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Calcium and mast cell activation

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Introduction

The clinical manifestations of allergic and anaphylactic reactions are produced by the release of chemical mediators from tissue mast cells and circulating basophil leucocytes (Lewis & Austen, 1981; Samuelsson, 1983; Siraganian, 1983). These mediators are either preformed and stored within the cell in association with characteristic secretory granules (histamine, neutrophil and eosinophil chemotactic factors, heparin or related glycosaminoglycans, and hydrolases) or are generated *de novo* by the oxidative metabolism of membrane-derived lipid components (leukotrienes, prostaglandins and thromboxanes). In total, these spasmogenic, vasoactive and chemotactic factors act on distinct effector cells to induce the immediate and late-phase reactions characteristic of asthma and other inflammatory responses.

Given the central role of the mast cell in immunopathology, intense effort has been directed towards elucidating the immunological and biochemical mechanisms involved in mediator release. Most work has been performed using pleural or peritoneal cells from the rat, since these can be easily isolated and purified, and this article is based primarily upon findings in these cell types. The release of histamine is often chosen as an indicator of secretion because there are well documented assay methods for this amine and, being the first mast cell mediator to be identified, comparatively more is known about its turnover than other granule-derived substances.

Stimulus-secretion coupling in the mast cell

The pathophysiological stimulus for the release of chemical mediators from the mast cell is provided by the combination of specific antigen with reaginic (IgE) antibody fixed to the cell surface. This leads to a dimerization of receptor sites, an event which may also be induced experimentally by lectins such as concanavalin A and by antibodies to IgE or to the IgE-receptor molecule (see Foreman, 1981). In addition, mediator release may be triggered by a variety of pharmacological stimuli (see Lagunoff *et al.*, 1983), which have provided the pharmacologist with a range of important tools with which to study the release process.

As in other secretory processes, the primary event appears to be a rise in the intracellular concentration of free calcium ions following cell activation. That an increase in free calcium is in itself a sufficient condition for secretion has been clearly demonstrated by experiments in which exocytosis and mediator release have been induced by direct introduction of the cation into the cell, either by microinjection (Kanno *et al.*, 1973), by means of calcium ionopores (Foreman, 1981), by fusion with liposomes containing the cation (Theoharides & Douglas, 1978), or by permeabilization of the cell with ATP (Bennett *et al.*, 1981) or Sendai virus (Gomperts *et al.*, 1983). What is less clear is the relative contributions of intracellular and extracellular stores of calcium to the release process.

Calcium pools involved in histamine secretion

Extracellular calcium

Non-cytotoxic activation of the mast cell is believed to open receptor-mediated gates or channels in the plasma membrane, thus permitting influx of calcium down its electrochemical gradient (Foreman *et al.*, 1977; Foreman, 1981). The first step in the internalization of calcium appears to involve combination of the ion with a specific membrane receptor (Grosman & Diamant, 1974; White & Pearce, 1981). Consistently, secretion is inhibited by ions of the

lanthanide series which are competitive antagonists of binding of this type (Foreman & Mongar, 1973; Pearce & White, 1981). Furthermore, stimulation of rat mast cells with IgE-directed and other ligands evokes a correlated uptake of radioactive calcium from the external medium (Foreman *et al.*, 1977), although this may simply reflect either (a) non-specific binding of calcium to membrane sites exposed by the degranulation process (Grosman & Diamant, 1978), or (b) an increased exchange of isotope across the membrane, rather than net accumulation of calcium. Direct evidence that the end result is a rise in intracellular free calcium has come from the recent studies of White *et al.* (1984). These authors used the fluorescent probe quin-2 to demonstrate directly an increase in cytoplasmic calcium following mast cell activation with either anti-IgE, compound 48/80 or the ionophore A23187.

Intracellular calcium

While maximal histamine secretion is dependent on the presence of calcium ions in the external medium, suboptimal responses may be obtained from rat mast cells in the absence of the added cation (see Pearce, 1982). Different agonists vary in their potency under these conditions. Most agents, including IgE-directed ligands and ionophores, induce some release of histamine but polycationic secretagogues are particularly effective (Pearce *et al.*, 1981).

