Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1045-1052

Augmentation of IgE-Mediated Release of Histamine by 5-Hydroxyeicosatetraenoic Acid and 12-Hydroxyeicosatetraenoic Acid

William F. Stenson†, Charles W. Parker and Timothy J. Sullivan

Department of Medicine, Divisions of Gastroenterology and Immunology and the Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO and the Department of Internal Medicine, Allergy and Clinical Immunology Section, University of Texas Health Science Center at Dallas, TX.

Received August 26,1980

SUMMARY

Highly purified rat mast cells converted 1.12% and 1.64% of exogenously added [1-^{14}C]arachidonic acid to 5-OH-6,8,11,14 eicosatetraenoic acid (5-HETE) and 12-OH-5,8,10,14 eicosatetraenoic acid (12-HETE) respectively during a three minute incubation at 37°. Both 5-HETE and 12-HETE (1-10 μM) augmented the histamine release response to goat anti-rat IgE antibody (a reverse anaphylaxis system). These results indicate that mast cells synthesize 5-HETE and 12-HETE and that these molecules can enhance mediator release.

INTRODUCTION

Stimulation of mast cells by antigen-IgE interactions, anaphylotoxins or other agonists activates a series of intracellular biochemical processes which very rapidly lead to the release of inflammatory mediators (1,2), including newly formed lipids such as slow reacting substance (SRS)[1] (3). Marked alterations in phospholipid metabolism have been detected (4,5,6) and there is evidence that at least three fusogenic substances accumulate in mast cell membranes; diglycerides, monoglycerides and certain unsaturated fatty acids (5,6). A possible role for newly formed metabolites of arachidonic acid (AA) in mediator release has been suggested by recent studies in isolated rat mast cells (7) and human peripheral blood leukocytes (8,9). In both

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† To whom correspondence should be addressed at Division of Gastroenterology, Washington University Medical School, St. Louis, MO 63110

1 Abbreviations used are SRS, slow reacting substance; AA, arachidonic acid, 5-HETE 5-OH-6,8,11,14 eicosatetraenoic acid; 12-HETE, 12-OH-5,8,10,14 eicosatetraenoic acid; ETYA, 5,8,11,14 eicosatetraynoic acid; MCM, mast cell medium; TLC, thin layer chromatography; HPLC, high pressure liquid chromatography; PG, prostaglandin; 13-HPLA, 13-hydroperoxylinoleic acid; HPETE, hydroperoxyeicosatetraenoic acid.

0006-291X/80/191045-08$01.00/0
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systems mediator release is inhibited by 5,8,11,14 eicosatetraynoic acid (ETYA), an acetylenic analog of AA capable of inhibiting both the cyclooxygenase and lipoxygenase pathways, with IC_{50} values of 17 to over 60 μM depending on the stimulus. Aspirin and indimethacin either fail to affect or enhance mediator release indicating that the inhibitory effect of ETYA probably involves the lipoxygenase pathway.

In this report we provide evidence that two lipoxygenase products of AA metabolism, 5-OH-6,8,11,14 eicosatetraenoic acid (5-HETE) and 12-OH-5,8,10,14 eicosatetraenoic acid (12-HETE), considerably enhance mediator release from isolated rat mast cells. The present studies also indicate that mast cells synthesize 5-HETE and confirm a previous report that these cells produce 12-HETE (10). Finally, mast cells incubated with ^{14}C-5-HETE are shown to esterify 5-HETE into triglycerides and phosphatidylcholine indicating a possible mechanism for its effect on mediator release. These results suggest that lipoxygenase products may exert an important influence on the release of inflammatory mediators from mast cells.

METHODS

Rat mast cell preparations. Highly purified mast cells from the pleural and peritoneal cavities of male Sprague Dawley rats (Camm Research Institute, Wayne, NJ) were obtained as described previously (11). The preparations used in these experiments were greater than 95% pure mast cells by morphologic criteria (12) and were greater than 98% viable as assessed by trypan blue exclusion. None of the experimental procedures reduced mast cell viability below 96%.

Reagents. The reagents used in these studies were from sources recorded in previous reports (7,9,11). Unlabeled 5-HETE and 12-HETE were prepared as described previously (9,13). [^{14}C]5-HETE was biosynthetically prepared by incubating human peripheral blood neutrophils with [^{14}C]AA as previously described (14).

Cell incubation conditions. Studies of histamine release were performed in 12 x 75 mm polypropylene tubes containing 10^5 mast cells in a final volume of 0.10 ml of mast cell medium (MCM)(7). Conditions for release of histamine by goat anti-rat IgE antibody are based on previous detailed studies (7). The sequence of addition is detailed in Results. Each experimental condition was performed in triplicate. Each experiment was repeated at least three times. Incubations were conducted at 37° C without agitation after mixing initially. After incubation 0.40 ml of ice cold medium was added and the tubes were centrifuged at 1400 x g for two minutes at room temperature. The supernatant solutions were obtained by decantation, frozen and stored at -20° C for later assay of histamine.

