The Inhibitory Effect of Histamine on Mast Cell Damage Induced by Antihistamines

The demonstration of mast cell damage by antihistamines by SMITH[1] and MOTA and DIAS DA SILVA[2] provided an effective means for the experimental verification of the existence of an antagonism antihistamine × histamine at the mast cell level.

Material and methods. Rats (200 g) and guinea-pigs (250–300 g) of either sex were killed by a blow on the head and bled from the jugular veins. The mesentery was dissected out and divided into pieces, each of which was used as a sample. All samples were preincubated at 37 °C for 15 min in tyrode with or without histamine. After adding the antihistamine, the incubation was continued for 40 min more. For observation of mast cell damage the pieces of mesentery were fixed and stained and the cell damage assessed as described by MOTA and DIAS DA SILVA[2]. For the rat the results are given in percentages of mast cells presenting granule extrusion (magnification 500). For the guinea-pig the results are given as the mean content of mast cells counted in 30 microscopical fields (magnification 130). The following drugs were used: diphenhydramine hydrochloride (Benadryl, Parke-Davis), promethazine hydrochloride (Phenergan, Rhodia), chlorpromazine hydrochloride (Amplictil, Rhodia), chlorcyclizine hydrochloride (Perazil, Borroughs Wellcome), and histamine diphosphate[3].

Results. Rat: when rat mesentery was pre-incubated with 0.001 *M* histamine followed by addition of diphenhydramine 0.001 *M* the percentage of mast cells disrupted was reduced to 22.6 % of that of the samples treated with the antihistamine alone. After pre-treatment with 0.0001 *M* histamine no inhibition of mast cell disruption was seen (Table I). The inhibitory action of 0.001 *M* histamine was also reduced to 5.7% when diphenhydramine 0.0015 *M* was used, as compared to a 77.4% inhibition found when equimolar concentrations of both drugs were used. Diphenhydramine 0.003 *M* totally abolished the protection given by histamine (Table I).

Guinea-pig: in the guinea-pig mesentery, histamine also exerted an action protecting the mast cells against the damage induced by diphenhydramine, when both drugs were in the same concentration (0.00066 *M*). When the concentration of diphenhydramine was increased to 0.00099 *M* the inhibitory action of histamine (0.00066 *M*) was totally overcome (Table II). With promethazine (0.0001 *M*) the concentration of histamine had to be raised to 0.001 *M* in order to obtain a complete protection of the mast cells. Concentrations of 0.0005 and 0.00075 *M* exerted a partial protection only (Table III). We have found that chlorcyclizine (0.0001 *M*) induced a disappearance of guinea-pig mast cells. Histamine (0.0005 *M*) gave a partial protection, while at a concentration of 0.001 *M* the effect of the antihistamine was totally abolished (Table III).

Discussion. Our findings show that histamine inhibits mast cell damage induced by antihistamines. This protective action of histamine indicates clearly that there occurs an antagonism antihistamine × histamine not only at the peripheral receptors but also at the mast cell level. These experiments do not supply any information as to

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[3] The author thanks Dr. I. MOTA for the histamine and diphenhydramine, to Rhodia for the promethazine and chlorpromazine, and to Borroughs Wellcome for the chlorcyclizine kindly supplied.

Table I. Effect of histamine on rat mast cell damage induced by diphenhydramine

Diphenhydramine									
Histamine	1 m*M*			1.5 m*M*			3.0 m*M*		
	Average	Range	% Inhibition	Average	Range	% Inhibition	Average	Range	% Inhibition
0.1 m*M*	39.6 (6)	9.2–75.0	17.0						
1 m*M*	10.9 (9)	2.2–26.4	77.4	44.0 (3)	37.6–53.8	5.7	56.7 (3)	54.0–58.6	0
0	47.7 (9)	17.4–83.8	0						

The results are given as percentages of disrupted mast cells. Values in brackets indicate the number of experiments performed.

