IMMUNOLOGIC RELEASE OF CHEMICAL MEDIATORS FROM HUMAN TISSUES

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The long-standing observation that the introduction of specific antigen into the skin of an appropriately sensitive individual produces an immediate allergic reaction characterized by wheal and flare formation may now be viewed in more physiologic terms. The interaction of antigen with IgE immunoglobulins specifically bound to the cell surface receptors of mast cells initiates a series of biochemical reactions within these cells resulting in the secretion of a variety of biologically active chemical mediators which in turn increase vascular permeability, contract smooth muscle, and selectively attract eosinophils (Figure 1).

This review focuses on the pharmacologic modulation of this immunologic reaction as well as the biochemical events comprising the activation and secretory phases. The data to be considered have been derived from an in vitro model of human immediate hypersensitivity employing human lung tissue and from related studies in analogous systems employing human peripheral leukocytes or nasal polyp fragments. With the exception of certain critical experiments with isolated purified rat mast cells, the reader is referred elsewhere for reviews concentrating on investigations in laboratory animals (1) and other tissues (2).

COMPONENTS OF IMMEDIATE HYPERSENSITIVITY REACTIONS

In 1941 Katz & Cohen demonstrated that peripheral leukocytes from allergic individuals would release the chemical mediator histamine upon interaction with appropriate antigen (3), and ten years later lung tissue from an asthmatic patient was observed to release histamine upon antigen challenge (4). These same tissues, as well as human nasal polyp (5) and skin (6) fragments, obtained from normal individuals

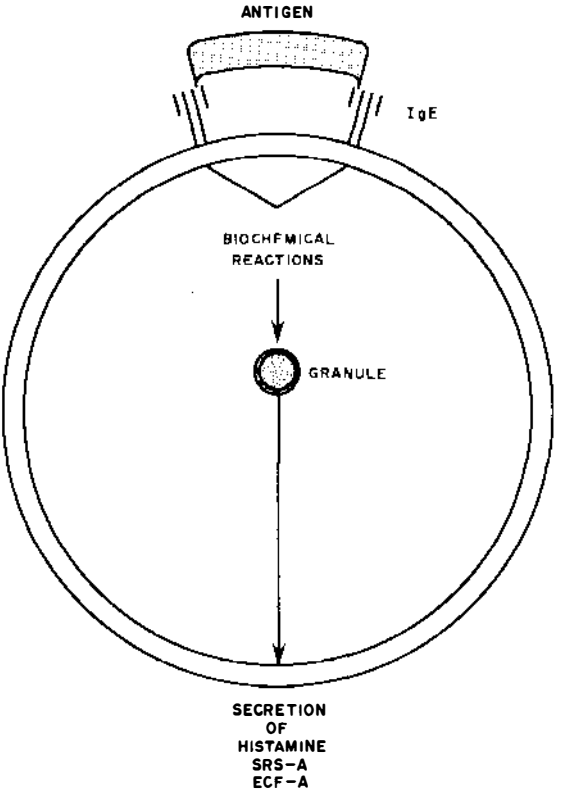

Figure 1 Simplified view of the reaction scheme leading to the release of chemical mediators.

have been shown to be capable of passive sensitization by incubation in serum from allergic patients so that specific antigen challenge induced the release of mediators (7–9).

IgE

The evidence that IgE (10) is the immunoglobulin class critical to acute allergic reactions in the human is functional, histological, and clinical. Thc functional evidence that the immunoglobulin responsible for the passive sensitization of human lung tissue is IgE includes 1. loss of sensitizing activity when IgE is deleted from serum by absorption with a monospecific antiserum directed against IgE so as to leave the concentrations of the other immunoglobulins unchanged (11); 2. inhibition of sensitization by the addition of IgE myeloma protein to serum but not myeloma proteins of the other immunoglobulin classes (12); and 3. mediator release from human lung tissue challenged with monospecific anti-human IgE, presumably by interaction with IgE molecules normally bound to target cells, but not with antisera

directed against other immunoglobulin classes (11). Histologic studies have revealed IgE immunoglobulin to be selectively present on mast cells and basophils as detected by autoradiography (13) and electron microscopy (14). Clinically there is an association between increased levels of circulating IgE and allergic diseases such as asthma (15) and between specific IgE antibody as determined by radio-allergosorbent assay (RAST), skin test positivity, IgE-dependent leukocyte histamine release, and clinical symptoms (16).

