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STUDIES ON HISTAMINE RELEASE FROM SKIN AND FROM PERITONEAL MAST CELLS OF THE RAT INDUCED BY HEAT

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Abstract. Rat cutaneous mast cells were degranulated in vivo by immersing the animals in water at 60–80°C for 20 sec. In vitro, histamine was released from minced skin and peritoneal mast cells exposed to temperatures above 52°C. The rate of release was markedly increased by raising the temperature from 56 to 60°C. The metabolic inhibitors 2,4-dinitrophenol and potassium cyanide did not inhibit the release process. The kinetics of the heat-induced release differed from those of the release caused by compound 48/80. It is concluded that the energy-dependent histamine release mechanism activated in for example anaphylaxis or by compound 48/80 is not involved in the release caused by heat.

Thermal injury evokes an inflammatory response in the exposed tissue and may thus be used for producing experimental inflammation in animals (1). Some observations indicate that mast cells and release of substances from these cells are involved in the inflammatory response. (*a*) The number of mast cells is decreased in the acute inflammation, but increased in chronic inflammatory states (6, 14, 16). (*b*) Histamine has long been claimed to act as a mediator in inflammatory reactions (13, 24, 26, 29) and is synthetized and stored in the mast cells (17, 22). (*c*) Proteolytic enzymes are also involved in the inflammatory mechanism (3, 4, 18, 19), and one such enzyme, chymotrypsin, is a major constituent of the mast cell granules (12, 15).

The release of histamine from mast cells is an enzymatic, energy-dependent process when induced by antigen, some basic histamine liberators, e.g. compound 48/80, or by some naturally occurring polypeptides (28). The aim of the present work was to find out whether these release mechanisms are activated in the mast cells also in thermal injury.

MATERIALS AND METHODS

Male Sprague-Dawley rats (300–400 g) were used in all experiments.

Experiments in vivo

The abdominal skin was shaved and the animals, anaesthetized with ether, were immersed in water at different temperatures (60–80°C) for 20 sec. Control animals were immersed in water at 22°C for the same period. After immersion they were kept at 37°C for 10 min and then killed by a blow on the head and bleeding from the carotids. Pieces of the abdominal skin measuring approximately 5 × 15 mm were removed and immediately placed in 3.7% formaldehyde in ethanol. After fixation for 24 hours the specimens were subjected to conventional histological processing. Paraffin sections, 8 μm thick, were cut perpendicularly to the skin surface and stained with toluidine blue. In each section the number of degranulated mast cells was determined and the result was expressed as the percentage of the total number of visible mast cells.

Experiments in vitro

Minced skin. The rats were bled under light ether anaesthesia. Pieces of shaved abdominal skin were removed, freed from subcutaneous fat, and minced with scissors. The mince was washed three times in an isotonic salt solution (NaCl, 154 mM; KCl, 2.7 mM; $CaCl_2$, 0.9 mM) containing 10% (v/v) Sörensen phosphate buffer ($Na_2HPO_4 + KH_2PO_4$, 6.7×10^{-2}M), pH 7.0, and divided into 0.25 g samples. These were placed in 25 ml Erlenmeyer flasks, suspended in 2.5 ml of the above salt solution, and incubated. At the end of the incubation the suspension was filtered through paper and the filtrate was collected for histamine assay. The tissue was rinsed on the

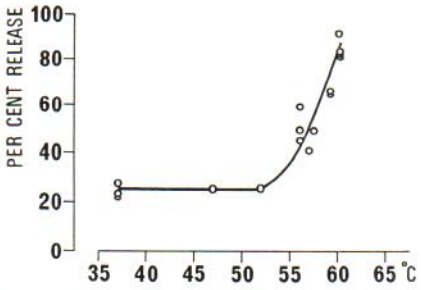

Fig. 1. Histamine release from minced skin after incubation at different temperatures for 30 min.

filter paper with buffered salt solution and then suspended in 0.1 N HCl and heated in a boiling water bath to extract the non-released histamine. Released and non-released histamine was determined, and the release was expressed as a percentage of the total amount of histamine present during the incubation.

