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LIPID METABOLISM DURING MEDIATOR RELEASE FROM MAST CELLS: STUDIES OF THE ROLE OF ARACHIDONIC ACID METABOLISM IN THE CONTROL OF PHOSPHOLIPID METABOLISM[1]

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Recent studies indicate that both arachidonic acid (AA) metabolism and phospholipid (PL) metabolism are markedly stimulated during the release of mediators from mast cells. The relationship between stimulated AA metabolism and stimulated PL metabolism in isolated rat mast cells was investigated and then correlated with the secretory process. ETYA (5,8,11,14-eicosatetraynoic acid, a known inhibitor of cyclooxygenase and lipoxygenase pathways of AA metabolism) inhibited $^{32}PO_4$ incorporation into phosphatidic acid (PA), phosphatidylinositol (PI), and phosphodidylcholine (PC) in both unstimulated mast cells and mast cells stimulated by cross-linking of surface IgE molecules. ID_{50} values for inhibition of mediator release and of basal and stimulated $^{32}PO_4$ incorporation into PL were 50 to 60 μM ETYA. Indomethacin (1 to 10 μM) and aspirin (10 to 100 μM) had no significant effect on $^{32}PO_4$ incorporation or on mediator release. AA (10 μM) inhibited PL labeling in resting mast cells and rendered the cells less responsive to secretory signals. Preincubation of the cells with indomethacin (1 μM) blocked both of these AA effects. When AA was added to stimulated mast cells, however, both PL labeling and mediator release were enhanced. Thus, each of the alterations in AA metabolism caused parallel changes in mast cell PL metabolism and in mediator release. Since both basal and stimulated PL metabolism were modified by ETYA and AA, some form of direct regulation of mast cell PL metabolism by AA metabolites seems likely. The close parallelism of effects on mediator release and on PL metabolism suggests that modulation of mast cell function by AA metabolites may be mediated at least in part by effects on lipid metabolism.

The release of inflammatory mediators from mast cells and basophils is of central importance in immediate hypersensitivity reactions and appears to contribute to complement and lymphocyte-mediated inflammation. Despite the well known importance of the mediator release process, knowledge of the precise mechanisms of release is incomplete. Activation of cell surface receptors by antigen-IgE reactions, C_{5a}, C_{3a}, or by a variety of other signals is known to lead to fusion of the plasma membrane with the membranes surrounding cytoplasmic granules (1). Preformed mediators such as histamine that are contained in these granules are then released into the extracellular space. Activation also leads to the formation and release of slow-reacting substances of anaphylaxis (SRS-A),[5] prostaglandin D_2 (PGD_2), and other arachidonic acid (AA) metabolites (1–3). The release of both preformed and newly formed mediators can be inhibited by pharmacologic agents that increase cellular cyclic 3′5′-adenosine monophosphate (cAMP) levels (1, 4), but until recently little was known about the actual biochemical processes leading from surface activation to mediator release.

Recent studies in this laboratory have provided evidence that the conversion of AA to some active moiety may be a necessary part of the release process in rat mast cells stimulated by anti-IgE, conconavalin A (Con A), or the calcium ionophore A23187 (5). Studies by Goth (6) indicate that similar AA metabolism appears to be necessary for release provoked by antigen, dextran, or 48/80. Since mediator release is inhibited by 5,8,11,14-eicosatetraynoic acid (ETYA) but not by aspirin or indomethacin, cyclooxygenase products of AA do not appear to be involved. The addition of AA to stimulated mast cells augments release as would be expected if AA metabolism were altered in activated mast cells and were involved in regulating the process. The active moiety has not yet been identified but is expected to be a product of the lipoxygenase pathway. A recent report by Marone and co-workers (7) indicates that AA also enhances mediator release from human basophils and that ETYA inhibits mediator release with ID_{50} values very similar to those reported in mast cells.

Separate studies in this laboratory have identified a 2nd change in lipid metabolism that occurs during mediator release; markedly stimulated incorporation of ^{32}P into phosphatidylcholine (PC), phosphatidic acid (PA) and phosphatidylinositol (PI) (8). These changes occur after mast cell activation by all stimuli examined and occur with early kinetics and dose-response curves very similar to those obtained for mediator-release kinetics and dose-response relationships. These observations suggest that altered lipid metabolism is in some way an intrinsic part of the secretory process.

