Ann. Rev. Pharmacol. Toxicol. 1981. 21:63–81

THE PHARMACOLOGICAL CONTROL OF IMMEDIATE HYPERSENSITIVITY

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Immediate hypersensitivity is a reaction to a foreign substance and is immunological in nature. Initial challenge with the foreign material stimulates, by interaction with T lymphocytes and macrophages, the immune system. The response is the generation of B lymphocytes which recognize the antigenic determinants of the foreign material; these B cells then differentiate into plasma cells which elaborate IgE-class antibodies directed against the antigens. The IgE antibodies so formed become fixed to tissue mast cells or circulating basophil leucocytes. In man, rat, and mouse the antibody which fixes to mast cells and basophils is of the IgE class whereas in other species such as guinea pig it appears to be a subclass of IgG antibodies. In either case the production of antibody which specifically binds to mast cells and basophils is fundamental to the immediate hypersensitivity reaction. Second and subsequent challenges with foreign antigen lead to an antigen-antibody reaction on the mast cell and basophil membrane. The immunological and the pharmacological control of IgE production has been the subject of earlier reviews (1–4).

Stimulation of the mast cell and basophil membranes initiates a sequence of cellular events leading to the release of the mediators of the immediate hypersensitivity reaction. The mediators have long been known to include histamine, slow reacting substance (SRS), and, in some species, 5-hydroxytryptamine. Other putative mediators include prostaglandins, kinins, chemotactic factors, and platelet activating factor. The smooth muscle contraction, glandular secretion, cell accumulation, increased capillary

0362-1642/81/0415-0063$01.00

permeability, and altered blood coagulation are together the manifestations of the mediators released in the immediate hypersensitivity reaction to foreign antigens.

Recent developments in the understanding of the immediate hypersensitivity reaction have involved the properties of the cellular receptor for IgE, phospholipid metabolism in mast cells, the role of calcium in stimulus-secretion coupling, and the structure of slow reacting substances. All of these areas are of interest to the pharmacologist and are the subject of this review.

CELLULAR RECEPTOR FOR IgE

The initial studies of the mechanism of antigen-antibody–induced histamine release were performed using guinea pig tissues where the antibody class which binds to mast cells is IgG (5). However, experiments in rats indicated that a special class of antibody which was both heat labile and destroyed by sulfhydryl reagents was responsible for sensitizing mast cells (6). The reaginic antibody, as it was called, also showed persistence at the site of skin injection in comparison with other antibody classes (7). Ishizaka demonstrated that reaginic antibody was a class separate from the immunoglobulins already described and he designated it IgE (8). The IgE class of antibody binds selectively to mast cells and basophil leucocytes with little binding to other cells such as macrophages, neutrophil, and eosinophil leucocytes (9). By using Fc and Fab fragments of IgE antibody in competition experiments it has been demonstrated that the Fc region of IgE inhibits the binding of IgE to mast cells and basophils (10). On the basis of this evidence it is presumed that the receptor for IgE on mast cells and basophils is an Fc receptor. Both autoradiographic experiments and radioassays of labeled IgE binding kinetics have indicated that rat mast cells and human basophils possess about 10^5 IgE receptors per cell. The basophilic leukemia of the rat has about 10^6 receptors per cell and it is this cell line which has been used to study the IgE receptor (11).

The binding of the IgE molecule to its receptor has a rate constant of 10^5 $M^{-1}sec^{-1}$ whereas the reverse reaction has a rate constant of about $10^{-7}sec^{-1}$. The equilibrium dissociation constant for the binding of IgE to its receptor has a value of about 10^{-11} M (11).

The valency of the receptor for IgE is believed to be unity and this is based on experiments in which free receptors were exposed to mixtures of IgE labeled either with rhodamine or with fluorescein. Anti-IgE treatment of such cells caused the two fluorescent colors to co-cap on the membrane, but antifluorescein produced only green cap formation indicating that receptors bearing IgE-fluorescein had no attached IgE-rhodamine (12). In addition

to suggesting univalency of the receptor, these experiments also demonstrate mobility of the receptor within the lateral plane of the membrane. Similar conclusions have been drawn from photobleaching experiments in which fluorescence recovery time is measured after bleaching of membrane bearing IgE-fluorescein and IgE-rhodamine. Immobilization of IgE-fluorescein prevented recovery of green fluorescence in a bleached area without altering the mobility of rhodamine-labeled IgE: The experiments provide evidence against receptor-receptor interactions and confirm the univalent nature of the receptor (13). In addition, the rate of recovery in photobleaching experiments provided an estimate of the diffusion coefficient of the receptor in the membrane: 2×10^{-10} cm^2 sec^{-1}. The diffusion coefficient of a lipid probe in the same membrane was found to be 8×10^{-9} cm^2 sec^{-1} indicating that the membrane is fluid at physiological temperatures. Interestingly, cytochalasin B, which inhibits microfilament function, reduces receptor mobility, whereas disruption of microtubules with colchicine has no effect on mobility.