The secretion of histamine from mast cells in a calcium-free medium is normally attributed to the mobilization of membrane-bound or intracellular reservoirs of the cation. Consistently, the response is abolished by prolonged treatment with chelating agents which are believed to deplete these stores (Douglas & Ueda, 1973; Pearce *et al.*, 1981). This treatment also produces a parallel reduction in the fluorescence signal due to the calcium-chlortetracycline complex in mast cells preloaded with the antibiotic, indicative of the removal of membrane-bound calcium (White & Pearce, 1983). More directly, White *et al.* (1984) have again used the probe quin-2 to demonstrate a release of intracellular calcium following anaphylactic stimulation in the absence of the added cation.

The location of the putative internal calcium stores involved in mediator release remains unknown although the plasma membrane itself appears a preferred candidate. Thus, histamine secretion in a calcium-free medium is blocked by prolonged incubation with lanthanide ions which are thought not to penetrate into the cytosol (Pearce & White, 1981). Also, the secretory response is potentiated by brief pretreatment of the cells with chelating agents (in contrast to the inhibitory effect of prolonged exposure to these compounds) and blocked by supramaximal concentrations of calcium. By analogy with other systems, it has been argued that these treatments may modulate the fluidity or stability of the membrane, thus respectively facilitating or preventing the release of bound calcium from this site (Pearce *et al.*, 1981).

Calcium efflux

In contrast to calcium influx into the mast cell, efflux of the cation has only recently been studied. The main mechanism for the extrusion of calcium in this system appears to be through the operation of a sodium-calcium antiporter (Pearce & White, 1984). Thus, the basal efflux of radioactive calcium from cells preloaded with the isotope is essentially unaffected by metabolic inhibitors, thereby excluding the immediate involvement of an ATP-dependent calcium pump, but is totally dependent on extracellular sodium ions. Most interestingly, anaphylactic stimulation of the cell causes a temporary cessation of calcium efflux. This effect would then act to enhance the rise in intracellular calcium produced by influx or mobilization of the cation and hence provide a cooperative mechanism for augmenting the secretory response.

Early biochemical events involved in mast cell activation and calcium mobilization

A number of rapid biochemical changes following rat mast cell activation have now been delineated. In many cases their precise role in the activation sequence, and in calcium mobilization, remains speculative. The next section will summarize the main events and indicate their possible importance in mediator release.

Phosphatidylinositol turnover

Throughout the last decade, it has become recognized that many agonists which employ calcium as a second messenger also evoke parallel changes in the metabolism of the endogenous membrane lipid phosphatidylinositol (PI), as discussed earlier in these proceedings by Triggle (1985) and Hesketh (1985). The PI response has been demonstrated following activation of mast cells by a number of diverse immunological and pharmacological stimuli (Cockcroft & Gomperts, 1977). However, there is now considerable argument as to whether the effect is a causal event in mediator release or merely represents an epiphenomenon of cell

activation (Cockcroft, 1981). Further work will be required to resolve the issue and the possible involvement in histamine secretion of polyphosphoinositide breakdown, recently proposed to be the initial reaction in receptor-mediated inositol phospholipid metabolism (Gil *et al.*, 1983), remains to be investigated.

Transmethylation of phospholipids

It is now widely recognized that methylation of phospholipids may play an important role in the transduction of biochemical signals through cell membranes (Hirata & Axelrod, 1980). Commonly, two membrane-bound enzymes (methyltransferases I and II) successively convert phosphatidylethanolamine (PE) to phosphatidyl-*N*-monomethylethanolamine (PME) and then to phosphatidylcholine (PC). This sequential methylation causes reorientation of the phospholipid to the outer surface of the membrane, an effect which is associated with an increase in membrane fluidity. This change in microviscosity may facilitate calcium fluxes. Alternatively, PC may be metabolized further by activation of a calcium-dependent phospholipase A_2, thus generating lyso-PC (a known fusogen) and, in the case of arachidonyl-PC, free arachidonic acid.

Transmethylation has now been demonstrated following anaphylactic stimulation of rat basophil leukaemic cells, human pulmonary mast cells and rat peritoneal mast cells (Hirata *et al.*, 1979; Crews *et al.*, 1981; Ishizaka, 1982; Ishizaka & Conrad, 1983). Immunological activation of these cells induces a rapid rise and fall in methylated lipid which is followed temporally by calcium influx and histamine secretion. Inhibitors of methyltransferases block all three processes in highly correlated fashion and also prevent IgE-mediated release from human basophil leucocytes. In total, these data strongly suggest that transmethylation is a primary event in immunological histamine release and calcium transport, though the phenomenon does not seem to apply to non-immunological activation (Hirata *et al.*, 1979).