The experiments presented in this report were designed to probe the relationship between these 2 newly recognized processes in stimulated mast cells. The influence of AA metabolism on phospholipid (PL) metabolism in resting and stimulated mast cells was studied and then correlated with effects on mediator release.

MATERIALS AND METHODS

Sources of materials. The reagents used and their sources were as follows: bovine serum albumin (BSA), acetylsalicyclic acid, AA, indomethacin, PI, and phosphatidylserine (PS) (Sigma, St. Louis, MO); heparin (ICN, Cleveland, OH); ^{32}P-orthophosphoric acid (carrier-free) in HCl-free water (New England Nuclear, Boston, MA); silica gel plates (20 x 20 cm) (Brink-

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[5] Abbreviations used in this paper: AA, arachidonic acid; PL, phospholipids; PI, 1-(3-sn-phosphatidyl)inositol; PC, 3-sn-phosphatidylcholine; PS, 3-sn-phosphatidylserine; PA, 3-sn-phosphatidic acid; TLC, thin-layer chromatography; MCM, mast cell medium; SRS-A, slow-reacting substances of anaphylaxis; PGD_2, prostaglandin D_2; ETYA, 5,8,11,14-eicosatetraynoic acid; IgE-PC, rat myeloma (IR-162) IgE haptenated with phosphorylcholine; 5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; cAMP, cyclic 3′5′-adenosine monophosphate.

mann Instruments, Westbury, NY); PA (Avanti, Birmingham, AL). The rat IgE myeloma IR-162 and the goat anti-rat myeloma IgE were prepared and characterized as previously described (9). ETYA was a gift from Hoffman LaRoche Laboratories. TEPC-15 myeloma IgA protein was prepared and characterized by the method of Chesebro and Metzger (10). IgE was substituted with phosphorylcholine (IgE-PC; 4.2 mols of phosphorylcholine/mol IgE) by the method of Isakson *et al.* (11). All other reagents were obtained from Fisher Scientific (St. Louis, MO). The kinetics of mediator release were determined using a Beckman Microfuge B.

Preparation of rat mast cell suspensions. Mast cells were obtained from male Sprague-Dawley rats (Camm Wayne, NJ) and purified by a BSA density gradient method that has been described in detail (12). Mast cell preparations were greater than 95% pure and 96% viable. No cytotoxicity was observed at the concentrations of reagents used in these experiments as assessed by trypan blue exclusion (13). Mast cell medium with phosphate (MCM-PO_4: 150 mM NaCl, 3.7 mM KCl, 5 mM Na_2HPO_4, 1.0 mM $CaCl_2$, 5.6 mM dextrose, 1 mg/ml BSA, 1 U/ml heparin, 2 mM piperazine-*N*-*N*'-bis(2-ethane sulfonic acid), pH 6.8) was used during isolation and purification. After these procedures, mast cell medium without phosphate (MCM-P) was used.

Phosphate depletion and $^{32}PO_4$ incorporation. Purified mast cells were incubated in the presence of IgE-PC (0.2 mg/ml) for 60 min at 37°C. The mast cells were then depleted of endogenous phosphate by washing twice in MCM-P (no added phosphate) followed by incubation for 60 min at 37°C in MCM-P. After a final wash in MCM-P, 3×10^5 cells were placed into individual tubes containing 40 μCi $^{32}PO_4$ in MCM-P. Incorporation of labeled phosphate was permitted to progress for 5 min at 37°C before experimental procedures were initiated. Experiments using $^{32}PO_4$-labeled cells were performed in a final volume of 0.25 ml. Reactions were halted by adding 2 ml ice-cold MCM-PO_4, centrifuging at 1000 × G at 4°C for 2 min, aspirating the supernatant, and immediately extracting the cell pellet (see below). All experimental conditions were performed in triplicate.

