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PHOTOLYSIS OF MAST CELLS IN PRESENCE OF PROTOPORPHYRIN

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Abstract. The effects of ultraviolet and visible irradiation in presence of protoporhyrin upon mast cells were investigated. Rat peritoneal mast cells were suspended in a medium containing varying concentrations of protoporphyrin and were irradiated using a mercury vapor lamp. The release of histamine is related to both the concentrations of protoporphyrin and the period of irradiation. Comparatively negligible amounts of histamine were released when the cells were irradiated in absence of protophyrin or when the cells were incubated in the dark in presence of protoporphyrin. When ^{51}Cr-labelled cells were employed, the release of histamine was paralleled by the release of ^{51}Cr. These results suggest that the release of histamine was due to the lysis of cells. It was found that 2-deoxyglucose, iodoacetate and carbonyl cyanide, m-chlorophenyl hydrazone at concentrations which are known to inhibit the secretory release of histamine or uncouple oxidative phosphorylation, had no effect on the photolysis of mast cells. When mast cells were irradiated in presence of protoporphyrin for 5 to 10 minutes and then kept in the dark, the release of histamine was greater than when the cells were irradiated but not subsequently kept in the dark. It is suggested that the initial site of photochemical lysis is at or near the cell surface.

The relationship between mast cells and porphyrins in human skin is important for two reasons, viz.: (1) to gain a better understanding of clinical photosensitivity reactions that occur in erythropoietic protoporphyria, (2) the treatment of lesions of urticaria pigmentosa with porphyrin and light. Several reports have been published concerning the treatment of tumors with porphyrin and light (2, 3, 6, 9, 11, 12).

Several porphyrins are associated with abnormal porphyrin metabolism resulting in accumulation of photosensitizing porphyrins in red cells and other cells. In erythropoietic protoporhyria, red cells contain large amounts of porphyrins. The photohemolysis of red cells sensitized with protoporphyrin (PP) has been studied in the past (4, 5). The cells sensitized with PP undergo hemolysis and the action spectrum of hemolysis corresponds to the absorption spectrum of PP. The hemolysis requires molecular oxygen and is inhibited by free radical inhibitors (8). A number of enzyme activities are destroyed in photosensitized cells. The product of membrane-bound cholesterol formed by singlet oxygen or free radical oxidation seems to cause hemolysis after catalytic decomposition which initiates the oxidation process in the membrane.

The dermal mast cells are known to play a role in urticaria. If mast cells are sensitized with PP, they may undergo photolysis and cause some of the early symptoms in photosensitive individuals. Although, at present, there is no evidence to implicate the mast cells in photosensitivity reactions, it is conceivable that these cells may be involved in certain dermal responses to light. The present studies were therefore undertaken to evaluate the photosensitivity of PP-treated mast cells.

MATERIAL AND METHODS

Protoporphyrin-IX-dimethyl ester (obtained from Sigma Chemical Company, St. Louis, Mo., USA) was dissolved in 12 N HCl to give a 1.0 mg/ml solution. After keeping the solution at room temperature for 3 hours, it was diluted 10-fold with distilled H_2O. This solution was distributed into small vials and stored in the dark at 4°C, for a maximum period of about one month. Prior to each experiment, the solution in one vial was diluted with 0.1 M phosphate buffer, pH 7.4, to give a concentration of 8.0 μg/ml. The pH of the solution was adjusted to 7.4 by the addition of 2–3 drops of 1.0 N NaOH. Further dilutions were done with phosphate-buffered saline, pH 7.4 (PBS).

Dimethyl-DL-2, 3 distearoyloxyl-propyl-2-hydroxyethyl ammonium acetate (DDPHA) reagent grade A was obtained from Calbiochem, USA. 2-Deoxy-D-glucose (2DG) reagent grade III and carbonyl cyanide, m-chlorophenyl hydrazone (CCCP) were obtained from Sigma. Sodium iodoacetate (IAA) was obtained from Fisher Scientific Company, New Jersy, USA and was of reagent grade.

Buffers. All chemical solutions were prepared in PBS. Histamine release studies were carried out in Tyrode solution, pH 7.4, containing 0.1% gelatin.