Arachidonic acid metabolism

Both the PI response and the transmethylation sequence provide potential routes to the generation of free arachidonic acid *in situ*. The latter may then be metabolized via the lipoxygenase or cyclooxygenase pathways to generate a number of potent inflammatory mediators, principally leukotrienes, prostaglandins and thromboxanes (Lewis & Austen, 1981; Samuelsson, 1983). In addition, the oxidative metabolism of the compound may generate intermediates essential to the release process itself, such as 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which may act prior to calcium translocation (Nemeth & Douglas, 1980; Peters *et al.*, 1981; Magro, 1982).

Changes in cyclic nucleotide levels

Immunological activation of the rat peritoneal mast cell produces a rapid, transient elevation in the level of adenosine 3′:5′-cyclic monophosphate (cAMP) which appears to be an integral part of the activation sequence (Holgate *et al.*, 1980a). Other changes in the intracellular levels of cyclic nucleotides also occur but appear to be secondary to the induced release of prostaglandins (Lewis *et al.*, 1979). It has been suggested that bridging of IgE receptors activates adenylate cyclase, the interaction between the receptors and the catalytic subunit being mediated (as in other systems) by combination of guanosine 5′-triphosphate (GTP) with a specific guanine nucleotide-binding protein (Ishizaka *et al.*, 1981). The elevation in cAMP is then thought to activate specific protein kinase isoenzymes with consequent phosphorylation of proteins essential to the release mechanism (Holgate *et al.*, 1980b). The proposed interrelationships between IgE receptor activation, adenylate cyclase activation and transmethylation of phospholipids, together with their combined effects on calcium translocation, are summarized in Figure 1.

Historically, elevated levels of cAMP have been associated with the prevention of exocytosis but, in the light of the data summarized above, it has been argued that cAMP may occur within discrete pools in the mast cell thereby leading to the preferential activation of protein kinase isoenzymes associated with either the initiation or inhibition of the response (Holgate *et al.*, 1980b). However, the close association between the early rise in cyclic AMP and secretion does not hold for all pharmacological stimuli nor for all mast cell types (Burt & Stanworth, 1983; Hughes *et al.*, 1983; Leoutsakos & Pearce, unpublished observations; MacGlashan *et al.*, 1983), and so further work is required to define the exact significance of the cAMP changes.

Calmodulin and histamine secretion

The precise mechanism whereby calcium induces secretion is not known in the mast cell nor indeed in any other system. However, many of the effects of calcium in non-contractile cells are mediated through its interaction with the specific

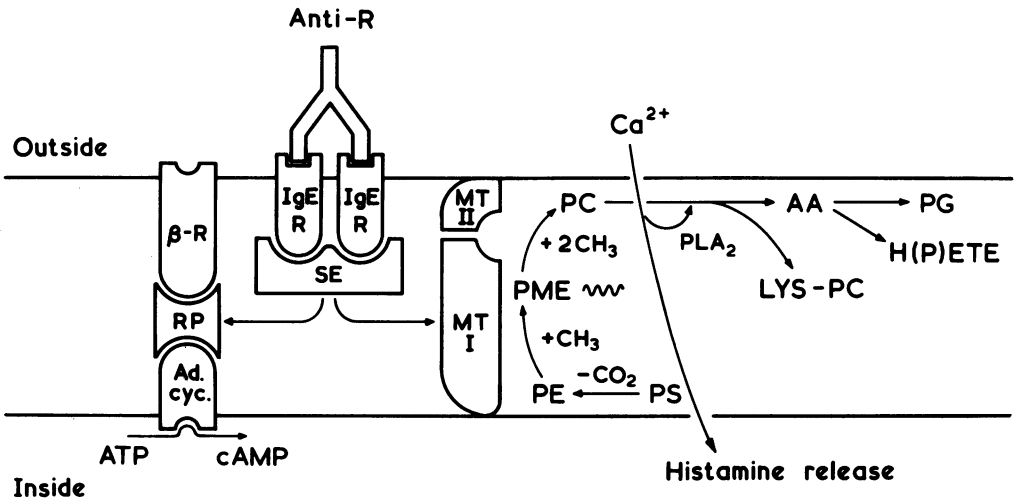

Figure 1 Schematic representation of the relationship between IgE-receptors, methyltransferases and adenylate cyclase in the mast cell membrane. Key: IgE receptor (IgE-R), anti-receptor antibody (Anti-R), β-adrenoceptor (β-R), GTP-dependent regulatory protein (RP), adenylate cyclase (Ad. cyc), methyltransferases I and II (MTI, MTII), putative serine esterase (SE), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidyl-*N*-monomethylethanolamine (PME), phosphatidylcholine (PC), lysophosphatidylcholine (LYS-PC), phospholipase A_2 (PLA_2), arachidonic acid (AA), prostaglandins (PG), hydr(oper)oxyeicosatetraenoic acids (H(P)ETE), and change in membrane fluidity (〰).