Preparation of reagents. All pharmacologic agents and media were prepared immediately before use. AA was dissolved in 0.1 M sodium carbonate and then diluted with MCM-P. Indomethacin was dissolved in absolute ethanol at a concentration of 10 mg/ml and then diluted with MCM-P. ETYA was dissolved in dimethylsulfoxide and then diluted with medium. Aspirin was made up to 10 mg/ml in ethanol, evaporated to dryness with N_2, dissolved in MCM-P, and used immediately. TEPC-15 was dissolved in MCM-P containing PS (100 μg/ml). PS was present in the final incubation media at 10 μg/ml. Diluent controls were performed for all of these reagents with no detectable effects observed at the dilutions used in these experiments.

Isolation of mast cell PL. Lipid extraction of the cell pellet was performed as previously described (8) by a modification of the method of Bligh and Dyer (14). After extraction, samples were centrifuged at 1000 × G for 10 min to remove insoluble material. The supernatant was decanted into tubes containing 0.5 ml $CaCl_2$ and 0.5 ml chloroform, 150 μg rat liver lipid extract, 20 μg PA, and 15 μg PI. Tubes were capped, shaken, and after centrifugation at 1000 × G for 5 min, the upper aqueous phase was discarded. The lower organic phase was evaporated to dryness under N_2 at room temperature. Fifty microliters of absolute ethanol were added to each tube, followed by drying under a stream of N_2. Sixty microliters of chloroform were then added to each tube, and samples were stored at −80°C until chromatographic analysis (1 to 2 days). Samples were chromatographed in a 2-dimensional thin-layer system shown to resolve all of the major mast cell PL (8, 15). Lipid-containing areas were identified after spraying with methanolic iodine spray (0.8% w/v), cut from the plate, and placed in counting vials with 3 ml of Scintiverse for measurement of radioactivity in a liquid scintillation counter (Searle Mark III-6880).

Histamine release. Histamine-release experiments were performed under reaction conditions identical to those used for PL-labeling experiments except that unlabeled sodium phosphate was used in place of $^{32}PO_4$. The cell pellets and supernatants obtained by the rapid sedimentation of cells were saved for an assay of histamine with a modification (4) of the method of Shore *et al.* (16).

Analysis of data. The results described represent the mean ± standard error of the mean (SEM) of at least 3 experiments, each done in triplicate. Statistical significance was determined using the independent 2-tailed Student's *t*-test.

RESULTS

The TEPC-15 releasing system was utilized in these experiments because of the homogeneity of the reagents and the highly reproducible functional and biochemical reactions obtained. TEPC-15 is a well-characterized mouse IgA myeloma protein with affinity for phosphorylcholine (10). By substituting rat myeloma IgE with phosphorylcholine and then cross-linking the IgE with TEPC-15, a reversed-anaphylactic reaction occurs that is high reproducible. The heterogeneity of anti-IgE and antigen activation and the less than physiologic activation caused by Con A, 48/80, or A23187 produce functional and related metabolic effects that are much less reproducible.

Kinetic analysis of TEPC-15-stimulated histamine release and PL metabolism (Fig. 1). Preliminary experiments indicated that near-maximal release was caused by challenge of IgE-PC-treated mast cells with 300 μg/ml of TEPC-15. In order to characterize the rate of mediator release in this system, IgE-PC-treated mast cells were stimulated with 300 μg/ml TEPC-15, and the reactions were allowed to proceed up to 30 min. A microcentrifuge was used to separate cell pellets from the supernatant solutions in order to accurately study the early kinetics of mediator release (9). The time from the removal of the tube from the waterbath to the time of aspiration was kept constant at 30 sec. The 30-sec value in Figure 1 represents the result when centrifugation and aspiration procedures were begun immediately after TEPC-15 addition. (All reagents were 37°C before addition.) The results are presented as the time of aspiration of media from cell pellets. TEPC-15-induced mediator release began within 30 sec, was half completed at 45 to 60 sec, and was halted 3 to 5 min after stimulation. Phosphate depletion had no detectable effect on TEPC-15-, anti-IgE-, concanavalin A-, or A23187-induced mediator release (data not shown). In order to study the kinetics of PL labeling (Fig. 1), cells were incubated in IgE-PC, phosphate depleted, labeled with $^{32}PO_4$ for 5 min, and then challenged with 300 μg/ml TEPC-15. PA labeling was increased approximately 4-fold above control values within 15 sec with stimulation ratios ($^{32}PO_4$ cpm stimulated/cpm unstimulated) constant through 5 min. Stimulated PI labeling was significantly ($p < 0.02$) greater than control within 2 min; peak labeling occurred at 5 min. PC demonstrated changes in labeling that were similar to changes in PI labeling, with significant changes detectable 1 min after stimulation. Maximum stimulation ratios occurred between 2 and 5 min. By 30 min stimulation ratios in all 3 PL declined to approximately 2.0 (not shown). Increased labeling of PA was detectable within 15 sec, suggesting that this response preceded the onset of mediator rlease, whereas increased labeling of PI or PC were not detectable until active mediator release was occurring.