Using the rat basophilic leukemia as a source of material, the IgE receptor has been solubilized and purified (14, 15). The most recent estimates reveal that the receptor is a glycoprotein with a molecular weight of about 80,000, and this protein appears to have two subunits. The part of the receptor which is labeled from the cell surface and which binds IgE is a subunit of molecular weight 50,000 (16). Binding of IgE to the 50,000 subunit is not influenced by the presence of the 30,000 subunit, the function of which is not known. The various methods of determining the size of the IgE binding protein are not in agreement. Polyacrylamide gel electrophoresis (PAGE) and amino acid analyses give values of 50,000, whereas radiation inactivation has yielded a value of 30,000. The question of further functional subdivision of the 80,000 molecular weight receptor is one which remains unanswered.

THE MEMBRANE SIGNAL

Simple binding of an IgE molecule to its receptor on a mast cell or basophil leucocyte does not activate the cell to secrete histamine. The feature of an immediate hypersensitivity reaction which brings about activation of the cell is the binding of antigen to the cell-bound IgE. It has been known for some time that the antigen must be bi- or multivalent in order to produce cellular activation: Monovalent antigens do not activate the cell when they bind to the IgE (17). The requirement for divalency may indicate that some type of cross-linking is the key to cellular activation. Further evidence in support of the cross-linking hypothesis is available from a variety of experimental approaches which are summarized in Figure 1. The most recent data

employing antibody (IgG) directed against the IgE receptor itself is compelling evidence in support of the hypothesis that the mechanical coupling of adjacent IgE receptors within the fluid mosaic of the membrane is the initial event in a sequence which ultimately brings about the secretion of histamine (18). Only complete antireceptor antibody with its two antigen combining sites (Fab) acts as a membrane signal. Antireceptor which has been dissociated into single Fab units still binds to the receptor but cannot act as a membrane signal because it is only monovalent. Figure 1*f* shows that chemically dimerized IgE binds to unoccupied IgE receptors in the membrane, cross-linking them and stimulating secretion. An important question relating to this type of experiment is: What is the activity of trimers and higher polymers? Asked in another way the question becomes: What is the nature of the membrane signal formed by cross-linking three or more receptors compared with cross-linking only two? Recent experiments comparing the secretory response to dimers, trimers, and higher polymers of IgE have revealed that in rat basophilic leukemic cells, trimers are more effective than dimers by at least two orders of magnitude (19). It appears, therefore, that cross-linking more than two IgE receptors is a more effective membrane signal than cross-linking two. In molecular terms we are not yet in a position to explain this phenomenon since the relationship between stimulus and later events in stimulus-secretion coupling has yet to be defined.

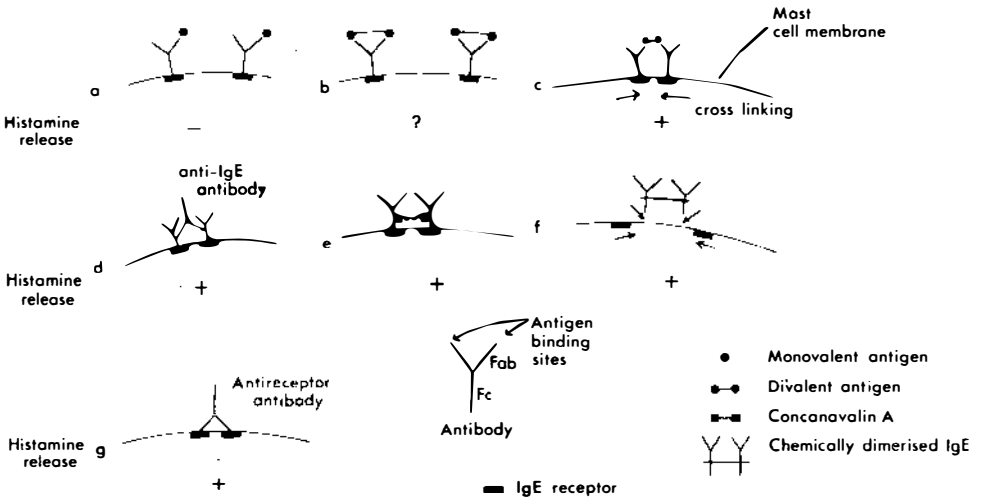

Figure 1 Diagrammatic representation of the various modes of cross-linking IgE receptors to initiate histamine secretion. (Reproduced by permission of Elsevier/North Holland.)

