THE EFFECTS OF LOCAL ANESTHETICS ON THE DEGRANULATION OF RAT PERITONEAL MAST CELLS

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ABSTRACT
The effects of local anesthetics on the degranulation of rat peritoneal mast cells induced by an intensive histamine liberator, compound 48/80 were examined under a phase contrast microscope (PCM) and a scanning electron microscope (SEM). The degranulation was reversibly inhibited by local anesthetics, lidocaine, procaine and tetracaine. It thus was revealed that mast cells share a susceptibility to anesthetics with sensory cells and neurons.

Morphologically, the mast cells treated with 0.5% lidocaine appeared slightly enlarged under the PCM and the microfolds on the surface disappeared or remarkably decreased in number under the SEM. Such mast cells were strongly resistant to stimulation by compound 48/80. After being washed in Krebs-Ringer solution, they recovered their size and microfold number. In this state the cells again became sensitive to compound 48/80. Similar morphological changes and inhibited responsibility to compound 48/80 were found also by the use of procaine (0.5%) and of tetracaine (0.02%). These findings suggest that the morphological changes on the mast cell surface induced by the anesthetics might be involved in the resistance against stimuli causing degranulation.

KEY WORDS mast cell / compound 48/80 / local anesthetics / microfold / phase contrast microscopy / scanning electron microscopy

Since Paul Ehrlich in 1877 revealed the occurrence of mast cells as a special element of connective tissue cells, an overwhelming mass of studies has been performed regarding their degranulation and histamine release (35, 40). However, there have been only a few reports which investigated the effects of local anesthetics on mast cell degranulation or histamine release.

According to Fujita and Kobayashi, the mast cell is included in the family of paraneurons which are recepto-secretory cells sharing the morphological, metabolic and functional features with neurons (15, 16). Furthermore, Douglas and his associates kept their eyes upon the mast cell as a recepto-secretory cell conformable to the concept of stimulus-secretion coupling, which they had applied to their studies on adrenal chromaffin cell and some neurons (8–10). The present study was designed to learn whether or not the mast cell might be susceptible to anesthetics like sensory cells and neurons.

This paper reports the effects of some typical and routinely available local anesthetics, lidocaine, procaine and tetracaine on mast cell degranulation induced by compound 48/80, a representative stimulator for this cell. Emphasis is put on describing the morphological changes in the mast cell treated with lidocaine. These changes are described in detail and their possible relation to the inhibited sensitivity of the cells will be discussed.
**MATERIALS AND METHODS**

**Preparation of Mast Cells**

Rats of both sexes weighing 250–400 g were bled by severance of a carotid artery after a blow on the head. Forty ml of a Krebs-Ringer bicarbonate buffer of 37°C containing 11.5 mM glucose and saturated with 95% O₂ and 5% CO₂ (pH 7.3–7.4) were injected into the abdominal cavity. After gentle massage for 3 min, the abdomen was opened and the peritoneal fluid was pipetted out and centrifuged (125 g, 5 min). The supernatant was gently decanted, leaving 2–3 ml of it and the sediment was resuspended in the remaining supernatant. The above procedure was carried out at a constant temperature of about 36°C. Each reaction was stopped by adding 1.25% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.4 (final concentration, 1%).

The morphological changes of mast cells in the samples indicated below were compared under a phase contrast microscope (PCM) and a scanning electron microscope (SEM) by the preparation techniques described below.

**Preparation of Local Anesthetic Solutions**

A crystal form of lidocaine was extracted from its 10% solution (Fujisawa Pharmaceutical Co., Osaka, Japan) with 2 N NaOH and ethylether. The melting point of the obtained lidocaine crystal was 68.0–69.5°C. The lidocaine crystal was dissolved in 1 N HCl to make 1 N lidocaine. One percent, 0.5% and 0.1% solutions of lidocaine were made by diluting the lidocaine in the Krebs-Ringer solution mentioned under ‘preparation of mast cells’. The pH value of 0.5% lidocaine thus prepared measured 7.3–7.4.