Dose-response relationships of mast cell mediator release and PL labeling induced by TEPC-15. Mast cells were challenged with TEPC-15 (0.1 to 300 μg/ml) for 15 min, and the amount of histamine released was determined (Fig. 2). PL labeling in parallel experiments with the use of the same cell preparations was ex-

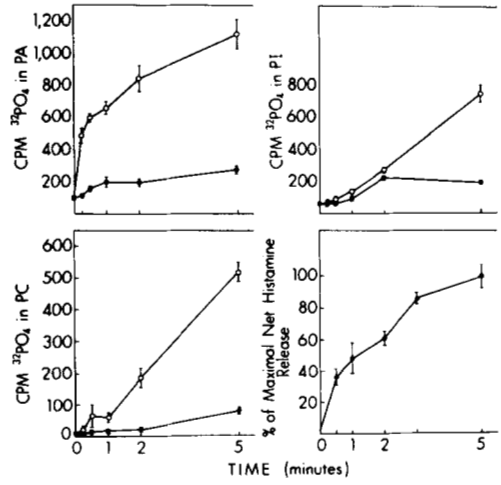

Figure 1. The kinetics of TEPC-15-stimulated histamine release and phospholipid labeling. Mast cells (3×10^5) prelabeled with $^{32}PO_4$ for 5 min were stimulated with TEPC-15 (300 μg/ml) + PS (10 μg/ml) (○——○) or PS alone (●——●). At the times shown, reactions were stopped by adding ice cold MCM-PO_4 followed by extraction of the cell pellet and separation of lipids by TLC. In studying the kinetics of histamine release, 1.5×10^5 mast cells were challenged with 300 μg/ml TEPC + PS 10 μg/ml (●——●), or PS alone. Data represent mean ± SEM of 3 experiments. Histamine release was determined at the indicated times as described in *Materials and Methods*. Data are presented as the mean ± SEM % of total histamine released in 3 experiments: maximal release was 43.4% (± 3.3 SEM) compared to a spontaneous release of 5.2% (± 1.1 SEM).

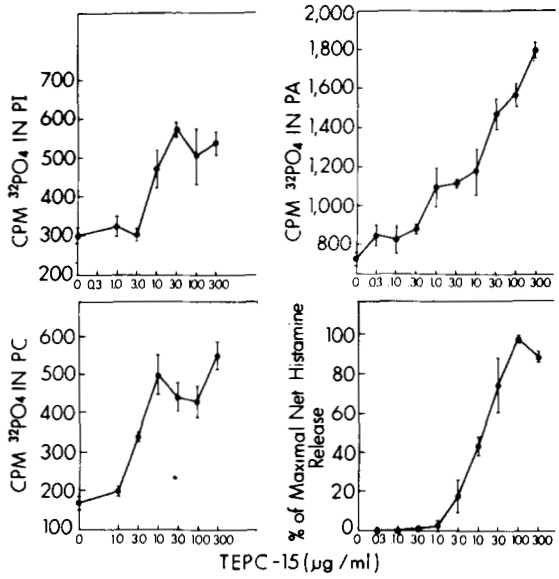

Figure 2. Effect of varying concentrations of TEPC-15 on mediator release and phospholipid labeling. 3×10^5 mast cells were prelabeled for 5 min with $^{32}PO_4$ and then challenged with varying concentrations of TEPC-15 (0 to 0.3 mg/ml) in the presence of 10 μg/ml of PS. Labeling was halted 5 min after stimulation by the addition of 250 μl of MCM-PO_4. In histamine release experiments 1.5×10^5 cells were used and reactions were halted 15 min after stimulation by the addition of 250 μl of MCM-PO_4 and rapid pelleting by centrifugation. Data are presented as the mean ± SEM of results in 3 experiments. Maximal histamine release was 41.0% (± 2.7 SEM) with a 3.8% (± 1.4 SEM) spontaneous release.

