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Inhibition of mast cell mediator secretion induced by protoporphyrin plus long-wave ultraviolet light: A morphometric and ultrastructural analysis

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It has been shown that treatment of rat peritoneal mast cells with protoporphyrin plus long-wave ultraviolet light (UVA) irradiation can suppress mediator secretion. In this study we conducted a morphometric and ultrastructural analysis of rat peritoneal mast cells to investigate possible alterations produced by this treatment before or after stimulation with calcium ionophore. Protoporphyrin plus UVA, at doses causing inhibition of mediator release, had no effect on either cell size or viability but increased cellular sphericity. There was a 43% reduction of the cell surface area, and qualitative inspection of the cells revealed that this change was associated with a reduction in microfolds on the cell surface. After 1 minute of incubation with calcium ionophore A23187 (1 μ mol/L), both cells that were pretreated with protoporphyrin plus UVA and control cells showed dramatic changes in granule structure. Although treated cells had an unchanged tendency to have granules that closely approached the plasma membrane, there was an inhibition of granule extrusion in response to ionophore stimulation. These observations may be relevant to the inhibitory effect of protoporphyrin plus UVA on the generation and release of mast cell mediators. (J ALLERGY CLIN IMMUNOL 1994;93:909-18.)

Key words: Mast cells, protoporphyrin, long-wave ultraviolet light, plasma membrane, morphology, morphometry

We have previously shown that the treatment of rat peritoneal mast cells (RPMCs) with the endogenous photosensitizer protoporphyrin, plus long-wave ultraviolet light (UVA) irradiation, can

inhibit the release of histamine and generation of prostaglandin D₂ induced by compound 48/80, calcium ionophore A23187, or antigen. This inhibitory effect is partially dependent on extracellular calcium, is noncytotoxic, and is apparently unrelated to the generation of reactive oxygen species.¹ We and others have also shown that another photosensitizer, methoxsalen, plus UVA, inhibits skin test responses to mast cell degranulators in human subjects.²⁻⁴ However, the mechanism by which photoactive substances inhibit mast cell activation remains unclear. Protoporphyrin plus UVA (PP/UVA) can inhibit mast cell mediator release stimulated by a phorbol ester, implying that the treatment acts on steps in the stimulus-

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Abbreviations used

BSA:	Bovine serum albumin
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
PP/UVA:	Protoporphyrin IX plus long-wave ultraviolet light irradiation
RPMCs:	Rat peritoneal mast cells
SE:	Standard error
Tyr:	HEPES-buffered Tyrode's solution
UVA:	Long-wave ultraviolet light

secretion pathway subsequent to activation of protein kinase C.⁵ Further, methoxsalen plus UVA has been shown to cause structural modifications of erythrocyte and lymphoblast membranes.^{6,7}

Because protoporphyrin is a lipophilic substance and would be expected to penetrate mast cell plasma and perigranular membranes, we hypothesized that the ability of PP/UVA to inhibit mast cell mediator release may be reflective of modifications in mast cell membrane structure, thereby modulating degranulation. To test this hypothesis, we used ultrastructural and morphometric techniques to examine PP/UVA-treated mast cells before and after activation with calcium ionophore A23187. We report here that PP/UVA does indeed have substantial effects on mast cell membrane structure, which may account for the ability of this treatment to reduce degranulation.

METHODS**Materials**

The following chemicals were purchased from Sigma Chemical Co., (St. Louis, Mo.): trypan blue, toluidine blue, bovine serum albumin (BSA), N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), and calcium ionophore A23187. Heparin solution (100 U/ml) was from Elkins-Sinn, Inc. (Cherry Hill, N.J.). Hanks' balanced salt solution was from Gibco Laboratories (Grand Island, N.Y.). Osmium tetroxide, propylene oxide, EM bed-812, uranyl acetate, and bismuth subnitrate were from Electron Microscopy Sciences (Ft. Washington, Pa.).

HEPES-buffered Tyrode's solution (Tyr), pH 7.4, had the following composition: 137 mmol/L NaCl, 1 mmol/L glucose, 10 mmol/L HEPES, 2.7 mmol/L KCl, 1 mmol/L, MgCl₂, 1 mmol/L CaCl₂, and 0.4 mmol/L NaH₂PO₄ and contained 0.1% BSA. Protoporphyrin IX was obtained from Porphyrin Products (Logan, Utah). Before use, 1 mg of protoporphyrin was dissolved in 1 ml of 11.6 mol/L HCl and then diluted in 1:10 in distilled water to yield a solution of 100 µg/ml. This solution was further diluted in Tyr to the desired concentration, and the pH was adjusted to 7.4.

