

EFFECT OF INHIBITORS OF ARACHIDONIC ACID METABOLISM UPON IgE AND NON-IgE-MEDIATED HISTAMINE RELEASE

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Abstract—Inhibitors of arachidonic acid metabolism were tested for their ability to block histamine release from human basophils. Eighteen inhibitors of lipoxygenases, cyclo-oxygenases, prostaglandin isomerases and thromboxane synthetases were tested. Agents inhibitory to the activity of lipoxygenases were effective blockers of IgE and non-IgE-mediated histamine release; agents antagonistic to cyclo-oxygenases, isomerases and thromboxane synthetases were not. These findings indicate that a functioning lipoxygenase pathway is essential for basophil activation and secretion and that the cyclo-oxygenase, isomerase and thromboxane synthetase pathways are not. Compounds antagonistic to phospholipase A also block histamine release, as does the intracellular Ca^{2+} antagonist TMB-8. The data are consistent with the idea that mast-cell and basophil activation and secretion involve phospholipase A generation of arachidonic acid, which is metabolized via the lipoxygenase pathway.

Generation of arachidonic acid (AA) by cellular phospholipase A acting on membrane phospholipids appears to be an integral part of cellular activation. The AA produced can be metabolized by cyclo-oxygenases, isomerases, synthetases and lipoxygenases to yield short and long-lived metabolites. Many aspects of the pathways of AA metabolism have been elegantly determined. Cyclo-oxygenase (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974) acting upon AA (Bergström, Danielsson & Samuelsson, 1964) can yield the transient endoperoxide intermediates PGG_2 and PGH_2 . These intermediates can then be isomerized to PGD_2 , PGE_2 or $\text{PGF}_{2\alpha}$ (Nugteren & Hazelhof, 1973; Hamberg & Samuelsson, 1973).

The intermediate endoperoxide PGH_2 can also be transformed into other metabolites, such as thromboxane A_2 (TxA_2) and hydroxyheptadecatrienoic acids (HHT), possessing non-prostanoate acid structures (Hamberg & Samuelsson, 1974; Hamberg, Svensson & Samuelsson, 1975). Additional metabolites of AA, including hydroxyeicosatetraenoic acids (HETE) and their corresponding peroxides (HPETE) (Hamberg, Svensson & Samuelsson, 1974), can be formed along with the leukotrienes by the lipoxygenase pathway, an enzyme unrelated to enzymes involved in prostaglandin biosynthesis (Jakschik, Falkenhein & Parker, 1977; Murphy, Hammarström & Samuelsson, 1979). The prostaglandin and non-

prostanoate acid-producing enzymatic pathways (Fig. 1), being well delineated, provide a rationale by which their effects can be analysed. The prostanoate fatty-acid metabolites possess a myriad of activities capable of affecting almost all biological functions. Much attention is currently being focused on cellular lipase generation of AA and the influence of the metabolites upon cellular activation. A number of laboratories, particularly in the pharmaceutical industry, have been actively pursuing enzymatic inhibitors which show specificity and can selectively inhibit the production of the various AA metabolites.

The present study was initiated in order to determine the effect of a number of these inhibitors upon IgE-mediated and non-IgE-mediated histamine release from human basophils.

EXPERIMENTAL PROCEDURES

Buffers and cell preparations

Peripheral blood was obtained by venipuncture from allergic donors. The blood was made relatively free of red cells by sedimentation at 1 g for 45 min at room temperature in dextran and EDTA. The leukocyte-rich supernatant was removed, and platelets and plasma constituents were washed from the leukocytes by two centrifugal washes for 8 min each at 275 g at 5°C in Tris buffer (pH 7.4). The leukocyte cell

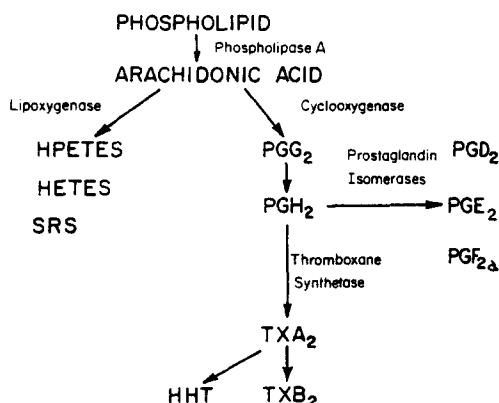


Fig. 1. Some enzymatic pathways of AA production and metabolism and their products. Inhibitors of the enzymatic systems, phospholipase A, cyclo-oxygenase, prostaglandin isomerase, thromboxane synthetase and lipoxygenase were utilized to determine whether inhibition of the enzymatic system blocks IgE and non-IgE-mediated histamine release from human basophils.