Although both experimental and clinical evidence suggest that IgE is the primary immunoglobulin involved in immediate hypersensitivity reactions, the presence of an immunoglobulin of the IgG class with similar function in a number of experimental animals (17) supports the possibility of a human counterpart. Indeed, an IgG homocytotropic antibody has been detected in the serum of a variety of patients, especially those with serum sickness. This antibody is capable of preparing the skin of humans or monkeys for an antigen-induced wheal and flare reaction, has a relatively brief period of functional persistence at these sites, and is selectively removed by immunosorbents directed against IgG and not IgE (18).

Target Cell and Chemical Mediators

The evidence relating the mast cell and basophil to IgE-dependent immediate hypersensitivity reactions includes the observations that most of tissue and leukocyte histamine is stored within the granules of these cells (19), the recent finding that eosinophil chemotactic factor of anaphylaxis (ECF–A) is also stored at the same site (20), the detection of IgE on the surface of these cells (13, 14), and the phase contrast microscopic demonstration of basophil degranulation in response to antigen challenge (21).

The mast cell was implicated as a source of slow reacting substance of anaphylaxis (SRS–A) because the deletion of this cell by chemical or specific immunologic pretreatment of the rat peritoneal cavity prevented release (22). Subsequently SRS–A has been detected in the diffusate after antigen challenge of an IgE-sensitized, basophil-rich fraction of human leukocytes (23) and a monkey lung-derived cell suspension containing mast cells (24). However, this mediator appears to arise from more than one cell type in that the release of SRS–A in the rat peritoneal cavity prepared with an IgG class homocytotropic antibody (IgGa) and challenged with specific antigen requires the presence of an intact complement sequence and polymorphonuclear leukocytes but not mast cells (25). Recent studies with human and guinea pig lung fragments and enzymatically derived human lung cell suspensions have revealed that the cellular formation of SRS–A after direct or reversed anaphylatic challenge occurs immediately and precedes its release by 30–60 sec (26). This fact affords an explanation for the consistent finding that the time course of SRS–A release lags behind that of the preformed mediators, histamine (27) and ECF–A (5), and may well permit definition of its cell source and separation of the biochemical characteristics of formation from those of release.

Mass spectrometric analysis of SRS–A by electron impact or chemical ionization and after various derivatization procedures has not yielded an identifiable mass spectrum (28). Additional characterization of highly purified SRS–A, molecular

weight approximately 500, was thus sought by spark source mass spectrometry of the elemental composition and susceptibility to inactivation by various enzymes. The functional activity of SRS–A has proved resistant to the proteases trypsin, chymotrypsin, and pronase (29, 30), to phospholipases A, B, C, D. (28, 31), to 15-hydroxyprostaglandin dehydrogenase (32), and to neuraminidase (28), but is susceptible to inactivation by mollusk arylsulfatases (33). The abundance of sulfur in the active principle determined by spark source mass spectrometry as compared to control material derived by the same steps but without initial antigen challenge of the sensitized tissue supports the view that SRS–A contains an esterified sulfate group (33).

The appreciation that neither histamine nor highly purified SRS–A is chemotactic for homologous eosinophils led to the discovery of ECF–A, a third primary chemical mediator of immediate hypersensitivity reactions (11, 34). ECF–A filtered on Sephadex G-25 with an apparent molecular weight of about 500, was stable to freeze-thawing, lyophilization, and heating in acid but not base. The ninhydrin positivity of the active material on paper chromatography and high voltage electrophoresis, its electrophoretic mobility, and its inactivation by crystallin subtilisin (20, 35) would indicate that ECF–A is a low molecular weight acidic peptide.

