

EFFECTS OF ARACHIDONIC ACID AND ITS METABOLITES ON ANTIGEN-INDUCED HISTAMINE RELEASE FROM HUMAN BASOPHILS *IN VITRO*¹

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We have studied the role of arachidonic acid (AA) metabolism in immediate hypersensitivity reactions. The AA analog, eicosa-5,8,11,14-tetraenoic acid (ETYA), which inhibits both the cyclo-oxygenase and lipoxygenase pathways of AA metabolism, was found to cause a dose-dependent inhibition of antigen-induced histamine release from human basophils; complete inhibition occurred at 10^{-4} M. In contrast, nonsteroidal anti-inflammatory drugs (NSAID) including indomethacin (10^{-9} to 10^{-6} M), meclofenamic acid (10^{-6} to 10^{-4} M), and aspirin (10^{-5} to 10^{-3} M), which block the cyclo-oxygenase pathway of AA metabolism, enhanced mediator release. AA (10^{-7} to 10^{-5} M) also enhanced basophil mediator release, an effect that was not affected by preincubation with indomethacin. Other fatty acids including linoleic, linolenic, and stearic acids were without effect. Indomethacin (and the other NSAID studied) reversed the inhibition caused by the AA metabolite, prostaglandin (PG) E₂, in a dose-dependent fashion. This reversal of inhibition was not unique to PG, but was observed with all inhibitors of histamine release studied that act via activation of adenylate cyclase including isoproterenol and the H₂ agonist, dimaprit. NSAID, however, did not reverse the inhibition caused by drugs that do not act on adenylate cyclase, i.e., dibutyryl cAMP, isobutylmethylxanthine, and 2-deoxyglucose. In the leukocyte preparations studied, the NSAID did not block the increase in the intracellular cAMP levels caused by agonists that act via adenylate cyclase. It appears that a product(s) of AA metabolism, generated via the lipoxygenase pathway, is involved in IgE-mediated histamine release and that the same or a different product(s) modulates the control mechanisms that are linked to adenylate cyclase.

Studies of arachidonic acid (AA)⁴ metabolism have become a main focus of those interested in immediate hypersensitivity reactions. Interest in this area is associated with the demonstration that the prostaglandins (PG) were capable of modulating antigen-induced histamine release in a number of systems (1-3). PG of the E series were found to be potent inhibitors of this response, whereas PG of the F series had either negligible effects or caused potentiation of the reaction (4). It was proposed in 1968 that aspirin-like compounds exerted their major therapeutic effects by antagonizing the action of PG (5). It was later shown, however, by Vane and his colleagues (6) that nonsteroidal anti-inflammatory compounds inhibited PG biosynthesis in a variety of tissue preparations. It is now appreciated that these agents act by inhibiting the cyclo-oxygenase pathway of AA metabolism (7). A more recently described antagonist, eicosa-5,8,11,14-tetraenoic acid (ETYA), an acetylene analogue of AA, blocks not only the cyclo-oxygenase pathway leading to PG synthesis but also the lipoxygenase pathway (8). ETYA has been shown to cause inhibition of mediator release in rat mast cells, presumably as a result of this metabolic block (9). This observation was interpreted as indicating that some product or products of AA metabolism were necessary for histamine release.

AA is a structural component of phospholipids, and recent work demonstrates that an increase in phospholipid turnover is associated with stimuli that lead to the secretion of histamine from rat mast cells (10, 11). The relationships between AA metabolism through pathways leading to PG synthesis and those leading to phospholipid synthesis are not, at present, totally understood. Also not clearly related, but of possible importance, is the recent observation that AA stimulated the production of slow-reacting substance of anaphylaxis (SRS-A), one of the mediators of allergic reactions (12), and may in fact be a precursor of this substance (13, 14), although this appears questionable in some systems (15).

As a result of these observations, we began an exploration of the role of AA and its metabolites in an *in vitro* model of human allergic reactions, antigen-IgE-induced histamine release from human basophils. The results indicate that a product of AA metabolism, presumably arising via the lipoxygenase pathway, is necessary for IgE-mediated histamine release. Furthermore, stimulation of the same pathway leads to a selective block of

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⁴ Abbreviations used in this paper: AA, Arachidonic acid; ETYA, eicosa-5,8,11,14-tetraenoic acid; Indo, indomethacin; PG, prostaglandin; iso, isoproterenol; dbcAMP, dibutyryl cyclic adenosine monophosphate; SRS-A, slow-reacting substance of anaphylaxis; ASA, acetylsalicylic acid; EDTA, ethylenediaminetetraacetic acid.

the inhibition of histamine release caused by PG and other agonists that act on adenylate cyclase.

MATERIALS AND METHODS

Reagents. Arachidonic, linoleic, linolenic, and stearic acids, indomethacin (Indo), DL-isoproterenol-HCl (Iso), acetylsalicylic acid (ASA), 3-isobutyl-1-methylxanthine (IBMX), dibutyl cyclic AMP (dbcAMP), 2-deoxyglucose, dimethyl sulfoxide (DMSO), and piperazine-*N,N'*-bis(2-ethane sulfonic acid) (PIPES) were purchased from Sigma Chemical Co., St. Louis, Mo. Human serum albumin was obtained from Miles Laboratories, Inc., Elkhart, Ind. Eicosa-5,8,11,14-tetraenoic acid (ETYA) was the gift of Dr. W. E. Scott, Hoffman-LaRoche, Inc., Nutley, New Jersey. PGE₂ was the gift of Dr. J. Pike, Upjohn Co., Kalamazoo, Michigan. Dimaprit was supplied by Dr. M. Parsons, Smith Kline and French, Herts, England. Sodium meclofenamate was obtained from the Parke-Davis Co., Ann Arbor, Mich. Ragweed antigen E was kindly provided by Dr. T. P. King (16).