Histamine assay. The radioenzymic assay developed by Snyder and co-workers (15) using rat kidney homogenates as a source of histamine N-methyltransferase was used to measure histamine. Total histamine content was determined on cell samples heated at 105° C for three minutes.

Arachidonate metabolism. Rat peritoneal mast cells were suspended in MCM with ovalbumin (1 mg/ml) and incubated for 5 minutes at 37° C. [^{14}C]arachidonic acid (final concentration 5 μM) was then added and the incubation continued for an additional three minutes. The final incubation volume was 7 ml and the final cell concentration

1.1 x 10^6/ml. The incubation was ended by briefly immersing the tubes in ice followed by a five minute centrifugation at 4° C. The mixture was extracted by adding 0.1 volume of glacial acetic acid and 2.4 volumes of ice cold chloroform:methanol (1:1). The extracted lipids were analyzed by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) (9,14). TLC was done on silicic acid plates (Brinkman Sil-G25) developed with solvent system I (benzene:ether:ethanol:acetic acid, 50:40:2:0.2), solvent system II (benzene:dioxane:acetic acid, 60:30:3), solvent system III (organic layer of ethyl acetate:acetic acid:2,4,4-trimethylpentane:water, 45:10:25:50), and solvent system IV (organic phase of ethyl acetate:2,2,4-trimethylpentane:water, 75:150:150). For studies using solvent system IV the samples were first converted to their methyl esters with diazomethane. The capabilities of these solvent systems in separating known AA metabolites has been discussed in detail previously (14). HPLC was performed with a preparative Knauer-Unimetrics C-18 column on a Varian 8550 instrument. An isocratic elution with methanol:water:acetic acid (75:25:0.1) was used. The flow rate was 1 ml/min and a total volume of 100 ml was collected in 2 ml individual fractions.

Incubation of mast cells with [^{14}C]5-HETE. Purified rat peritoneal mast (1 x 10^6/ml) were incubated for 20 minutes at 37° C with [^{14}C]5-HETE (2.5 μM) in a volume of 2 ml. Lipids were extracted as described above and subjected to two dimensional TLC (14). To determine whether radiolabel in the triglyceride and phosphatidylcholine bands were indeed esterified 5-HETE the bands were scraped, eluted, and subjected to transesterification in anhydrous methanol containing 0.2 N NaOH (14). The liberated fatty acid methyl esters were then chromatographed using solvent system IV and compared with known standards.

RESULTS

Effect of 5-HETE and 12-HETE on histamine release. Both 5-HETE and 12-HETE at concentrations of 1 μM and 10 μM considerably augmented anti-IgE induced histamine release (Figure 1). Anti-IgE plus 5-HETE (1 μM) released 158% of the histamine released by anti-IgE alone. The data in Figure 1 are expressed as percent of control because of the variations in anti-IgE-induced release. Anti-IgE (10 μg/ml) alone released 32.6 ± 4.7% of histamine (mean ± S.E. for four experiments), while anti-IgE plus 5-HETE (1 μM) released 50.7 ± 6.9% ($p < .05$), and anti-IgE plus 12-HETE (10 μM) released 60.7 ± 4.6% ($p < .02$). Neither 5-HETE nor 12-HETE had any effect on histamine release when used without anti-IgE (Figure 1). 5-HETE (1 μM) enhanced histamine release whether added before or up to 5 minutes after anti-IgE (Table I) indicating that enhancement can occur even after release is under way. 12-HETE enhanced release when added before or simultaneously with anti-IgE but not when added after. 5-HETE (1 μM) augmented histamine released at low, intermediate and high concentrations of anti-IgE (Figure 2). A representative experiment is shown because of variations in the anti-IgE dose response curves between experiments, but in three separate experiments the percent release in the presence of