Peritoneal cell suspension. Peritoneal cells were obtained as described in a previous paper (8). The salt solution used for washing and suspending the cells had the same composition as that used for the minced skin, except that human serum albumin, 1 mg/ml, was added. The suspension was divided into 2 ml aliquots, which were incubated in 15 ml centrifuge tubes. The incubation was terminated by cooling in iced water, and the samples were centrifuged at 350 *g* for 10 min. The non-released histamine was extracted by suspending the sediment in buffered salt solution and heating in a boiling water bath. Histamine was determined in both the supernatant and the sediment, and the release was expressed as the percentage of the total amount.

Mesentery. Pieces of mesentery were prepared and processed as described by Högberg & Uvnäs (11), and the percentage of degranulated mast cells was estimated using the light microscope.

Histamine assay. The histamine was assayed by the fluorometric method of Shore et al. (23). In the experiments with peritoneal cells the purification procedure was omitted and *o*-phthalaldehyde was added directly to the alkalinized samples (8). The *bee venom phosphatidase A*, identical to "F I" (7), was obtained by gel filtration on Sephadex G 50 of bee venom precipitated with picric acid and chromatographied on Amberlite IRC-10.

RESULTS

Mast cell degranulation in vivo

In the abdominal skin of rats immersed in water at 60°C for 20 sec, the percentage of mast cells which had undergone degranulation ranged between 25 (equal to the degranulation in control animals) and 70. Heating at 80°C for 20 sec invariably caused degranulation of about 70% of the mast cells.

Histamine release from minced skin

The "spontaneous" release of histamine from minced skin was high. About 35% of the skin histamine was lost during the mincing and washing procedures. Of the remaining histamine, 20–35% was recovered in the filtrate after 30 min incubation at 37°C.

Histamine release at different temperatures. The histamine release at different temperatures was measured after 30 min incubation. Below 52°C no more histamine was liberated than that "spontaneously" released. Above this temperature there was a steep increase in the release to a maximum of 85–90% at 60°C (Fig. 1).

Time course of the histamine release. When the minced skin was incubated at 56°C, the histamine release was almost linear during the first 60 min. During this period about 70% of the histamine was released. At 60°C the major part of the release occurred during the first 20 min and after 60 min more than 90% of the histamine had been released (Fig. 2).

Influence of inhibitors. The effect of two substances interfering with oxidative metabolism, 2, 4-dinitrophenol and potassium cyanide, was studied. Prior to the incubation at 60°C the skin samples were incubated for 20 min with the inhibitor in concentrations known to inhibit the histamine release mechanism activated by, for example, compound 48/80 (9, 11, 21). In no instance was an inhibition observed (Fig. 3). The spontaneous histamine release was not influenced by the presence of the inhibitors.

The possible release of a mast cell degranulating factor. If the heat-induced histamine release were mediated by a humoral factor, released or produced in the skin during heating, it might be

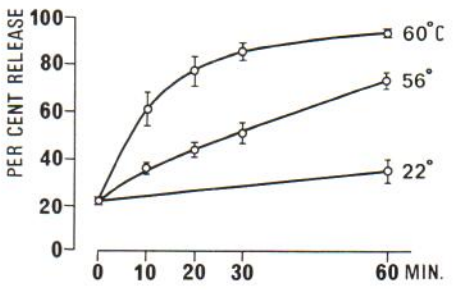

Fig. 2. Time course of the histamine release from minced skin during incubation at 22°, 56° and 60°C. 3 expts. The bars represent standard errors.