Preparation of mast cells

Sprague-Dawley male rats (200 to 450 gm) purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Del.), were used throughout the study. Rats were killed by CO₂ inhalation. RPMCs were obtained by an established method, as previously reported.⁸ Briefly, the peritoneal cavity was lavaged with 20 ml ice cold Tyr containing heparin (10 U/ml). The cell suspension was centrifuged at 300g for 5 minutes at 4° C, and the mast cells were then purified over a 38% BSA gradient (38% BSA in Hanks' balanced salt solution), yielding a final population of greater than 90% mast cells, which were more than 95% viable as determined by trypan blue staining.

Pretreatment of RPMCs with PP/UVA

Four General Electric F40BL tubes (GE Medical Systems, Waukesha, Wis.) were used as the source of UVA light. They emitted photons over a wavelength range of 320 to 450 nm, with a peak emission at 350 nm. The output of the lamps over the wavelength range of 400 to 410 nm was 60 µW/cm², measured at a distance of 15 cm, with an IL Research Radiometer, an SEE 400 sensor, an NB 405 filter, and a W1968 diffuser (International Light, Newburyport, Mass.). A 3 mm window glass filter was used during irradiation to eliminate any photons of wavelengths below 320 nm.

Five hundred microliters of a suspension of RPMCs (2×10^6 /ml) was added to polystyrene tubes containing 500 µl of either protoporphyrin (6 ng/ml) in Tyr or Tyr alone. The tubes were placed at 37° C in the dark for 10 minutes, and the cells, held at 37° C, were then irradiated in the same tubes at a distance of 15 cm from the light source for 10 minutes and 40 seconds to achieve UVA at a dose of 0.038 J/cm², or kept in the dark. The cells were washed twice with Tyr and assessed for viability by trypan blue exclusion (all cells studied were greater than 90% viable). The cell pellets were then fixed by the addition of 500 µl of modified Karnovsky's fixative (0.5% glutaraldehyde, 1.5% paraformaldehyde in sodium phosphate buffer, pH 7.4), as routinely used in our laboratory for cell suspensions. In some experiments treated and untreated cells were stimulated with calcium ionophore A23187 before fixation. The cells were resuspended in Tyr after washing, brought to 37° C, then mixed with 500 µl of calcium ionophore A23187 (2 µmol/L) or Tyr as control, and then further incubated at 37° C for another minute. Ice cold Tyr was added to each tube to stop the reaction and the cells were pelleted by centrifugation (300g, 4° C, 5 minutes); the supernatant was removed, and 500 µl of Karnovsky's fixative was added.

Preparation of RPMCs for transmission electron microscopy

The cell pellets fixed in modified Karnovsky's fixative were rinsed in 0.1 mol/L sodium phosphate buffer. They were postfixed for 2 hours in a 1% solution of

TABLE I. Effect of protoporphyrin, UVA, or PP/UVA on the diameter of mast cells in suspension and the cell cross-sectional area after preparation for electron microscopy

Treatment		Diameter (μm)*		Area (μm^2)†
Protoporphyrin	UVA	Longest	Shortest	
—	—	13.9 $\pm$ 0.3	13.0 $\pm$ 0.2	64.3 $\pm$ 3.6
+	—	14.0 $\pm$ 0.3	13.1 $\pm$ 0.3	65.6 $\pm$ 4.1
—	+	14.1 $\pm$ 0.2	13.2 $\pm$ 0.2	67.9 $\pm$ 4.0
+	+	14.3 $\pm$ 0.1	13.5 $\pm$ 0.2	66.8 $\pm$ 5.1

All data are means $\pm$ SE. Doses of protoporphyrin and UVA used were 3 ng/ml and 0.038 J/cm², respectively.

*Diameters of mast cells in suspension were measured from at least 30 cells for each experimental condition with an image analyzer.

†Cross-sectional area was measured for at least 60 cells for each experimental condition.

osmium tetroxide in 0.1 mol/L sodium phosphate buffer, dehydrated in increasing concentrations (30% to 95%) of ethanol, rinsed in propylene oxide, and embedded in EM bed-812. One-micrometer sections were cut on a Sorvall Porter Blom MT2 Ultramicrotome I (Du Pont Co., Wilmington, Del.) and stained with 0.1% aqueous toluidine blue. Ultrathin sections (70 nm) were stained in ethanol-saturated uranyl acetate for 30 minutes in the dark. After rinsing, the sections were further stained with bismuth subnitrate (0.04% in water) for 12 minutes and then rinsed. Micrographs for morphometry were taken on 70 mm films with a Zeiss EM 10 electron microscope (Carl Zeiss, Inc., Thornwood, N.Y.). Micrographs of a carbon grating replica (E.F. Fullam, Inc., Schnectady, N.Y.) were recorded for calibration on each film.

