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HISTAMINE RELEASING ACTION OF POLYMYXIN B AND ITS ANALOGS

D. I. Bairamashvili, V. G. Voitenko, I. S. Gushchin, A. A. Zinchenko, A. I. Miroshnikov, and A. I. Zebrev

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Among mast cell activators special importance is attached to the peptide antibiotic polymyxin B (PB) and its analogs. First, it is important to establish the connection between the histamine-releasing activity (HRA) of the polymyxins with their structure and, correspondingly, to determine sites on the antibiotic responsible for triggering secretion. Second, PB and its derivatives can be used as standard activators of the target cells of allergy and inflammation in order to discover changes in the reactivity of these cells systems in patients with allergy. Third, PB can be useful to analyze the basic principles of the secretory process. In the modern view, triggering of the secretory process in the mast cell activator molecule requires the presence of free positive charges and of lipophilic regions capable of fixation on the cell membrane [2].

In this investigation, to study relations between HRA of a mast cell activator and its hydrophobic properties and charge, we used PB and its analogs, differing with respect to these parameters.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-300 g were used. Mast cells (90-95% purity) were isolated from a cell suspension obtained from the peritoneal and pleural cavities [5]. The experimental conditions, sources of reagents, and compositions of the solutions used to incubate the cells were described previously [3]. Histamine was determined by a fluorometric method [5]. Polymyxin B_1 (PB_1) was obtained by the HPLC method on an Ultrasphere C8 column (Alltex, USA) measuring 9.5 × 250 mm in a system of 0.1 M NaCl–HCl, pH 2.0, in water-methanol 25:75. Deacylated derivatives of PB – decapeptide (DPB), nonapeptide (NPB), and heptapeptide (HPB) – were obtained by enzymic hydrolysis of the original antibiotics [1, 4, 10]. The structure of the peptides was determined by amino acid analysis after hydrolysis with 5.6 N HCl, and confirmed by identification of the 11-terminal amino acid residues in the form of their dansyl derivatives. The individuality of the compounds tested was characterized by reverse-phase HPLC as described previously [4].

EXPERIMENTAL RESULTS

The compounds studied had the following structure: R–Dab–Dab–D–Phe–Leu–Dab–Dab–Thr, where for PB_1, R = $CH_3CH_2CH(CH_3)(CH_2)_4CO$–Dab–Thr–Dab, for DPB R = Dab–Thr–Dab, for NPB R = Thr–Dab, and for HPB R = H. PB [9] is a mixture of PB_1 and PB_2; for PB_2 R = $CH_3CH(CH_3)(CH_2)_4CO$–Dab–Thr–Dab. A characteristic feature of the ploymyxins is their amphilicity, due on the one hand to the presence of positively charged diaminobutyric acid (Dab) residues, and on the other hand to fatty acid, leucine, and phenylalanine residues. By using the chosen series of compounds (PB, PB_1, DPB, NPB, and HPB) it is possible to assess the contribution of all functionally important fragments of the molecule to its biological activity.

Institute of Immunology, Ministry of Health of the USSR. All-Union Antibiotics Research Institute, Ministry of the Medical and Biological Industry of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 447-449, April, 1989. Original article submitted March 15, 1988.

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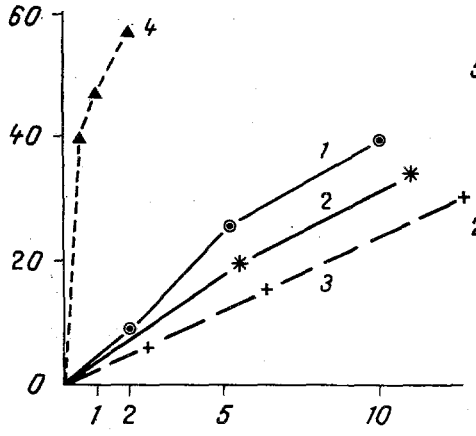

Fig. 1

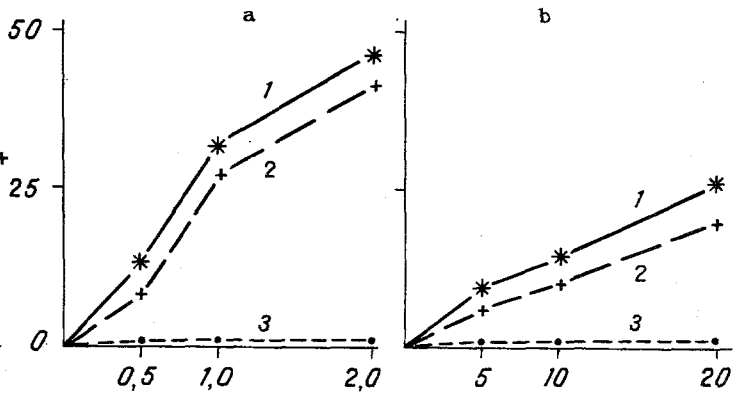

Fig. 2

Fig. 1. Histamine-releasing action of PB_1 (4), DPB (1), NPB (2), and HPB (3) on rat mast cells. Here and subsequently data are given with correction for the level of spontaneous histamine release, not exceeding 10%. Abscissa, concentration of PB_1 and its analogs (in μM); ordinate (here and subsequently), level of histamine release (in percent).