Procaine and tetracaine solutions were made by respective dilution of procaine hydrochloride (Hoei Yakuko, Osaka, Japan) and tetracaine hydrochloride (Kyorin Pharmaceutical Co., Tokyo, Japan) in the Krebs-Ringer solution. The pH value of both the 0.5% procaine and 0.05% tetracaine was 7.3–7.4.

**Design and Method**

In order to examine the effects of the local anesthetics upon the degranulation reaction of mast cells to compound 48/80, the suspensions of mixed cells obtained as above-described were, in each case, divided into four parts of an equal volume (0.5 ml) (samples 1, 2, 5 and 6). Moreover, in the process of this study, a marked morphological change of mast cells was noticed after treatment with 0.5% lidocaine. In order to investigate this point in detail, another two samples were added (samples 3 and 4).

**Untreated samples (first control)—1**

The suspension was added to an equal volume (0.5 ml) of Krebs-Ringer solution containing sucrose in the same molecular concentration as that of the local anesthetics to be used in the corresponding experiment to make the medium equal in osmolarity to that of the experimental samples, and this mixture was centrifuged and fixed after 5 min.

**Samples treated with the histamine liberator (second control)—2**

Use was made of compound 48/80 (Sigma, St. Louis, U.S.A.) dissolved in the Krebs-Ringer solution to a concentration of 5 μg/ml. One ml of this solution was added to the mixture of 0.5 ml of the peritoneal cell suspension with 0.5 ml of the Krebs-Ringer solution containing sucrose used in the first control, and fixed after 5 min (final concentration of compound 48/80, 2.5 μg/ml).

**Samples treated with 0.5% lidocaine—3**

The cell suspension was added to an equal volume (0.5 ml) of 1% lidocaine and fixed after 5 min.

**Samples treated with 0.5% lidocaine and washed by Krebs-Ringer solution—4**

The cell suspension was washed by Krebs-Ringer solution after treatment with lidocaine and subsequently exposed to the histamine liberator—6

A half ml of the cell suspension was added respectively with 0.5 ml of 1%, 0.5% and 0.1% solutions of lidocaine, the final concentration of the anesthetic then being 0.5%, 0.25%, 0.05%. After 5 min, this 1 ml mixture was added by 1 ml of a 5 μg/ml solution of compound 48/80, and fixed after 5 min.

**Samples washed in Krebs-Ringer solution after treatment with lidocaine and subsequently exposed to the histamine liberator—6**

A half ml of the cell suspension was added to 0.5 ml of each of the above-mentioned concentrations of lidocaine. After 5 min, this suspension was washed for 10 min with a large volume of
Krebs-Ringer solution. A half ml of the suspension was added to 0.5 ml of the Krebs-Ringer solution containing sucrose. After 5 min, 1 ml of the compound 48/80 was added to the mixture and fixed after 5 min.

Besides the above experiments, the effects of other local anesthetics, procaine and tetracaine, on mast cell degranulation evoked by compound 48/80 were preliminarily examined under the PCM.

Quantitative Study

In samples 1, 2, 5 and 6, 200 mast cells were counted at ×400 magnification under a PCM and the ratios of degranulated mast cells were calculated and treated statistically. In samples 5 and 6, only the lidocaine treated and not the procaine or tetracaine treated specimens were used for this quantitative analysis. Cells with possible but very slight degranulation, i.e., showing only a few granules on cell surface (as the right-hand cell in Fig. 1c), were not regarded as 'degranulated' cells.

The difference between the means in each two samples was statistically analyzed by Student's t-test.

Preparation for the SEM

The mast cell suspension in each of the sample groups was fixed by 1% glutaraldehyde (in a 0.1 M phosphate buffer, pH 7.4) for 30 min. The suspension was centrifuged, lightly washed in a 0.1 M phosphate buffer, and centrifuged again. The sediment thus obtained was dropped on small pieces of glass slides which were previously coated with 0.1% poly-L-lysine hydrobromide (Sigma, St. Louis, U.S.A.) in a 0.1 M phosphate buffer to ensure adhesion of cells to them (31, 38). After 5 min, the specimens were lightly washed in a 0.1 M phosphate buffer. Then they were postfixied for 30 min in a 1% solution of OsO₄ dissolved in a 0.1 M phosphate buffer and dehydrated through a graded series of ethanol.