Mast cells. Rat peritoneal cells were obtained from male Wistar rats weighing around 400 g. Mast cells were iso-

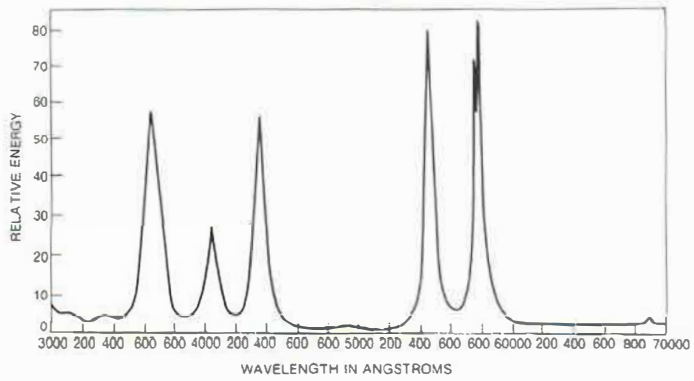

Fig. 1. Emission spectrum of mercury vapor lamp, provided by the manufacturer.

lated according to the method described by Ranadive & Ruben (10).

Histamine assay. Histamine was determined by bioassay on a guinea pig ileum, suspended in Tyrode solution, with 1×10^{-5}M atropine, through which oxygen was bubbled. The contractions induced by the samples were compared with those induced by standard amounts of histamine.

Chromium labelling of mast cells. Mast cells (1×10^{7} cells) in 1 ml PBS containing 5% lamb serum were incubated with 5 μ Ci of ^{51}Cr (sodium chromate) for 30 min at 37°C. The cells were centrifuged at 225 *g* and washed four times with 5 ml Tyrode solution. The cells were then suspended in the desired volume of Tyrode solution (to get 1–2$\times10^{6}$ cells/ml). The amount of chromium released by cell lysis was determined by placing the chromium-loaded cells in boiling water for 5 minutes. The chromium released in the supernatant was determined by a gamma counting system (Intertechnique). It was found that normally 8–10% of the radioactive chromium from the medium was taken up by the cells. The amount of chromium released due to the treatment is expressed as the percentage of the total released from the lysed cells.

Irradiation of mast cells. The cells were irradiated with a Westinghouse (400 W) mercury vapor lamp ((H33C)-400), consisting of an arc operating in an atmosphere of argon containing traces of mercury at a maximum pressure of 8 atmospheres; the arc was enclosed within an outer envelope which was not covered with any phosphor. The emission spectrum of the lamp is given in Fig. 1.

1.0 ml cell suspensions were taken in test tubes which were placed in a metabolic shaker at 37°C. The test tubes were kept centrally under the lamp at a distance of 20 cm from the outer envelope of the lamp to the cell samples.

Release of histamine and chromium from chromium-loaded mast cells on irradiation. Mast cells (5×10^{5}) were incubated with protoporphyrin in a total volume of 1 ml Tyrode solution. The concentration of protoporphyrin was varied between 25 and 200 ng. After irradiation for 10 minutes at 37°C, the cells were centrifuged down at 225 *g* and the supernatants were assayed for histamine. The degree of histamine released is expressed as percentages of the total histamine present in the cells. The total histamine in a given number of cells was obtained by heating the same number of cells in a boiling water bath for 5 min.

The control tubes were treated exactly as in the experiment, except that they were incubated covered with aluminum foil (in the dark). The experiments were performed in duplicates or quadruplicates.

Inhibition of histamine release by 2 DG, IAA, DDPHA and CCCP. Mast cells (5×10^{5}) were incubated with various concentrations of 2DG (1×10^{-2} M and 1×10^{-3} M), with IAA (1×10^{-3} M), with DDPHA (1×10^{-5} M, 1×10^{-6} M and 1×10^{-7} M) and CCCP (1×10^{-5} M and 1×10^{-6} M) at room temperature for 20 min. Protoporphyrin (400 ng) was added to the cells and the cell suspensions were irradiated at 37°C for 10 min. The cells were spun down and the supernatants were used for histamine assay.

Release of histamine as function of duration of irradiation. Mast cells (5×10^{5}) were irradiated in presence of 200, 400 and 800 ng protoporphyrin for different periods. The cells were spun down and the supernatants were used for histamine assay.

Release of histamine in dark from mast cells irradiated in presence of protoporphyrin. Mast cells (2.5×10^{5} cells) were irradiated in presence of either 200 ng or 400 ng of PP for 5 and 10 min as before. The cells were then allowed to stand in the dark for 0 to 20 min. At the end of the desired time interval the cells were centrifuged off and the histamine release in the supernatant was assayed.

