190. A Fluorescent Study of Histamine Release from a Single Mast Cell of the Rat Mesentery

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Microelectrophoresis has been used for the study of the site of action of drugs.1,2) Tasaka et al.3,4) have recently reported that when histamine releasers, such as compound 48/80, toluidine blue, sinomenine and ATP, were applied microelectrophoretically to the isolated rat mast cell, extracellular application of these compounds induced the extrusion of granules, while no degranulation was observed in their intracellular application. These observations indicate that the site of action of these histamine releasers is on the cell membrane as far as degranulation is concerned. The present experiment was intended to see if the same assumption can be held in the release of histamine by these compounds from the mast cell, using a single mast cell of the rat mesentery.

Materials and methods. Histamine releasers, compound 48/80, sinomenine, toluidine blue, \( \alpha \)-chymotrypsin and \( n \)-de cylamine were dissolved in 0.9% NaCl to make the concentrations of 0.0125, 7, 0.3, 0.025, and 0.45%, respectively. ATP was made in 0.3 M in distilled water. These solutions were filled in micropipettes after the method of Tasaki et al.5) The tips of the micropipettes were 0.1 to 1.5 \( \mu \) in diameter. The microelectrode was made as previously reported.3) Electric resistance of the microelectrodes used ranged from 9 to 200 M\( \Omega \). The electrodes were connected to an anode except for an ATP electrode that was connected to a cathode.

Wistar rats were stunned and exanguinated. The mesentery was excised and gently washed in normal saline. Mechanical trauma was avoided to minimize the non-specific release from the mast cells. A small piece of mesentery was stretched over a filter paper which was cut out in a small ring (15 mm in outside diameter). This was transferred into a small glass-bath (18 mm in diameter, 3 mm depth), made of a round holed slide glass pasted with cover glass on its bottom side, and submerged in oxygenated Tyrode solution. By the aid of a micromanipulator, a micropipette was placed very close to the surface of cell membrane or impaled into the cell, under an invert-type phase contrast microscope (Olympus PMB \( \times 480 \)). By applying
D. C. or repeating square waves to the electrodes, histamine releasers were ejected from the micropipettes.

The histochemical study for detecting histamine was performed with a modification of the original method of Shelley et al. After application of histamine releasers to the mast cell, the mesentery piece was blotted with a filter paper and dried using an electric fan at room temperature. A drop of 1% o-phthalaldehyde dissolved in ethylbenzene was applied to the mesentery piece, which was then transferred into the 100% humidity chamber and allowed to stand for 4 min at room temperature. The mesentery piece was re-dried as before, and mounted in tetrahydrofurfuryl alcohol. Fluorescence microscopy was performed using a high pressure mercury lamp (Toshiba SH200) equipped with an excitation filter (Nikon-V). Bright yellow fluorescence was sharply localized on the mast cell against a blue background. After complete removal of tetrahydrofurfuryl alcohol, the same specimen was fixed with Carnoy solution and stained in alcohol thionine. Metachromatic granules were seen in dark violet color in the control cell. This was done for the purpose of studying the sequential difference in the release between histamine and heparin from the mast cell.

**Results.** By the extracellular application close to the cell membrane using a fine micropipette, compound 48/80, toluidine blue, sinomenine and α-chymotrypsin all induced degranulation localized near the tip of the micropipette as seen previously in the isolated mast cells. The fluorescence of the mast cell was confined in accordance with the outline of the cell viewed under phase-contrast microscope but was missed in the area where local degranulation took place (Fig. 1). No fluorescence of the extruded granules was seen at all. When ATP was applied to a cell through a coarse pipette, the cell became markedly swollen usually accompanying the degranulation all over the cell surface. The fluorescence was scarcely seen in those cells, but thionine staining revealed the whole cell structure composed of many swollen metachromatic granules, although cellular swelling may partly be due to the effect of staining procedure (Fig. 2). This indicates that heparin was still retained in the granules of the rat mast cell from which histamine had been exhausted. In a few cases, fluorescence of the mast cell was faint without showing a marked degranulation after a diffuse extracellular ATP application. Extracellular diffuse application, using a wide tipped micropipette, of α-chymotrypsin to a mast cell likewise induced a remarkable swelling and drastic degranulation. No fluorescence was displayed in these cells, but thionine staining again clearly disclosed amassed swollen metachromatic granules replicating the whole cell somewhat deformed
Differing from these histamine releasers, n-decylamine applied either extra- or intracellularly induced no extrusion of granules, although the cell was markedly swollen and pale and obscure in its phase-contrast cytoplasmic structures. In such a cell, however, fluorescence of histamine was never observed. When the mesentery piece having those cells was treated with thionine, metachromatic staining of the mast cell granules were still retained.