amined 5 min after challenge with TEPC-15 (0.1 to 300 μg/ml). Half-maximal stimulation of PL labeling and of histamine release were observed at 10 to 18 μg/ml of TEPC-15. Concentrations of TEPC-15 less than 0.3 μg/ml caused no histamine release or PL changes compared with control values. PA labeling was nearly linear with the TEPC-15 concentrations from 1 to 300 μg/ml, whereas maximal PC and PI labeling were achieved by TEPC-15 concentrations of 10 and 30 μg/ml, respectively. Thus the concentrations of TEPC-15 causing threshold and half-maximal mediator release and PL labeling were very similar.

Effects of ETYA, indomethacin, and aspirin on PL labeling. $^{32}PO_4$-labeled mast cells were preincubated at 37°C for 10 min with MCM-P, indomethacin (1 to 10 μM), or ETYA (10 to 100 μM) and then challenged with 300 μg/ml TEPC-15 (Fig. 3 and see Table II). At the concentrations studied, indomethacin and aspirin caused no significant changes in $^{32}PO_4$ incorporation into PA, PI, or PC in unstimulated or stimulated cells. In marked contrast, ETYA inhibited PL labeling in both unstimulated and stimulated mast cells, with nearly complete inhibition observed at 100 μM ETYA and 50% inhibition at approximately 60 μM ETYA for PA, PI, and PC.

Effects of AA on PL metabolism. These pharmacologic modification studies suggested a potent ability of AA metabolites to regulate mast cell PL metabolism. The effects of exogenous AA itself were then studied. Mast cells were labeled with $^{32}PO_4$ for 5 min, and then challenged with TEPC-15 or medium alone. AA was added at varying times in relation to TEPC-15 challenge, and the effects of these manipulations on PL metabolism were determined (Table I). Incubation of unstimulated mast cells in the presence of AA for 15 min caused a modest decrease in labeling of PA, PI, and PC. There was a marked decrease in PL labeling in TEPC-15-stimulated mast cells when the cells were preincubated with AA for 10 min. When AA was added simultaneously with TEPC-15, no alteration in PA labeling was noted. PI and PC labeling were significantly less than control values after addition of AA simultaneously with TEPC-15 in all of the 3 experiments performed. The addition of AA 1 or 2 min after TEPC-15 stimulation resulted in a modest but definite enhancement of $^{32}PO_4$ incorporation into PA. AA inhibition of PI and PC labeling was not observed when AA was added 2 min after TEPC-15. Thus AA added to resting cells inhibited PL responses, but when added to activated cells AA enhanced PA formation and no longer inhibited PI and PC labeling.

Effects of AA on mast cell PL metabolism in the presence and absence of indomethacin. In order to probe the role of cyclooxygenase metabolites in these AA effects on PL metabolism, the effect of AA was examined in the presence or absence of the potent cyclooxygenase inhibitor indomethacin. Mast cells were preincubated with 1 μM indomethacin or medium alone for 10 min followed by the addition of AA (10 μM) or medium for an additional 10 min. The cells were then challenged with TEPC-15 (300 μg/ml) or medium alone, and the $^{32}PO_4$ labeling was determined after 5 min (Table II). Indomethacin blocked the AA-induced decrease in $^{32}PO_4$ incorporation into PA, PI, and PC, but did not reverse the enhancement of PA labeling caused by AA added 2 min after TEPC-15 challenge.