Table II. Effect of histamine on guinea-pig mast cell damage induced by diphenhydramine

Diphenhydramine						
Histamine	0.66 m*M*			0.99 m*M*		
	Average	Range	% of control	Average	Range	% of control
0.66 m*M*	35.4[a]	32.0–38.9	100	8.8	8.3–9.4	3.2
0	0.2	0.2– 0.2	0.7			
Control[b]	27.1	26.8–27.5	100			

The results are given as the mean count of mast cells in 30 microscopical fields. [a] Mean of 2 experiments. [b] Incubated in tyrode alone.

Table III. Effect of histamine on guinea-pig mast cell damage induced by promethazine or chlorcyclizine

Histamine	Promethazine 0.1 m*M*			Chlorcyclizine 0.1 m*M*		
	Average	Range	% of control	Average	Range	% of control
0	3.6 (6)	0.3–10.5	14.3	1.4[a]	1.4–1.4	5.4
0.1 m*M*	5.1 (4)	0.9–10.7	20.3	2.4	1.3–3.5	9.3
0.25 m*M*	5.7 (4)	1.0–18.2	22.7			
0.5 m*M*	12.8 (4)	2.4–23.2	51.0	5.3	4.6–6.1	20.6
0.75 m*M*	14.0 (2)	7.0–17.0	55.7			
1 m*M*	24.2 (6)	15.6–29.6	96.4	25.9	23.1–28.8	100
Control[b]	25.1 (6)	15.2–38.6	100	25.7	24.5–26.9	100

The results are given as the mean count of mast cells in 30 microscopical fields. Values in brackets indicate the number of experiments performed. [a] Mean of 2 experiments. [b] Incubated in tyrode alone.

whether the site of this antagonism is at the granule, cell membrane or some other cell site. However, since the high concentration of histamine normally found within the mast cell granule does not prevent the demaging action of antihistamines, these drugs are probably disrupting the mast cells and releasing histamine by a mechanism other than a direct displacement of the amine from the granules; exogenous histamine would thus be antagonising the antihistamines at a different site of the cell. Antihistamines with different chemical structures showed different ratios antihistamine:histamine (1:1 for diphenhydramine, and 1:10 for promethazine and chlorcyclizine) necessary for a complete protection of the mast cells. Preliminary experiments showed that chlorpromazine (0.0001 *M*), which has a much lower antihistamine activity than promethazine, damaged 100% of guinea-pig mast cells, but a concentration of 0.005 *M* of histamine was necessary for a complete protection of the mast cells, 5 times that necessary for promethazine.

The fact that the inhibitory action of histamine was rather easily overcome – it was sufficient to raise the ratio diphenhydramine:histamine from 1:1 to 1.5:1 for the protection to disappear – suggests that the histamine added is not very firmly bound to the cell. Our results do not imply as yet the existence of a relationship between chemical structure or potency of the antihistamine and antagonism by histamine at the mast cell level. More experiments are being performed to verify any such relationship and the nature and specificity of this antagonism.

Resumen. Se demonstra la capacidad de la histamina para inhibir las alteraciones de los mastocitos de la rata y cobayo producidas por diversos antihistamínicos (difenhidramina, prometazina, clorciclizina, clorpromazina). Diferentes concentraciones de histamina fueron necesarias para antagonizar los distintos antihistamínicos. Se sugiere la existencia de un antagonismo antihistamínico – histamina a nivel mastocitario.

I. Vugman

Departamento de Morfologia, Faculdade de Medicina, Ribeirao Prêto (Sao Paulo, Brasil), 30th December 1966.

Severe Alterations in Myelin Structure in Experimental Lymphogenous Encephalopathy

It has been reported in a series of papers that, in striking contrast to the text book opinion, lymphatics play a fundamental role in the fluid circulation of the brain[1-8]. A blockade of cervical lymph vessels and glands results in an experimental disease – lymphogenous encephalopathy – characterized by various neuropathological, biochemical and clinical signs.

Submicroscopic alterations in the grey substance of the brain are conspicuous 3 days after surgery; swelling of mitochondria, enlargement of the perivascular cisternae and the appearance of lysosomas. No alterations were found in the white matter at this time, however[9].

Further examinations of rats suffering from lymphogenous encephalopathy revealed that 7 days after surgery severe alterations in myelin structure became apparent.

Instead of geometrically regular concentric organization of the lamellae constituting the normal myelin

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