Prostaglandins of the E_1, E_2, and $F_{2\alpha}$ classes have been detected in the perfusate of antigen-treated, IgE-sensitized human lung tissue (36). However, as prostaglandins have also been released by gentle agitation of lung tissue, the appearance of this mediator class during an allergic reaction may be secondary to the effects of the release of the primary chemical mediators. Disodium cromoglycate suppressed the release of primary chemical mediators (37) and prostaglandins in response to antigen challenge but did not decrease prostaglandin appearance during agitation (36). Indomethacin, an inhibitor of the synthesis of the prostaglandins (38), suppressed their appearance after either antigen challenge or agitation (36) while failing to inhibit primary mediator release. There is, however, a principle, possibly lipid in nature (39), that is specifically released by IgE-antigen interaction on rabbit or human basophilic leukocytes, namely the platelet-activating factor (PAF). The latter elicits secretion of histamine and serotonin from rabbit platelets (40).

The chemical mediators whose release directly reflects the interaction of antigen with cell-fixed IgE are histamine, SRS–A, ECF–A, and PAF. The biologic effects of these chemical agents relevant to human allergic diseases include increased vascular permeability, induction of bronchial smooth muscle contraction, stimulation of parasympathetic afferent nerve endings, increased mucous gland secretion, selective attraction of eosinophils, and activation of a platelet release reaction.

PHARMACOLOGICAL CONTROL OF THE IMMUNOLOGIC RELEASE OF MEDIATORS

Suppression by Increased Concentrations of Cyclic AMP

More than three decades ago it was observed that epinephrine suppressed the reagin (IgE)–dependent wheal and flare reaction in human skin (41) and the immunologic

release of histamine from guinea pig lung tissue (42). The mechanism underlying this effect was attributed in 1968 by Lichtenstein & Margolis to elevation of the tissue concentration of cyclic adenosine 3',5'-monophosphate (cyclic AMP) (43); this conclusion was based on the demonstration that β-adrenergic agents, which increase cyclic AMP by activation of adenylate cyclase (44), and methylxanthines, which protect cyclic AMP from breakdown by phosphodiesterase, suppressed histamine release from antigen-challenged mixed human leukocytes. Subsequent studies with human lung tissue and nasal polyp fragments, peripheral human leukocytes, and isolated rat mast cells have demonstrated an inverse relationship between the tissue concentrations of cyclic AMP and the capacity for immunologic induction of the secretion of chemical mediators (Figure 2).

The evidence that an increase in the tissue levels of cyclic AMP in human lung is responsible for the suppression of the antigen-induced release of mediators includes 1. the kinetic relationship between increases in cyclic AMP and inhibition of mediator release after stimulation with isoproterenol (45), prostaglandin E_1 (46), or cholera toxin (47); 2. the close dose-response relationships between elevations in the cellular concentrations of cyclic AMP and suppression of mediator release appreciated after treatment of the tissues with β-adrenergic stimuli (12, 45), prostaglandins E_1 and $F_{2\alpha}$ (46), methylxanthines (48), and dibutyryl cyclic AMP (48); 3. the parallel rank order of potency of isoproterenol = epinephrine > norepinephrine > PGE_1 > $PGF_{2\alpha}$ (46) (range of concentrations from nanomolar to micromolar respectively) in terms of increasing cyclic AMP and preventing mediator release; 4. the synergism expressed between stimulators of adenylate cyclase, whether catecholamine (45) or prostaglandins (46), and inhibitors of phosphodiesterase upon the occurrence of both phenomena; and 5. the capacity for exogenously added dibutyryl cyclic AMP to inhibit mediator release (48). That each of these effects is manifest upon the secretion of three chemically and biologically distinct mediators (histamine, SRS–A, ECF–A) and is elicited by different pharmacologic agents with different sites of action implies that the changes in the concentration of cyclic AMP appreciated in the mixed cell population of intact lung fragments reflect the changes occurring within the subpopulation of target cells stimulated by the immunologic challenge.