![Fig. 1 Phase contrast micrographs of mast cells in each sample. a: Untreated mast cells (sample 1). Note that they possess a refractile cell margin. b: Mast cells exposed to a 2.5 μg/ml solution of compound 48/80 (sample 2). c: Mast cells exposed to a 2.5 μg/ml solution of compound 48/80 after treatment with 0.5% lidocaine (sample 5a). Note that they are enlarged as compared with the control cells in Fig. a and have lost the refractile cell margin. The right-hand cell is degranulated slightly. d: Mast cells treated with 0.5% lidocaine, washed in Krebs-Ringer solution, and subsequently exposed to a 2.5 μg/ml solution of compound 48/80 (sample 6). Note the intensive degranulation. All, ×1,200](image-url)
MAST CELL DEGRANULATION AND LOCAL ANESTHETICS

Next they were immersed in isoamyl acetate, and critical point dried using liquid CO₂. Specimens were evaporation-coated with carbon and gold-palladium and viewed in a field-emission type SEM, HFS-2 at 10 kV accelerating voltage.

RESULTS

Untreated Mast Cells—1

Observation under the PCM  The mast cells were easily distinguished from other cells by their rounded shape and large size (10–25 μm in diameter). They were characterized by a brightly refractile cell margin. Also the granules densely filling the cytoplasm appeared more or less refractile. The nucleus located in the middle of the cell generally appeared as a darker round area. It was very rare to see degranulated mast cells (Figs. 1a and 2).

Observation under the SEM  The cell surface was covered with numerous microfolds and a small number of microvilli (Fig. 3, a and b). Among the microfolds and microvilli, swellings of underlying secretory granules were often visible. Essentially all mast cells assumed this shape and it was rare to see a so-called raspberry-type cell, totally devoid of microfolds, which had been described in the previous SEM studies (2, 3, 7, 26, 42).

Mast Cells Treated with Compound 48/80—2

Observation under the PCM  More than 95% of the mast cells showed striking degranulation (Fig. 2). The cells were enlarged about twofold. Most of the released granules adhered to the cell surface, but some of them were free from the cell and showed a Brownian movement (Fig. 1b). They no longer had a refractile cell margin, though the deeper portion of the cell was refractile because of remaining granules.

Observation under the SEM  The cell surface was attached by numerous released granules, singly or in fusion. Furthermore, orifices of tunnel-like cavities were often noticed beneath them. Microvilli and microfolds were visible.

Fig. 2  Frequency of degranulated cells, as counted in 200 mast cells. Control samples—untreated or compound 48/80 (‘48/80’)-treated—and samples treated with 0.05%, 0.25% or 0.5% lidocaine (‘L’) and, either after washing (‘W’) in Krebs-Ringer solution or without being washed, exposed to compound 48/80 are compared. The figure in or above each column indicates the sample number (see the text in Results). Each column is presented as mean ± SD (n = 5). The difference in mean values is significant (P<0.01) between samples 2 and 5b, between samples 5b and 6, and between samples 5a and 5b.
Fig. 3  Scanning electron micrographs of mast cells and their reaction to compound 48/80 in control and
lidocaine-treated samples.  a, b: Untreated cell (sample 1). Note numerous microfolds and a small number
of short microvilli on the cell surface. c, d: Cell simply exposed to compound 48/80 (sample 2). Note the
figures indicating intensive degranulation.  e, f: Cell treated with lidocaine (0.5%) and then exposed to
compound 48/80 (sample 5a). Note that only a few granules are released. Underlying granules are visible
on the surface devoid of microfolds.  g, h: Lidocaine (0.5%)-treated, washed and then compound 48/80-
exposed cell (sample 6). Note the degranulation is essentially the same as in sample 2.  a, c, e, g: ×10,000
b, d, f, h: ×20,000
among them (Fig. 3, c and d).