button was then resuspended in a high Tris reaction buffer containing 0.03% gelatin, 120 mM Tris (pH 7.4), 25 mM Na^+ , 5 mM K^+ , 1 mM Mg^{2+} and 0.8 mM Ca^{2+} .

Inducing agents

The purified allergenic protein antigen E, derived from ragweed pollen (King, Norman & Connell, 1964) was supplied by National Institutes of Health, Bethesda, MD. Calcium ionophore A23187 (Reed & Lardy, 1972; Foreman, Mongar & Gomperts, 1973) was obtained from Calbiochem-Behring Corp., La Jolla, CA.

Reaction mixtures

The cell suspension in high Tris buffer was aliquoted at 4°C into 12 × 75 mm Falcon plastic tubes. Twenty-one compounds affecting the activity of the arachidonic acid cascade (Table 1) were added to human leukocytes at concentrations from 10^{-7} to 10^{-3} M at 2 points per log₁₀. To maintain a pH of 7.4 at the higher concentrations, the compounds were solubilized in the high Tris buffer. The cells were warmed for 4–5 min at 37°C and subsequently challenged with either ionophore A23187 (0.1 µg/ml) or antigen E (5×10^{-10} M). The total reaction volume of cells, inhibitor and inducing agent was 0.6 ml. The reaction mixtures were incubated at 37°C for 40 min.

Each tube contained $3\text{--}4 \times 10^6$ leukocytes/ml, approximately 1% basophils, corresponding to a total histamine content of 75–100 ng/ml. Depending upon the donor, the percentage of total histamine release in the absence of inhibiting compound ranged

from 40 to 80%. The ability of each compound to block histamine release was obtained by comparing the quantity of histamine released in the presence of the compounds of the paired leukocyte samples from the same donor in the absence of the compound.

Histamine assay

After 40 min of incubation at 37°C, the tubes were iced and centrifuged at 1000 g at 5°C for 15 min. The supernatants were assayed for histamine content by an automated spectrofluorometric technique (Siraganian, 1974). The extraction and fluorometric procedures were modified versions of the techniques reported by Shore, Burkhalter & Cohn (1959). Blank tubes lacking antigen released less than 5% of the total histamine content. The percentage histamine release was calculated in excess of the blank. All compounds utilized to affect the activity of the arachidonic acid cascade were analysed for fluorescence and whether they affected the spectrofluorometric analysis of histamine standards (100 ng/ml).

RESULTS

Compounds antagonistic to cyclo-oxygenase activity were not inhibitory to histamine release within the range of concentrations at which they block cyclo-oxygenase activity (Table 1). Although indomethacin and meclofenamic acid did inhibit histamine release at 10^{-4} – 10^{-3} M, these concentrations are about 1000-fold higher than those required to inhibit cyclo-oxygenase activity. The higher concentrations correspond to the range at which some acidic cyclo-oxygenase inhibitors block lipoxygenase activity. Phenylbutazone also is a cyclo-oxygenase inhibitor which requires higher concentrations to block histamine release than to inhibit cyclo-oxygenase activity. Phenylbutazone is an analog of 1-phenyl-3-pyrazolidone, a reported lipoxygenase inhibitor, and may have similar histamine release inhibitory mechanisms. Naproxen, which inhibits cyclo-oxygenase activity at much higher concentrations than indomethacin, meclofenamic acid, flurbiprofen or phenylbutazone, did not block histamine release.