Morphometry

The shortest and longest diameters (cell diameter) of treated and control mast cells suspended in Tyr buffer were measured with an image analyzer (Videometric 150; American Innovision, Inc., San Diego, Calif.). At least 30 cells were measured per experimental condition. They were selected by systematic sampling of the cells placed in a hemocytometer and examined with a Leitz Ortholux microscope at a magnification of $\times 40$. Final magnification on the screen of the image analyzer was $\times 1800$.

The average cross-sectional area of mast cells in 1 μm sections was also measured by image analysis at a final magnification of $\times 1800$. At least 60 cells were measured for each experimental condition. They were selected by systematic sampling.

Fifteen electron micrographs (2 to 4 cell portions per micrograph) were obtained by systematic sampling from the same block of each experimental condition. Contact prints of the electron micrograph films were projected on a 144-point square test grid at a final magnification of $\times 14,000$ with a microfilm reader (Dokumator DL 2; Jenoptic, Jena, Germany). Standard point counting techniques were used to assess the cell surface area relative to total cell volume and the

relative volume of three types of granules (intracellular electron-dense, intracellular with reduced electron density, and free extracellular granules) per cell volume.⁹ Point counts were accumulated, stored, and processed with the aid of an Apple II⁺ computer (Apple Computer, Inc., Cupertino, Calif.). In addition, the shortest distance between the outermost ring of intracellular granules and the plasma membrane was measured with a videometric 150 image analyzer in the same micrographs examined at a final magnification of $\times 38,000$.

Statistical analysis

Data are expressed as means $\pm$ standard error (SE). The SE of the estimates of cell surface area and the volume fraction of granules relative to total cell volume was calculated by applying the formula for the SE of ratios.¹⁰ Each picture frame (micrograph) was taken as the primary sampling unit. Therefore the SE indicates the variability between micrographs for each experimental condition. The SE of the estimates of cell diameter, cell sectional area, and distance between the outermost intracellular granule and cell membrane indicates the variability between individual measurements. Statistically significant differences between group means obtained for different treatments were assessed with Student's *t* test or analysis of variance, as appropriate.

RESULTS

Effects of PP/UVA treatment on resting mast cell ultrastructure

Size and shape of RPMCs in suspension. The shortest and longest diameters of untreated RPMCs were 13.01 $\pm$ 0.23 μm and 13.92 $\pm$ 0.26 μm , respectively, yielding an average ellipsoidal volume of a single cell of about 1240 μm^3 . There was no significant difference among the sizes of RPMCs after treatment with protoporphyrin alone, UVA alone, or PP/UVA (Table I). The image analyzer provides a shape factor for the cells,

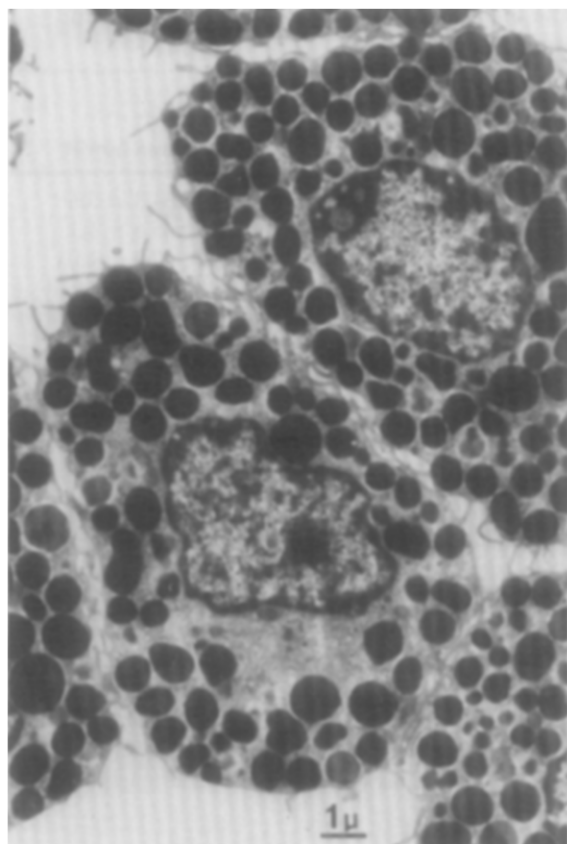


FIG. 1. Electron micrograph showing normal RPMCs. The majority of the cells were elongated. Many electron-dense granules and a few altered granules are present in the cytoplasm. Numerous traces of microfolds are distributed on the cell surface.

