Mast Cell Heterogeneity in Response to Cholinergic Stimulation

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Abstract

Mast cell heterogeneity in response to acetylcholine has been evidenced by the virtual lack of sensitivity or by the full reaction to nanomolar concentrations of acetylcholine, observed in samples of serosal mast cells isolated from the same animal species. The incubation with IgE of isolated rat mast cells renders the originally heterogeneous response homogeneous, the release of histamine evoked by acetylcholine being proportional to the IgE concentration. The histamine release induced by acetylcholine is due to the activation of muscarinic receptors, since it is blocked by atropine, not reproduced by acetylthiocholine and potentiated by exposure of the cells to the specific antigen.

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Introduction

The heterogeneity of mast cells within the same order (rodents) is well evidenced by the differences in size, proteoglycan and monoamine content, life span and releasability by various secretagogues, which occur between rat, guinea pig and hamster mast cells [Mota, 1966]. Within the same species (rat), evidence for heterogeneity of mast cells was provided by the different characters of mucosal mast cells (MMC) and connective tissue mast cells (CTMC) [Bienenstock et al., 1982]. Within CTMC (i.e., serosal mast cells isolated in the rat by pleural and peritoneal lavages), heterogeneity in response to acetylcholine has been envisaged by the virtual lack of sensitivity or by the full reaction to nanomolar concentrations of acetylcholine, observed in different samples. The present experiments demonstrate that the incubation with IgE of mast cells isolated from 'nonreactor' Wistar albino rats increases the sensitivity of mast cells to acetylcholine rendering the initially heterogeneous response homogeneous.

Methods

Mast cells were isolated through gradient centrifugation in Fi-coll after collection from abdominal and thoracic cavities of male albino Wistar rats (200–250 g), according to the method of Than and Uvnäs [1959]. Peritoneal and pleural mast cells from at least two animals were pooled and resuspended in a solution of the following composition: NaCl 145 mM; KCl 2.4 mM; CaCl₂ 0.9 mM; MgCl₂ 0.45 mM; glucose 0.1%; human serum albumin 0.1%, adjusted at pH7 with 10% (v/v) Sörensen phosphate buffer. Monoclonal hybridoma anti-egg albumin or anti-(DNP)2-lysine

mouse IgE were dissolved in distilled water and added to obtain a final protein concentration of 50, 75 and 100 µg/ml. The cells were incubated with the given IgE concentration for 60 min at 37 °C and washed twice by centrifugation. The pellet was resuspended in 2 ml of the solution described before and 50 µl of cell suspension (containing 0.5×10^5 cells) was added to test tubes containing various concentrations of drugs. After incubation for 10 min at 37 °C the reaction was stopped by chilling the tubes and the cells were harvested by centrifugation. Histamine was measured fluorimetrically [Kremzner and Wilson, 1961], and histamine release (supernatant histamine) was expressed as a percentage of the total present in the cells plus supernatant. Spontaneous histamine release ranged between 1 and 8% and was subtracted from all values.

Results and Discussion

IgE binding to mast cells was evidenced by the release of histamine occurring when the cells were challenged with the specific antigen (egg albumin or DNP2-lysine; fig. 1). Under these circumstances, a dose-dependent response to acetylcholine is present, the cholinergic histamine release paralleling the IgE content in the incubation medium (fig. 1). Cholinergic histamine release from passively sensitized mast cells

IgE in Rat Mast Cells and Cholinergic Histamine Release

30
20
10
-U

Fig. 1. Pattern of response to acetylcholine and to the specific antigen in isolated purified rat mast cells passively sensitized with different concentrations of mouse IgE antibody. The values are the means $\pm$ SE of 5 experiments performed in duplicate.

-10 -9 -8 -7 Acetylcholine, log M
100
µg
IgE
Ovalbumin 20 µg or DNP 10^{-6} M

is due to the specific stimulation of muscarinic receptors, since (1) acetylthiocholine does not evoke histamine release even at high concentrations (10^{-4} M); (2) atropine inhibits the cholinergic histamine release in a dose-dependent fashion (10^{-8} – 10^{-5} M); (3) acetylcholine (10^{-5} – 10^{-6} M) potentiates significantly the anaphylactic histamine release, confirming the hypothesis that the receptors involved in the release of histamine are different.

The experiments here reported show that cholinergic histamine release from isolated rat serosal mast cells is an IgE-mediated phenomenon which is due to the activation of muscarinic receptors; the intensity of the release is correlated with the content of reaginic antibodies presumably bound to mast cell membranes. Tentatively, these data may solve the discrepancies on cholinergic histamine release evidenced by those who failed to substantiate any histamine-releasing properties of acetylcholine [Kazimierzczak et al. 1980], and are in keeping with former experiments showing that acetylcholine releases histamine in mast cells isolated from actively sensitized individuals [Schmutzler et al., 1978]. This may be relevant in the pathogenesis of

similar allergic disorders implicating a cholinergic step, such as exercise-induced asthma and cholinergic urticaria.

References

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