It should be noted, however, that the nature of the cyclic AMP effect is not limited to control of secretion of the preformed mediators, histamine and ECF–A, but extends to determination of the quantity of intracellular SRS–A formed. Thus, the nucleotides modulate specific immunologic activation of at least two sites: formation of SRS–A and release of SRS–A and preformed mediators (26).

The postulated effect of alterations in the cyclic AMP concentration of target cells upon mediator release has been directly confirmed with purified populations of rat peritoneal mast cells. Agents such as dibutyryl cyclic AMP, aminophylline, and PGE_1 suppressed the reversed anaphylactic secretion of histamine while increasing the cyclic AMP levels of these cells. Further, the combination of threshold concentrations of PGE_1 and aminophylline synergistically increased cyclic AMP levels and inhibited histamine release (49).

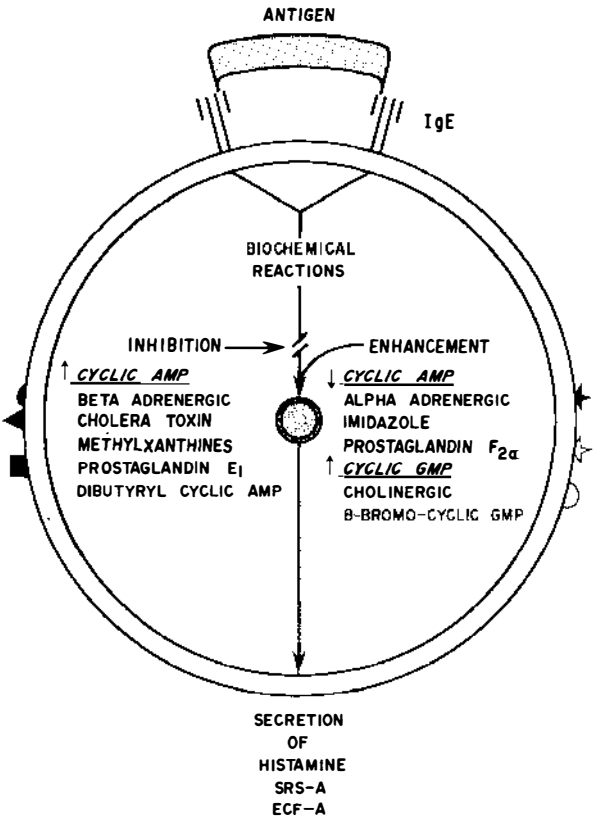

Figure 2 Relationship between concentrations of the cyclic nucleotides and release of chemical mediators.

Enhancement by Decreased Concentrations of Cyclic AMP

Treatment of lung tissue with phenylephrine (50) or with natural catecholamines such as epinephrine or norepinephrine in combination with the β-adrenergic blocking agent propranolol (45) to achieve α-adrenergic predominance consistently decreased total tissue concentrations of cyclic AMP in association with augmentation of the antigen-induced release of mediators. Low concentrations of PGE_1 and $PGF_{2\alpha}$ also reduced cyclic AMP levels and enhanced mediator release (46). Whereas the prostaglandins and α-adrenergic agents apparently produce their effects by stimulating membrane receptor sites, imidazole reduces cyclic AMP by augmenting the activity of the cytoplasmic enzyme phosphodiesterase; imidazole stimulation of lung tissue produced a simultaneous reduction in cyclic AMP levels and enhancement of the release of mediators (51). None of the enhancing agents induces mediator release alone and their effect is only to augment immunologically

activated release. These data support the concept that a reciprocal relationship exists between the intracellular levels of cyclic AMP and the ability of the cells to release mediators upon immunologic activation (Figure 2).

Enhancement by Cholinergic Stimulation

Parasympathetic stimulation employing either acetylcholine or carbamylcholine, at concentrations ranging from nanomolar to picomolar, produced an enhancement of antigen-induced release of histamine, SRS–A (5, 50), and ECF–A (47) from IgE-sensitized tissues without effecting a consistent or significant alteration of the tissues' cyclic AMP concentrations. The enhancement induced by these cholinomimetic agents is preventable by pretreatment of the tissue with atropine and thus appears to be effected through a muscarinic receptor. Enhancement of mediator release was also achieved by introducing exogenous cyclic 8-bromoguanosine 3',5'-monophosphate (50). The accompanying knowledge that cholinergic activation of a variety of tissues, including lung tissues (52), selectively stimulates guanylate cyclase with resultant increases in cyclic GMP levels (53, 54) indicates that elevations in this cyclic nucleotide enhance the immunologic release of mediators and function in opposition to increases in cyclic AMP (Figure 2).