There was some lot to lot variability in the inhibition of histamine release by ETYA. The ID₅₀ (concentration required for 50% inhibition of histamine release) varied from 1.4×10^{-5} M to 5×10^{-5} M. This may be due to binding of ETYA to albumin or to slight lot to lot variation of ETYA. AA at concentrations above 10^{-5} M (and occasionally at 10^{-5} M) inhibited release. At a concentration of 10^{-4} M, cell lysis occurred. This inhibitory effect of 10^{-5} M to 10^{-4} M AA was not blocked by 1μ M Indo.

Indo (10^{-3} M) was prepared in buffer, sonicated, and stored in the dark at 4°C. A fresh preparation was made biweekly. ETYA (10^{-1} M) was dissolved in DMSO and further diluted in buffer before use. DMSO at the concentrations used had no effect on histamine release. All other drugs were prepared immediately before use.

Buffers. a) PA, which contains 25 mM PIPES, 110 mM NaCl, 5 mM KCl, and 0.03% human serum albumin, was used to wash leukocytes; b) PACM, used for release studies, contained, in addition to the above, 0.6 mM CaCl₂·6H₂O and 1.0 mM MgCl₂. The buffers were adjusted to pH 7.3 before use.

Leukocyte donors. Venous blood was obtained from adult, atopic volunteers who had given informed consent. The donors had no history of aspirin idiosyncrasy and were not symptomatic when their blood was used. The donors had not taken any systemic medications for at least 48 hr nor any anti-inflammatory drugs for at least 1 week before venesection.

Histamine release assay. Blood was drawn into dextran-EDTA,⁴ allowed to sediment for 90 min, washed twice in PA, and the leukocytes were suspended in PA or PACM before challenge (17). Individual assay tubes usually contained the leukocytes from 0.5 ml of whole blood. In experiments involving AA, Indo, meclofenamic acid, and ASA, a 5-min preincubation of cells with drug was allowed before the addition of a second drug. ETYA was preincubated with cells for 10 min. The release reaction was then initiated by addition of antigen E and allowed to proceed for 45 min at 37°C. The total reaction volume was 1.0 ml. All experiments were carried out in duplicate or triplicate with the cells from three or more different donors, in separate assays, for each experimental design. When the two stages of histamine release were studied (18), cells were incubated for 2 min with antigen, with or without drug, in the absence of Ca²⁺ or Mg²⁺, washed twice in PA, and resuspended in PACM for release to occur. Following the release reaction, the leukocytes were removed by centrifugation (1000 × G for 1 min), and the

supernatants were assayed for histamine content with an automated fluorometric technique (19). The difference between replicate histamine measurements was less than 5%. Aliquots of cells in the same reaction volume were lysed with 2% perchloric acid to evaluate total histamine. Cells incubated in buffer (blanks) were included to estimate spontaneous release (2 to 5%), which was subtracted from all values. The histamine release was then calculated as a percentage of the total histamine content. None of the drugs used interfered with the histamine assay; lytic effects of the drugs are noted in the text. Inhibition or enhancement of histamine release caused by one drug was calculated as the percent decrease or increase in release *vs* release by antigen alone. In experiments with two drugs, in which drug 1 enhanced release (e.g., Indo), the inhibition or enhancement of release by drug 2 (e.g., PGE₂) was calculated as the percent change in histamine release with the value obtained with antigen in the presence of drug 1 as the control for release.

Cyclic AMP assay. The saturation, protein-binding assay of Brown *et al.* (20) was used with minor modifications (21). Agonists and leukocytes were allowed to react for 10 min, the cells were quickly centrifuged, the supernatants were decanted, and the cells were lysed with 5% trichloroacetic acid before cAMP measurements. The supernatants were found to contain <1% of the cAMP content of the cell pellets; therefore only the cellular cAMP was measured.

RESULTS

In the first series of experiments based on the work of Sullivan and Parker in the rat mast cell (9), we tested the effect of ETYA on histamine release from human basophils. Confirmatory experiments were necessary since in many instances the effects of drugs on rat mast cells and human basophils are quite different (22-24). Figure 1 shows that ETYA, which blocks both the lipoxygenase and the cyclo-oxygenase pathways of AA metabolism, causes a dose-dependent inhibition of histamine release. Inhibition begins at 5×10^{-6} M; 50% inhibition is usually observed between 1.4 and 5×10^{-5} M and is complete at 10^{-4} M. Similar dose response curves were obtained in eight separate experiments.

Although ETYA inhibited histamine release when present during antigen challenge, its inhibitory effect was entirely reversible: thus, it had no effect when it was preincubated with leukocytes for 10 min and then removed by washing before antigen challenge (Table I). Similar results were obtained when the cells were incubated with ETYA for 30 min before the wash procedure. These data suggest that a product of AA metabolism is actively involved in the release process rather than that the inhibition by ETYA is simply a toxic effect.