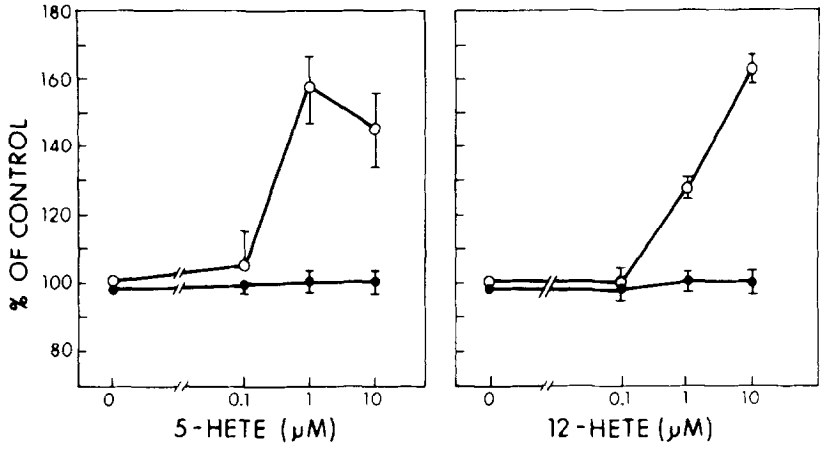

Figure 1 Effect of 5-HETE and 12-HETE on mediator release from mast cells. Anti-IgE (10 μg/ml, o——o) or medium as a control (●——●) and the indicated concentrations of 5-HETE and 12-HETE were added to mast cell cultures at the same time. After a 20 minute incubation at 37° C the amount of histamine released was determined as described in the text. For each experiment the data for conditions containing 5-HETE or 12-HETE are expressed as a percent of control. Each data point represents the mean ± S.E. for four experiments done in triplicate. All data is corrected for spontaneous release of histamine which was 2-3%.

Table I

Influence of the Time of Addition on the Enhancement of Anti-IgE Induced Mediator Release by 5-HETE and12-HETE

Addition	Time of Addition[a]	Percent Control Histamine Release[b]
5-HETE	-15 minutes	143 ± 10.4
12-HETE		167 ± 22.7
5-HETE	-05 minutes	146 ± 12.3
12-HETE		168 ± 21.7
5-HETE	Simultaneous	136 ± 10.3
12-HETE		116 ± 4.7
5-HETE	+05 minutes	136 ± 5.8
12-HETE		112 ± 5.0

a. Mast cells were preincubated for 15 minutes at 37° C and then challenged with 1 μg/ml of anti-IgE for 15 minutes at 37° C. At various times during the preincubation or after anti-IgE addition either 5-HETE (final concentration 1 μM) 12-HETE (final concentration 1 μM) or medium alone were added. The times of addition relative to anti-IgE challenge are presented.

b. The effects of 5-HETE and 12-HETE on net anti-IgE induced histamine release were normalized as the percent of release observed when cells were challenged with anti-IgE in medium. The data are presented as the mean ± S.E.M. of results in three experiments. Addition of medium alone or as a control at varying times did not significantly affect the release process. Spontaneous release of histamine was 4.4 ± 1.7%. Net anti-IgE induced release was 32.4 ± 5.3%.

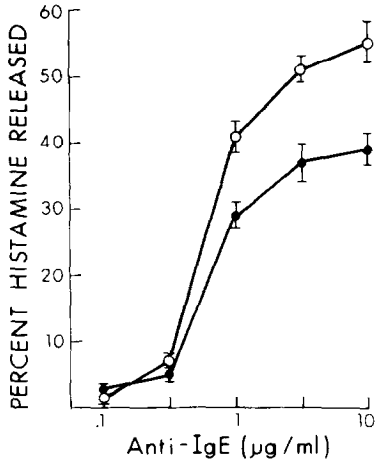

Figure 2 The effect of 5-HETE on the release of mediators induced by varying concentrations of anti-IgE. 5-HETE (1 μM, o——o) or medium as a control (●——●) and the indicated concentrations of anti-IgE were added to mast cell suspensions. After 20 minute incubation the amount of histamine released was determined. The data are presented as the mean ± S.E.M. of the results in one experiment. Similar results were obtained in two other identical experiments. Spontaneous release of histamine was 2.0%.

5-HETE was increased for every anti-IgE concentration. When these three experiments were analyzed by paired t-test the percent histamine release was significantly higher, ($p < .0$ in the presence of 5-HETE with the three highest concentrations of anti-IgE.

Arachidonate metabolism in mast cells. Over a 3 minute period mast cells converted 1.2% of exogenous ^{14}C-AA (5 μM) to 5-HETE, a previously unreported arachidonate metabolit in mast cells, as determined chromatographically in solvent systems I and II (Table II). They also converted 1.64% to 12-HETE, another lipoxygenase product originally described in platelets and more recently in mast cells by Roberts et al. (10). Cyclooxygenase products formed included PGD_2, PGE_2, $PGF_{2\alpha}$ and thromboxane B_2. The solvent systems used (I and II) do not separate 6-keto $PGF_{1\alpha}$ from phospholipid and so we cannot comment on its production. There were also as yet unidentified products that had Rfs on TLC similar to other HETEs and diHETEs. To confirm that 5-HETE was indeed present, the band on TLC in solvent system I comigrating with authetic 5-HETE was scraped and eluted with methanol. One-third was subjected to TLC in