binding protein calmodulin (for reviews, see Cheung, 1980; Means *et al.*, 1982).

A number of neuroleptic drugs, principally those of the phenothiazine family, are able to combine with the calcium-calmodulin complex. These compounds prevent interaction of the complex with its target proteins and so inhibit calmodulin-dependent reactions. Most significantly, these agents prevent histamine release from mast cells and basophil leucocytes (Douglas & Nemeth, 1982; Marone *et al.*, 1983; Peachell & Pearce, 1984). The characteristics of the inhibition are consistent with a specific action of the drugs distal to calcium influx and the rank order of potency of different compounds correlates at least approximately with their reported anti-calmodulin activity. These results provide preliminary evidence for the involvement of calmodulin in histamine release. The precise nature of this involvement remains uncertain but several enzymes involved in mast cell biochemistry have in other systems been shown to be regulated by calmodulin: cyclic nucleotide phosphodiesterase, adenylate and guanylate cyclase, calcium/magnesium-ATPase, phospholipase A_2, tryptophan hydroxylase, methyltransferases, myosin light chain kinase, phosphorylase *b* kinase, glycogen synthetase kinase, and a variety of kinases involved in the phosphorylation of membrane proteins (Cheung, 1980; Means *et al.*, 1982). The possible role of calmodulin in the control of exocytosis has been further discussed elsewhere (Pearce, 1982).

Drugs which inhibit histamine release; the effect of calcium antagonists

A variety of pharmacological agents are able to inhibit secretion in mast cells, to varying extents depending largely on the cell type and experimental conditions which are adopted e.g. phosphodiesterase inhibitors, β-adrenoceptor agonists, cyclic nucleotide analogues and sodium cromoglycate.

Given the essential nature of stimulus-secretion coupling, it is natural that attempts have been made to account for the action of inhibitory compounds in terms of their effects on calcium homeostasis. The first coherent hypothesis was that proposed by Foreman, Mongar and coworkers (Foreman *et al.*, 1976, 1977), namely that such agents act directly on the receptor-mediated calcium channels operative during the anaphylactic reaction to prevent influx of the cation from the external environment. Evidence against this view has been accumulated in the author's laboratories and recently summarized (Pearce, 1982; Pearce & Clements, 1982; White & Pearce, 1982). We have suggested that existing antiallergic and cAMP-active drugs may exert an

ill-defined, general stabilizing effect on the mast cell membrane. An increased stability or reduced fluidity of the membrane would have manifold effects on histamine release, potentially protecting against the disruptive effect of detergents, or prevent the fusional changes involved in exocytosis, the mobilization of membrane-bound calcium and the opening of calcium channels.

The recent findings of White *et al.* (1984) are in accord with this view. These workers showed that cromoglycate and inhibitors of methyltransferase and phosphodiesterase prevented both the antigen-induced release of histamine and the increase in quin-2 fluorescence in mast cells preloaded with the calcium indicator. These effects were manifest in the presence and absence of extracellular calcium, indicating that the test compounds are indeed able to prevent both the stimulus-dependent influx of calcium and the release of the bound cation from intracellular stores.

With the advent of the calcium antagonist group of drugs, a few investigators have examined their influence on mediator release from mast cells. Thus far, the results are not very encouraging. While verapamil and nifedipine do prevent IgE-mediated histamine release from rat mast cells (Ennis *et al.*, 1983), the concentrations required to do so are very much higher than those active in smooth muscle. The inhibition is unaffected by elevated levels of external calcium and the compounds are additionally active against the ionophore A23187. Like cromoglycate and cAMP, the calcium antagonists may thus also have non-specific effects on the mast cell membrane. In this context, it should of course be appreciated that the term 'membrane-stabilization' is one of pure convenience and the biochemical basis of the effect remains to be established.

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