Interestingly, Indo, which blocks only the cyclo-oxygenase pathway of arachidonic metabolism (25), has a distinctly different effect. At high concentrations ($>10^{-4}$ M) it also causes inhibition (data not shown) but at lower concentrations, obtainable therapeutically *in vivo* (26), it enhances antigen-induced histamine from human basophils (Fig. 2). Enhancement begins at concentrations of 10^{-9} to 10^{-8} M and increases in a dose-dependent fashion to peak at about 10^{-6} M (Fig. 2a). This effect is extremely consistent, having been observed in each of 15 experiments. The percentage of enhancement observed is variable from experiment to experiment and depends on the amount of histamine released in the absence of drug. Thus, when low concentrations of antigen are used, and the level of histamine release is in the range of 10 to 15%, Indo may enhance release

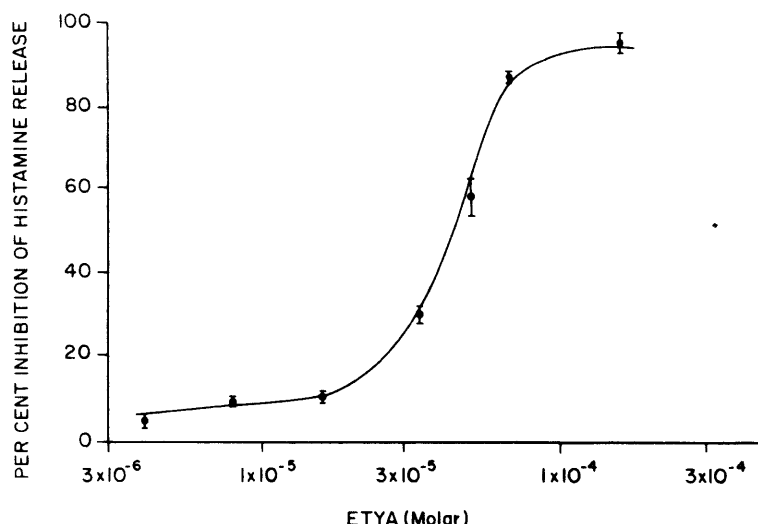


Figure 1. Dose response curve of inhibition of antigen-induced basophil histamine release by an arachidonic acid analog, ETYA. The cells were preincubated with ETYA for 10 min before challenge. Control release by antigen E was 62%. The means $\pm$ S.E.M. of triplicate determinations are indicated ($\bullet$).

TABLE I
Reversibility of ETYA inhibition of antigen-induced histamine release^a

First Incubation	Second Incubation	% Histamine Release
Buffer	Antigen E ^b	65
ETYA, 7.5×10^{-6} M	Antigen E	60
ETYA, 1.25×10^{-5} M	Antigen E	57
ETYA, 2.5×10^{-5} M	Antigen E	62
ETYA, 5×10^{-5} M	Antigen E	57
Buffer	ETYA, 7.5×10^{-6} M + Antigen E	58
Buffer	ETYA, 1.25×10^{-5} M + Antigen E	55
Buffer	ETYA, 2.5×10^{-5} M + Antigen E	19
Buffer	ETYA, 5×10^{-5} M + Antigen E	9

^a After a 10 min incubation at 37°C in the presence of buffer or ETYA, the cells were washed twice with ice-cold PA, resuspended in PACM, and challenged with antigen alone or together with several concentrations of ETYA. Histamine release was allowed to proceed for 45 min at 37°C.

^b Antigen E concentration: 1×10^{-3} μ g/ml.

by 2- to 4-fold. At higher levels of histamine release, smaller degrees of enhancement are noted but, as shown in Figure 2b, enhancement occurs at all points of the dose response curve, extending onto the plateau region of mediator release.

To explore the specificity of Indo's effect on histamine release, we studied other drugs known to have an effect on the cyclo-oxygenase pathway of AA metabolism. Table II lists the results of one of a series of experiments comparing the effects of Indo, meclofenamic acid, and aspirin. Each of these inhibitors of PG synthesis potentiated histamine release. Their activity ratio, Indo > meclofenamic acid > aspirin, was the same as that observed in other tissues for the inhibition of cyclo-oxygenase activity (7).

These experiments suggested that when AA metabolism was diverted from the cyclo-oxygenase to the lipoxygenase pathway, a substance was produced that potentiated histamine release. It was, therefore, of interest to study the addition of AA itself. Figure 3a shows that AA also potentiated the release of histamine (at concentrations as low as 10^{-7} M) in a dose-dependent fashion that peaked at 10^{-6} to 10^{-5} M. At concentrations of 10^{-4} M and above, cell lysis occurred. As with Indo, the amount of enhancement by AA was variable from experiment to experiment but was observed in each of 22 experiments. The degree

of enhancement again depended upon the amount of antigenic stimulation, ranging from 2- to 3-fold at low levels of histamine release to lesser effects when the antigenic stimulus and, therefore, the release of histamine, was maximal. Enhancement by AA occurred, however, over the entire extent of the dose response curve (Fig. 3b).

The specificity of action of AA was studied by comparing its effect on antigen-induced histamine release to that of other fatty acids. Table III presents the results of two experiments comparing the effect of several concentrations of arachidonic, linoleic, linolenic, and stearic acids in the basophil system. Only AA caused highly significant enhancement of histamine release (Student *t*-test; $p < 0.001$). Other fatty acids tested at 10^{-6} to 10^{-5} M neither significantly inhibited nor enhanced release.

The effects of AA do not seem to depend upon the integrity of the cyclo-oxygenase pathway. As shown in Table IV, incubation of cells with Indo before addition of AA does not impair the enhancement by the latter. The addition of AA and Indo together causes only slightly more enhancement than that observed with either agent alone. At high concentrations of one or the other drug, little or no additional effect is observed when the other is added. These results are compatible with the suggestion that both agents are acting through the same pathway.

