Electron Microscopic Observations on the Morphological Changes of Rat mesenteric Mast Cells Induced by Morphine HCl*

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ABSTRACT

The effects of morphine HCl on the rat mesenteric mast cells were studied with the electron microscopy.

The materials were prepared for electron microscopy by osmium tetroxide fixation and embedding in Epon. The rat mesenteric mast cells showed no distinct morphological changes due to morphine HCl, but the mast cell granules were changed in various ways. For instance, they formed clusters, showed granular lysis, and an appearance of electron transparency. Frequently, some granules appeared in the extracellular space and the boundary of the granules was not evident. From the results mentioned above, it was suggested that rat mesenteric mast cell granules were affected by morphine HCl in the shape, the granular matrix, and the granular boundaries.

INTRODUCTION

It is well known that the release of histamine from mast cells following stimulation with certain basic histamine liberators is generally accompanied by a degranulation.

A number of morphological studies regarding morphine induced mast cell changes have been reported. Lee and Pak(1969) reported that a significant degranulation of the mesenteric mast cells of albino rats occurred following the intravenous injection of morphine HCl and meperidine HCl. Shin et al.(1971) have demonstrated the effect of morphine HCl in its ability to induce a degranulation of mesenteric mast cells in vitro.

Since the first electron micrographs of degranulation were obtained by Smith and Lewis in 1957, the ultrastructure of tissue mast cell has received a great deal of attention, and thus much information concerning the cytological aspect of the cell has been gained by the electron microscope.

However, the ultrastructural configuration of the mast cell, particularly the definition of the mast cell granules is not completely elucidated.

In the present study, morphological changes of rat mesenteric mast cells and their granules were examined with the electron microscopic after treatment with morphine HCl.

MATERIALS AND METHODS

The materials for study were healthy

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mature male albino rats (Sprague–Dawley) weighing approximately 200 Gms.

The experimental rats were killed by a blow to the head 4 hours after being injected in the tail vein with 12 mg./kg. of morphine HCl in normal saline sol.

The animals in the control group were killed in the same way. The rat mesenteries were placed in a drop of cold fixative, cut into small cubes and fixed in phosphate (pH 7.4) buffered 1% osmium tetroxide for 2 hours. After dehydration in grade ethanol, the materials were embedded in Epon 812. Sections were cut on the Sorvall MT-2 porter-Blum ultramicrotome, doubly stained in uranyl acetate and lead citrate and examined in a Hitachi HU-11 E electron microscope.

Thick sections(1-2u) were stained in puff solution for the light microscope.

RESULTS

In this electron microscopic study of mast cells, normal mast cells in the untreated specimens were surrounded by collagen bundles. They were generally round, oval, spindle-like, or polygonal in shape in the section. The free surface of the cell showed short villous processes, (Fig. 1).

The nucleus was usually round or oval with a few indentations, but sometimes irregularly shaped.

The cytoplasm was densely packed with the specific granules. The mitochondria were few in number; round and ovoid-shaped ones were scattered in the cytoplasm.

The Golgi apparatus was usually found in the vicinity of the nucleus and the centriole located in the center of the perinuclear Golgi area(Fig. 2).

The cytoplasmic granules were variable in size, shape and electron density. They were round, oval or gourd-shaped in section(Fig. 3, 4).

The matrix of specific granules was homogenous(Type 1), reticular(Type 2), and occasionally showed a compound form(Type 3)(Fig. 3, 4).

In the specimens injected with 12 mg./kg. morphine HCl intravenously, most mast cells showed no morphological change and possessed an intact nucleus around which were loosely scattered granules having a higher density comparatively at the periphery. The free surface of the cell showed villous processes(Fig. 5).

Particularly in the fine granular matrix, most of them were homogenous in texture (Type 1) and those of higher density were at the periphery(Fig. 5). Some granular substance formed clusters(Fig. 6), some granular lysis(Fig. 7). Some showed an appearance of electron transparancy, and vacuole formation around individual granules was observed(Fig. 8). Some granules appeared in the extracellular space(Fig. 9), and were irregular in shape. Usually a granular boundary was not evident. A few mitochondria were scattered in the cytoplasm and other cellular organelles could not be shown.