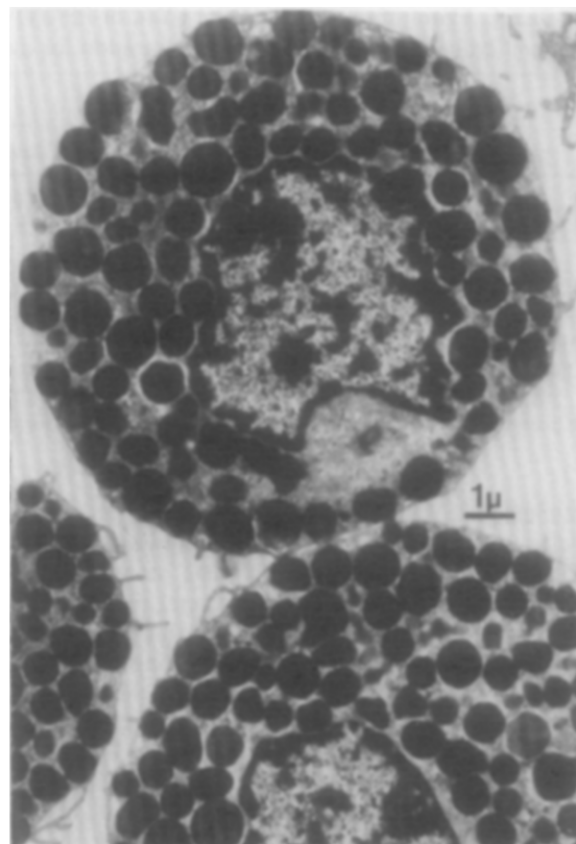


FIG. 2. Electron micrograph of PP/UVA-treated RPMCs. There is a reduction in the number and length of microfold traces on the cell surface as compared with control cells (Fig. 1). The cells appear uniformly spherical.

TABLE II. Effect of protoporphyrin, UVA, and PP/UVA on cell surface area and granule volume relative to total cell volume

Treatment*		Surface area/cell volume (cm ² /cm ³)	Granule volume/cell volume (%)
Protoporphyrin	UVA		
—	—	11183 ± 1045	51 ± 2
+	—	10456 ± 734	45 ± 1
—	+	9387 ± 925	55 ± 3
+	+	6343 ± 655†	50 ± 3

All data are means ± SE obtained by counting 10 fields of randomly taken micrographs (magnification, ×2000) for each experimental condition.

*The doses of protoporphyrin and UVA used were 3 ng/ml and 0.038 J/cm².

†Significantly lower than that in control cells, $p < 0.01$ as determined by analysis of variance.

which is a parameter reflecting cellular sphericity. This shape factor ranges from 0 to 1, and the more uniformly spherical the cells are, the more closely the factor will approach a value of 1. The presence of either irregularly shaped or ellipsoidal cells will reduce the value of this factor. PP/UVA-treated cells had a significantly higher

shape factor than untreated cells (0.81 ± 0.01 vs 0.71 ± 0.01 , $p < 0.001$, $n = 50$), suggesting that the treatment increases cellular sphericity.

Transmission electron microscopy. Untreated RPMCs were generally oval in shape. Low rounded elevations were seen on the cell surface, reflecting the presence of granules just beneath

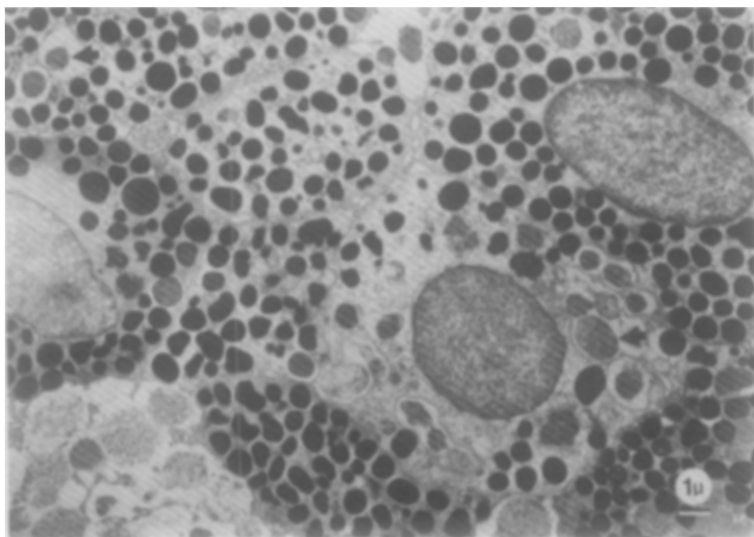


FIG. 3. Electron micrograph of control RPMCs (not treated with PP/UVA) 1 minute after activation by calcium ionophore ($1 \mu\text{mol/L}$). Note the large number of altered granules, many of which reside in common cavities (*arrow*). Extracellular granules can also be seen.