Electron microscopic observation of the aggregation of IgE on the membrane of stimulated mast cells has failed to reveal a relationship between aggregate size and the magnitude of the secretory response. Perhaps this is not surprising since only large changes in receptor distribution are observable on electron micrographs and the changes necessary to stimulate the cells may amount only to the cross-linking of a few adjacent receptors (20). No relationship between aggregation of receptors, patching or capping, and either activation or inactivation of secretory response has been observed (21).

CALCIUM

The requirement of antigen-induced histamine secretion for extracellular calcium was first demonstrated in chopped lung and has been confirmed in basophil leucocytes and rat mast cells (22–24). Calcium 0.1 to 1 mM activates antigen-induced histamine release but in the presence of zero extracellular calcium some antigen-stimulated histamine secretion still occurs. The histamine secretion which is not dependent on extracellular calcium is not inhibited by di (2-aminoethoxy)-ethane tetracetic acid (EGTA), a calcium chelating agent (24, 25).

Douglas has drawn attention to the similarities between the excitation-contraction coupling of muscle and stimulus-secretion coupling (26). It was proposed that a rise in free cytosolic calcium ion concentration was the second messenger coupling the membrane receptor-ligand interaction with the subsequent release of preformed granular material. Several pieces of evidence are available which are consistent with such a model for the activation of histamine secretion. First, the calcium ionophores A23187 and ionomycin transport calcium from the extracellular medium into the cell, and this is associated with histamine secretion (27, 28). It is interesting to note that the calcium inonphore A23187 can apparently also be made to release intracellular stores of calcium and induce histamine secretion in the absence of calcium (25, 29–31). Second, published reports concerning microinjection of calcium into mast cells with a calcium-filled glass micropipette are in conflict: One group (32) reported activation of degranulation by injection of calcium into mast cells whereas another group failed to obtain such a result (33). Third, it has recently been shown that fusion of calcium-loaded liposomes with rat mast cells can lead to histamine secretion, presumably as a result of calcium being released from liposomes into the cell interior (34). Finally lanthanum, which competes for calcium binding sites in a number of biological systems and prevents calcium transport across membranes, inhibits histamine secretion induced by antigen

stimulation. Inhibition of histamine secretion by lanthanum is the result of competition between calcium and lanthanum at an extracellular site which is thought to be the calcium channel (35).

Accepting evidence that intracellular calcium is associated with activation of secretion and knowing that antigen-stimulated histamine secretion requires extracellular calcium, it might be expected that the antigen-antibody reaction on the mast cell membrane would increase the permeability of the membrane toward calcium. Figure 2 shows that stimulation of purified rat mast cells with an antibody directed against IgE receptors (see also Figure 1) causes histamine secretion and an uptake by the cells of ^{45}calcium. It has been shown that the uptake of calcium measured in this way has several components including binding to the cell surface and to released granular material. However, about one third of the total uptake observed in stimulated cells represents passage of ^{45}calcium across the cell membrane and this occurs in the absence of secretion in stimulated cells treated with antimycin A (36, 37). It is concluded, therefore, that cross-linking signals increase the membrane permeability of mast cells to calcium.

It has already been mentioned that some experiments on mast cells have indicated the presence of intracellular stores of calcium which may be

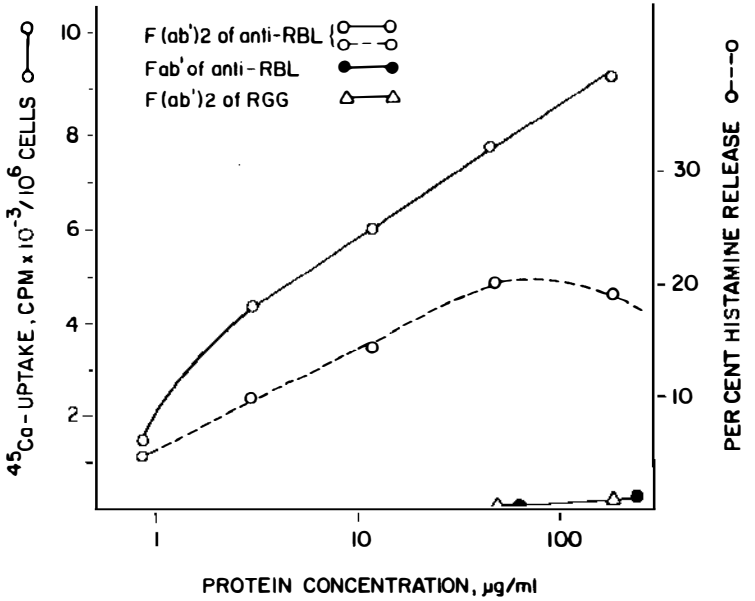

Figure 2 Histamine release and ^{45}calcium uptake induced by antibody to the IgE receptor (anti-RBL). Monovalent antibody (Fab') or nonspecific rabbit gamma globulin [F(ab')2 RGG] are inactive.