An additional complexity in these relationships is illustrated by the effects of Indo on the inhibition of histamine release caused by PGE₂. Figure 4 shows data that indicate that Indo reverses, in a dose-dependent fashion, the inhibition caused by PGE₂. These experiments are complex since the addition of Indo alone, as indicated above and shown in Figure 4, potentiates release. It is clear, however, that Indo not only reverses PG-induced inhibition of histamine release but at higher concentrations (e.g., 10^{-6} M) brings the release to levels above that found in the presence of antigen alone and often to the control levels seen with antigen and Indo together (in the example cited, 74 vs 80%). Other cyclo-oxygenase inhibitors including meclofenamic acid and aspirin are also capable of reversing the inhibition caused by the PG (Table V). Although meclofenamic acid caused little enhancement of histamine release at 10^{-4} M, it completely reversed the inhibition by 10^{-6} M PGE₂. Aspirin caused marked enhancement of release at 10^{-3} M, but even at lower concentrations (e.g., 3 to 10×10^{-5} M) at which no enhancement occurred, it was quite effective in reversing the PGE₂ inhibition.

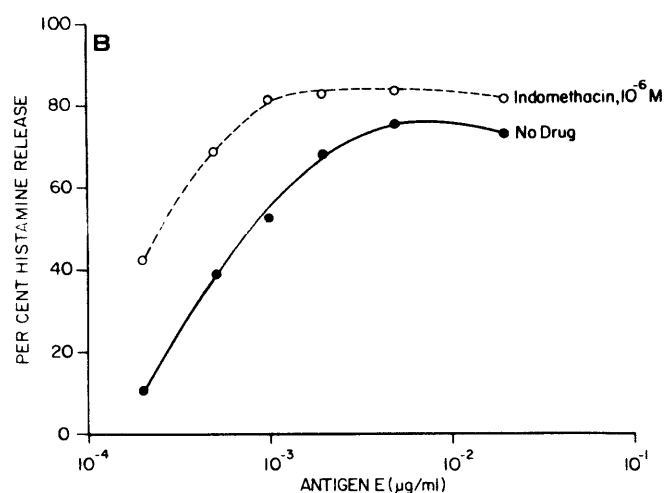
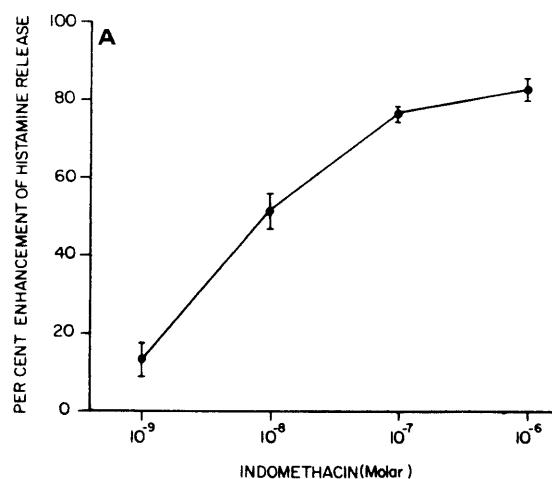


Figure 2. A, dose response curve of enhancement of histamine release by indomethacin. The leukocytes were preincubated with indomethacin for 5 min. Secretion was induced by antigen E; control release was 29%. B, comparison of a histamine release curve without indomethacin (●) and one obtained after a 5-min preincubation with 10^{-6} M indomethacin (○). Enhancement of release by indomethacin was observed at each concentration of antigen E used for release.

The reversal of the inhibition of histamine release by the nonsteroidal anti-inflammatory drugs was not unique to the PG. In fact, Indo reversed the inhibition caused by all of the drugs studied that are thought to act by stimulating cell membrane-bound adenylate cyclase. This includes the β agonist, isoproterenol (iso), and the histamine type-2 agonist, dimaprit (27). These two drugs probably act via their own receptors linked to adenylate cyclase (2, 4, 27) to inhibit histamine release from human basophils. As shown in Figure 5a, Indo, at concentrations that effectively reverse the effect of 10^{-6} M PGE_2 , also reversed inhibition induced by 10^{-6} M iso. In this experiment the effects of the drugs and antigen were studied only in the first stage of histamine release. In other experiments, Indo, added in the second or release stage, neither augmented release nor reversed the inhibition induced by PGE_2 added in the first stage (data not shown). With dimaprit alone, as shown in Figure 5b, there was 78% inhibition of histamine release. In the same experiment, Indo (10^{-8} to 10^{-6} M) both enhanced antigen-induced histamine release and reversed the inhibition caused by dimaprit. At 10^{-6} M Indo, the level of release was almost the same whether or not dimaprit was present.

TABLE II

The effect of acetylsalicylic acid, meclofenamic acid, and indomethacin on antigen-induced histamine release from human basophils

Drug	$\mu\text{g/ml}$	% Enhancement of Histamine Release ^a
Acetylsalicylic acid	18.0	96.0 ± 3.0^b
	59.6	163.3 ± 1.3
Meclofenamic acid	2.4	53.0 ± 2.9
	7.9	91.7 ± 2.9
	24.0	118.7 ± 1.6
Indomethacin	0.0036	26.3 ± 4.4
	0.036	52.5 ± 9.0
	0.36	80.7 ± 1.3

^a Control release = 29%.

^b $\pm$ S.E.M.