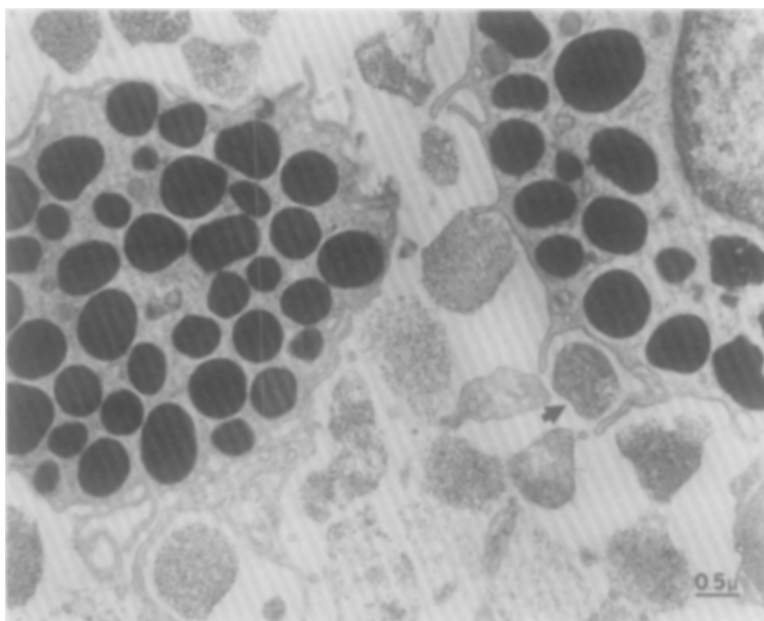


FIG. 4. Higher power electron micrograph of control RPMCs, 1 minute after activation by calcium ionophore. At points of granule extrusion, long membrane processes embrace the released granules (*arrow*).

the plasma membrane. These granules were densely packed in the cytoplasm. They were electron-dense, usually round or oval, and varied somewhat in size. A few granules of lower electron density were found in some cells. Numerous microfolds were on the cell surface (Fig. 1). These ultrastructural features of resting RPMCs are in agreement with reports by others.¹¹⁻¹³ Neither UVA nor protoporphyrin alone

had any obvious effect on RPMC ultrastructure.

Structural alterations were apparent in PP/UVA-treated mast cells (Fig. 2). There was an obvious reduction in microfolds on the cell surface. Many cells appeared uniformly spherical, and there were fewer rounded elevations on the cell surface than in control cells. In contrast, neither UVA alone nor protoporphyrin alone had any obvious effect on RPMC ultrastructure.

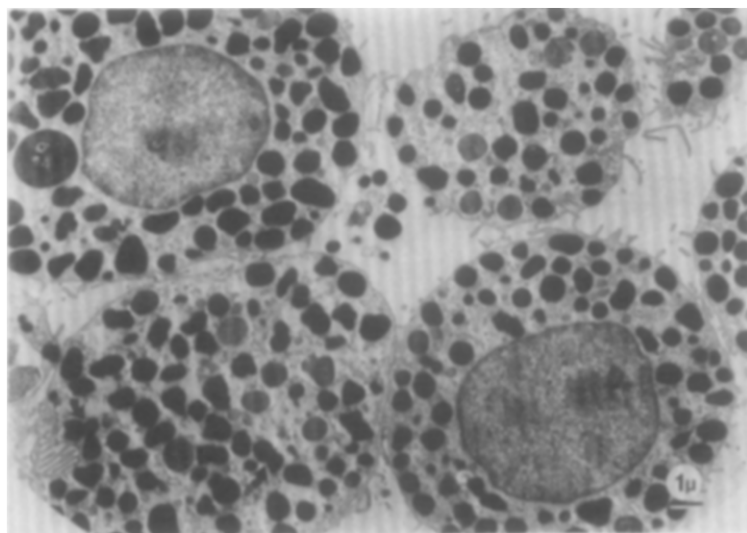


FIG. 5. Electron micrograph of PP/UVA-treated RPMCs, 1 minute after activation by calcium ionophore, showing the variability in the responses of individual cells to the ionophore. Note the large number of altered granules. Compared with Fig. 3, fewer granules are observed in the extracellular space.