utilized for coupling a stimulus to histamine secretion. Some ligands such as compound 48/80, peptide 401, and polylysine produce histamine secretion in the absence of extracellular calcium (35, 38). Furthermore histamine secretion by these agents is not sensitive to inhibition by lanthanum at concentrations which prevent antigen-stimulated histamine release, indicating that it is not an external membrane-bound source of calcium ions being utilized in histamine secretion by these agents. However, incubation of mast cells with EGTA to chelate calcium prevents the action of compound 48/80 and the other basic ligands but secretion may be restored by adding extracellular calcium (39). It has recently been shown that A23187 can induce histamine secretion in the absence of calcium, presumably by releasing an intracellular pool of calcium. Changes in extracellular pH in an acid direction increase the proportion of histamine secretion which is independent of extracellular calcium when the cells are stimulated either with antigen or A23187 (Figure 3). The calcium-independent release induced in this way is not sensitive to extracellular chelating agents and is presumed, therefore, to be due to an action of extracellular hydrogen ions resulting in the release of intracellular calcium. In fact, EGTA increases the histamine secretion from antigen-stimulated cells in the absence of external calcium at acid pH (Figure 3) (25, 30).

Histamine secretion can, therefore, result from a ligand-receptor interaction at the membrane causing entry of calcium from extracellular to intracellular compartments or by causing release of calcium from intracellular stores. The evidence for internal stores is, however, indirect,

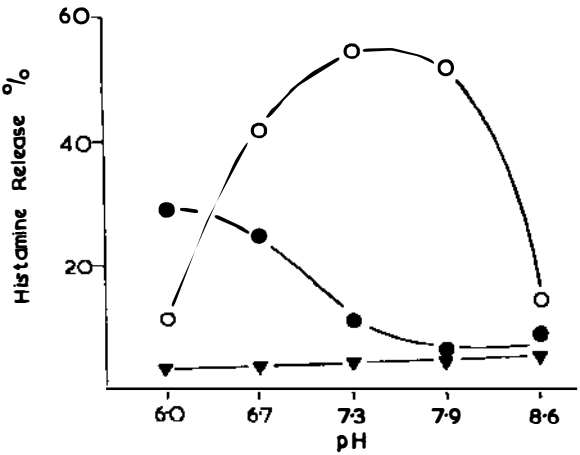

Figure 3 Effect of extracellular hydrogen ion concentration on histamine release induced by A23187. ▼—▼ no A23187; ●—● A23187 0.6 μM in the absence of Ca^{2+}; ○—○ A23187 0.6 μM in the absence of Ca^{2+} but with EGTA 0.1 μM.

although an alternative explanation of the data available would require separate mechanisms of secretion: one calcium-coupled and one not.

A further point to be made about calcium and histamine secretion concerns secretion in the absence of a ligand-receptor interaction: so-called spontaneous secretion. Spontaneous histamine secretion from rat mast cells does not increase with time over periods of 1–2 hr incubation at 37°C in the presence of calcium (40). Furthermore, the extracellular calcium concentration does not influence spontaneous histamine release. If IgE receptor cross-linking induces increased membrane permeability to calcium, perhaps by forming calcium channels, it would not seem unreasonable to predict a certain frequency of spontaneous aggregation of receptors. Calcium-dependent spontaneous secretion would be expected to ensue from this only if the amount of calcium entering through the spontaneously opened channel was sufficient to activate secretion. The experimental data indicates that this is not so. However, it has been shown that the antigen-operated calcium channel has greater permeability to strontium compared with calcium (41, 42) and so this other alkaline earth ion can be used as a more sensitive probe of calcium channels. Replacement of extracellular calcium with extracellular strontium ions increases spontaneous secretions, and the mechanism of this spontaneous secretion appears to be similar to the mechanism of antigen-mediated secretion in the presence of calcium (40). Also, while the resting mast cell membrane shows virtually no permeability to calcium (8 f mole cm^{-2} sec^{-1}) it has a permeability to strontium (38 f mole·cm^{-2} sec^{-1}) which allows the ion to accumulate in the cells and thus stimulate secretion (40).