*Mast Cells Singly Treated with 0.5% Lidocaine—3*

*Observation under the PCM*  The cells were round in shape and appeared slightly enlarged. The refractile margin as observed in untreated mast cells decreased conspicuously. It seemed likely that the original spherical shape of mast cells was flattened to some extent on the glass slide (Fig. 4a).

*Observation under the SEM*  The microfolds on the cell surface became smooth and the contour of underlying granules was clearly visible. A small scattered number of short microvilli were recognized (Fig. 4, b and c).

*Mast Cells Washed in Krebs-Ringer Solution after Treatment with 0.5% Lidocaine—4*

*Observation under the PCM*  The mast cells re-

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Fig. 4  a, b, c: Mast cells singly treated with 0.5% lidocaine (sample 3), by PCM (a) and in lower (b) and higher magnification (c) of SEM.  a: The cells are enlarged and their refractile cell margin is inconspicuous.  b: Note that the cell surface is smooth and completely devoid of microfolds. Round protrusions by underlying granules are prominent.  d, e, f: Mast cells washed in Krebs-Ringer solution after treatment with 0.5% lidocaine (sample 4).  Note that their appearance is recovered to the state of the untreated cells.  a, d: ×1,200.  b, e: ×10,000.  c, f: ×20,000
covered their size and marginal refractility to the level of the untreated sample (Fig. 4d), though irregularity of the cell shape was often noticed, possibly due to washing and second centrifugation.

Observation under the SEM The cell surface configuration recovered to that comparable with untreated mast cells. Namely, the surface was covered by many microfolds mingled with a small number of microvilli (Fig. 4, e and f).

Mast Cells Treated with Lidocaine and then with Compound 48/80—5

Pretreatment with 0.5% lidocaine—a

Observation under the PCM After previous exposure of the cell suspensions to 0.5% lidocaine for 5 min, approximately 95% of the mast cells were found resistant to stimulation by a 2.5 μg/ml solution of compound 48/80 (Fig. 2). A few cells underwent degranulation which, however, was slight to moderate in grade and never as intensive as found in the case without the pretreatment of the anesthetic. Degranulated as well as not degranulated cells in this sample appeared slightly enlarged and their refractile cell margin was more or less unclear (Fig. 1c).

Observation under the SEM The microfolds characteristic of the untreated mast cells disappeared completely or decreased remarkably in number, whereas some microvilli remained. The cell surface was markedly undulated by the underlying granules. A few of the granules were strongly projected on the cell surface. Judgement as to whether a given granule had been released or not was often difficult under the SEM (Fig. 3, e and f).

Pretreatment with 0.25% lidocaine—b

Observation under the PCM About 50% of the mast cells were strongly resistant to stimulation by compound 48/80, but the remaining of them showed moderate degranulation by compound 48/80 (Fig. 2). No typical degranulation was recognized. The cells seemed to be slightly enlarged and the refractile margin of the cells was inconspicuous.

Pretreatment with 0.05% lidocaine—c

Observation under the PCM More than 95% of the mast cells showed an intense degranulation by compound 48/80 (Fig. 2). It is evident that this concentration of lidocaine exerts few inhibitory effects on the compound 48/80-induced degranulation.

Mast Cells Washed in Krebs-Ringer Solution after Treatment with Lidocaine and Subsequently Exposed to Compound 48/80—6

Observation under the PCM Regardless of the concentrations of lidocaine used, 90% or more of the mast cells showed typical degranulation by a 2.5 μg/ml solution of compound 48/80 (Figs. 1d and 2).

Observation under the SEM The surface morphology was essentially the same as that of the cells exposed to the histamine liberator without pretreatment with the anesthetics (Fig. 3g, h).