Effects of AA, ETYA, indomethacin, and aspirin on mediator release induced by TEPC-15. The effects of these pharmacologic agents on TEPC-15-induced release were studied under same conditons that were used for PL studies. Since the results were not significantly different from results previously reported for release provoked by cross-linking of IgE by goat anti-IgE antibody (5), a summary of the results will be presented. Preincubation of mast cells in the presence of 10 μM AA for 10 min inhibited subsequent TEPC-15- (300 μg/ml) induced release by 31% (±5.6% SEM). Addition of AA (10 μM) to mast cells 2 min after TEPC-15 (300 μg/ml) enhanced mediator release 20% (±4.3% SEM). The inhibition but not the enhancement was abrogated by preincubation of the cells in the presence of indomethacin (1 μM).

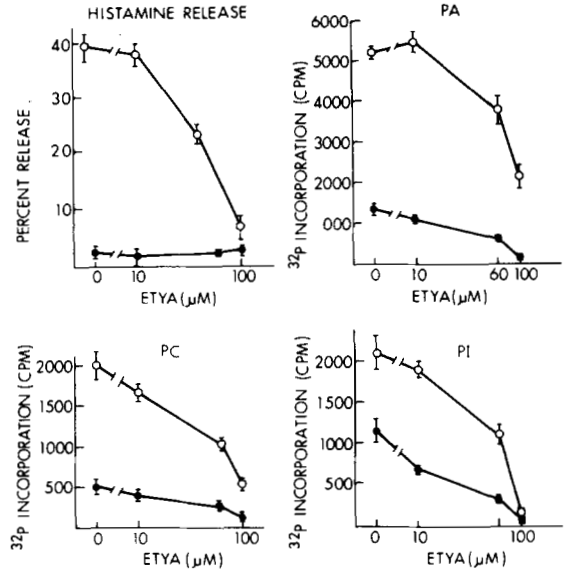

Figure 3. Effects of ETYA on phospholipid metabolism. After a 5-min prelabeling with $^{32}PO_4$, mast cells were incubated for 10 min with ETYA (10 to 100 μM) or MCM-P and challenged with TEPC-15 (300 μg/ml) + PS (10 μg/ml) or PS alone. Labeling was allowed to proceed for 5 min before halting the reaction. The data represent the mean ± SEM of triplicate observations in 1 of 3 identical experiments that produced very similar results.

TABLE I

Kinetics of arachidonic acid effects on mast cell phospholipid turnover

Time of AA Addition[a]	Agonist	% Control cpm $^{32}PO_4$ in[b]		
		PA	PI	PC
min				
−10	MCM-PS	74.8 ± 7.2	87.5 ± 7.5	80.0 ± 2.4
	TEPC-15	79.1 ± 2.7	58.0 ± 1.6	63.8 ± 2.1
Simultaneous	MCM-PS	89.1 ± 8.8	89.0 ± 3.4	111.0 ± 4.8
	TEPC-15	113.1 ± 9.2	82.5 ± 5.4	78.1 ± 6.2
+1	MCM-PS	105.0 ± 13.4	103.4 ± 9.0	131.5 ± 5.3
	TEPC-15	119.8 ± 1.6	82.3 ± 4.3	86.0 ± 2.0
+2	MCM-PS	118.2 ± 7.2	102.1 ± 6.1	110.5 ± 5.4
	TEPC-15	117.8 ± 10.8	103.8 ± 6.7	104.6 ± 3.6

[a] Times shown represent the time that AA (10 μM) was added either before (−10 min), during (simultaneous), or after (+1 and +2 min) TEPC-15 (300 μg/ml) + PS or PS only stimulation.

[b] Data represented mean ± SEM of 3 experiments.

TABLE II

Effect of arachidonic acid on mast cell phospholipid labeling in the presence and absence of indomethacin

Preincubation[a]	Addition	Time of Addition in Relation to TEPC-15 Stimulation	% of Control Labeling[b]		
			PA	PI	PC
		min			
MCM	AA	−10	63.7 ± 2.7	54.8 ± 6.4	83.8 ± 6.9
Indo 1 μM	MCM		101.7 ± 4.1	82.5 ± 12.6	135 ± 4.5
Indo 1 μM	AA		82.9 ± 3.8	85.9 ± 4.0	111.3 ± 0.8
MCM	AA	+1	110.8 ± 14.4	67.3 ± 3.9	69.2 ± 14.2
Indo 1 μM	MCM		112.0 ± 17.6	96.3 ± 6.7	94.0 ± 3.5
Indo 1 μM	AA		108.2 ± 2.3	86.0 ± 2.1	100 ± 1.1
MCM	AA	+2	127.0 ± 13.4	105.9 ± 9.8	110.8 ± 2.9
Indo 1 μM	MCM		125.1 ± 10.4	116.1 ± 6.7	117.0 ± 4.3
Indo 1 μM	AA		126 ± 13.4	100.7 ± 5.5	102.1 ± 13.0