Finally, the control of secretion by regulation of membrane calcium permeability should be discussed. A permanent and uncontrolled increase in membrane permeability to calcium would be expected to be lethal to a cell since so many cellular processes depend upon low intracellular calcium levels and precise buffering mechanisms. The increased membrane permeability of mast cells following a cross-linking stimulus appears to be a transient phenomenon. If mast cells or basophils are stimulated with antigen in the absence of extracellular calcium, little or no histamine secretion occurs until calcium is added back. The cells show a decreasing response to the readdition of calcium as the interval between stimulus and calcium addition is increased (43–45). The secretory response decays or desensitizes with respect to calcium. Cells whose response to calcium has decayed in this way are still responsive to stimulation with the calcium ionophore A23187. Furthermore, the response to calcium of cells treated with A23187 does not demonstrate inactivation of calcium response. The experiments support the view that activation of secretion involves transient increase of membrane permeability induced by the stimulus, and this has been confirmed by measurement of the membrane permeability using ^{45}calcium (36). Stimula-

tion causes increased permeability to calcium which decreases with time after stimulation; the time course of decay in the permeability of the membrane to calcium parallels the time course of the inactivation of secretion. Mast cells and basophils appear, then, to preserve cellular homeostasis by limiting stimulus-induced calcium entry.

PHOSPHOLIPIDS

Michell and colleagues (46, 47) have accumulated evidence in support of the hypothesis that breakdown of membrane phosphatidyl inositol is an essential step in the formation and control of membrane calcium channels. In view of the known role of calcium ions in histamine secretion and the hypothesis relating movement of calcium across the mast cell membrane to activation of histamine secretion, it is not surprising that much attention has recently been focused on phospholipid metabolism in mast cells.

It has been shown independently by Gomperts (48) and by Sullivan (49) that stimulating rat mast cells with antigen, anti-IgE, concanavalin A, compound 48/80 and the ionophore A23187 produces increased incorporation of either [^{32}P] P_i or [^{3}H] inositol. In both studies the time course of both phosphatidyl inositol turnover and histamine secretion was similar but there was a consistent difference between the concentrations of ligand causing histamine secretion compared with the concentrations required to stimulate phosphatidyl inositol turnover. Phosphatidyl inositol turnover was detectable at concentrations up to an order of magnitude lower than those needed for stimulation of histamine secretion. The increased phosphatidyl inositol turnover in stimulated mast cells does not require calcium in the extracellular medium which would be consistent with the hypothesis that the breakdown of this phospholipid is involved in calcium channel formation. However, it is not clear why compound 48/80 initiates increased phosphatidyl inositol turnover since it appears not to trigger secretion by forming membrane calcium channels. Phosphatidyl inositol is not the only phospholipid to turn over in stimulated mast cells: Phosphatidic acid and phosphatidyl choline also show stimulated turnover whereas phosphatidyl serine, phosphatidyl ethanolamine, and sphingomyelin do not (49, 50). It is interesting that phosphatidyl serine potentiates both histamine secretion and phosphatidyl inositol turnover induced by immunological stimulation of mast cells. Furthermore, theophylline and dibutyryl cyclic AMP inhibit both antigen-stimulated phosphatidyl inositol turnover and also histamine secretion. A close parallel therefore exists between secretion and phosphatidyl inositol turnover but the experiments published thus far have failed to show that phosphatidyl inositol turnover is an essential link between receptor-ligand binding and the opening of calcium channels. It is possible that phosphatidyl inositol turnover is an epiphenomenon of mast cell stimula-

tion. With this in mind and also the observation that phosphatidyl inositol is not the only phospholipid to show increased turnover in stimulated cells, it has been suggested that the important phospholipid metabolite is diacylglycerol. Diacylglycerol is a fusagen and is also a product of phospholipid turnover in mast cells (51). It has been suggested, therefore, that diacylglycerol formation might have a role to play in histamine secretion at a stage, much later than calcium entry into the cytosol, when granule and cell membranes are fusing to release the granule contents. Once again, the experiments available so far do not establish a functional role in secretion for either phosphatidyl inositol turnover or diacylglycerol formation since it is not clear whether or not these events are merely the *result* of secretion. It would be interesting to know whether diacylglycerol formation and phosphatidyl inositol turnover are increased when cells are stimulated under conditions where secretion itself is prevented, say by metabolic inhibitors such as 2-deoxy-D-glucose or antimycin A. If the phospholipid changes occur in stimulated cells in the absence of secretion, the change cannot then be said to be a *result* of the membrane fusions occurring during secretion.

Apart from increased turnover of phospholipids in stimulated mast cells, a number of methylation reactions involving membrane phospholipids have recently been described by Axelrod and colleagues (52). Mast cells stimulated by concanavalin A show increased incorporation of [^{3}H] methyl groups from methionine into phospholipid (53). It has been suggested that phosphatidyl ethanolamine formed from membrane or exogenous phosphatidyl serine acts as a substrate for a methyltransferase designated *I* which generates N-monomethyl phosphatidyl ethanolamine. The methylated phosphatidyl ethanolamine then appears to be converted by methyltransferase *II* into phosphatidyl choline. The reaction sequence is shown diagrammatically in Figure 4. Ishizaka and colleagues have attempted to demonstrate the relationship between these methylations of mast cell phospholipids and the calcium transport across the membrane which is believed to be one of the early events in the activation of histamine secretion (54).

