EFFECT OF ACETYLCHOLINE ON MAST CELLS OF THE DURA MATER

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The effect of various doses of acetylcholine (100, 200, and 500 mg/kg) on the mast cells of the albino rat dura mater was studied electron-microscopically and by Falck's luminescence method. Qualitative and quantitative differences were found in the accumulation and elimination of monoamines depending on the dose of acetylcholine used, from which it is concluded that acetylcholine has an activating effect on monoamine secretion.

KEY WORDS: dura mater; mast cells; acetylcholine.

Stimulation of the parasympathetic nervous system has a significant effect on the monoamine content in the tissue depots. Some workers consider that acetylcholine activates [1, 3], whereas others, on the contrary, claim that the mediator inhibits secretion of monoamines [5, 6]. There is also a third point of view, according to which small doses stimulate whereas large doses block the liberation of biogenic amines [4].

The object of the present investigation was to study the response of mast cells (MC) and their content of monoamines to acetylcholine.

EXPERIMENTAL METHOD

The dura mater of sexually mature noninbred albino rats weighing 180-200 g was studied. Two groups of animals (with 20 rats in each group) served as the control: a) a group of intact rats (control 1) and b) a group of animals receiving an intraperitoneal injection of 1 ml physiological saline (control 2). The experiments were carried out on three groups of rats, with ten animals in each group, which received an intraperitoneal injection of acetylcholine made up in physiological saline. The rats of group 1 received a therapeutic dose (100 mg/kg), the rats of group 2 a toxic dose (200 mg/kg) and those of group 3 a lethal dose (500 mg/kg) of acetylcholine. The animals of the 1st and 2nd groups were killed 30 min after the injection of acetylcholine. Samples of tissue from rats receiving the lethal dose were studied after death of the animals, which took place in convulsions 5-10 min after injection of the drug. The dura mater was processed by Falck's method: It was stretched out on a slide, dried, and kept in standardized paraformaldehyde for 1 h at 80°C. The specimens were then examined under the LM-2 luminescence microscope, using DS-1 and SZS-7 filters. Pieces of dura for electron-microscopic investigation were fixed in 2.5% glutaraldehyde solution then stained with osmium in Millonig's mixture, dehydrated in alcohols of increasing concentration, and embedded in Épon-812. The sections were examined in the ÉMMA electron microscope and photographed.

The number of intact and degranulated cells was counted in 1 mm^2 of the dura processed by Falck's method. The mean area of cross sections of MC was calculated by the equation $S = LB$, where L is the length and B the breadth of the cell. The content of biogenic amines (serotonin, histamine, catecholamines) in MC was determined quantitatively *in toto* by means of the FMÉL-1A photometric attachment with a 0.5-mm probe for one cell. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

In the animals of both control groups the number, the area of cross section, and the intensity of luminescence of MC were identical (Table 1). The dimensions of most cells did

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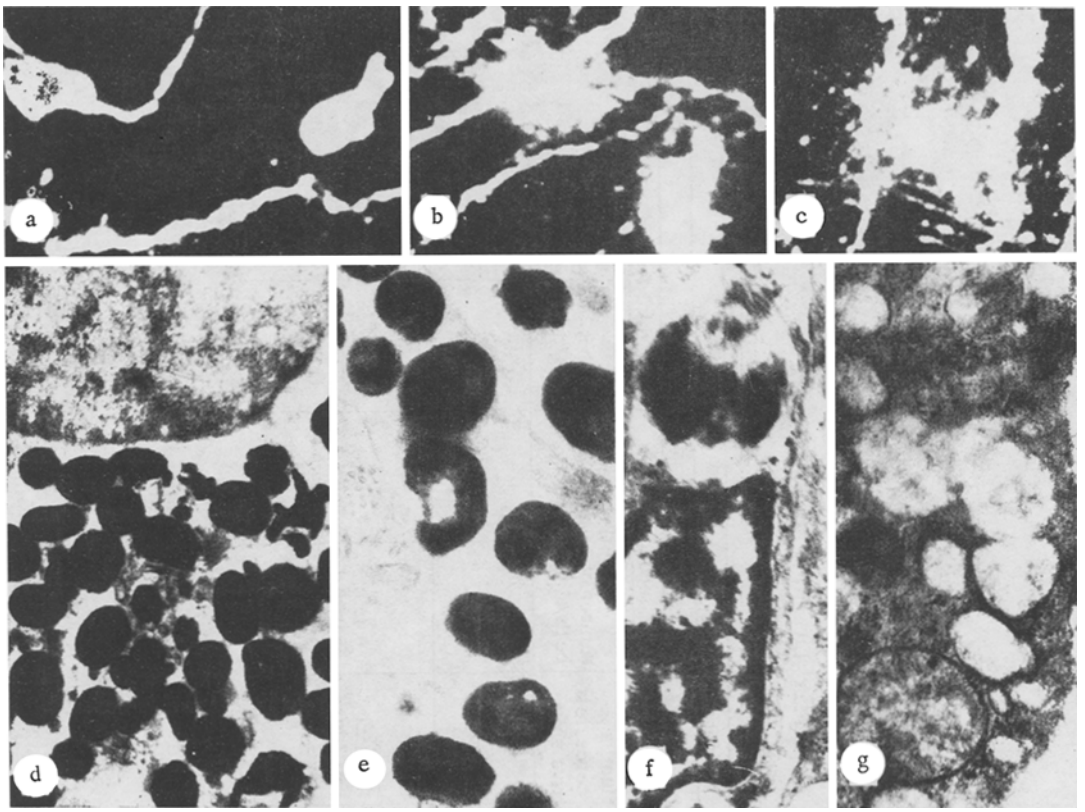

