Histamine Releasers and Rat Mast Cells

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Abstract

The release of histamine from rat tissues rich in mast cells, induced by antigen, concanavalin A, dextran, compound 48/80, A 23187, phosphatidic acid and chlortetracycline, has been compared with that from isolated peritoneal mast cells of rats. Whereas most of the agents were more active than dextran in in vitro experiments, the reverse was found when they were injected intradermally into the skin, or subcutaneously into the paws. In fact, A 23187 and chlortetracycline (both calcium ionophores), as well as phosphatidic acid (another **non-cytotoxic** releaser), failed to release significant amounts of histamine when injected into the animal. Thus, the experimental conditions in which comparisons of the activities of histamine releasers are made should always be stated.

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Recently, the antibiotic chlortetracycline has been shown to be an effective histamine releaser from isolated peritoneal mast cells of rats [1], and this calcium ionophore has been used in attempts to determine the site of action of anti-anaphylactic agents. Several compounds, including those which elevate intracellular levels of cyclic AMP, were reported to inhibit secretion evoked by the anaphylactic reaction, but not that induced by ionophores, which usually provide a more persistent signal for secretion.

Histamine release from isolated mast cells may not always accurately represent events taking place within the body, and large differences have already been reported in the relative activities of some histamine-releasing agents when tested under in vitro conditions and in the whole animal [3]. For example, concanavalin A was about 100 times more active than clinical dextran when tested on isolated cells, yet it was about 20 times less active when injected into rats. Furthermore, phosphatidyl serine enhanced the release of histamine induced by both these agents from isolated mast cells, though it did not do so in the whole animal.

We have now analysed the histamine-releasing activity of chlortetracycline in both in vitro and in vivo systems, and made comparisons with those of other agents including antigen.

Concanavalin A and dex-

tran were chosen as they initiate non-cytotoxic secretion through an interaction with specific polysaccharide receptors, and are models involving cross-linking of glucose receptors on the mast cell surface. Phosphatidic acid, the simplest of the glycerophosphatides found in cell membranes, was chosen as it was considered to be the activator of calcium gates, and anaphylactic release of histamine is triggered by an increased level of ionized calcium in the cell cytoplasm. Recent studies [4] have shown that histamine release induced by extracellular phosphatidic acid resembles that evoked by a basic secretagogue like compound 48/80, and so these two compounds were also included in the comparison. As the receptor-mediated mechanism of histamine release can be circumvented by the use of calcium ionophores, the widely used A 23187 served as a control calcium carrier.

Groups of 4 male Wistar rats (150–250 g) secured from Tucks Ltd., Rayleigh, Essex, and from the NELP colony, were used in all experiments. Mast cells were harvested from the peritoneal cavity using 5 ml calcium-free and protein-free mast cell medium (MCM) [2]. They were incubated either in MCM, or in MCM containing ImMcalcium, at 37°C for 5 min to equilibrate, and the appropriate amount of releaser was then added. Secretion was allowed to proceed for 15 min before the reaction was stopped by adding ice-

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cold MCM. After centrifugation, the supernatants were assayed for histamine using a spectrofluorimeter. Total histamine content was measured by heating cell samples at 100°C for 15 min. Histamine release was calculated as a percentage of the total content, allowing for spontaneous release in each case. Phosphatidyl serine (PS, Lipid Products) was included in some experiments at a concentration of 10 µg ml⁻¹. When antigen was used as the histamine releaser, rats were sensitised 10 days before use with 10 mg bovine serum albumin and 0.25 ml Bordetella pertussis vaccine injected intraperitoneally. The releasers tested were chlortetracycline hydrochloride (CTC, Sigma), A 23187 (Lilly), phosphatidic acid dipalmitoyl

sodium salt (PA, Sigma), concanavalin A (Con A), compound 48/80, clinical dextran (mol. wt. 110,000), and bovine serum albumin (BSA, Sigma).

For the *in vivo* comparisons, the releasers were injected either intradermally or intrapedially, and the responses measured either as the accumulation of albumin-bound dye (μg) in the skin after intravenous azovan blue, or as increases in paw volume on a volume differential meter over 24 h. The doses producing significant threshold responses were determined, using Student's *t* test for significance ($p < 0.05$).

All the agents produced dose-related releases of histamine from isolated peritoneal mast cells of both colonies of rat, but dextran was, as expected, about 40 times less active on cells of NELP rats (termed NR rats) than on cells of Tuck rats (termed R rats). Figure 1 shows the releases using corresponding doses of each agent. It is clear that PS potentiated release induced by Con A, BSA and dextran in R rats, and by Con A and BSA in NR rats. Furthermore, glucose was an effective inhibitor only of the releases by Con A, BSA and dextran. The non-cytotoxic release induced by compound 48/80, A 23187 and PA (but not by Con A, BSA and dextran), occurred in the absence, as well as in the presence, of calcium (1mM).

A comparison is made in table I of the concentrations of agents required to produce comparable threshold histamine release from isolated peritoneal mast cells of R rats, and the doses needed to produce threshold histamine release in the skin (increased vascular permeability) and in the paw (oedema). Antigen and Con A are much less active on intact cells than they are on isolated cells, the ratio being over 100, whereas the reverse is found for dextran (a ratio of 0.1). Histamine release induced by Con A, BSA and dextran *in vivo* was not enhanced by PS, suggesting Fig. 1. Comparison of histamine release (%) induced by different agents ($\mu\text{g ml}^{-1}$) from isolated peritoneal mast cells of R rats (upper diagram) and NR rats (lower diagram), using unwashed cells maintained in MCM. Shaded histograms show releases in the presence of PS ($10 \mu\text{g ml}^{-1}$). Values are the means $\pm$ SEM of four separate experiments.

Table I. Amounts of agents required to produce threshold histamine release ($p < 0.05$) from isolated peritoneal mast cells ($\mu\text{g ml}^{-1}$) and in the skin and paws (μg) of R rats (values are means of four experiments)

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that different mechanisms are involved in the intact animal from those operating with isolated cells. The two ionophores and PA were ineffective on intact mast cells.

When cells of NR rats were tested, comparable results were obtained though, as expected, dextran was much less effective in all systems.

Thus, the release of histamine induced by CTC, A 23187 and PA resembled that evoked by a basic se-cretagogue like compound 48/80, which is independent of exogenous calcium and glucose, and unaffected by PS. The study also shows that the non-cytotoxic release of histamine induced by these three agents is distinct from those by Con A, BSA and dextran.

In other experiments, CTC in doses of up to 60 mg kg^{-1} , failed to elicit an anaphylactoid reaction in rats. However, within the dose range of $10\text{--}60 \text{ mg} \cdot \text{kg}^{-1}$, it inhibited the dextran anaphylactoid reaction in a dose-dependent manner when the two agents were injected simultaneously.

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