Oxatomide protects against degranulation of rat peritoneal mast cells during in vitro challenge with antigen or compound 48/80. Ultrastructural aspects

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Abstract

The ultrastructure of isolated rat peritoneal mast cells was evaluated after in vitro degranulation and treatment with oxatomide, a new anti-allergic compound.

In a first series of experiments, mast cells of rats infected with Trichinella spiralis larvae were incubated with Trichinella larvae somatic antigen to produce histamine release. The release was visualized in the electron microscope by exocytosis of the peripheral amine-containing granules, which resulted from fusion between several perigranular membranes and fusion of these membranes with the plasma membrane.

A more drastic degranulation was provoked in a second series by incubation of unsensitized mast cells in the presence of the amine liberator compound 48/80. This treatment led to a complete extrusion of the granules in most of the cells, while in a smaller number of cells, only large vacuoles containing remnants of several granules were seen. The plasma membrane of these cells however was intact and there were no signs of exocytosis.

The effect of oxatomide against mast cell degranulation was dose-dependent and comparable for the two types of histamine release. After incubation with high doses (10^{-4} M, 5 \cdot 10^{-5} M) granule liberation was rarely observed in antigen-challenged and compound 48/80-challenged cells. Protection was apparently situated at the level of the plasma membrane which seemed to be unable to fuse with the perigranular membranes while fusion of perigranular membranes of individual granules was still possible. None of the tested concentrations of oxatomide induced spontaneous degranulation. High doses, however, led in a number of cells to some ultrastructural alterations such as partial disappearance of plasmalemmal folds, slight cytoplasmic oedema and the appearance of intranuclear microtubules. The latter were also seen in oxatomide-treated challenged mast cells.

Introduction

Oxatomide or R 35 443 (1-{3-[4-(diphenylmethyl)-1-piperazinyl]propyl} -1,3-dihydro-2H-benimidazol-2-one) is a potent inhibitor of both the release and the effects of allergic mediators [1]. Histamine secretion from mast cells can be triggered by antigens reacting with IgE antibodies fixed on the mast cell membrane or by a number of polymer compounds. The morphologic appearance of mast cells in both degranulation processes is well documented [2-4]. The aim of this study is to investigate the effect of varying doses of oxatomide on the ultrastructure of isolated rat peritoneal mast cells after in vitro challenge with antigen or with compound 48/80 and on the ultrastructure of unchallenged mast cells. At the same time the histamine release from peritoneal mast cells was studied in a quantitative approach [5].

Materials and methods

1. Isolation and incubation of peritoneal mast cells

The procedures are described in detail by DE CLERCK et al. [5]. For ultrastructural investigation one half of the mast cell suspension was used. The other half served for histamine assays.

1a. Sensitized cells (from rats infected with Trichinella spiralis larvae). Degranulation was performed by incubation with Trichinella larvae somatic antigen (TLH) for 1 and 10 min. Preincubation with oxatomide was done at pH 6.4 at a dose of 10^{-4} M for 5 and 25 min. Control cells were obtained after incubation in the solvents of both oxatomide and TLH during the same time periods. For evaluation of spontaneous release, cells were incubated in 10^{-4} M oxatomide while TLH was replaced by its solvent.

1b. Unsensitized cells (from normal rats). Degranulation was performed by incubation with Trichinella larvae somatic antigen (TLH) for 5 and 10 min. Oxatomide preincubation at pH 6.4 was done at a dose of 10^{-4} M for 5 and 25 min. Control cells were obtained after incubation in the solvents of both oxatomide and TLH during the same time periods. For evaluation of spontaneous release, cells were incubated in 10^{-4} M oxatomide while TLH was replaced by its solvent.

1b. Unsensitized cells (from normal rats). Degranulation was performed by incubation with compound 48/80 (0.5 ng/ml) for 10 min. Oxatomide preincubation at pH 6.3 was done for 5 min at doses of 10^{-4} M, 5 \cdot 10^{-5} M and 10^{-5} M. Oxatomide preincubation at pH 7 was done for 5 min at doses of 5 \cdot 10^{-5} M, 2.5 \cdot 10^{-5} M and 10^{-5} M. Control cells were again obtained after incubation in the solvents. Spontaneous release was evaluated at the above-mentioned doses.
2. Electron microscopy

At the end of the appropriate incubation period, an identical volume of 4% glutaraldehyde in 0.1 M Sörensen phosphate buffer was added to one half of the mast cell suspension. Fixation lasted 2 h at room temperature. Washing proceeded overnight at 4°C in the same buffer, supplemented with 0.22 M sucrose. After post fixation in 1% osmium tetroxide buffered with 0.05 M veronal acetate (pH 7.4) for 1 h at 4°C, the cells were impregnated with 0.5% uranium acetate of pH 5.2 for 20 min, dehydrated in graded series of ethanol, and routinely embedded in Epon. Mast cell rich areas were identified on semithick sections stained with a polychrome stain [6] or with toluidine blue. Pale gold ultrathin sections, briefly stained with uranium acetate and lead citrate, were examined in a Philips EM-300 electron microscope.

Results

1. Control cells (Fig. 1)

The aspect of the mast cells was similar in all of the examined control samples. The cells were round and had at their contours a large number of pseudopod-like plasmalemmal folds. The nucleus was centrally located and the cytoplasm was filled with numerous dark mature granules, each surrounded by a single membrane. The appearance of the granules was uniform although several cells also contained some less electron-dense granules or granules with a shrunken aspect. In addition to the closely packed granules, the cytoplasm contained normal mitochondria, scarcely developed strands of rough endoplasmic reticulum and several microfilaments and microtubules. The Golgi apparatus was located in the vicinity of the nucleus. Microtubules were never encountered in nuclei of control cells. No connections were seen between the perigranular membranes of the neighbouring granules or between perigranular membranes and cell membrane. Except for some rare necrotic cells in each sample, there were no signs of granule exocytosis.

2. Untreated-challenged cells

2a. Antigen-challenge (Fig. 2). The granules were swollen and accumulated in large vacuoles at different foci in the peripheral cytoplasm. These vacuoles originated by fusion of adjacent perigranular membranes. These membranes as well as single granular membranes fused with the plasmalemma, which resulted in expulsion of the altered granules in the pericellular space. The nucleus, as well as several surrounding amine-containing granules, remained normal. Unaltered cells of the control type were rare.

2b. Compound 48/80 challenge (Fig. 3). As after antigen-challenge, almost all cells were altered. The degranulation was obviously more drastic. Intracytoplasmic granules with a normal appearance were rarely seen. In most of the mast cells, an explosive complete degranulation was seen (Fig. 3a). All granules were swollen and had lost their normal electron density. Most of them were extruded after disappearance of their limiting membrane. The nucleus, however, and the

Figure 1

Control mast cell. The cytoplasm is filled with numerous electron-dense granules (g), each surrounded by a membrane. Mitochondria are relatively scarce (arrowheads). The Golgi apparatus (G) is located closely to the nucleus (n). At the periphery the plasmalemma shows several pseudopod-like folds (arrows). (×6150).

Figure 2

Untreated antigen-challenged mast cell. The degranulation is obvious at the cell periphery and is characterized by a swelling of the granules which become electron-lucent (g). Many of them are accumulated into vacuoles (v) but the largest part is extruded after fusion of the perigranular membranes with the plasmalemma (arrows). The nucleus (n) and a number of surrounding granules (g) are unaltered. (×4000).