Whereas histamine release from peripheral human leukocytes sensitized with human IgE is inhibited by agents capable of stimulating adenylate cyclase such as β-adrenergic agents, prostaglandins, histamine, and cholera toxin (55, 56), enhancement by neurohormone treatment has not been observed. Neither α-adrenergic nor cholinergic stimulation augments mediator release in this system, whereas the microtubule aggregation-enhancing agent heavy water (D_2O) enhances the immunologic release of histamine (57). The discrepancy between the enhancement noted in studies of human lung tissue and confirmed with similar studies of nasal polyp fragments (5) and the lack of such an effect on peripheral leukocytes may mark enhancement difference between human tissue mast cells and circulating basophils.

BIOCHEMICAL EVENTS ESSENTIAL TO OR CONCOMITANT WITH THE IMMUNOLOGIC RELEASE OF MEDIATORS

Sequence of Biochemical Events for Mediator Release from Human Lung Fragments

The release of chemical mediators after immunologic activation of mast cells or basophils is recognized to be a secretory process (21, 58, 59). The biochemical prerequisites for the reaction to proceed to completion encompass the discrete phases of activation, SRS–A formation, and release of the mediator group. The data available are, however, based predominantly upon the quantity of mediator released. In this regard the biochemical requirements for the immunologic release of mediators from human lung tissue are consistent with those of various other tissues (2) and consist of the necessity for an intact glycolytic pathway, the availability of calcium ions, and the activation of a serine esterase from its precursor state; these steps are inhibited by 2-deoxyglucose (2-DG), ethylenediamine tetraacetate (EDTA), and diisopropyl fluorophosphate (DFP), respectively (12, 60).

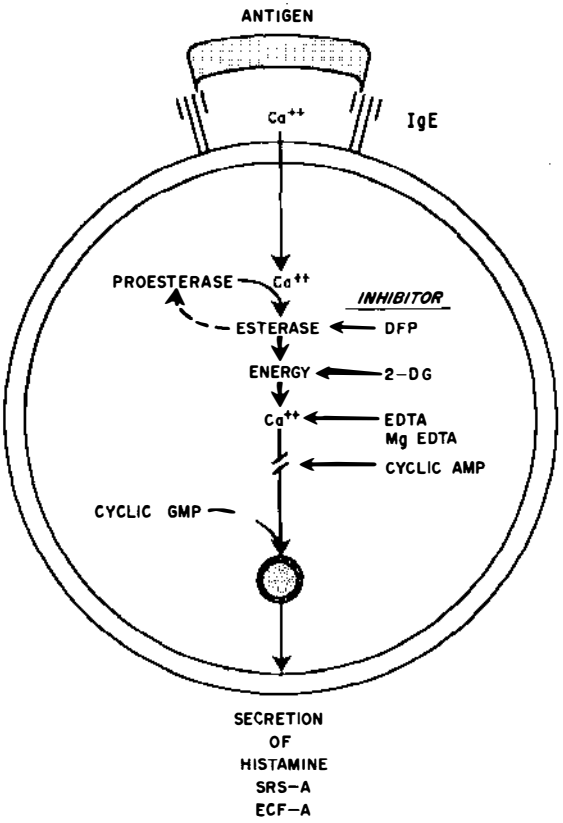

Figure 3 Sequence of biochemical events leading to the release of chemical mediators.

The order in which these biochemical prerequisites are required in the reaction sequence has been partially delineated and appears to proceed as follows: the union of antigen with two IgE antibodies upon the surface of the target cells; the extracellular calcium ion-requiring activation of a serine esterase from its precursor form to its DFP-inhibitable state; the further autocatalytic feedback activation of the proesterase; an energy-requiring stage inhibitable by 2-DG; an intracellular calcium ion-requiring step suppressible by EDTA; and a cyclic AMP–inhibitable step (61) (Figure 3).