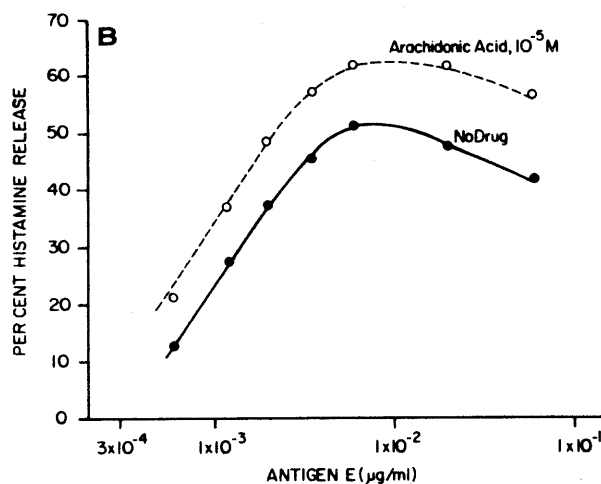
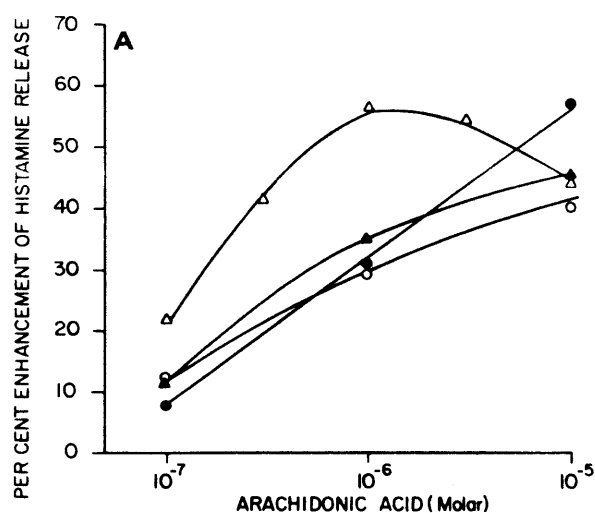


Figure 3. A, dose response curves showing enhancement of antigen-induced histamine release by arachidonic acid using the leukocytes of four donors (Δ , $\blacktriangle$, $\circ$, $\bullet$). The control release ranged from 30–54%. The cells were preincubated for 5 min with arachidonic acid before addition of antigen E. B, comparison of histamine release curves obtained in the absence ($\bullet$) and presence ($\circ$) of 10^{-5} M arachidonic acid. Enhancement was observed at each antigen E concentration tested.

In order to ascertain whether this reversal of inhibition was simply the result of a balance between inhibition and enhancement, we examined the effects of Indo on other pharmacologic agents that act by increasing cyclic AMP levels, but that do not involve adenylate cyclase activation (Fig. 6). Even high concentrations of Indo did not block the inhibition caused by dibutyryl cAMP, but actually potentiated the effect. Similarly, the inhibition caused by the phosphodiesterase inhibitor, IBMX (28), was not reversed by Indo. These experiments were repeated with several concentrations of dibutyryl cAMP and IBMX, as well as lower concentrations of Indo. In some instances, potentiation of inhibition by these compounds was observed. In no instance was there any evidence that Indo reversed the inhibition caused by exogenous dibutyryl cAMP, and little effect on

TABLE III

The effect of various fatty acids on antigen-induced histamine release from human basophils

	Expt. 1: % Histamine Release	Expt. 2: % Histamine Release
	Mean $\pm$ S.E.M.	Mean $\pm$ S.E.M.
No drug	24.8 $\pm$ 1.6	27.2 $\pm$ 2.2
Arachidonic acid, 10^{-6} M	54.7 $\pm$ 3.3 ^a	33.7 $\pm$ 0.6 ^b
Arachidonic acid, 3×10^{-6} M	56.7 $\pm$ 0.8 ^c	43.7 $\pm$ 1.2 ^c
Arachidonic acid, 10^{-5} M	46.7 $\pm$ 1.4 ^c	45.3 $\pm$ 0.3 ^c
Linoleic acid, 10^{-6} M	25.3 $\pm$ 1.2 ^d	23.3 $\pm$ 1.2 ^d
Linoleic acid, 10^{-5} M	29.3 $\pm$ 1.2 ^d	28.7 $\pm$ 2.9 ^d
Linolenic acid, 10^{-6} M	22.3 $\pm$ 1.4 ^d	22.0 $\pm$ 1.5 ^d
Linolenic acid, 10^{-5} M	27.0 $\pm$ 1.0 ^d	29.3 $\pm$ 2.0 ^d
Stearic acid, 10^{-6} M	21.5 $\pm$ 0.5 ^d	24.7 $\pm$ 1.6 ^d
Stearic acid, 10^{-5} M	22.5 $\pm$ 0.5 ^d	25.3 $\pm$ 1.4 ^d

^a $p < 0.01$ when compared with control group (no drug) by Student's *t* test.

^b $p < 0.05$ when compared with control group (no drug) by Student's *t* test.

^c $p < 0.001$ when compared with control group (no drug) by Student's *t* test.

^d Not significantly different from control group (no drug) by Student's *t* test ($p > 0.05$).

TABLE IV

The effect of indomethacin and arachidonic acid, alone or in combination, on antigen-induced histamine release from human basophils

	No Drug	% Histamine Release		
		Indomethacin		
		10^{-8} M	10^{-7} M	10^{-6} M
Experiment 1				
No drug	44	57	57	57
Arachidonic acid, 10^{-7} M	47	60	60	60
Arachidonic acid, 10^{-6} M	51	62	62	61
Arachidonic acid, 10^{-5} M	53	58	57	58
	No. Drug	2×10^{-7} M	2×10^{-6} M	
Experiment 2				
No drug	30	37	43	
Arachidonic acid, 10^{-6} M	36	40	47	
Arachidonic acid, 3×10^{-6} M	41	40	42	
Arachidonic acid, 10^{-5} M	34	36	45	
	No. Drug	10^{-8} M	10^{-7} M	10^{-6} M
Experiment 3				
No drug	39	48	51	55
Arachidonic acid, 10^{-7} M	39	49	51	49
Arachidonic acid, 10^{-6} M	54	52	56	56