Methylated phospholipids are formed within 15 sec of stimulating mast cells (54), and the stimulated increase in phospholipid methylation is over within 1 min of stimulus application. It clearly precedes in time both histamine secretion and ^{45}calcium uptake. S-isobutyryl-3-deaza-adenosine, 1–30 μM, was used to inhibit S-adenosyl-L-methionine–dependent methylation reactions and it was shown that this compound inhibited phospholipid methylation, ^{45}calcium uptake, and histamine secretion in mast cells stimulated with anti (IgE receptor) antibody. Methylation and ^{45}calcium uptake were also inhibited by 3-deaza-adenosine, 0.1–6.4 μM,

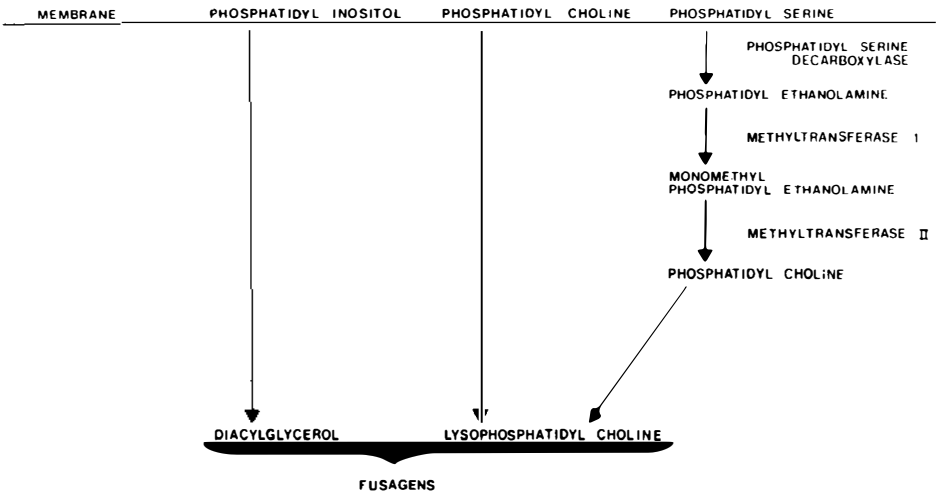

Figure 4 Methods of forming potential fusagens from membrane phospholipids.

and inhibition by this latter drug was enhanced in the presence of L-homocysteine-thiolactone 1–10 nM. The observations have been used to support the hypothesis that phospholipid methylation is a reaction which couples the membrane stimulus (anti-receptor-induced receptor aggregation) with calcium channel formation. While the time courses of the events appear compatible with this, no experiments with inhibitors of calcium transport have been performed to see whether they interfere also with the methylation reactions. Also, it would be interesting to know whether the calcium ionophore A23187 stimulates phospholipid methylation. One would predict that artificial calcium carriers should not require phospholipid methylation to initiate calcium transport and histamine secretion. It must also be pointed out that the concentrations of S-isobutyryl-3-deazaadenosine required to produce 60% inhibition of phospholipid methylation are about ten times less than the concentrations required to inhibit histamine secretion and about three times less than those to inhibit ^{45}calcium uptake (54). The actions of this inhibitor may, therefore, not be at a single site.

It has been suggested many times [see (55) for review] that one of the events coupling the membrane stimulus to histamine secretion is the activation of phospholipase. Indirect evidence showing that rat mast cells produce prostaglandins makes it very likely that arachidonic acid can be liberated from mast cell phospholipids by a phospholipase, and since the discovery (56) that slow reacting substance (SRS-A) is a metabolite of arachidonic acid (see Figure 5) more attention is again focused on a role for phospholipase in the secretory response of mast cells. Unfortunately no specific

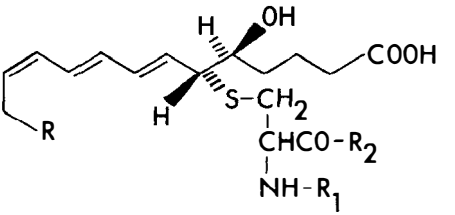

Figure 5 Structure of the leukotrienes (90) which have slow reacting substance activity. R_1 - Glu, R_2 - Gly Leukotriene C; R_1 -H, R_2 - Gly Leukotriene D; R_1 - H, R_2 - H Leukotriene E.

inhibitor of phospholipase A is available and so no clear picture emerges from the various experimental approaches.

Labeled arachidonic acid becomes incorporated into material which has the biological activity of SRS (56) and it has been shown that the lipoxygenase metabolites of arachidonic acid, the leukotrienes, have some or possibly all of the biological activity of SRS (57, 58). Mast cells also metabolize arachidonic acid by the cyclooxygenase pathway to produce prostaglandins, principally PGD_2, but also PGE_2 and $PGF_{2\alpha}$ as well as thromboxanes. While the mediator role of SRS is accepted and has been supported by the use of the selective antagonist of SRS, FPL 55712 (59), the mediator role in immediate hypersensitivity of the prostaglandins, and thromboxanes remains to be determined.