The experimental design that permitted this analysis was based on the capacity to maintain the immunologically activatable serine esterase in its DFP-resistant precursor state despite antigen challenge and to arrest reversibly the active intermediates of the reaction sequence by deletion of a glucose source or calcium ions. The former permitted determination of those conditions essential to the activation of the proesterase such as extracellular calcium ions and the autocatalytic feedback action

of the activated esterase, while the latter positioned the subsequent intracellular events. Antigen challenge of sensitized tissue in the presence of DFP does not permit mediator release, while removal of DFP before antigen challenge or within 1–2 min of challenge yields full release. Thus, antigen-IgE interaction activates a proesterase essential to mediator release over a finite interval. If calcium ions are deleted from the medium, antigen challenge in the presence of DFP does not result in activation of the proesterase in that removal of DFP and restoration of the calcium ion concentration gives full release 15–25 min later without reintroduction of antigen. As calcium ion deletion alone is not inhibitory, the maintenance of the proesterase state after antigen challenge requires inactivation of any esterase formed by DFP in order to prevent an autocatalytic effect. As any inhibitor operative after this step would not maintain the proesterase state in the presence of antigen challenge, it was established that the calcium ion-dependent activation of the proesterase was the earliest definable step (61).

A second and subsequent intracellular calcium ion-requiring step was then recognized. The presence of 2-DG in a glucose-free medium suppressed the antigen-induced release of mediators, while removal of the 2-DG and antigen by washing the tissue and then restoring glucose reversed the inhibition with resultant mediator release. However, removal of the 2-DG and restoration of glucose in the presence of EDTA maintained the inhibition until the EDTA was removed and calcium ions replenished. Conversely, reversal of EDTA-induced inhibition of mediator release was not suppressed by the absence of glucose and presence of 2-DG, indicating that the energy-requiring stage must precede the intracellular calcium ion-requiring step inhibitable by EDTA. In similar experiments with EDTA- or 2-DG-inhibited tissues, the β-adrenergic agent isoproterenol increased the tissue concentrations of cyclic AMP and inhibited the release of mediators despite reversal of either inhibitory condition. Thus the cyclic AMP-suppressible step either coincides with or follows the second, EDTA-inhibitable calcium-requiring stage (61).

The immunologic release of histamine from human peripheral leukocytes, as had previously been noted for contraction of guinea pig ileal strips (62) or histamine release from guinea pig lung tissue (63), may be divided into two stages relating to the calcium content of the tissues (64). Antigen challenge in the absence of calcium apparently activates the reaction sequence so that after washing and restoring calcium ions histamine is released. Isoproterenol and other cyclic AMP active agents exert their inhibitory effects in the initial or calcium-independent phase, while 2-DG suppresses release in the second or calcium-dependent phase (64). As this is the only system in which activation of a proesterase has not been demonstrable (65), the calcium-independent phase may well represent an intracellular stage in a reaction sequence differing from that defined in human lung tissue.

Histamine release from rat peritoneal mast cells by cationic band 2 protein derived from rabbit leukocytes demonstrated an early requirement for the activation of a DFP-sensitive esterase, the necessity for an intact glycolytic pathway suppressible by dinitrophenol or iodoacetate, and a calcium-requiring step appreciable only after the reaction sequence has been initiated at 0° C and the activated cells then transferred to 37°C buffer containing EDTA (66). The secretion of serotonin from

rabbit platelets after treatment with a number of agents appears to involve the early activation of a serine esterase inhibitable by DFP and later stages suppressible by EDTA and increased concentrations of cyclic AMP (67). Thus, the sequence data available for these latter two systems are consistent with the more detailed analysis of mediator release from human lung fragments.