Additionally, the mast cells treated with other local anesthetics and compound 48/80 were observed under the PCM: Mast cells pretreated with 0.5% or 0.25% procaine appeared slightly enlarged under the PCM and were resistant to stimulation by compound 48/80 (2.5 μg/ml), though the inhibitory effect on the mast cell degranulation was lower than that of the same concentrations of lidocaine. After washing in the Krebs-Ringer solution, they recovered their sensitivity to compound 48/80 again and degranulated intensively. On the other hand, mast cells treated with 0.05% or 0.025% tetracaine appeared heavily enlarged and were non-sensitive to stimulation by compound 48/80, and their degranulation by compound 48/80 was irreversibly inhibited. Treatment of mast cells with these concentrations of tetracaine for over 10 min caused the rupture of the cells. Even after a 1 min incubation with 0.025% tetracaine, they still showed the irreversible reaction. It was difficult to decide the concentration and treatment time of tetracaine, by which clear reversible inhibition upon the mast cells was caused. However, it was found that 5 min treatment of mast cells with 0.02% tetracaine reversibly inhibited the responsibility to compound 48/80 in the majority of the cells. A 0.01% solution of tetracaine showed no detectable suppression to the cells at all.

DISCUSSION
The present study revealed that the degranulation of mast cells induced by an intensive histamine liberator, compound 48/80 was reversibly inhibited by local anesthetics, lidocaine, procaine and tetracaine.

Various chemicals including antihistaminics have been known to inhibit the mast cell reaction to compound 48/80 (6, 11, 14, 45). However, only a few researchers have examined local
anesthetics in this respect (25, 45). VanArsdel and Bray (45) examined the inhibitory effect of procaine, besides many other substances which could not be categorized as anesthetics, upon rat peritoneal mast cells releasing histamine in response to compound 48/80. According to their result, the histamine release of mast cell was not inhibited by $1 \times 10^{-3}$M procaine. However the present study showed that procaine of 0.25% (about $1 \times 10^{-2}$ M) or higher concentrations inhibited mast cell degranulation by compound 48/80. It appears that the negative finding by VanArsdel and Bray on the inhibitory effect of procaine on mast cell histamine release is due to the one order lower concentration of the anesthetic they used.

The present morphological study is most closely related to the histamine bio-assay investigation by Kazimierczak et al. (25) on the effect of local anesthetics on the mast cells. The anesthetics used in their study coincide with those in the present study. Namely, they examined the inhibitory effect of lidocaine, procaine and tetracaine on compound 48/80-induced histamine release from rat peritoneal mast cells. Their results correspond to the present findings with regard to the effective concentrations and the order in potency of these three anesthetics to inhibit histamine release. However, Kazimierczak et al. wrote that the inhibitory action of the anesthetics upon the mast cells remained after the cells were washed and did not notice that the effect might be reversible.

Recently, it was reported that mast cells were derived from bone marrow (20, 27, 28). Mast cells differ in this point from neurons whose origin is neuroectodermal. However, it is noticed that mast cells possess characters common to neurons in several cell-biological aspects. They are typical recepto-secretory cells and when they receive adequate stimuli, they release their secretions in response to them. The secretory granules are released by exocytosis and the mechanism is Ca^{2+}-dependent (10, 22, 23, 24). The secretions contain biogenic monoamines, histamine and serotonin. It has been reported that mast cells contain the neuropeptide, vasoactive intestinal polypeptide (VIP) in their secretory granules (5), though the author could not confirm it immunohistochemically. Moreover, mast cells have been demonstrated to proliferate remarkably by the stimulation of the nerve growth factor (1) which has been shown to elicit a growth effect on sympathetic ganglia and sensory ganglia (30). Thus it seems reasonable to regard the mast cell as a member of para-neurons, although its origin seems mesenchymal. Neurons generally possess a pharmacologically well-known property in which their activity is reversibly inhibited by various kinds of anesthetics. Therefore it seems worthwhile to examine the occurrence of the same property in the mast cell, with regard to its close cell-biological relation to neurons.

A few researchers have examined the effects of local anesthetics on cells which today are included in the group of paraneurons. Rubin et al. (37) demonstrated in the cat that tetracaine inhibited the secretagogous effect of acetylcholine on the adrenal medulla. In the same organ of the gerbil, Douglas and Kanno (12) confirmed the same effect of tetracaine. Miele and Rubin (32) showed the similar effect of other local anesthetics on the cat adrenal medulla. Fujita et al. (17) reported that lidocaine infused into the dog duodenum inhibited the effect of intraduodenal amino acids causing a pancreozymin release from endocrine cells, whose apical receptor sites protrude into the duodenal lumen.