Intracellular applications with a fine pipette, of these histamine releasers except n-decylamine, induced a swelling of the cytoplasm and a slight enlargement of the granules. In these cells, brightness by α-chymotrypsin.

Fig. 1. Local histamine release from rat mesentery mast cell due to extracellular iontophoretic application of compound 48/80 (0.0125%). Electric resistance of micropipette, 200 MΩ. A: Before application; B: Repeating square wave current (0.05 µA, 0.2 sec duration, 3 cps for 6 sec) induces local degranulation near the tip of micropipette; C: Histamine fluorescence of the mast cell. The fluorescent area coincides with the rest of the cell escaping degranulation. No fluorescence of the extruded granules can be seen. Magnif. ×750
of the histamine fluorescence was almost the same as in the untreated controls. Intensities of the current to the electrode for the intracellular applications were virtually identical to those used for the extracellular applications which induced a local degranulation. Ca$^{2+}$ (0.5 M) as well as Na$^+$ (1 M) given intracellularly did much affect neither cell morphology nor histamine fluorescence.

Discussion. It has previously been demonstrated that basic histamine releasers, α-chymotrypsin and ATP all evoked local degranulation of the isolated mast cell at the site of topical application, while the rest of the cell, both the membrane and the inside structures, was apparently normal.$^{3,4}$ In accordance with these observations, the histamine fluorescence was missed only in the area where local degranulation took place, and in the rest of the cell the intensity of fluorescence was not much different from that of the untreated control. For a complete loss of cellular histamine, the releasers should have
been applied diffusely to the cell membrane. This was also the condition required for the generalized degranulation. All these observations may give the impression that the histamine release is a phenomenon inevitably associated with the degranulation. However, it is not our intention to emphasize that the degranulation is prerequisite to the histamine release, since histamine release could have been evoked without accompanying degranulation with toluidine blue.\(^7,8\)

\(n\)-Decylamine, unlike the other releasers, released histamine without showing degranulation in either intracellular or extracellular application.\(^9\)

In the cell which lost histamine fluorescence with these releasers, metachromatic granules were still clearly seen after thionine restaining. The metachromatic reaction may occur with a number of macromolecular acid polyelectrolytes which do not contain sulfate groups, but the only material known to be responsible for the reaction in the mast cell granules is sulfated mucopolysaccharide (heparin).\(^9\)

Consequently, the finding can be interpreted as meaning that even after histamine release, heparin was still retained in the granules. This explanation is consistent with the observation that in rats injections of compound 48/80 and stilbamidine always produced bursting of mesentery mast cells while there was no prolongation of the blood coagulation time.\(^10\)

Uvnäs, Åborg and Bergendorff\(^11\) made an assumption, based on ample experimental evidences, that in the granules heparin links firmly to protein while histamine makes a weak ionic binding to the protein COO\(^-\)-group of protein-heparin complex. The histochemical finding in the present experiment favors this hypothesis.

None of the releasers, except \(n\)-decyllamine, did affect histamine fluorescence of the mast cell when applied intracellularly. This also indicates that these releasers are able to release histamine only by triggering a local reaction at the site on the cell membrane. Intracellular applications of Ca\(^2+\) and Na\(^+\) did not evoke histamine release even in high concentrations. This observation makes no longer tenable the theory of the intracellular ionic exchange formerly proposed for the action of basic releasers. And now it becomes evident that the occurrence of some structural changes in the granules is necessary for the realization of the ionic exchange between cations and histamine bound in the granules. This view is also supported by a recent electron microscope study\(^12\) reported from this laboratory.

**Summary.** Microelectrophoretic application of histamine releasers was made to a single mast cell of the rat mesentery. When topically applied close to the cell membrane, compound 48/80, sinomenine, toluidine blue, ATP and \(\alpha\)-chymotrypsin all induced a local degranulation associated with the loss of histamine fluorescence in the degranulated area. Extruded granules showed no fluorescence.
Intracellular application of these compounds, and also of Ca$^{2+}$ and Na$^{+}$, did not affect histamine fluorescence of the mast cell, although $n$-decanilamine caused disappearance of the fluorescence with either intra- or extracellular application.

Metachromatic granules were revealed by re-staining with thionine in the mast cell which had lost histamine fluorescence after a diffuse extracellular application of these releasers.

References