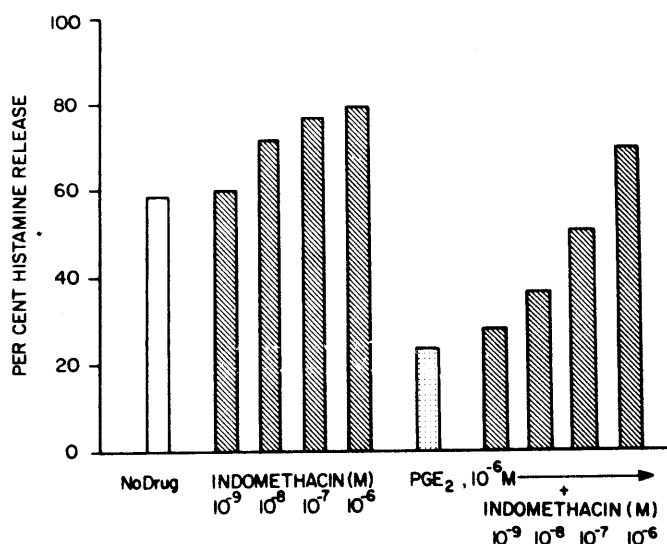


Figure 4. Reversal of PGE₂-induced inhibition of histamine release by indomethacin. As shown on the left, indomethacin alone (■) (10^{-8} – 10^{-6} M) caused a dose-dependent increase in release above control (□). PGE₂ alone (▨) caused 66% inhibition of release; this effect was reversed in a dose-dependent fashion by the addition of indomethacin (10^{-9} – 10^{-6} M).

TABLE V

The effect of meclofenamic acid and acetylsalicylic acid on PGE₂ induced inhibition of antigen-induced histamine release from human basophils

Meclofenamic Acid							
No Drug		1×10^{-6} M	3×10^{-6} M	1×10^{-5} M	3×10^{-5} M	1×10^{-4} M	
% histamine release							
Control	48	51	52	54	57	57	
PGE ₂ , 10^{-6} M	37	41	44	50	53	60	
PGE ₂ , 10^{-5} M	20	26	29	35	38	45	
Acetylsalicylic Acid							
		10^{-5} M	3×10^{-5} M	1×10^{-4} M	2×10^{-4} M	5×10^{-4} M	1×10^{-3} M
% histamine release							
Control	50	50	51	53	59	68	71
PGE ₂ , 10^{-5} M	17	21	26	36	44	58	64

IBMX was observed. A summary contrasting the effects of Indo on inhibition by PGE₂, dimaprit, dibutyryl cAMP, and IBMX is shown in Table VI. It is clear that 10^{-6} and 10^{-7} M Indo consistently reversed inhibition of histamine release by PGE₂ and dimaprit, whereas it had little effect on the inhibition caused by dibutyryl cAMP or IBMX. In other experiments we studied the effect of Indo on another inhibitor that does not act via the cAMP system, 2-deoxyglucose (data not shown). Indo had no effect on inhibition of histamine release by this compound, which acts in part by blocking aerobic glycolysis.

Experiments were next designed to test whether AA which acts like Indo in potentiating antigen-induced histamine release, could also block the inhibition caused by adenylate cyclase agonists. In preliminary experiments, AA reversed the inhibition caused by PGE₂. Rather than studying all such drugs, we concentrated on the H₂ agonist, dimaprit. In each of four

experiments, one of which is shown in Figure 7, AA reversed the inhibition caused by dimaprit in a dose-dependent fashion. This effect was first observed at approximately 10^{-7} M and was complete by 10^{-5} M. This figure also provides data showing reversal by Indo of the inhibition caused by dimaprit.

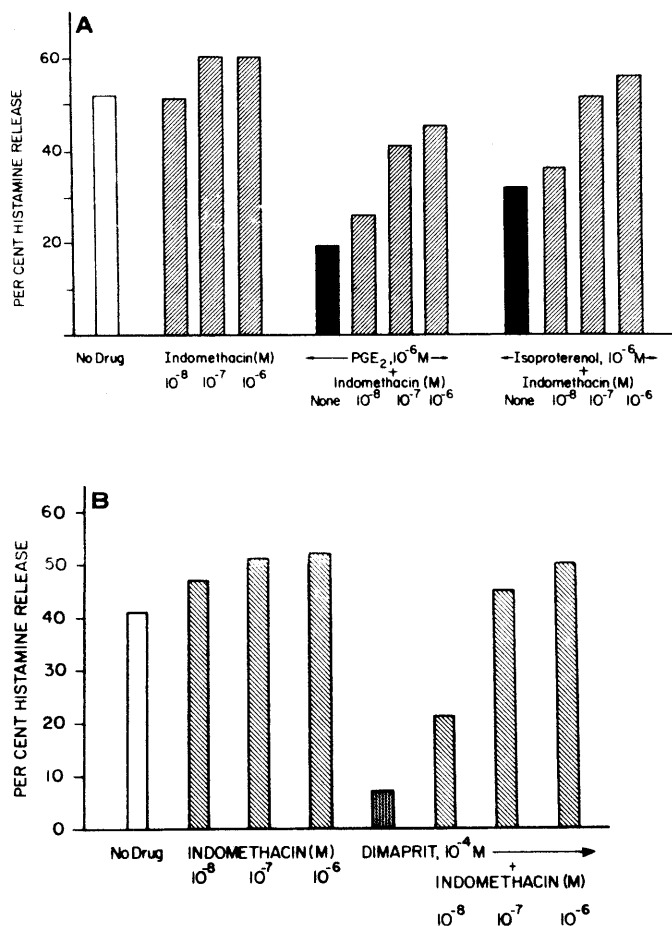


Figure 5. A, reversal by indomethacin of the inhibition of antigen-induced histamine release induced by PGE₂ and Isoproterenol. This experiment was done on the first stage of histamine release (see *Materials and Methods*). The leukocytes were preincubated with buffer or indomethacin (10^{-8} , 10^{-6} M) for 5 min, after which the agonists were added and the reaction begun with antigen E. B, reversal by indomethacin of the inhibition caused by an H₂ agonist, dimaprit. On the left is shown enhancement by indomethacin (▨) of histamine release above control (□).