DISCUSSION

Morphine HCL is well known as a histamine releasing agent. The effect of morphine HCL on mast cells has been examined mainly in vivo using rat mesenteric mast cells. Bhattacharya and Lewis (1956) studied the degranulation of granules of tissue mast cells after the injection of morphine. Lee and Pak (1969) stated that fairly significant deg-
ranulation of the rat mesenteric mast cell occurred after injection morphine HCl.

At the electron microscopic level, the mesenteric mast cell treated with morphine HCl did not show any morphological changes, but one or two granules were degranulated in the mast cell as observed under the light microscope. This phenomenon was thought to be due to the effects of sectioning.

On the other hand, the cytoplasmic granules showed various changes. Some granular substance formed clusters, others granular lysis and others showed an electron transparency.

In the present study folds or vacuole formation around individual granules was observed occasionally. Frequently, at the cell periphery some granules were close to the extracellular space and similar findings were found by several authors. Fugita et al. (1960) reported that in some cases these electron lucid spaces and amorphous substances seem to be due to artifacts.

Bloom et al. (1965, '67, '70), Chakravarty et al. (1967), Horsfield (1965), and Mann (1969) reported that electron microscope observations of the degranulation process describe changes in the structure of the granules, formation of vacuoles around the granules, fusion of vacuoles to larger ones, and their opening to the cell surface. Johnson and Moran (1969) emphasized that mast cell degranulation did not destroy the normal outward impermeability of the plasma membrane. Röhlich et al. (1971) suggested that all the 'intracellular' cavities, formed by degranulation, were shown to communicate with the extracellular space; consequently, granules lying in these cavities must be considered as biologically extracellular.

The homogenous or reticular substances of the granules in the experimental group were the same as in the control group. Moriyasu (1969) demonstrated that a reticular filamentous texture within the granules may represent changes or discharge of the intragranular highmolecular substances and it can be considered that a clear halo or vacuole around each granule originates from the release of proteolytic enzymes.

In the study of Smith (1963) perigranular membranes were first clearly depicted to be present as unit membranes separating the granules from the cytoplasm. Lagunoff (1972) stated that this membranous investment of the granule is particularly difficult to preserve but almost certainly occurs in all mast cells. Electron micrographs showed that although the granules had lost their membranes, they still bound most of the original histamine of the cell. No distinct membrane was shown in the present study.

Frequently, the outline of the granules in the experimental group treated with the morphine HCl was not observed clearly and did not show the cellular organelles.

REFERENCES


Bloom, G.D., and Haegermark, Ö.: A study on morphological changes and histamine release induced by compound 48/80 in rat peritoneal mast
EM of Rat Mesenteric Mast Cell due to Morphine HCl


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Explanation of Figures

Fig. 1. Electron micrograph of normal mesenteric mast cell. The cytoplasm was filled with granules having homogenous or reticular structures. The plasma membrane of each cell possesses short villous processes(V1). Note the centriole(C), the Golgi apparatus(G), and deposits of lead stain scattered in the cytoplasm. ×9,000

Fig. 2. The Golgi apparatus was usually found in the vicinity of the nucleus and a centriole located in the center of the perinuclear Golgi area. A few mitochondria were scattered in the cytoplasm. ×20,000

Fig. 3. The cytoplasmic granules were variable in size, shape and electron transparency. They were round, oval, or gourd-shaped in section. The matrix of specific granules consisted of homogenous(T1), or reticular substances(T2) and showed occasionally a compound-form(T3). ×20,000

Fig. 4. In the cytoplasm, the specific granules were homogenous(T1) and reticular(T2). ×20,000

Fig. 5. A mast cell in rat mesentery treated with 12 mg./kg. morphine HCl intravenously. The free surface of the cell showed short villous processes and intact nucleus. Mast cells did not degranulate and the granules occurred loosely in the cytoplasm. Deposits of lead stain scattered in the cytoplasm. ×9,000

Fig. 6. Some granular substance formed a cluster(arrow). ×20,000

Fig. 7. A granule showed granular lysis(arrow). ×16,000

Fig. 8. A granule seems to appear electronically transparent(arrow). ×20,000

Fig. 9. A granule appears in the extracellular space(arrow). ×20,000

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