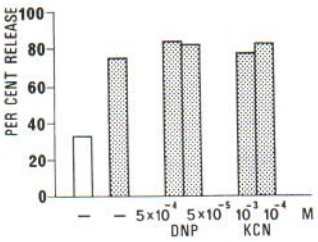

Fig. 3. Influence of dinitrophenol (DNP) and potassium cyanide (KCN) on the histamine release from rat skin caused by heating at 60°C for 30 min. Inhibitors were added 20 min before the temperature was raised. Shaded columns represent heated samples. The blank column represents samples at 0°C.

possible to demonstrate that factor in the suspension fluid after exposure of minced skin to heat. Therefore we carried out a series of experiments in which mesentery pieces were incubated in the filtrate from skin samples heated at 57°C for 30 min. However, there was no significant difference in degranulation of the mast cells in mesenteries treated with filtrate from heated skin and skin incubated at 22°C (Table I).

Histamine release induced by chemical agents. For comparison, two agents known to release histamine from rat skin, phosphatidase A from bee venom and compound 48/80, were tested. The releasing capacity of these agents was low, especially that of compound 48/80, which liberated maximally 30–35% of the histamine. The release induced by 48/80 was completed within the first 5 min of exposure, while phosphatidase A caused a slow release very much like that induced by moderate heat (Figs. 4, 5).

Table I. *Degranulation of mesentery mast cells exposed to the filtrate from heat-treated skin.*

Samples of minced skin (0.5 g in 5 ml of salt solution) were heated at 57°C for 30 min. The samples were filtered and pieces of mesentery were incubated in the filtrate for 20 min. Control pieces were incubated in the filtrate from minced skin kept at 22°C for 30 min

	Percentage degranulation	
Experiment no.	Treated with 57°-filtrate	Treated with 22°-filtrate
1	16	5
2	13	13
3	6	12
M	12	10

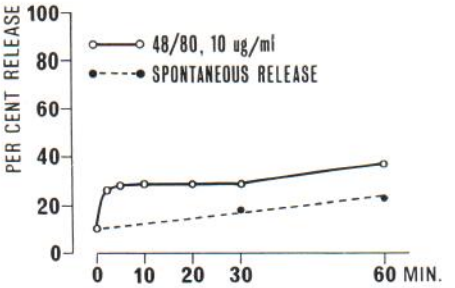

Fig. 4. Time course of histamine release from minced skin induced by compound 48/80. Temp. 37°C.

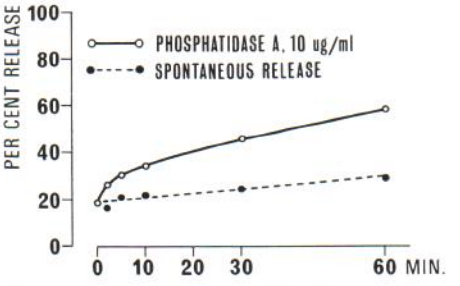

Fig. 5. Time course of histamine release from minced skin induced by phosphatidase A. Temp. 37°C.

Experiments with peritoneal cells

The heat-induced histamine release from *suspended peritoneal mast cells* is illustrated in Fig. 6. The temperature-response curve had virtually the same appearance as in the experiments with rat skin (Fig. 1); the threshold temperature for histamine release was 52–55°C, and total histamine depletion was obtained at 60°C. The time course of the heat-induced release was dependent on the temperature, but the "explosive" release found to occur on exposure to certain histamine liberators, e.g. 48/80 (5, 8), was never observed (Fig. 7).

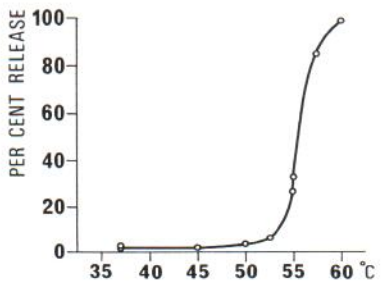

Fig. 6. Histamine release from peritoneal mast cells after incubation at different temperatures for 30 min.

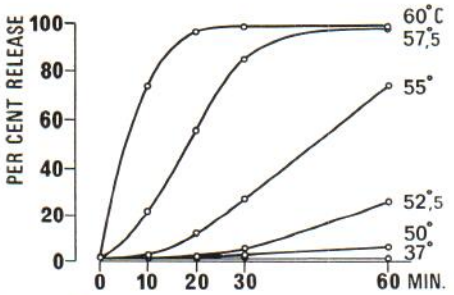

Fig. 7. Time course of the histamine release from peritoneal mast cells during incubation at different temperatures.