[a] Mast cells were incubated with either medium or indomethcin (1 μM) for 10 min followed by the addition of AA at various times (−10, +1, +2 min) in relation to PS or TEPC-15 stimulation.

[b] Data presented are triplicate determinations in 1 representative experiment. Nearly identical results were obtained in 2 other experiments.

ETYA inhibited TEPC-15-induced release with an ID_{50} of 60 μM (±5.3 SEM). Aspirin and indomethacin had no effect on the stimulated release.

DISCUSSION

The results of these experiments provide new evidence that alterations in lipid metabolism are tightly linked to the release of mediators from mast cells. Previous studies in this laboratory have shown that mediator release provoked by anti-IgE, Con A, 48/80, or the ionophore A23187 is associated with selective stimulation of the incorporation of ^{32}P into PA, PI, and PC (8). The dose-response curves for stimulated PL formation and for mediator release were nearly identical. Kinetic analysis of the anti-IgE response revealed that increased labeling of PA occurred within 3 to 8 sec after anti-IgE challenge and that PI and PC formation were increased within 30 to 45 sec. Conditions that enhanced or inhibited mediator release were found to cause parallel changes in the stimulated labeling of PL. Cross-linking of surface IgE molecules with anti-hapten antibody in the present studies led to PL responses that occurred before or during mediator release over the same TEPC-15 concentration range that caused mediator release. Inhibition or enhancement of anti-hapten antibody-induced release by ETYA or AA caused parallel changes in stimulated incorporation of ^{32}P into PL. The diversity of secretory agonists causing the same selective changes in mast cell PL formation, with concentration dependence and kinetics very similar to those observed for functional responses, and the covariance of these metabolic and functional responses when mediator release is pharmacologically enhanced or inhibited, strongly indicate that these are intrinsically related processes.

ETYA, an acetylenic derivative of AA known to inhibit cyclooxygenase and lipoxygenase enzymes (17) and rat mast cell diglyceride lipase (18), inhibited the incorporation of $^{32}PO_4$ into PA, PI, and PC in unstimulated mast cells, indicating that ETYA can affect PL metabolism independent of effects on cell activation. The mechanisms of these effects are not yet clear, although the failure of aspirin or indomethacin to alter basal PL labeling suggests that ETYA is not acting through inhibition of cyclooxygenase enzymes (19). These results are compatible with the notion that mast cell PL metabolism may be stimulated by some lipoxygenase product of AA constantly being formed in resting mast cells. Recent studies (3, 20) have demonstrated the ability of rat mast cells to synthesize 5-OH-6,8,11,14 eicosatetraenoic acid (5-HETE) and 12-OH-5,8,10,14-eicosatetraenoic acid (12-HETE). Both of these molecules have been reported to augment rat mast cell secretion (20). Thus interruption of endogenous production of 5-HETE and 12-HETE in resting or stimulated mast cells could contribute to or potentially account for the ETYA effects. The concentrations of ETYA required to modulate mediator release and PL metabolism in this study would be expected to inhibit diglyceride lipase activity and thereby to inhibit liberation of the precursor AA (18) while also inhibiting lipoxygenase modification of AA (17). Adcock and colleagues (21, 22) have reported that 15-hydroperoxy arachidonic acid and 13-hydroperoxy linoleic acid augment mast cell mediator release in guinea pig lung, raising the possibility that a variety of endogenous products of the actions of lipoxygenases on fatty acids may influence mast cell mediator release. ETYA may inhibit the formation of this entire group of molecules by inhibiting fatty acid liberation (18). The concentrations of ETYA causing dose-related inhibition of mediator release (10 to 100 μM), inhibition of stimulated PL labeling, and inhibition of basal PL labeling were very similar, in keeping with a possible common mechanism.