Concomitants to Histamine Release from Purified Rat Mast Cells

Studies of the intracellular changes which accompany histamine release in a homogeneous cell population are limited to purified rat peritoneal mast cells. Further, to achieve a percent release sufficient to conclude that the intracellular measurements reflect changes in those cells releasing histamine, the activating agents have been diverse. Reversed anaphylactic challenge with rabbit anti-rat Fab (49) in glucose-containing buffer reduces cellular ATP and cyclic AMP in association with histamine release. Direct anaphylactic challenge with ^{14}C-pyruvate as substrate has been associated with a fall in cellular ATP and the elaboration of CO_2, while ATP did not decrease in the presence of glucose (68). Activation with 48/80 has revealed a fall in cyclic AMP (69) as well as a reduction in ATP, providing that oxidative phosphorylation was inhibited by the presence of antimycin A (70). The fall in ATP could reflect not only an uncoupling of oxidative phosphorylation (68, 70) but other mechanisms as well and need not be directly responsible for the critical diminution in cyclic AMP. Activation of histamine release by a calcium ionophore (71) has been related to an influx of radiolabeled calcium, while impaired uptake was momentarily present during the reversed anaphylactic release reaction (49). Calcium ion flux in both directions is conceivable, as two calcium ion-dependent steps have already been noted for mediator release from human lung fragments. Influx could relate to activation and efflux to an excess of free intracellular calcium ions as part of the secretory phase.

CONCLUDING COMMENTS

The presence of IgE receptors on mast cells and basophils (13, 14, 72) serves to concentrate this trace protein by placing several hundred thousand molecules on a cell (72). The bridging of two adjacent IgE molecules activates the cell, perhaps through a steric perturbation of the cell surface. It seems possible that the receptor for IgE represents a binding portion of a multiunit proenzyme which undergoes a calcium ion-dependent activation to a DFP-sensitive serine esterase (61). The esterase then initiates the secretory phase through action on an as yet unidentified substrate.

The evidence that microtubules are involved in the secretory phase includes the inhibitory effects of the microtubule-binding agent colchicine upon both human leukocyte (57, 73) and rat mast cell (74) histamine release, the enhancing effect of the microtubule stabilizing agent D_2O (75) upon both systems (57, 74) and the identification of microtubules by electron microscopy in rat mast cells (76). Cytochalasin B suppressed immunologic histamine release from rat mast cells (77) but enhanced release from human leukocyte suspensions (78) and thus a microfilament requirement for release

Although direct evidence for activation of a membrane ATPase in mediator release is lacking, such an activity has been conjectured (49, 79) on the basis of 1. the consistent association of ATPases with secretory reactions in general (80); 2. the specific enhancement of mediator release by phosphatidylserine (79, 81), which is known to augment certain ATPase activity (82, 83); 3. the fall in cellular ATP during reversed anaphylactic histamine release in glucose-containing buffer (49); and 4. the inhibition of rat mast cell histamine release by oligomycin (79), an ATPase inhibitor (84). ATPases are also involved in the generation of intracellular calcium fluxes (85) and in mitochondrial-poor cells such as mast cells (86) might produce an appreciable, although transient, depletion of ATP levels.

A hypothetical sequence of events might then be as follows: the calcium ion-dependent activation of the serine proesterase linked to the IgE receptor site with further autocatylytic activation; the assembly and function of microtubules and the activation of cellular ATPases, both energy-requiring events perhaps associated with transient depletions of ATP stores and suppressible by colchicine, EDTA, and metabolic inhibitors; possibly the contraction of myofilaments facilitated by increased intracellular levels of calcium ions and suppressible by cytochalasin and EDTA; the movement of the secretory granules to the cell surface along channels set down by the microtubules; fusion of granular and plasma membranes; and finally the release of the granules by exocytosis (87). The inhibitory action of increased intracellular cyclic AMP might relate either to phosphorylation of microtubular protein or of myofilaments (88, 89). Alternatively, increases in cyclic AMP may lead to impaired fusion of granular and plasma membranes as even cytolytic reactions of mast cells are suppressed (90). It seems likely that release will also prove to be associated not only with a fall in cyclic AMP but also with a transitory rise in cyclic GMP.

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