The arachidonic acid which acts as a substrate for SRS and prostaglandin formation is assumed to come from mast cell or basophil membrane phospholipid by the action of phospholipase. Use has been made of the inhibitors of cyclooxygenase such as aspirin, meclofenamic acid, and indomethacin, together with drugs such as 5,8,11,14-eicosatetraynoic acid (ETYA) and BW 557 which inhibit lipoxygenase and cyclooxygenase enzymes, to investigate the role of arachidonic acid metabolites in the process of histamine secretion.

Evidence that aspirin inhibited histamine secretion predated the knowledge of its role as an cyclooxygenase inhibitor (60). The inhibitory action of aspirin on histamine release is manifest at concentrations much higher than those which inhibit cyclooxygenase and such drugs may inhibit secretion by acting either as calcium antagonists (61) or by preventing ATP synthesis (62). Concentrations of aspirin and indomethacin up to 60 μM do not inhibit histamine secretion. However, ETYA does inhibit histamine secretion induced by cross-linking stimuli (63, 64). In addition arachidonic acid inhibits stimulated secretion, this inhibition being prevented by aspirin (63, 64). It has been proposed therefore, that lipoxygenase products are necessary for the activation of secretion and that cyclooxygenase products act to inhibit secretion. It has been established for some time that PGE_2 inhibits antigen-stimulated histamine release (65).

No general conclusion about the role of phospholipids and arachidonic acid metabolites in histamine secretion is currently possible but the available evidence indicates that these molecules play a central role in the biochemistry of the immediate hypersensitivity reaction.

CYCLIC NUCLEOTIDES

The original observation that epinephrine inhibited antigen-induced histamine secretion (66) was confirmed using a human basophil leucocyte model (67). From those starting points research on the role of cyclic nucleotides in histamine secretion has followed many paths but no clear picture emerges. There is good evidence that a β-adrenergic receptor in guinea pig and human lung can, when activated, inhibit antigen-induced histamine release (68), but the evidence for such a β-receptor is less good in human basophils where quite high concentrations of isoprenaline (isoproterenol) are needed for inhibition of histamine secretion (69). The quantitative data for pA_2 measurement is also lacking for the human basophil. In the case of the rat mast cell, it is fairly clear that a β-adrenergic receptor does not influence histamine secretion but it is not clear whether these cells have no receptor or possess an uncoupled receptor (70, 71).

Apart from β-receptor agonists, other agents which cause increased levels of cyclic AMP within the cell have been shown to inhibit antigen-induced histamine release from mast cells and basophils. Cholera toxin, theophylline, dibutyryl cyclic AMP, and adenosine phosphorothioate all inhibit antigen-induced histamine release from mast cells and basophils (67, 72–74).

A recent development in understanding how the phosphodiesterase inhibitor theophylline inhibits histamine release has arisen from the report that adenosine enhances histamine release from mast cells (75, 76). The mechanism of action of adenosine in this respect is not known but it enhances antigen- and A23187-stimulated secretion. Because theophylline is a competitive antagonist of adenosine, it has been suggested that it inhibits histamine release by preventing the enhancing effect of adenosine which may be present at the mast cell membrane (77). Some doubt about this hypothesis has been raised by the observation that in basophils adenosine itself inhibits antigen-induced histamine secretion (78).

Changes in cyclic AMP levels which result from stimulating cells have been measured and the experiments show a rapid fall in cyclic AMP level following stimulation; the levels recover to basal values with the same time course as the inactivation process (see above) (79). Other experiments show a similar rapid fall of cyclic AMP level followed by much slower return to resting level (80).

Rat mast cells contain two protein kinase enzymes, only one of which is

cyclic AMP-dependent (81). The cyclic AMP-dependent protein kinase is activated by immunological stimulation of the cells and accounts for the majority of the total protein kinase activity. The relationship betweeen these protein kinases and the phosphorylation of membrane proteins when cells are stimulated with A23187 or 48/80 is not established (82) but will no doubt be an interesting area for further experiments.

The observation that cyclic AMP is associated with inhibition of antigen-stimulated histamine secretion but does not affect A23187-induced secretion (83) led to the hypothesis that cyclic AMP inhibits histamine secretion by blocking calcium transport across the mast cell membrane. Direct evidence for this has been obtained with ^{45}calcium uptake experiments (36). However, the histamine secretion induced by A23187 can under certain conditions be inhibited by dibutyryl cyclic AMP which suggests that cyclic AMP may have more than one action (84). In basophils, drugs which elevate cyclic AMP levels can both inhibit and enhance histamine secretion depending on how the cells are stimulated (85). Cross-linking stimulation results in a type of secretion whose maximum level is reduced by cyclic AMP but the *rate* of secretion is increased (86). The ionophore A23187-induced secretion and spontaneous secretion are not reduced in amount by cyclic AMP but again the rate of secretion is increased (86). Cyclic AMP may, therefore, exert actions on the rate and on the magnitude of secretion.