RESULTS

Release of histamine and chromium-51 from labelled mast cells irradiated in presence of PP. When mast cells irradiated for 20 min in presence of 25 to 200 ng of PP, the relase of histamine occurred. It will be seen from Fig. 2 that the histamine release was paralleled by the release of ^{51}Cr in the extracellular medium. These results suggest that the release of histamine was due to the lysis of the cells. The optimal lysis of the cells irradiated for 20 minutes occurred in presence of 50 ng of PP. The mast cells exposed to light in the absence of PP released

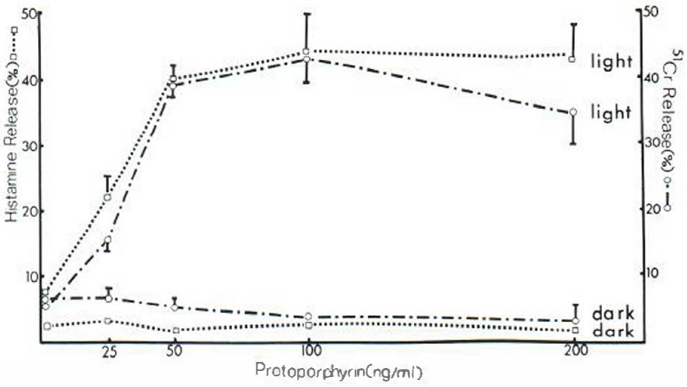

Fig. 2. Release of histamine and ^{51}Cr from ^{51}Cr-loaded mast cell irradiated in the presence of protoporphyrin. Bar represents ± S.E.M. ($n=4$).

approximately 6% histamine, in comparison with 3% released in the dark.

Effects of inhibitions of energy metabolism on release of histamine from mast cells exposed to light. It has been observed that the lysis of mast cells by anti-rat mast cell antibody in presence of complement requires permissive levels of intracellular ATP (12). An attempt was made to see whether a similar energy requirement is necessary for the lysis of mast cells by light in presence of PP. It was found that 2 DG, IAA and CCCP at the concentrations which are known to inhibit the secretory release of histamine or uncouple oxidative phosphorylation from mast cells, had no effect on the photolysis of mast cells (Fig. 3).

Effect of DDPHA on photolysis of mast cells. DDPHA, an inhibitor of phospholipase A, inhibited the photolytic release of histamine from mast cells at concentrations as low as 1×10^{-7} M (Fig. 4). It is not possible, however, to ascertain from these results whether this inhibition was due to its specific effect on the enzyme.

Time-course of photolysis of mast cells. It will be seen from the data shown in Fig. 5 that the photolysis of mast cells in presence of PP was dependent on both the concentration of PP and the duration of irradiation. It was noted that the time-course of the mast cell lysis was different from that of photohemolysis of red blood cells in that the former showed virtually no lag period and the photolysis was gradual; on the other hand the photohemolysis of red blood cells had an initial lag period but a steep subsequent rise.

Lysis of irradiated mast cells in dark. The results in Fig. 6 shows that the cells initially damaged by photoreaction continues to undergo lysis in the dark. The amount of histamine released from mast

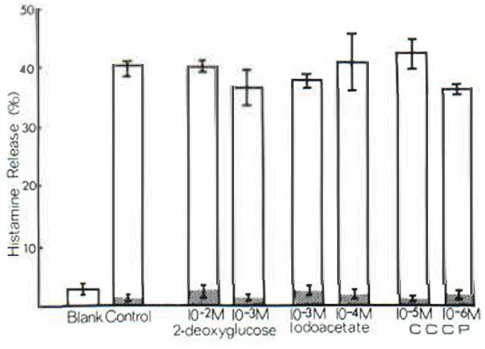

Fig. 3. Effects of metabolic inhibitors on the release of histamine from mast cells irradiated in the presence of protoporphyrin. Release in the dark is shown by hatched area of the columns. Bar represents ± S.E.M. ($n=4$).

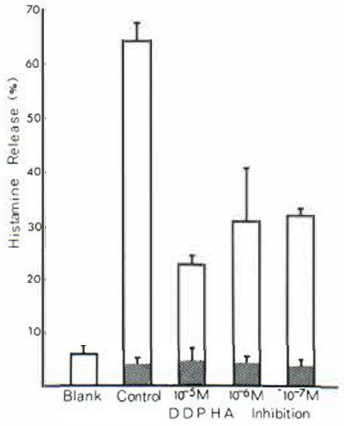

Fig. 4. Effect of dimethyl-DL-2, 3-distearoyloxyl-propyl-2-hydroxyethyl ammonium acetate on the release of histamine from mast cells irradiated in the presence of protoporphyrin. Release in the dark is shown by hatced area. Bar represents ± S.E.M. ($n=4$).