It is worthwhile to note that the cat and gerbil adrenal medullary cells (12, 31, 36) are at least one order more sensitive to the anesthetic (0.33 mM or lower concentrations of tetracaine being effective to suppress the cell reaction) than rat mast cells (about 1 mM tetracaine being effective to suppress it). There seems to be a difference in sensitivity to local anesthetics between both cells. It may be reasonable to postulate a spectrum, like the one for neurons (36), with regard to the sensitivity to anesthetics among different paraneurons. Furthermore, it is noteworthy that the order in potency to mast cells of the three kinds of anesthetics as revealed in the present study (tetracaine $>$ lidocaine $>$ procaine) is valid in the case of the routine local anesthesia of nerves (4).

Djaldetti et al. (7) examined the morphological changes of rat peritoneal mast cells during degranulation induced by calcium chloride and its prevention by anti-allergic drugs, salbutamol sulfate (SS), chlorpheniramine maleate (CM), and disodium cromoglycate (DSCG) under the SEM and the transmission electron microscope, and reported that SS and DSCG induced a decreased number of membrane folds and an increased number of microvilli under the SEM. The present investigation revealed marked morphological changes of the mast cell by anesthetics. The mast cells treated with lidocaine (0.5%) and the other anesthetics of appropriate concentrations appeared enlarged under the PCM and their microfolds on the surface dis-
appeared or decreased remarkably in number under the SEM. Such mast cells were strongly resistant to stimulation by compound 48/80, whereas the cells which had recovered their size and microfold number after being washed were degranulated intensively by compound 48/80. Thus, the reversibility in the morphological changes of plasma membrane paralleled that in the inhibitory effect of the anesthetics on degranulation of the cell. Therefore, it seems reasonable to postulate that the changes in the molecular structure of the plasma membrane by local anesthetics might have caused the morphological changes of mast cells on one hand, and also on the other hand, caused the inhibited reactivity of the cells to compound 48/80 which acts upon the mast cell.

Some studies on the fine surface structure of the mast cells by the use of SEM are available (2, 3, 7, 26, 29). Especially the report by Burwen and Satir seems valuable for the interpretation of the present results. They assumed that the receptors to adequate stimuli are on the plasma membrane of the microfolds and therefore mast cells possessing more microfolds might be able to respond more easily to stimuli, while the so-called raspberry-type cell, a mast cell totally devoid of folds on its surface, is resistant to stimulation. This assumption of Burwen and Satir may well account for the reason why mast cells devoid of microfolds after treatment with 0.5% lidocaine were resistant to stimulation by compound 48/80.

It has been suggested that local anesthetics might affect the molecular structure of the biological membranes (21) which may cause an expansion of the membranes (4, 39). Some authors postulated that local anesthetics may compete with calcium binding to its membrane binding sites either in nerves (4) or in mast cells (25). On the other hand, compound 48/80 has been presumed to affect mast cells by activation of lytic enzymes in the plasma membrane and secretory granule membrane (18, 41, 43, 44). Thus, the common action site of these two substances seems to be in the membrane.

However, the entity of the inhibitory effect upon the degranulation mechanism of mast cells may not solely be attributed to the changes in the plasma membrane, but at least partly to those in the cell internal structure such as microtubules. It is reported that local anesthetics reversibly inhibit the polymerization of microtubules (19, 33, 34) which may be involved in the process of secretory granule release (13).

The possibility that the colloid osmotic effect of lidocaine (10^{-2} M) might have caused the cell swelling and disappearance of microfolds can be excluded by the fact that in every experiment the control cells were treated with the Krebs-Ringer solution containing sucrose to the same molar concentration as that of the anesthetic to be used in the counterpart of the experiment. The possibility is also negated by the fact that tetracaine of a much lower concentration (10^{-3}M) produced similar morphological changes of mast cells.

The morphological changes of mast cells by anesthetics demonstrated in this study are worthy of further detailed study as they seem to provide some clues for the elucidation of the action mode of anesthetics and of the granule release mechanism of neurons and paraneurons.

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