The various sites of action of cyclic AMP, the functions of the protein kinases, and the nature of phosphorylated membrane proteins will no doubt lead to a better understanding of antigen-stimulated histamine secretion.

There have been reports that cholinergic receptor activation may potentiate antigen-stimulated secretion and it was suggested that the second messenger for such potentiation was cyclic GMP (87). The experiments were performed in chopped lung where 8-bromo-cyclic (GMP) potentiated antigen-stimulated histamine secretion. There are no reports confirming these observations in human basophils or rat mast cells. In fact, experiments designed to confirm them have failed in the author's own laboratory and also elsewhere (L. M. Lichtenstein, personal communication). Recent accounts of the histamine releasing action of acetylcholine (88, 89) have also not been reproduced in other laboratories (J. C. Foreman, unpublished; J. L. Mongar, personal communication; B. D. Gomperts, personal communication; L. M. Lichtenstein, personal communication).

This review sets the most recently reported experiments in the context of some of the more firmly established knowledge about the immediate hypersensitivity reaction. The model depicted in Figure 6 is an attempt to synthesize the available material though, as with most models, it is incomplete and, in places, speculative. It will, I hope, provoke further experiments.

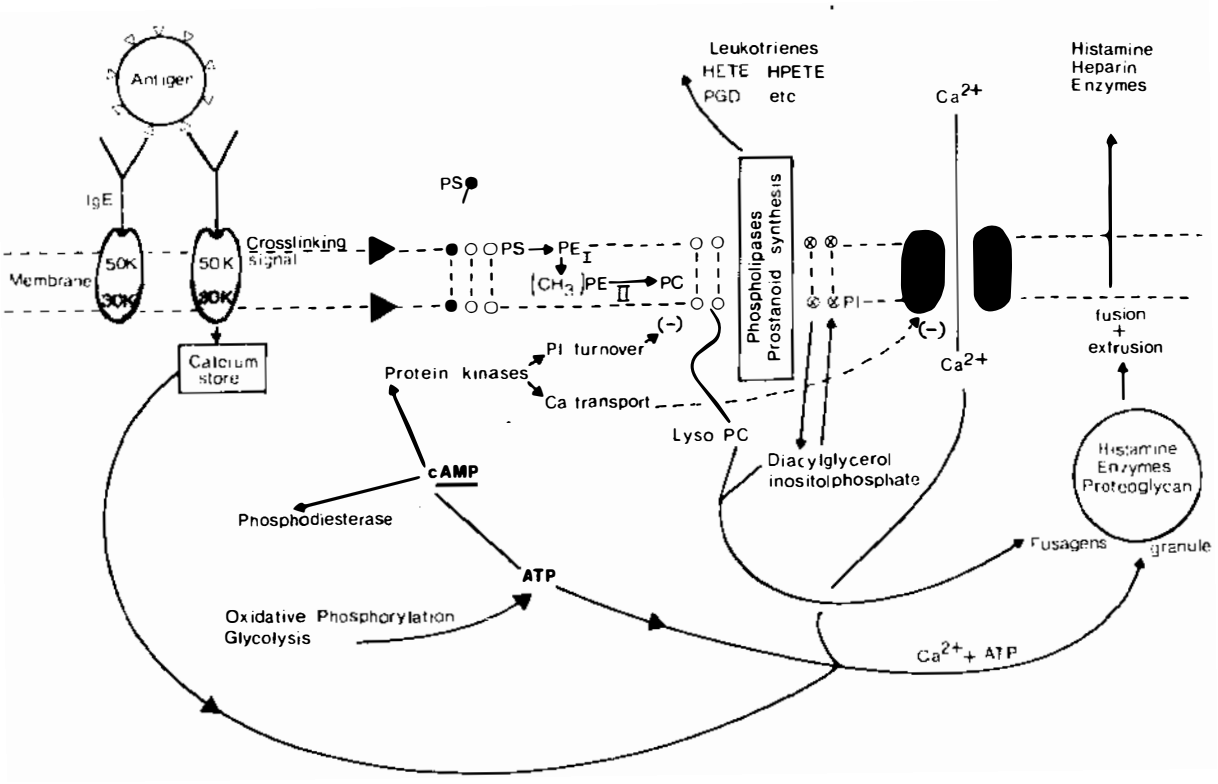

Figure 6 Model for histamine secretion (see text for explanation).

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