HISTOCHEMICAL PROPERTIES OF THE CARBOHYDRATE COMPONENT OF MAST CELLS OF THE DOG GASTRIC MUCOSA AFTER PROLONGED HISTAMINE STIMULATION

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During prolonged administration of histamine, three varieties of mast cells are found in the mucous membrane of the dog stomach. The histochemical characteristics of the first variety are the same as those of mast cells in the normal dog gastric mucosa. The second variety is characterized by accumulation of the water-soluble form of heparin, all the functional groups of which are ionized, in the granules. The third variety of mast cells is characterized by the presence of blocked anionic groups in the mast cell haloes. These anionic groups could be screened by exogenous histamine.

It is well known that exogenous histamine stimulates gastric acid secretion, but the mechanism of this process has not yet been explained. In experiments on dogs, rats, and guinea pigs receiving histamine over long periods [6, 10, 13, 18], attention was concentrated on the dynamics of structural elements in the fundal glands and the indices of gastric secretion; the effect of exogenous histamine on the mast cells (MC) of the mucous membrane of the dog's stomach was not examined in these papers. The writer considers that this is a vital aspect of the problem of the stimulating action of exogenous histamine, for, as many workers believe [1-3, 8, 11], it is histamine from the MC which is the ultimate chemical stimulator of the secretory cells of the gastric glands. The processes of accumulation and liberation of histamine are unquestionably connected with the nature and reactivity of the polyanionic component of the MC.

The object of the present investigation was to study the effect of exogenous histamine on MC of the gastric mucosa by examining the histochemical characteristics of their carbohydrate component.

EXPERIMENTAL METHOD

Experiments were carried out on four adult male dogs weighing 14-18.9 kg with a Basow gastric fistula. A histamine-wax mixture was given daily, every evening for 11-60 days, in a dose equivalent to 30 mg histamine base [7].*

Before the administration of the histamine-wax mixture began and throughout the period of chronic histamine stimulation, gastric biopsy specimens were taken from the whole thickness of the mucous membrane of the body of the stomach through the fistula every 3-6 days. Pieces were fixed in Hamperl's fluid and embedded in paraffin wax in the usual way. The material described in this paper also includes the results of investigation of MC of the gastric mucosa of 25 healthy dogs (control group).

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TABLE 1. Results of Histochemical Investigation of Carbohydrate Component of MC of Dog Gastric Mucosa during Prolonged Histamine Administration

Variety of mast cells	Basic brown	Testicular hyaluronidase + basic brown	Alcian blue, pH 1.0	PAS reaction	Azure I, pH 1.5	Distilled water + azure I, pH 1.5	Testicular hyaluronidase + azure I, pH 1.5	CCE method: alcian blue, pH 1.0 +NaCl (concn. in moles)						
								0	0,05	0,2	0,5	0,9	1	1,5	2
First															
Granules	2—3	2—3	1—3	0	2—3	2—3	2—3	2—3	2—3	2—3	2—3	3	3	2—3	0
Intergranular cytoplasm and haloes	1	0—1	1—2	0	1—2	0	0	1	1	1	1	1	0	0	0
Second															
Granules	2—3	2	1—3	0	2—3	1	1	3	3	1	1	1	1	1	0
Intergranular cytoplasm and haloes	1	0	1—2	0	1—2	0	0	2	2	1	1	1	0	0	0
Third															
Granules	2—3	2—3	1—3	0	2—3	2—3	2—3	2	2	2	2	3—4	3—4	2	0
Intergranular cytoplasm and haloes	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0

Legend: 0) no staining; 1) weak staining; 2) average intensity; 3) strong staining; 4) very strong.

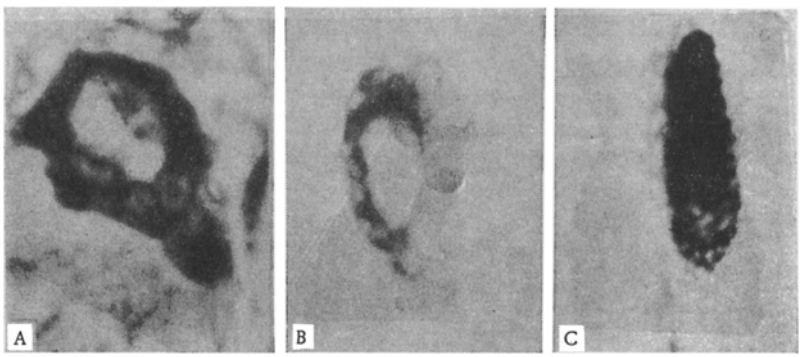

Fig. 1. Mast cells of dog gastric mucosa (azure I, pH 1.5). A, B) Mast cells of a dog receiving histamine injections daily: A) stained with azure I without preliminary incubation of the sections in distilled water; B) stained with azure I after preliminary treatment of sections with distilled water; C) mast cells of gastric mucosa of a dog not receiving histamine, stained with azure I after incubation in distilled water. Here and in Fig. 2, MBI-6 microscope, objective 100×, ocular 6×, total magnification 4000×.