Table II

Metabolism of Exogenous [1- ^{14}C]arachidonic Acid by Mast Cells

Product	Percentage of total recovered radioactivity
Unmetabolized arachidonic acid	61.25
Phospholipids and 6-keto $PGF_{1\alpha}$	11.43
Triglycerides	3.80
12-HETE	1.64
5-HETE	1.12
Unidentified HETE's	3.80
di-HETE	1.12
PGD_2	11.78
TxB_2	0.77
PGE_2	0.53
$PGF_{2\alpha}$	2.76

Mast cells (1.1 x 10^6 ml) were incubated at 37° for 5 minutes with [1-^{14}C]AA (5 uM) in a final volume of 7 ml. The incubation was ended by immersing the incubation mixture in ice. Lipids were extracted and analyzed by TLC and HPLC as described in Methods.

solvent system III, comigration with authentic 5-HETE was again observed. The second third was converted to its methyl ester and subjected to TLC in solvent system IV in which it comigrated with authentic 5-HETE-methyl ester. The final third was analysed by HPLC as described in Methods. This compound had the same retention volume, 40 to 42 ml, as authentic 5-HETE.

Incubation of mast cells with [^{14}C]5-HETE. We have previously reported that 5-HETE and 12-HETE are incorporated covalently into neutrophil phospholipids and triglycerides. To determine if mast cells also have this capability they were incubated with exogenous [^{14}C]5-HETE; while much of the radiolabel remained in the med as [^{14}C]5-HETE, 1.2% of the radiolabel was incorporated into phosphatidylcholine and 1.0% in triglycerides as determined by TLC of the lipid extracts of the cells. After transesterification of the phosphatidylcholine and triglyceride band from the TLC plates and rechromatography on TLC in solvent system IV, a single band comigrating with authentic 5-HETE methyl ester was observed.

DISCUSSION

In this study we have demonstrated that rat peritoneal mast cells make the lipoxygenase product, 5-HETE, and have confirmed that these cells make 12-HETE (10). The synthesis of 5-HETE by mast cells is of interest in regard to their previously

demonstrated ability to make SRS (3). SRS and 5-HETE are both products of the lipoxygenase pathway and are derived from the same intermediate 5-hydroperoxy-6,8,11,14 eicosatetraenoic acid (5-HPETE) (16). Other cells that synthesize SRS, including neutrophils and rat basophilic leukemia cells, also synthesize 5-HETE; whereas human platelets, which make 12-HETE but not 5-HETE, produce little, if any, SRS. Thus, the apparent absence of 5-HETE synthesis by mast cells in the study of Roberts et al. (10) represented a contradiction which has now been resolved. Both the present study and earlier work (10) described the presence of other mast cell AA products that have chromatographic characteristics consistent with HETEs in addition to the 5-HETE and 12-HETE described. In addition we found a product on TLC that comigrates with 5,12 di-HETE. Work is in progress on the identification of these products.

The augmentation of histamine release by 5-HETE and 12-HETE is consistent with studies from our own laboratories on specific granule release in human peripheral blood neutrophils (9). In this earlier work we found that low μM concentration of 5-HETE and 12-HETE can directly induce release of specific granules but not azurophilic granules. In the neutrophil experiment 5-HETE and 12-HETE induced degranulation directly, in the absence of other stimuli, whereas in mast cells the HETEs alone do not induce degranulation but do considerably augment degranulation in response to anti-IgE. Taking the results in these two systems together it is apparent that the role of hydroxylated fatty acids in other secretory responses deserves careful study.

The esterification of 5-HETE into mast cell triglycerides and phospholipids is also in accord with the results of similar experiments done in neutrophils (13,14). It is not certain that the incorporation of these hydroxy fatty acids into complex lipids is related to their effects on degranulation, but is easy to imagine how the presence of hydroxy fatty acids in the phospholipid bilayer could promote membrane fusion or otherwise alter membrane stability.

There have been suggestions from previous work that lipoxygenase products may have a role in mast cell mediator release. Adcock and colleagues (17) showed that

exogenous 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and 13-hydroperoxylinoleic acid (13-HPLA) enhanced anaphylactic release of histamine and SRS from guinea pig isolated perfused lungs. These are hydroperoxy fatty acids similar to 5-HPETE, the precursor of 5-HETE. The hydroperoxy fatty acids may be active in themselves or they may be spontaneously or enzymatically converted to the corresponding hydroxy compounds which are more stable. In addition to the instability of the hydroxy compo there are two major differences between the work of Adcock et al. and that presented here. 15-HPETE and 13-HPLA are not naturally occurring mast cell products whereas 5-HETE and 12-HETE are. In the isolated perfused lung system, 15-HPETE and 13-HPLA may be exerting effects on vascular permeability or on one of the other cell types rather than on mast cells.

Acknowledgements We gratefully acknowledge the expert technical assistance of Ms. Elizabeth Lobos and Edward Lewis. This work was supported in part by NIH grants AI 17298 and AI 00414.

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