It would be of considerable interest to know whether the effects of Indo and AA, both in potentiating histamine release and reversing the inhibition caused by adenylate cyclase active agonists, were paralleled by changes in the intracellular cAMP level. This cannot be studied directly since the basophils cannot be purified sufficiently to study relevant cAMP levels. Indirect measurements on the peripheral leukocytes used in these experiments can, however, be carried out; in previous studies the results of such assays were found to be consistent with the pharmacologic effects of various agonists on basophil histamine release (29). The results of one of several experiments directed to this question are shown in Table VII. In this experiment we measured the cAMP levels in both mixed leukocytes and purified lymphocytes from the same donor. Indo was found to cause a small but significant decrease in the basal cAMP level in the lymphocyte preparation, but had no effect on the baseline levels of cAMP in mixed leukocytes, the cell population used for the release studies. Indo did not affect the increase in cAMP caused by PGE₂ in the mixed leukocyte preparation; and had only a slight, but significant, effect in the lymphocyte preparation (analysis of variance with the Studentized Range test for individual comparisons).

DISCUSSION

These experiments suggest that AA metabolism plays a role in the regulation of antigen-IgE-induced mediator release from human basophils. Assuming that the mechanism of action of ETYA is to block both the lipoxygenase and cyclo-oxygenase pathways of AA metabolism (8, 25), then the inhibition of histamine release caused by this drug suggests that products of AA are necessary for antigen-induced release of histamine. The reversibility of the ETYA effect argues against the results being due simply to nonspecific cell damage, and further supports the concept that a product of AA metabolism is involved in mediator release. Together with the enhancement of histamine release caused by Indo, these results suggest that it is a product(s) of AA metabolism, produced via the lipoxygenase pathway, that plays this role. This conclusion is supported by the observation that AA itself specifically potentiates histamine release, and that this effect is fully expressed whether Indo is present or absent. Thus, any of the stimuli studied that lead to increased utilization of the lipoxygenase pathway potentiated mediator release.

The argument that Indo is acting as an inhibitor of the cyclo-oxygenase pathway is supported by the fact that two different drugs, meclofenamic acid and aspirin, which also block the

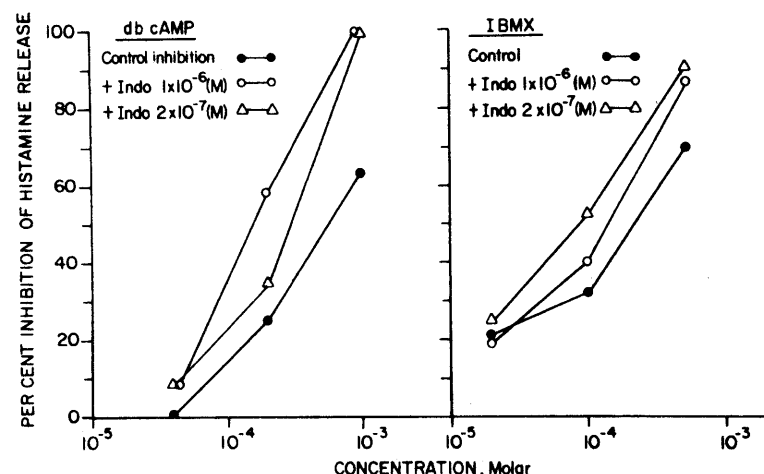


Figure 6. The effect of indomethacin on the inhibition of histamine release by dbcAMP (left panel) and IBMX (right panel). Control release by antigen E was 44%. Note that indomethacin potentiated or had little effect on the inhibition by both drugs. (See *Materials and Methods* for the calculation of inhibition in the presence of two drugs.)

cyclo-oxygenase pathway (25), have effects that are qualitatively similar to that of Indo. The quantitative relationships to those reported for their inhibition of the cyclo-oxygenase pathway that leads to the production of PG in other tissues (30, 31). Although this effect is presumed to be the basis for the mechanism of action of this class of compounds, recent reports indicate that nonsteroidal anti-inflammatory agents also inhibit some of the actions of PG (32, 33). Moreover, it has been shown recently that such agents inhibit the stimulation of cAMP formation by PGE and iso in cultured human astrocytoma cells (34).

It is interesting that both Indo and AA selectively antagonize the inhibition of histamine release caused by a variety of agonists whose only known similarity is that they activate adenylate cyclase. Each of the agonists we studied acts through separate basophil receptors, i.e., the H₂-receptor (27), the β adrenergic receptor, and, presumably, a PG receptor (4). Thus, the effects of Indo and AA are unlikely to be exerted at the level of the hormone-receptor interaction. Rather, their effect on basophil secretion seems to occur after the interaction between diverse receptors and adenylate cyclase. No direct evidence bearing on this question is presently available. It was found, however, that whereas Indo at high concentrations slightly lowers cAMP levels in leukocyte preparations, its ability to block the increase in cAMP levels caused by the adenylate cyclase agonist, PGE₂, is marginal, at best. Thus, it seems likely that the effects of Indo and AA occur at some later stage, after the generation of cAMP. This concept is supported by the recent finding that Indo in submicromolar concentrations inhibits cAMP-dependent protein kinase and endogenous protein phosphorylation (35). A paradox remains, however, since Indo does not affect the inhibition of histamine release that is caused by dibutyl cAMP or by IBMX, both of which raise the cAMP level of mixed leukocyte preparations. It is possible to postulate that the agonists in question cause their effect on histamine secretion by influencing localized pools of cAMP, perhaps in or near the cell membrane. Such a hypothesis has been suggested by others (36, 37), but it is quite difficult to test adequately. Another possibility is that the agonists inhibit histamine release by a local production of PG that are actually responsible for the inhibition noted, and that the observed increase in intracellular cAMP levels is merely a secondary effect. Others have