The carbohydrate component of the TC was detected and its characteristics revealed by the PAS reaction, staining with basic brown at pH 1.0 [5], with alcian blue 8 gS at pH 1.0 [12] and at pH 2.7 [14], and staining with azure I at pH 1.5 [17]. As enzyme control testicular hyaluronate-liase was used, a working solution being made up in physiological saline [9]. To verify the specificity of action of the hyaluronate-liase, some sections were incubated for 3 h at 37°C (the same conditions as were used for treatment of the preparations with the enzyme) in distilled water, and other sections were incubated in physiological saline. To study the salt resistance of the biopolymer complexes and obtained information about the reactivity of the anionic groups of these complexes, the method [4, 16] of critical concentration of the electrolyte (CCE) was used.

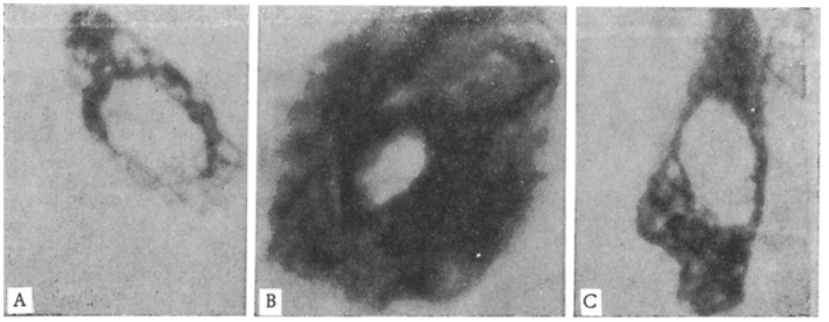

Fig. 2. Mast cells of mucous membrane of dogs receiving daily injections of histamine (stained by the critical electrolyte concentration method: 0.01% alcian blue solution, pH 1.0). A) No salt present in alcian blue solution; B) solution of alcian blue contains 0.05 M NaCl; C) solution of dye contains 0.9 M NaCl.

EXPERIMENTAL RESULTS

The results of the histochemical investigation are given in Table 1.

Sulfonate groups of the water-resistant fraction of heparin were found in the TC granules of the gastric mucosa of dogs of the control group. Sulfonate groups also were found in the intergranular cytoplasm and in the haloes of the mast cells (areas of stained amorphous substance located around the MC); these belonged to the water-soluble fraction of heparin. In the gastric mucosa of some dogs MC with mainly ionized functional groups of cytoplasmic heparin were found, while in others most MC had a large proportion of the anionic groups of their heparin screen in the cytoplasm. In the haloes only ionized anionic groups were found.

During chronic histamine stimulation three varieties of MC were found in the gastric mucosa of the dogs, two of which differed in their histochemical properties from the characteristics of normal MC.

Mast cells whose histochemical characteristics fully agreed with those of the MC in the normal gastric mucosa (MC of the first variety) were found in the dog Druzhok on the 5th, 13th, and 43rd day of chronic histamine administration, in the dog Mars on the 31st day, in the dog Kruzhok on the 3rd, 5th, and 6th day. and in the dog Gigant on the 11th, 16th, 21st, 38th, 56th, and 61st days of investigation.

Changes in the histochemical properties of the second variety of MC, found in the dog Druzhok on the 3rd day of chronic histamine administration and in the dog Gigant on the 26th, 34th, 45th, and 47th days, were essentially as follows. 1) Preliminary treatment of the sections with distilled water not only prevented staining with azure I (pH 1.5) of the haloes and intergranular cytoplasm of the MC (as normally), but also sharply reduced the intensity of staining of the cytoplasmic granules, which now have the appearance of weakly stained structures with indistinct boundaries (Fig. 1). 2) On investigation by the CCE method, a sharp decrease in the intensity of staining of the MC cytoplasm in 0.2 M alcian blue solution was found. The sharp decrease in the intensity of cytoplasmic staining was evidently due to breakdown of the complex of the dye and water-soluble heparin located in the granules.

No blocked sulfonate groups could be found in the cytoplasm of these MC.

The following features were characteristic of the third variety of MC, found in the dog Druzhok on the 28th day and in the dog Kruzhok on the 10th day of chronic histamine administration: azure I (pH 1.5) and alcian blue (pH 1.0) did not stain the intergranular cytoplasm or haloes of the MC. Loss of free functional groups could be due either to their disappearance from these structures or to blocking by tissue cations. The problem was solved by the CCE method: the mast cell haloes were detectable even with low concentrations of salt – 0.05 M NaCl (Fig. 2) – indicating that the haloes contain anionic groups blocked by tissue cationic substances. Besides ionized groups, the cytoplasm of these MC also contains screened SO_3H groups.

The histochemical characteristics of the first variety of MC found during chronic histamine stimulation thus agree with the histochemical characteristics of the MC in the normal gastric mucous membrane. This variety of MC is characterized by the accumulation of the water-soluble form of heparin, all

functional groups of which are ionized, in the granules, thus facilitating the formation of complexes between histamine and cytoplasmic biopolymers of the MC. The third variety of MC, which is rare during chronic histamine administration, is characterized by the presence of blocked anionic groups in the mast-cell haloes which dissociate in the presence of very low salt concentrations (0.05 M NaCl). These anionic groups are very probably screened by exogenous histamine.

The facts described above are evidence of the active response of the mast-cell apparatus of the gastric mucous membrane to administration of exogenous histamine.

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