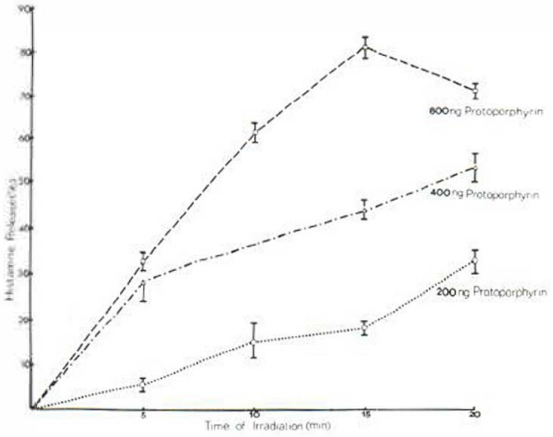

Fig. 5. Effect of time of irradiation and concentration of protoporphyrin on histamine release from mast cells. Bar represents ± S.E.M. ($n=4$).

cells irradiated for 10 min with 200 ng of PP was approximately 10% whereas the cells which were allowed to stand in the dark after irradiation showed an increase in histamine release, with a maximum release of 50% after 20 min in the dark. Those cells exposed to light for 15 min showed an initial release of 30% which rose to 55% after 10 min in the dark. The cells in presence of 400 ng of PP released 50% histamine during initial exposure of 5 min and released 65% after 5 min in the dark. The cells exposed to light for 10 min in presence of 400 ng PP did not show any further increase in histamine release on standing in the dark for up to 20 min.

DISCUSSION

The investigations reported here show that rat peritoenal mast cells subjected to visible and long-wavelength ultraviolet irradiation in presence of protoporphyrin undergo lysis. The lytic activity is related to both the concentration of the sensitizer and the dose of radiation. Unlike photohemolysis of red blood cells, there is no lag period. The significance of this observation is difficult to interpret at present. It is apparent from the data in Fig. 5 that the initial damage occurs within the first few minutes of irradiation. The subsequent cell lysis appears to be due to the initial lesion on cell membrane. The time lag for the optimal lytic activity in the dark is related to the extent of initial damage.

Although the photolysis of mast cells is independent of cellular metabolic energy, DDPHA, an inhibitor of phospholipase A, inhibited the lysis at a concentration as low as 1×10^{-7} M. The mechanism by which this protection is afforded is not known.

Changes in cell surface properties and intracellular enzymes by photoactivated porphyrins in leukemia L1210 cells have been investigated by Kessel (6). The first step in cytotoxicity appears to be binding of porphyrins to the cell surface and this property is related to the partition coefficient of a porphyrin between octanol and water. A variety of cell surface effects such as inhibition of transport of nucleosides and amino acids across the membrane, perturbation of permeability barriers to actinomycin D uptake, altered cell surface properties etc., were observed on irradiation of photosensitized cells. These surface changes were evident at concentrations only one-tenth of those required for inhibition of intracellular nucleoside kinase. It was therefore suggested that the initial site of photoactivated porphyrin toxicity is at or near the cell surface. The results reported here suggest that the release of histamine from mast cells is due to the cell lysis. The cytotoxicity appears to be due to the action of photoactivated protoporphyrin on the cell membrane.

In summary, histamine—and presumably other mediators—is released from rat peritoneal mast cells after irradiation with ultraviolet light in the presence of protoporphyrin. This action on mast cells could explain some of the clinical reactions of the skin that occurs in cutaneous porphyria. In addition, in view of the destruction of mast cells by

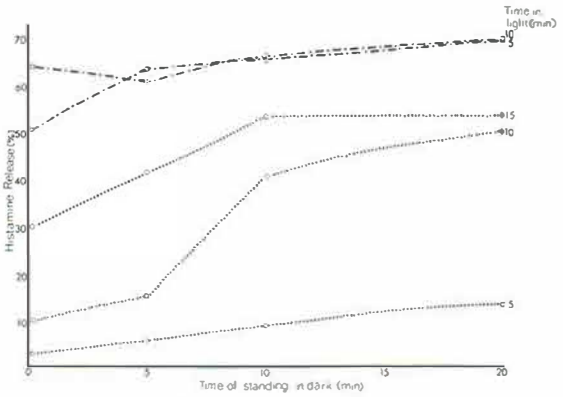

Fig. 6. Release of histamine from mast cells irradiated in the presence of protoporphyrin and allowed to stand in the dark for various lengths of time. Each point represents mean of two observations. ---, 400 ng PP; . . . ., 200 ng PP.

porphyrin and light, it seems possible that porphyrin and light could be useful in treating cutaneous lesions of mastocytosis, especially in view of the encouraging reports of psoralen and less penetrating long wavelength ultraviolet radiation for the treatment of mastocytosis (1).

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