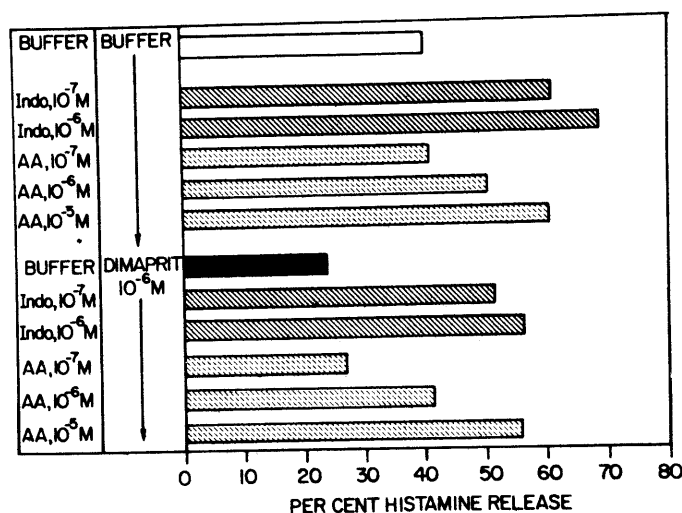


Figure 7. An experiment showing the dose-dependent enhancement of histamine release by AA and the reversal by AA of the inhibition induced by the H₂ agonist, dimaprit. Included for comparison are the effects of indomethacin on these two parameters.

TABLE VII

Effects of indomethacin alone or with PGE₂ on the cAMP content of human leukocytes^a

	pmoles/10 ⁷ cells cAMP	
	Lymphocytes ^b	Mixed leukocytes
No Drug	34.4 ± 0.4	15.1 ± 0.7
Indo, 10 ⁻⁸ M	27.9 ± 2.3	15.5 ± 1.0
Indo, 10 ⁻⁷ M	28.9 ± 1.0	14.1 ± 0.8
Indo, 10 ⁻⁶ M	22.7 ± 1.8 ^c	14.4 ± 0.8
Indo, 10 ⁻⁵ M	22.0 ± 3.0 ^c	15.2 ± 2.2
PGE ₂ , 10 ⁻⁶ M Alone	262.3 ± 19.3	109.6 ± 8.7
+ Indo, 10 ⁻⁸ M	219.3 ± 6.3 ^d	122.4 ± 3.4
+ Indo, 10 ⁻⁷ M	220.7 ± 10.4 ^d	110.2 ± 3.4
+ Indo, 10 ⁻⁶ M	193.8 ± 6.3 ^c	89.6 ± 2.8
+ Indo, 10 ⁻⁵ M	198.8 ± 3.6 ^c	92.8 ± 1.6

^a Results are expressed as mean ± S.E.M. of triplicate determinations. Indo, indomethacin.

^b Mononuclear cells were isolated from venous peripheral blood by Ficoll-Hypaque density gradient (21).

^c Significantly different from control without indomethacin ($p < 0.01$).

^d Significantly different from cells treated with PGE₂ alone ($p < 0.05$).

^e Significantly different from cells treated with PGE₂ alone ($p < 0.01$).

TABLE VI

The effect of indomethacin on inhibition of histamine release induced by PGE₂, dimaprit, IBMX and dibutyl cAMP

Agonist	% Inhibition ^a	% Reversal of Inhibition by Indomethacin		N ^b
		1 × 10 ⁻⁶ M	1 × 10 ⁻⁷ M	
PGE ₂ , 1 × 10 ⁻⁶ M	53 ± 5	94 ± 5	89 ± 7	7
Dimaprit 1 × 10 ⁻⁵ M	67 ± 8	86 ± 9	80 ± 7	4
1 × 10 ⁻⁶ M	32 ± 3	101 ± 10	102 ± 7	4
IBMX 5 × 10 ⁻⁴ M	85 ± 5	7 ± 5	9 ± 5	4
1 × 10 ⁻⁴ M	49 ± 5	20 ± 8	23 ± 14	6
dbcAMP 2-3 × 10 ⁻³ M	71 ± 8	2 ± 2	0	6
0.5-1 × 10 ⁻³ M	17 ± 2	0	12 ± 6	4

^a Mean ± S.E.M.

^b Number of experiments.

produced data in several systems that are in accord with this suggestion (38-40). Although this concept is compatible with the data with regard to the effects of Indo, it does not explain the ability of AA to reverse the inhibition caused by agonists that activate adenylate cyclase. Thus, the situation is complex and many unanswered questions remain.

We have, nevertheless, the firm observation that Indo and other nonsteroidal anti-inflammatory agents both potentiate mediator release and impair the homeostatic and pharmacologic control mechanisms that are represented by hormones and drugs that interact with adenylate cyclase. These facts must be accommodated into any theory on the mechanism of antigen-IgE-mediated histamine release. Furthermore, if these data can be extrapolated to the *in vivo* experience, they would seem to have important implications for both aspirin-induced

asthma (41-43) and the pharmacologic management of conditions that are associated with mediator release (44-46).

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