Fig. 2. Action of antimycin A on histamine release induced by PB_1 (a) and NPB (b). Mast cells were preincubated in 200 μl of medium at 37°C for 10 min without (1) and in the presence of antimycin A with 10 mM glucose (2) or without glucose (3). PB_1 or NPB was then added to the cells in concentrations indicated along the abscissa (in μM) and incubation continued for 5 min.

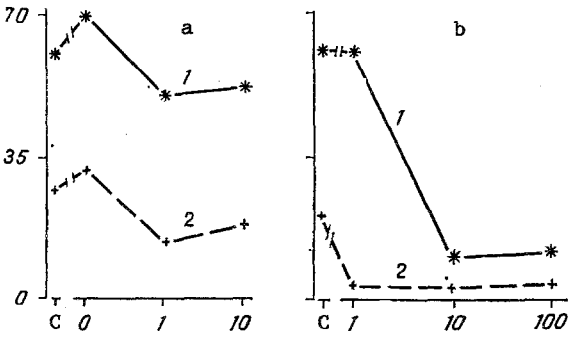

Fig. 3. Effect of LPS on histamine secretion from mast cells induced by PB_1: 1 μg/ml (1), 0.5 μg/ml (2). C) Control level of PB_1-induced histamine release, a) dependence of inhibitory action of LPS (1 μgβml) on duration of preincubation with PB_1 (in min, abscissa); b) dose-dependence of action of LPS, preincubated with PB_1 for 10 min before addition of the cells. Abscissa, LPS concentration (in μg/ml).

The dose-dependent histamine-releasing action of PB_1 and its analogs is illustrated in Fig. 1. Two circumstances deserve attention. First, successive shortening of the polymyxin chain leads to inhibition of HRA, but does not block it altogether. The dose–response curve is simply shifted toward an increase of concentration, but the character of the curve remains the same (reaching the same level of maximal histamine release) as for the original PB molecule. Hence it follows that the cyclic peptide part of the PB molecule plays an important role in the manifestation of HRA of the whole molecule. Second, the HRA level is largely determined by the presence of a hydrophobic acyl residue in the PB molecule. Since the acyl residue itself does not carry any free positive charges, but possesses marked lipophilic properties, it can be tentatively suggested that the hydrophobic regions of the polymyxins determine the level of their effector action. This hypothesis is confirmed by the inverse relationship discovered between the hydrophobicity constants of the substances tested, calculated by Rekker's method [7], and their HRA, expressed as the concentration of the substances required to release 30% of the mediator (ED_{30}) from the mast cells (Table 1).

TABLE 1. Dependence of HRA of PB and Its Analogs on Hydrophobicity and Charge

Polymyxin	ED_{30}, μM	Rekker's constant	Number of charges
PB	0,4	11,41	5
DPB	7,34	6,83	6
NPB	10,2	6,31	4
HPB	13,4	5,79	3

As Table 1 shows, positive correlation between the degree of HRA and the number of free positive charges was observed only in a number of deacylated derivatives of PB. It cannot be deduced from the results what is responsible for the HRA of the cyclic part of PB: the hydrophobicity of the amino acids, their charge, or the conformation of the ring, producing a definite spatial arrangement of functionally important groups.

Special experiments showed that PB_1 and NPB possess a selective (noncytotoxic) energy-dependent type of HRA (Fig. 2). Histamine release induced by PB_1 and NPB was inhibited in the same manner by the metabolic inhibitor antimycin A, i.e., under conditions leading to exhaustion of the ATP reserves in the mast cells [6]. Addition of glucose to the medium, restoring the intracellular ATP level through glycolysis [6], terminated the inhibitory action of antimycin A in both cases. These findings suggest that modification of the PB molecule does not lead to any change in its fundamental property as an activator of mediator secretion by the mast cells.

We know that PB is a specific antagonist of lipopolysaccharide (LPS), and binds with the A lipid of the LPS molecule [8]. Interaction of LPS with PB was found to inhibit the HRA of the antibiotic (Fig. 3). This modulation depended on the duration of preincubation and on the concentrations of PB and LPS. LPS had no HRA of its own, but in a dose of 10-100 mg/ml it blocked the HRA of PB. LPS had a similar inhibitory action in the case of preincubation with another mast cell activator – the polyamine substance 48/80. Meanwhile no inhibition of histamine release was found during preincubation of LPS with the calcium ionophore A23187. These data are in agreement with information published previously on the ability of polyamines to interact with LPS [11] and on the basic similarity of the mechanisms of action of PB and substance 48/80 [2].

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