All tested compounds antagonistic to lipoxygenase activity inhibited both IgE and non-IgE-mediated histamine release. Nordihydroguaiaretic acid appears to be the most effective blocker of antigen-induced histamine release. Overall, there is some degree of rank order of effectiveness between the concentration ranges at which compounds are antagonistic to lipoxygenase activity and to histamine release. This is demonstrated by the rank order of effectiveness of 3,4-dimercaptotoluene, 1,10-phenanthroline and 2,2'-dipyridyl. Of these three com-

Table 1. Inhibitors of AA metabolism and their capacity to block histamine release

Compound (source)	Enzymatic inhibition Pathway*†	Range (mM)	Histamine release inhibition			
			Ionophore Range (mM)	IC ₅₀ ‡	Antigen Range (mM)	IC ₅₀
Indomethacin (Sigma)	Cyc ^{1,4}	0.0005–0.005	0.1–1.0	0.2	0.1–1.0	0.5
Meclofenamic acid (Warner-Lambert)	Cyc ^{4, 5}	0.0001–0.001	0.1–1.0	0.2	0.1–1.0	0.3
Flurbiprofen (Boots)	Cyc ^{6, 7}	0.00005–0.0005	0.1–1.0	0.3	0.1–1.0	0.8
Naproxen (Syntex)	Cyc ²	0.05–0.5	Non-inhib.§		Non-inhib.	
Phenylbutazone (Sigma)	Cyc ⁴	0.001–0.1	0.03–0.3	0.05	0.1–1.0	0.5
	Pro ⁵	0.1–1.0				
1-Phenyl-3-pyrazolidone (Sigma)	Lip ⁸	0.1–1.0	0.1–1.0	0.5	0.1–1.0	0.4
	Cyc ⁸	0.1–1.0				
5,8,11,14-Eicosatetraynoic acid (Hoffman LaRoche)	Lip ⁹	0.01–0.1	0.005–0.05	0.02	0.03–0.3	0.2
	Cyc ⁹	0.01–0.1				
Benoxaprofen (Lilly)	Lip ^{10, 11}	0.1–1.0	0.1–1.0	0.2	0.1–1.0	1.0
BW755C ¹¹ (Wellcome)	Lip ¹²	0.005–0.05	0.005–0.05	0.03	0.005–0.05	0.03
	Cyc ¹²	0.001–0.01				
Nordihydroguaiaretic acid (Sigma)	Lip ^{13, 14}	0.01–0.1	0.01–0.1	0.02	0.003–0.03	0.01
3,4-Dimercaptotoluene (Aldrich)	Fe ³⁺ lip ¹⁵	0.01–0.1	0.03–0.3	0.1	0.03–0.3	0.05
1,10-Phenanthroline (Sigma)	Fe lip ¹⁵	0.1–1.0	0.1–1.0	0.4	0.08–0.8	0.2
2,2'-Dipyridyl (Aldrich)	Fe non-lip ¹⁵		Non-inhib.		Non-inhib.	
Imidazole (Sigma)	Thr ¹⁶	0.5–5.0	Non-inhib.		Non-inhib.	
N-0164 (Nelson)	Thr ¹⁷	0.05–0.5	Toxic, Non-inhib.¶		Toxic, Non-inhib.	
Benzydamine (Sigma)	Pro ⁵	0.1–1.0	Toxic, Non-inhib.		Toxic, Non-inhib.	
	Thr ¹⁸	0.1–1.0				
	Cyc ¹⁸	0.5–5.0				
L-8027 (Labaz)	Thr ¹⁹⁻²¹	0.0001–0.01	Non-inhib.		Non-inhib.	
	Cyc ¹⁹⁻²¹	0.001–0.01				
9,11-Azoprostano-5,13- dienoic acid** (Upjohn)	Thr ²²	0.0005–0.005	0.01–0.1	0.05	0.04–0.4	0.08
Tetracaine (Sterling Winthrop)	PhA ²³	0.03–0.3	0.05–0.5	0.2	0.05–0.5	0.2
p-Bromophenacylbromide (Sigma)	PhA ^{24, 25}	0.01–0.1	0.008–0.08	0.02	0.008–0.08	0.02
TMB-8†† (Dr. R. Feinman, Downstate Medical Center, NY)	Ca ²⁺ Ant ²⁶	0.1–1.0	0.01–0.1	0.02	0.1–1.0	0.3

* Abbreviations indicate enzymatic pathways as follows: Cyc, cyclo-oxygenase; Pro, prostaglandin isomerase; Lip, lipoxigenase; Fe³⁺ lip, Fe³⁺ chelator, lipoxigenase; Fe lip, non-specific Fe chelator, lipoxigenase; Fe non-lip, non-specific Fe chelator that does not inhibit lipoxigenase; Thr, thromboxane synthetase; PhA, phospholipase A; Ca²⁺ Ant, intracellular Ca²⁺ antagonist.