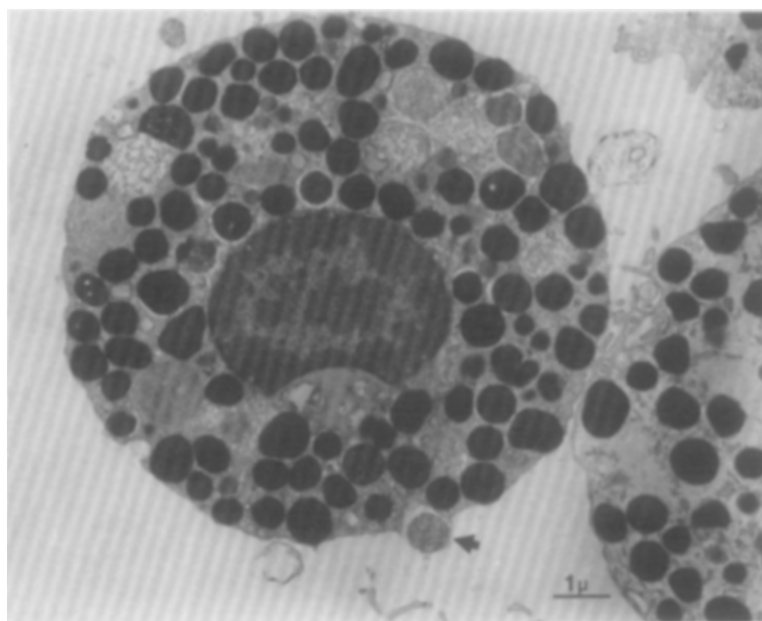


FIG. 6. Higher power electron micrograph of PP/UVA-treated RPMC, 1 minute after activation by calcium ionophore. The cell is more uniformly spherical than control cells with less abundant microfolds (Fig. 4). A released granule, not embraced by long processes, is seen sitting in a shallow pit with blunted borders (arrow).

Cell surface area per unit cell volume was decreased by approximately 43% after treatment with PP/UVA compared with the control value (Table II), which is consistent with our qualitative observation of micrographs. The proportion of total cell volume occupied by granules was $51\% \pm 2\%$ in control cells and was not affected

by PP/UVA treatment. Neither cell surface area nor ratio of granules to total cell volume was significantly altered after treatment with PP or UVA alone (Table II). The mean cross-sectional areas of RPMCs were very similar for each experimental condition (Table I), indicating that the processing for electron microscopy did not differ-

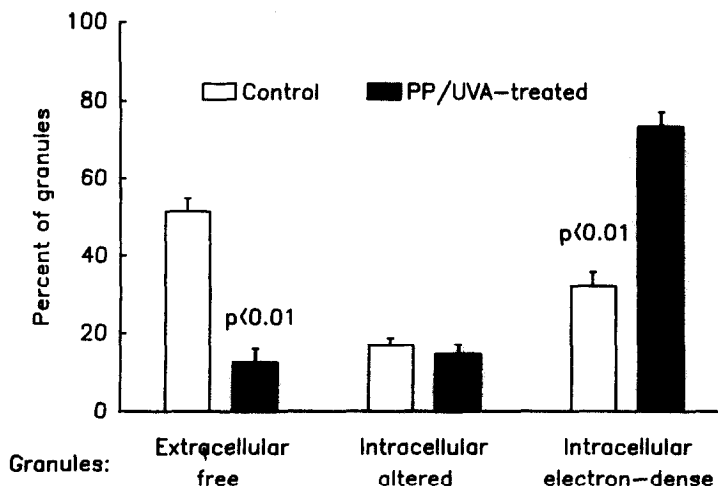


FIG. 7. Effect of PP/UVA on the relative proportion of three types of granules in cell preparations stimulated for 1 minute with calcium ionophore (1 μ mol/L).

entially alter cell size under the various experimental conditions (treatment with buffer only, protoporphyrin alone, UVA alone, or PP/UVA).

Effect of PP/UVA pretreatment on ultrastructure of activated mast cells

Control cells displayed a spectrum of responses to ionophore (1 μ mol/L) at the time point examined in the study. The vast majority of cells demonstrated ultrastructural features of degranulation consistent with those previously reported.¹¹⁻¹³ There was an increase in the number of granules with reduced electron density, and many such granules resided in common cavities. These cavities communicated with the extracellular space, and extrusion of granules through openings was frequently observed. Extracellular granules were also seen (Fig. 3). At points of granule extrusion, long membrane processes embraced the released granule (Fig. 4).

RPMCs treated with PP/UVA showed different responses to stimulation with calcium ionophore A23187. Although many cells had large numbers of altered granules and fusions between the perigranular membranes, direct evidence for extrusion of granules was less apparent, and fewer granules were found in the extracellular space than under control conditions. As observed for nonactivated cells treated with PP/UVA, some cells had a uniformly spherical shape, and all cells had less abundant microfolds (Fig. 5). When granule extrusion was seen, the released granule was not embraced by long processes, but rather sat in a shallow pit with blunted borders (Fig. 6).

Ionophore treatment slightly increased cell sur-

face area both in control cells (13343 ± 1235 cm^2/cm^3 vs 11183 ± 1045 cm^2/cm^3 , $n = 10$) and PP/UVA-treated cells (7990 ± 357 cm^2/cm^3 vs 6343 ± 655 cm^2/cm^3 , $p < 0.05$, $n = 10$), although the effect in control cells did not achieve statistical significance. However, cell surface area remained significantly greater in control cells than in treated cells ($p < 0.001$). Although control cells had fewer electron-dense cytoplasmic granules and were surrounded by a larger number of extracellular granules than PP/UVA-pretreated cells, there was no significant difference in the proportion of intracellular granules with reduced electron density between control and PP/UVA-treated cells stimulated with calcium ionophore (Fig. 7).