Fig. 1. Histochemistry and ultrastructure of mast cells from the dura mater. a) Intact mast cells (Falck's method; 400 ×); b) mast cells (1st type) after administration of toxic dose of acetylcholine (Falck's method, 400×); c) mast cell (3rd type) after injection of lethal dose of acetylcholine (Falck's method, 400×); d) intact mast cell (electron micrograph, 800×); e) massive liberation of granules from mast cell after injection of lethal dose of acetylcholine (electron micrograph, 10,000×); f) partial proteolysis of granules of mast cell after injection of therapeutic dose of acetylcholine (electron micrograph, 10,000×); g) total proteolysis of granules of mast cell after injection of toxic dose of acetylcholine (electron micrograph, 10,000×).

not exceed 8.5 ± 0.7 μ^2; their round or spindle-shaped body had distinct outlines and gave yellowish green luminescence (Fig. 1a). These undegranulated, conventionally intact cells, with a plasmalemma well defined over its whole extent, had a high concentration of granules with homogeneous electron-dense material (Fig. 1d). Fewer of the MC, with an area of cross section of 10.3 ± 0.5 μ^2, were degranulated, with small granules containing monoamines liberated into the intercellular space. In the degranulating MC the brightness of fluorescence was reduced and the perigranular spaces were widened and were partly confluent. The membrane surrounding these spaces joined with the plasmalemma to form channels through which single granules were eliminated.

Under the influence of acetylcholine the number of intact MC was considerably reduced and the number of degranulating cells increased proportionally (Table 1). According to several features (area of cross section, activity of liberation of granules, intensity of fluorescence), the degranulating MC could be subdivided into three types. MC of the first type were largest (16.2 ± 0.4 μ^2) and gave the brightest fluorescence. Their outlines were indistinct and irregular, with projecting granules, some of which were in the intercellular space (Fib. 1b). In the cells of the second type, with an area of cross section of 14.2 ± 0.4 μ^2, signs of degranulation were more marked. In the third type of MC (measuring 5.6 ± 0.2 μ^2), sharply reduced fluorescence was associated with the greatest liberation of granules: Numerous luminescent granules were concentrated around the cells (Fig. 1c). Under the luminescence microscope cells which had "completely disintegrated" into separate granules could be seen. Ultrastructurally, these MC evidently corresponded to cells with multiple "defects" of the plasmalemma through which, meanwhile, granules were expelled in large numbers (Fig. 1e). Be-

TABLE 1. Number of Mast Cells in Dura Mater and Intensity of Their Luminescence (M ± m)

Conditions	Mast cells									
	intact		degranulating							
			control				experiment			
					first type		second type		third type	
	number of cells/mm²	luminescence, relative units	number of cells/mm²	luminescence, relative units	number of cells/mm²	luminescence, relative units	number of cells/mm²	luminescence, relative units	number of cells/mm²	luminescence, relative units
Control 1	20,3±0,4	28,7±0,3	2,3±0,3	16,5±0,2	—	—	—	—	—	—
Control 2	20,5±0,5	28,7±0,3	2,1±0,9	16,7±0,3	—	—	—	—	—	—
Acetylcholine, mg/kg										
100	8,31±0,2	28,7±0,3	—	—	2,5±0,3	45,1±0,5	6,4±0,2	27,0±0,4	3,6±0,2	8,0±0,5
200	4,5±0,7	28,7±0,3	—	—	4,6±0,5	84,7±0,4	5,9±0,2	34,6±0,5	5,6±0,4	8,0±0,5
500	1,7±0,2	28,7±0,3	—	—	1,9±0,2	40,2±0,5	4,3±0,2	30,0±0,2	12,9±0,4	8,0±0,5

sides degranulating cells, MC with proteolysis of their granules, sometimes total, were found (Fig. 1f, g).

Administration of the therapeutic dose of acetylcholine (Table 1) led to the accumulation of biogenic amines in MC ($P < 0.01$), with a significant increase in the number of degranulating cells ($P < 0.001$). Both these phenomena increased in severity when acetylcholine was given in a toxic dose. The lethal dose led to abrupt exhaustion of the monoamine reserves with a decrease in the number of luminescent MC ($P < 0.01$) and a decrease in the intensity of their luminescence ($P < 0.001$).

Injection of different doses of acetylcholine thus led to substantial changes in the content of biogenic amines in MC, the depot of these substances in the tissues. When a lethal dose of acetylcholine was given the liberation of granules from MC increased and the reserves of monoamines in the cells were reduced, i.e., the experiment showed that large doses of acetylcholine do not have a blocking action on the liberation of biogenic amines as some workers have suggested [4-6], but lead to exhaustion of their reserves both in the tissue depots and in adrenergic nerves [2].

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