The ability of exogenous AA to enhance mediator release and PA labeling when added to stimulated mast cells provides additional evidence that AA or its metabolites may be involved in the activation of lipid metabolism and mediator release. The inability of indomethacin to block this enhancement suggests that lipoxygenase products of AA may be the active forms. Exogenous AA is known to be converted to 5-HETE and 12-HETE in rat mast cells, and these molecules in turn would be expected to augment mediator release (20). Alternatively, Irvine and co-workers (23) have presented evidence that free AA can activate rat liver microsomal phospholipase C activity. Mediator release would be expected to be stimulated if rat mast cell phospholipase C activity also is activated by free AA (18, 24).

The possible roles of AA in controlling mast cell lipid metabolism and mediator release do not appear to be restricted to activation, however, since the addition of AA to unstimulated mast cells partially inactivated the cells to TEPC-15 stimulation of PL metabolism and histamine release. Indomethacin blocked these biochemical and functional effects of AA, indicating that these actions are mediated by the formation of cyclooxygenase pathway derivatives of AA. Cyclooxygenase products can increase rat mast cell cAMP levels (12). Increased cAMP levels in turn can inhibit mediator release (4) and can suppress alterations in PL metabolism in stimulated cells (25, 26). Incorporation of ^{32}P into PL in resting mast cells was inhibited by exogenous AA, suggesting an affect on lipid metabolism independent of cell activation, as was observed with ETYA. Exogenous AA can inhibit basal and stimulated PL labeling and mediator release, apparently through the actions of cyclooxygenase products, but much work remains to be done to determine whether or not AA effects on lipid metabolism account for the effects on mediator release.

These experiments provide evidence that endogenous cyclooxygenase products can inhibit lipid metabolism and mediator release in mast cells, but leave unanswered the question of whether or not this potential action is important in normal mast cell regulation. The inability of aspirin or indomethacin (up to 60 μM) to modify rat mast cell mediator release *in vitro* (5) indicates that this potential mechanism is not detectably active under the conditions examined. Indomethacin does augment mediator release from guinea pig mast cells in a perfused lung preparation (22) and augments mediator release from basophils in mixed human peripheral blood leukocyte preparations (7), suggesting that this system may be active in complex tissues of other species. Rat mast cells do form cyclooxygenase products (e.g., PGD_2) and lipoxygenase products in the resting state, however, and form increased amounts of AA metabolites from both of these pathways after stimulation (2, 3). The inhibitory effect of cyclooxygenase products on lipid metabolism and mediator release could therefore be active *in vivo*.

The results of this study suggest the hypothesis that mast cell lipid metabolism and mediator release are regulated at least in part by opposing actions of different AA metabolites: these processes are stimulated by some AA metabolites, presumably lipoxygenase products, and are inhibited by certain cyclooxygenase products of AA. This hypothesis and the relationship of the observed changes in lipid metabolism to mediator release must be systematically examined to establish the precise role of lipid metabolism in mediator release from mast cells. These reactions also must be examined to determine their relationship to the lipid methylation reactions that are associated with cross-linking of IgE receptors (27).

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GLUCOCORTICOID RECEPTORS AND *IN VITRO* CORTICOSENSITIVITY OF PEANUT-POSITIVE AND PEANUT-NEGATIVE HUMAN THYMOCYTE SUBPOPULATIONS[1]

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In 6 human thymus glands, the immature subset of thymocytes was separated from the more mature one, by differential peanut lectine agglutination. These 2 cell subpopulations were analyzed for glucocorticoid receptor content by using a whole cell assay, with (^{3}H)-triamcinolone acetonide as tracer. The unagglutinated thymocytes (peanut negative) contained about 2 times more receptor sites per cell than agglutinated (peanut positive) ones (7650 ± 1550 S.D. *versus* 3195 ± 896 S.D.). The affinity for steroid was similar in both cell subsets, as was the stereospecificity for glucocorticoids, the time-course of steroid-receptor association, and cytoplasmic to nuclear translocation. Despite the greater number of glucocorticoid receptor sites, the peanut-negative thymocyte subpopulation did not differ from the peanut-positive one in its sensitivity to the inhibitory effects of

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