† Superscript numbers indicate references as follows: ¹ Vane (1971); ² Tomlinson & Ringold (1972); ³ Smith & Lands (1971); ⁴ Flower, Gryglewski, Herbaczynsak-Cedro & Vane (1972); ⁵ Flower, Cheung & Cushman (1973); ⁶ Crook & Collins (1975); ⁷ Ford-Hutchinson, Walker, Connor, Oliver & Smith (1977); ⁸ Blackwell & Flower (1978); ⁹ Hamberg & Samuelsson (1974); ¹⁰ Walker & Dawson (1979); ¹¹ Meacock & Kitchen (1979); ¹² Higgs, Flower & Vane (1979); ¹³ Tappel, Lundberg & Boyer (1953); ¹⁴ Hamberg (1976); ¹⁵ Aharony *et al.*, (1980); ¹⁶ Needleman, Raz, Ferrendelli & Minkes (1977); ¹⁷ Kulkarni & Eakins (1976); ¹⁸ Moncada, Needleman, Bunting & Vane (1976); ¹⁹ Chignard, Vargaftig, Sors & Dray (1978); ²⁰ Gryglewski *et al.* (1977); ²¹ Deby, Descamps, Binon & Bacq (1971); ²² Gorman *et al.* (1977); ²³ Scherphof, Scarpa & Toorenbergen (1972); ²⁴ Mitchell, Poyser & Wilson (1977); ²⁵ Volwerk, Pieterse & Haas (1974); ²⁶ Charo, Feinman & Detwiler (1976).

‡ Concentration (mM) required to inhibit 50% of the quantity of histamine released in the absence of inhibiting compound.

§ Compounds which did not inhibit by 10⁻³ M were termed non-inhibitory.

¹¹ BW755C at concentrations above 3 × 10⁻⁵ M interferes with assay of histamine.

¶ Compounds which released histamine before demonstrating any inhibitory capacity were termed toxic, non-inhibitory.

** Toxic at 10⁻⁴ M.

†† Toxic at 10⁻³ M.

pounds the Fe^{3+} chelator 3,4-dimercaptotoluene is the most effective lipoygenase inhibitor (Aharony, Smith & Silver, 1980) and the most effective inhibitor of histamine release. 1,10-Phenanthroline, an Fe chelator not specific for Fe^{3+} , inhibits lipoygenase activity less effectively and likewise does not block histamine release as well. 2,2'-Dipyridyl, a non-specific Fe chelator which has no capacity to inhibit lipoygenase activity, does not block histamine release.

The prostaglandin isomerase inhibitors and the thromboxane synthetase inhibitors shown in Table 1 are generally ineffective in blocking histamine release. L-8027, which blocks thromboxane synthetase in intact cells (Gryglewski, Zmuda, Korbut, Krecioch & Bieron, 1977), does not block histamine release. Likewise, the thromboxane synthetase inhibitor imidazole is totally ineffective in blocking histamine release. 9,11-Azoprostanoic acid inhibits thromboxane synthetase in intact cells (Gorman, Bundy, Peterson, Sun, Miller & Fitzpatrick, 1977). It also inhibits histamine release but at a concentration range about 100-fold higher. In addition, this azo compound is most likely competitive for peroxide analogs other than or similar to the prostaglandin endoperoxide PGH_2 (Gorman, 1979).

Both of the phospholipase A inhibitors, *p*-bromophenacylbromide and tetracaine, block histamine release within the range of phospholipase A inhibition. The intracellular Ca^{2+} antagonist TMB-8 also inhibits histamine release but is about 15-fold more effective as an inhibitor of ionophore-induced release than of IgE-mediated release. Phenylbutazone and 5,8,11,14-eicosatetraynoic acid were also significantly more effective in inhibiting ionophore-induced histamine release.