The mean distance between the outermost ring of intracellular granules and the plasma membrane was significantly shorter in control cells after activation with calcium ionophore (0.116 ± 0.006 μm , $n = 214$) compared with unactivated cells (0.137 ± 0.007 μm , $p < 0.05$, $n = 204$). It was not significantly different between control and PP/UVA-treated cells stimulated with calcium ionophore A23187 (0.116 ± 0.006 , $n = 214$ vs 0.117 ± 0.007 μm , $n = 203$). A parallel experiment showed that PP/UVA suppressed calcium ionophore-induced histamine release from RPMCs by 69% (net release: PP/UVA-treated cells, 13%; untreated cells, 42%).

DISCUSSION

In this study we have focused on an ultrastructural analysis of RPMCs exposed to protoporphyrin and UVA. Our discussion attempts to correlate the present results with our previous obser-

variations regarding functional inhibition of RPMCs by PP/UVA and with related findings reported by others.

In our previous studies, protoporphyrin at the same dose as that used in this study (3 ng/ml) plus UVA significantly inhibited secretagogue-induced generation and release of mediators from RPMCs without significant changes in cell size or viability, as assessed by fluorescence-activated cell sorter analysis. We have also shown that treatment of RPMCs with protoporphyrin, at a dose 10 times higher than that used in this study plus the same dose of UVA, not only suppressed the ability of RPMCs to release histamine in response to stimuli but also produced an increase in cell size as assessed by fluorescence-activated cell sorter analysis. The increase in size was followed by cytolysis, which gradually occurred during the next 24 hours.^{1, 5} Thus it was important to determine whether the lower dose of PP/UVA used in this study had an effect on cell size. The data obtained support our previous contention that mast cells treated with low doses of PP/UVA are still viable but functionally inhibited. A more detailed ultrastructural analysis appeared to be justified to clarify the functional alterations.

Both qualitative examination and morphometric analysis by electron microscopy suggest that the most striking effect of PP/UVA treatment on mast cell ultrastructure is the reduction of microfolds. Using scanning electron microscopy, others have shown that a distinct subpopulation of resting RPMCs devoid of surface folds are resistant to stimulation by polymyxin B.¹⁴ In keeping with this observation, we found that PP/UVA treatment, which decreased the number and length of microfolds in RPMCs, was also associated with an inability of the cells to respond to a number of stimuli. Changes in surface topology at the level of the microfolds can have a dramatic effect on cell surface area, as shown by our morphometric data. Such a marked transformation of the plasma membrane would almost certainly be expected to result in the rearrangement of plasma membrane components, as found in erythrocyte and lymphoblast membranes.^{6, 7} Because PP/UVA treatment had no effect on cell size, the ratio of the granule volume to total cell volume can serve as an indirect measurement of alterations in the volume of granules. Protoporphyrin, UVA alone, and PP/UVA have no significant effect on this ratio. Thus the lipophilic agent, protoporphyrin, more likely affected the plasma membrane rather than the perigranular membranes of mast cells.

Untreated RPMCs in suspension were not uniformly spherical in shape. The ellipsoidal volume of an untreated single cell, calculated from the average shortest and longest diameters, was similar to that reported by others.¹⁵ The comparison of shape factors revealed that PP/UVA-treated RPMCs were more uniformly spherical than control cells. This could be an indication that PP/UVA treatment may influence the fluidity of the cell membrane. Such a change may result in functional alterations, because mast cell exocytosis is dependent on membrane fluidity.^{16, 17}

To obtain cells exhibiting various degrees of exocytosis after exposure to the calcium ionophore, we chose 1 minute for the period of incubation with the stimulus. Although the response of individual cells to the calcium ionophore differed considerably, striking features emerged from both qualitative and quantitative examination of the micrographs. PP/UVA treatment apparently did not influence the ability of granules to fuse together after stimulation with the calcium ionophore, but a decreased percentage of granules was externalized. In addition, a higher percentage of unchanged intracellular electron-dense granules was seen in treated cells stimulated with calcium ionophore A23187 than in control cells. This again demonstrated that PP/UVA inhibits mast cell exocytosis induced by the calcium ionophore.

