Short Communication

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Calcium Channels and Histamine Release from Mast Cells

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Abstract

Using isolated peritoneal mast cells from two types of genetically different rats, histamine release induced by allergic stimuli was inhibited, in all cases except one, by significantly lower concentrations of an intracellular calcium antagonist than those required to inhibit release induced by a calcium ionophore. The exception related to inhibition of histamine release induced by clinical dextran in NR rats (which are relatively less sensitive to dextran than are control rats) as the concentrations required were of a similar order as those needed to inhibit release by ionophores. The data further support the suggestion that there are different calcium channels involved in the different release processes.

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The non-cytotoxic release of histamine from isolated peritoneal mast cells of rats induced by antigen, concanavalin A (Con A) and some polysaccharides like dextran is considered to be initiated through a cross-linking with specific antibody or specific poly-saccharide receptors located on the mast cell surface [1]. This receptor-mediated mechanism can be circumvented by the use of calcium ionophores such as compound A23187. Using both systems, information on the site of action of new anti-anaphylactic agents can be gained. However, when comparisons were made of the activities of many histamine-releasing agents using both in vitro experiments (incubation with isolated mast cells) and in vivo experiments (injection in paws), large differences were found. For example, Con A was about 100 times more active than clinical dextran on isolated cells, yet about 20 times less active when injected into the paws of rats. These releasers requiring calcium ions in the medium for activity in in vitro experiments were always potentiated by the presence of phosphatidylserine. These results were obtained using both R rats secured from Tucks Ltd. (Rayleigh, Essex, UK), which respond with an ana-phylactoid oedema when clinical dextran is injected, and NR rats from the NELP colony which respond only with difficulty.

The essential event which links stimulation of mast cells to histamine secretion is the increase in level of free calcium ions in the cytosol (from about 0.1 μ M to over 0.5 μ M) and this may be derived from the extracellular fluid or from mobilisation of intracellular calcium stores. The calcium then interacts with the low-molecular weight protein calmodulin, each molecule of which can bind four of calcium. When two or more calmodulin-binding sites are occupied, a series of intracellular enzyme systems is activated and mediator release takes place. In attempts to characterise the nature of the release, calcium ionophores which form lipid-soluble complexes with calcium ions have been shown to be transported through mast cell membranes. Blockers of the entry of calcium ions into the cell such as 3,4,5-trimethoxybenzoic acid diethylamino octyl ester (TMB-8) have also been used to explore the role of calcium in the release process and in the pathophysiology of immediate hypersensitivity reactions. However, TMB-8 inhibits both

antigen-induced histamine release from rat mast cells and release induced by A23187, and so the biochemical sequence of events leading to release have not yet been fully solved.

Last year, it was shown [2] that different calcium channels through the plasma membrane and the granular membrane of mast cells probably exist, as TMB-8 (a recognised intracellular calcium antagonist) exhibited different inhibitory activities as a calcium entry blocker (involving allergic histamine re-

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Table I. Inhibition by TMB-8 (threshold concentration) of his-tamine release from isolated unwashed peritoneal mast cells of R and NR rats induced by threshold concentration of different his-tamine releasers

lease) and as a calcium store blocker (involving non-allergic histamine release). Higher concentrations were required to reduce histamine release from rat peritoneal mast cells induced by calcium ionophores than those needed to reduce release induced by antigen. As only R rats were used in the initial study, similar experiments have now been completed using mast cells taken from rats genetically much less sensitive to clinical dextran (the so-called NR rats) which respond to antigen challenge after sensitisation equally as well as do those from R rats. Groups of 4 male Wistar R and NR rats (150–250 g) were used. Briefly, mast cells were harvested from the peritoneal cavity using 5 ml of protein-free mast cell medium (MCM) per rat [2]. They were incubated at 37 °C for 5 min to equilibrate and then the appropriate amount of releaser was added. Secretion was allowed to proceed for 15 min and then the reaction was stopped by adding cold MCM. After centrifugation, the supernatant was biochemically assayed for histamine using a spectrofluorimeter. Total histamine content was measured after heating samples at 100°C for 15 min. Histamine release was calculated as a percentage of the total content, allowing in each case for spontaneous release. When antigen was used as the histamine releaser, rats were sensitised 10 days before use with 10mg bovine serum albumin (BSA) and 0.25 ml Bordetella pertussis vaccine injected intraperitoneally. The releasers tested were BSA, Con A (Pharmacia), clinical dextran (molecular weight 110,000 daltons) and A23187 (Lilly). All the agents produced dose-related releases of histamine from isolated peritoneal mast cells using

MCM containing 1 mM calcium. Release by dextran from cells of NR rats required 25 times the concentration needed for release from cells of R rats (table I). When TMB-8 (gift of Dr. N. Grosman, Copenhagen) was included in the incubation mixtures of releasers exerting threshold effects, inhibition of release was found in all cases. As in earlier experiments, higher concentrations of inhibitor were required to reduce the activity of A23187 than those needed to reduce the releases induced by antigen, Con A and dextran (table I). Even more striking, however, was the finding that the concentration of TMB-8 required to inhibit histamine release induced by dextran in NR rat mast cells (400 µM) exceeded that needed to inhibit release induced by A23187 in both R and NR rat cells (300 µM). This result supports the suggestion that different calcium channels may be involved in histamine release from rat mast cells.

Free calcium ions play a vital role in most examples of mediator release from mast cells, and hence the channels by which these ions pass through the plasma and granular membranes could provide useful information for future experimentation. The compound TMB-9 is the octyl ester of a trimethoxyben-zoic acid derivative and it would be interesting to test in rats corresponding

esters of both lower and higher alcohols. Already, optimal activity has been shown to be present at the heptyl/octyl carbon length of amino acids [3] and of acetic acid [4], as well as in ethyl esters of straight-chain fatty acids [5], when tests were made against the effects of agents producing inflammation.

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