All 21 compounds were also tested for their ability to inhibit histamine release from peritoneal rat mast cells. The data were so remarkably similar to those for the human basophil system that they are not included here. Finally, the compounds were tested for their ability to inhibit IgE-mediated release in the presence of 3×10^{-6} M exogenous AA. Exogenous AA does potentiate IgE-mediated histamine release (Sullivan & Parker, 1979) but caused no detectable difference in the ability of any compound to inhibit histamine release.

DISCUSSION

The rôle of AA metabolites is not limited to vasoactive and inflammatory processes. Platelet aggregation and secretion studies have shown that AA metabolites generated by the cell can also regulate the cell's response to stimuli. (Smith & Willis, 1971;

Smith, Ingberman, Kocsis & Silver, 1974; Willis, Vane, Kahn, Scott & Petrin, 1974; Hamberg *et al.*, 1974). Much attention has been focused on their ability to mobilize intracellular Ca^{2+} during the activation process (Gorman, 1979). In many cell types other than platelets the cell is activated to generate AA and to mobilize Ca^{2+} by membrane-bound immunoglobulins. The ability of such immunoglobulins to translate messages of antigen presence to the cell to mobilize Ca^{2+} is a phenomenon of major importance. IgE-mediated histamine release offers a convenient system for studying this phenomenon and the histamine release system is becoming recognized as a paradigm for the study of immunoglobulin-mediated cellular activation (Metzger, 1978).

Virtually every tissue studied has some capacity to metabolize AA. Histamine-releasing cells can generate both prostaglandin and non-prostaglandin metabolites. By the cyclo-oxygenase pathway resting mast cells can produce significant quantities of PGE_2 (Tolone, Bonasera & Tolone, 1978). Stimulated cells can produce both thromboxanes and prostaglandins, with PGD_2 being the major prostaglandin metabolite (Roberts, Lewis, Lawson, Sweetman, Austen & Oates, 1978). By the lipoygenase pathway mast cells and basophils can produce various hydroxyeicosatetraenoic acids (HETE) and the corresponding hydroperoxides (HPETE) (Roberts *et al.*, 1978), along with slow reacting substance (SRS) (Bach, Brashler & Gorman, 1977; Jakschik *et al.*, 1977; Murphy *et al.*, 1979) and related leukotrienes (Lewis, Austen, Drazen, Clark, Marfat & Corey, 1980).

The postulate that AA metabolites govern mast-cell and basophil activation was made feasible by the demonstration that exogenous AA potentiates histamine release and that the acetylenic competitive analog of AA, 5,8,11,14-eicosatetraynoic acid, inhibits histamine release (Sullivan & Parker, 1979). Recently it has been demonstrated that 5,8,11-eicosatrienoic acid, which inhibits lipoygenases more effectively than cyclo-oxygenases, blocks histamine release (Marone, Hammarström & Lichtenstein, 1980).

It was the rationale of the present study to investigate the effect of a high number of inhibitors of AA metabolism in order to determine which enzymatic pathways are essential for basophil activation. The data lend support to the idea that the lipoygenase pathway of AA metabolism plays a central rôle in mast-cell activation and secretion. All of the agents antagonistic to lipoygenase activity were effective inhibitors of histamine release. Conversely, a majority of the cyclo-oxygenase, isomerase and thromboxane synthetase inhibitors tested were not effective in inhibiting histamine release. Cyclo-

oxygenase, isomerase and thromboxane synthetase inhibitors that did block histamine release required concentration ranges higher than those at which they were effective enzyme inhibitors. The mechanisms of basophil activation, being independent of thromboxane synthetase activity, are dissimilar to platelet activation where intracellular cyclic AMP levels, Ca^{2+} mobilization and the resultant secretion, are reported to be associated with thromboxane synthetase activity (Gorman *et al.*, 1977; Gorman, 1979).

Our data as a whole are consistent with the idea that basophil and mast-cell activation involves phospholipase A generation of AA and that the AA generated is metabolized via the lipoxygenase pathway, resulting in Ca^{2+} mobilization. In addition, although the histamine-releasing cells have the capacity to generate AA metabolites by pathways other than the lipoxygenase, these pathways do not appear to be necessary for the functioning of the cellular activation and secretory processes.

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