that significant conversion of exogenous EPA to leukotrienes occurs as a result of FMLP stimulation. Nonesterified fatty acids, depending upon the nature of the fatty acid, are likely to modulate the metabolism of arachidonic acid to leukotrienes, the proinflammatory products. Experiments are currently under way in our laboratory to establish a correlation between stimulus-induced responses, which include superoxide anion generation, phosphoinositide turnover, intracellular calcium mobilization, eicosanoid precursor fatty acids release, and the metabolism of these fatty acids to eicosanoids in human neutrophils.

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REFERENCES

1. Samuelsson B: Science 220:568–575, 1983.
2. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN: Science 237:1171–1175, 1987.
3. Feuerstein G, Hallenbeck JM: FASEB J 1:186–192, 1987.
4. Ford-Hutchinson AW, Bray MA, Dolg MV, Shipley ME, Smith MJH: Nature 286:264–265, 1980.
5. Goetzl EJ, Picket WC: J Exp Med 153:482–487, 1981.
6. Feinmark SJ, Lindgren JA, Claessan H, Malmsten C, Samuelsson B: FEBS Lett 136:141–144, 1981.
7. Goetzl EJ, Brindley LL, Goldman DW: Immunology 50:35–41, 1983.
8. Dyerberg J, Bang HO, Stofferson G, Moncada S, Vane JR: Lancet II:117–119, 1978.
9. Whitaker MO, Wyche A, Fitzpatrick F, Sprecher H, Needleman P: Proc Natl Acad Sci USA 76:5919–5923, 1979.
10. Weber PC, Fischer S, Schacky CV, Lorenz R, Strasser T: Prog Lipid Res 25:273–276, 1986.
11. Goldman DW, Pickett WC, Goetzl EJ: Biochem Biophys Res Commun 117:282–288, 1983.
12. Lee TH, Menica-Huerta J-M, Shih C, Corey EJ, Lewis RA, Austen KF: J Biol Chem 259:2383–2389, 1984.
13. Terano T, Salmon JA, Moncada S: Prostaglandins 27:217–232, 1984.
14. Borgeat P, Samuelsson B: Proc Natl Acad Sci USA 76:2148–2152, 1979.
15. Clancy RM, Dahinden CA, Hugli TE: Proc Natl Acad Sci USA 80:7200–7204, 1983.
16. Marcus AJ, Broekman MJ, Safier LB, Ullman HL, Islam N, Serhan CN, Rutherford LE, Karchak HM, Weissmann G: Biochem Biophys Res Commun 109:130–137, 1982.
17. Godfrey RW, Manzi RM, Clark MA, Hoffstein ST: J Cell Biol 104:925–932, 1987.
18. Ramwell PW: Arch Intern Med 141:275–278, 1981.
19. Irvine RF: Biochem J 204:3–16, 1982.
20. Rittenhouse-Simmons S: J Clin Invest 63:580–587, 1979.
21. Bell RL, Kennerly DA, Stanford N, Majerus PW: Proc Natl Acad Sci USA 76:3238–3241, 1979.
22. Mahadevappa VG, Holub BJ: Biochem Biophys Res Commun 134:1327–1333, 1986.
23. Bills TK, Smith JB, Silver MJ: J Clin Invest 60:1–6, 1977.
24. Broekman MJ: J Lipid Res 27:884–891, 1986.
25. Mahadevappa VG, Holub BJ: J Lipid Res 28:1275–1280, 1987.
26. Walsh CE, Waite BM, Thomas MJ, DeChatelet LR: J Biol Chem 256:7228–7234, 1981.
27. Walsh CE, DeChatelet LR, Chilton FH, Wykle RL, Waite BM: Biochim Biophys Acta 750:32–40, 1983.
28. Chilton FH, O'Connel TR: J Biol Chem 263:5260–5265, 1988.

29. Sellmayer A, Strasser TH, Weber PC: Biochim Biophys Acta 927:417–422, 1987.
30. Haines KA, Giedd KN, Rich AM, Korchak HM, Weissmann G: Biochem J 241:55–62, 1987.
31. Serhan CN, Broekman MJ, Korchak HM, Marcus AJ, Weissmann G: Biochem Biophys Res Commun 107:951–958, 1982.
32. Wynkoop EM, Broekman MJ, Korchak HM, Marcus AJ, Weissmann G: Biochem J 236:829–837, 1986.
33. Mahadevappa VG: Biochem Biophys Res Commun 153:1097–1104, 1988.
34. Prescott SM: J Biol Chem 259:7615–7621, 1984.
35. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese III J, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF: N Engl J Med 312:1217–1224, 1985.
36. Strasser T, Fischer S, Weber PC: Proc Natl Acad Sci USA 82:1540–1543, 1985.
37. Prescott SM, Zimmerman GA, Morrison AR: Prostaglandins 30:209–227, 1985.
38. Victor M, Weiss J, Klempner MS, Elsbach P: FEBS Lett 136:298–300, 1981.
39. Franson R, Weiss J, Martin L, Spitznagel JK, Elsbach P: Biochem J 167:839–841, 1977.
40. Bormann BJ, Huang CK, Mackin WM, Becker EL: Proc Natl Acad Sci USA 81:767–770, 1984.
41. Matsumoto T, Tao W, Sha'fi RI: Biochem J 250:343–348, 1988.
42. Balsinde J, Diez E, Schuller A, Mollinedo F: J Biol Chem 263:1929–1936, 1988.
43. Boyum A: Scand J Clin Invest 21(Suppl 97):77–89, 1968.
44. Bligh EG, Dyer WJ: Can J Biochem Physiol 37:911–919, 1959.
45. Powell WS: Anal Biochem 164:117–131, 1987.
46. Borgeat P, Pickard S: Anal Biochem 171:283–289, 1988.
47. Elsbach P: Semin Hematol 9:227–239, 1972.
48. Rubin RP, Sink LE, Shrey MP, Day AR, Liao CS, Freer RJ: Biochem Biophys Res Commun 90:1364–1370, 1979.
49. Sekiguchi ML, Tsukuda M, Ase K, Kikkawa U, Nishizuka Y: J Biochem 103:759–765, 1988.
50. Lee TH, Mencia-Huerta J-M, Shih C, Corey EJ, Lewis RA, Austen KF: J Clin Invest 74:1922–1933, 1984.
51. Jakschik BA, Sams AR, Sprecher H, Needleman P: Prostaglandins 20:401–409, 1980.
52. Ochi K, Yoshimoto T, Yamamoto S, Taniguchi K, Miyamoto T: J Biol Chem 258:5754–5758, 1983.
53. Stenson WF, Prescott SM, Sprecher H: J Biol Chem 259:11784–11789, 1984.
54. Culp BR, Titus BG, Lands WEM: Prostaglandins Med 3:269–278, 1979.
55. Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H: Proc Natl Acad Sci USA 76:944–948, 1979.
56. Corey EJ, Shih C, Cashman JR: Proc Natl Acad Sci USA 80:3581–3584, 1983.