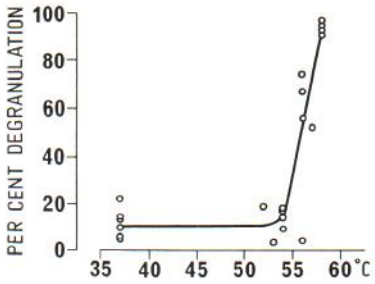

Fig. 9. Degranulation of mesentery mast cells after incubation at different temperatures for 20 min. The dots represent mean values of duplicate samples.

Peritoneal cells pretreated with dinitrophenol or potassium cyanide were exposed to 57°C. Neither of these agents had any influence on the amount of histamine released (Fig. 8).

Morphological alterations—degranulation—in *mesentery mast cells* were found to occur at temperatures above 55°C (Fig. 9). Dinitrophenol, 5×10^{-4} M, or potassium cyanide, 10^{-3} M, caused no inhibition of the morphological changes.

DISCUSSION

Histamine may play a role in inflammatory reactions including burns (26). Thus, the histamine antagonist mepyramine maleate can protect rats against the increase in capillary permeability caused by chemical as well as thermal injury (25). Since the histamine in all probability comes from the tissue mast cells, these might be expected to be degranulated in such injured states. As shown earlier (2) and confirmed in the present work, this is also the case.

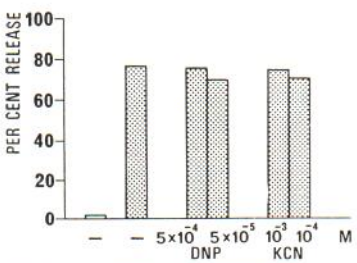

Fig. 8. Influence of dinitrophenol (DNP) and potassium cyanide (KCN) on the histamine release from peritoneal mast cells caused by heating at 57.5°C for 30 min. Inhibitors were added 20 min before the temperature was raised. Shaded columns represent heated samples. The blank column represents samples kept at 0°C.

The mast cell degranulation may be accomplished by a direct effect of heat on the mast cells or be caused by some chemical mediator appearing in the skin tissue during the exposure to heat. Biologically active agents are known to appear in these circumstances. For instance, a proteolytic enzyme is released from rat skin by thermal injury (3), and bradykinin appears in the perfusates from burned rat paws (20, 27). The hypothesis that histamine release is mediated by a humoral factor is supported by the observation (10) that exposure of rats to 58–62°C for 30 min leads to—among other symptoms—degranulation of the *peritoneal* mast cells. The results of the present study, however, do not support the concept of a chemical mediator of histamine release in heat injury. Histamine was released equally well from washed peritoneal mast cells and from skin pieces, and no mast cell degranulating activity was detectable in a filtrate of the fluid in which skin pieces had been heated. However, a short-lived compound, like bradykinin, would have escaped discovery in this test system.

Some histamine releasing agents, e.g. compound 48/80, act by triggering an energy-dependent, enzymatic process in the mast cells (28). The effect of heat might be of this type, or it might be a denaturation of essential components of the cells, resulting in leakage of histamine. Compound 48/80 has a weak histamine releasing effect on rat skin in vitro (9, 21), but this effect differs in important respects from that caused by heat. The time course for the 48/80-induced release is quite different from that for the heat-induced. The effect of compound 48/80 on rat skin can be blocked by dinitrophenol and potassium cyanide (9, 21), while the heat-induced release from skin

or from peritoneal mast cells was not inhibited by these substances. Moreover, the increase in the histamine release with increasing temperature is steeper than should be expected for an enzymatic reaction, and seems more compatible with the high temperature coefficients for protein denaturation. Thus in conclusion our data point to an unspecific mechanism of histamine release in thermal injury of the skin.

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