An expansion of the surface area of mast cells on stimulation has been reported¹⁸ and related to histamine secretion.¹⁹ It has been postulated that the increase in surface area may reflect fusion of granule membranes to the plasma membrane.¹⁴ Our finding of a slightly increased surface area of control mast cells after stimulation is consistent with this. Interestingly, although there was also an increase in the surface area of PP/UVA-treated cells after activation above that of resting cells, the final cell surface area was still significantly lower than that of control cells. The large mast cell surface area, which provides an expanded area for granule fusion, may be an important determinant of the extent of degranulation in untreated cells.

We found that intracellular granules moved closer to the plasma membrane on activation of mast cells. This finding and the increased percentage of intracellular granules of reduced electron density were displayed by both control and PP/UVA-treated cells after activation. In contrast, the percentages of intracellular electron-dense and extracellular free granules were mark-

edly different between the two groups of cells. It is likely therefore that PP/UVA inhibits calcium ionophore-stimulated degranulation by acting on granule extrusion, probably because of alterations in the plasma membrane.

Because high doses of PP/UVA have a cytolytic effect on mast cells,¹ it is important to determine whether some of our findings could simply reflect PP/UVA-induced cell injury or death. However, a number of points argue against cell injury or death in our preparations. First, before fixation, the cells were viable as assessed by trypan blue exclusion, and PP/UVA treatment did not directly cause histamine release. Second, the cellular adenosine triphosphate content was not decreased after PP/UVA treatment, suggesting that the observed inhibition of mast cell secretory function is not due to a defect in cellular energy generation (Yen et al. Unpublished data). Third, no direct evidence of cell apoptosis or necrosis was seen by electron microscopy (e.g., swollen mitochondria or condensation of chromatin into sharply delineated masses).²⁰ Fourth, under the same experimental conditions, we have demonstrated that PP/UVA-treated cells are viable not only immediately after treatment but also after 24 hours in cell culture, although this culture period does not result in a recovered ability to release histamine in response to compound 48/80.¹ Fifth, after exposure to calcium ionophore, granule extrusion was inhibited in PP/UVA-treated cells, even though other intracellular responses to stimulation, including granule fusion and the tendency of granules to approach the plasma membrane, were unchanged. Finally, using mouse bone marrow-derived mast cells, we have shown that PP/UVA treatment has a similar inhibitory effect on mediator release but has no effect on the phospholipid signaling pathways.²¹ We therefore conclude that the inhibitory effect of PP/UVA is not based on cell death, but rather reflects a membrane modification.

In summary, treatment of RPMCs with PP/UVA did not significantly influence the response of intracellular granules to calcium ionophore stimulation, at least as assessed ultrastructurally. However, there was clear evidence that the treatment modified the properties of RPMC plasma membranes. The changes observed in plasma membrane ultrastructure can be explained by an effect of PP/UVA on membrane fluidity. Thus our findings shed further light on the mechanism by which PP/UVA treatment is able to suppress mediator release stimulated by receptor-

linked stimuli, such as antigen and compound 48/80, as well as agents that bypass normal signal transduction events, such as calcium ionophore A23187 and phorbol esters. Our findings may also have more general applicability to the mechanisms by which photoactive substances interact with, and modulate properties of, other cells of the immune system.

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Association of class II sequences encoding DR1 and DQ5 specificities with hypersensitivity to chironomid allergen *Chi t I*

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*A panel of 188 unrelated Caucasian subjects who were exposed to the larvae of Chironomus thummi (Diptera, nonbiting midges) was HLA-typed by polymerase chain reaction amplification of the second exons of the DRB, DQA1, and DQB1 genes followed by dot-blot hybridization with sequence-specific oligonucleotide probes. Type I sensitization to the allergen Chi t I and a large number of other inhalant allergens was determined by RAST and skin testing. Sixty-one individuals were found to be sensitized to Chi t I, of whom 24 were sensitive to this allergen and to no other allergens tested. Statistical analyses showed that only in the latter group were the HLA-D genes DRB1*0101, DQA1*0101, and DQB1*0501 associated with IgE-responsiveness to Chi t I. These results suggest that HLA associations with responsiveness to certain allergens may be more striking in monosensitized subjects. (J ALLERGY CLIN IMMUNOL 1994;93:918-25.)*

Key words: Allergy, HLA-D typing, polymerase chain reaction, sequence-specific oligonucleotides

Usually, in genetic studies of disease susceptibility (e.g., for autoimmune disorders), no clear information exists about the antigen involved. This is not the case for human IgE-mediated atopic allergy in which the antigens or allergens

Abbreviations used

Chi t I: Group I allergen from *Chironomus thummi* (nonbiting midges)
GM: Geometric mean
SSO: Sequence-specific oligonucleotide

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implicated in causing the disease can be identified. Studies with several highly purified allergens have shown significant associations between IgE-mediated immune responsiveness and specific HLA-D type.¹⁻⁵ Such studies can serve as models for understanding the mechanisms of specific immune responsiveness in human beings. In certain