CHARACTER OF THE HISTAMINE-LIBERATING ACTION OF MCD-PEPTIDE FROM BEE VENOM

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The histamine-liberating action of MCD-peptide and of mellitin, isolated from bee venom, and of substance 48/80 was studied on isolated rat mast cells. The action of MCD-peptide is similar to that of the noncytotoxic agent 48/80 in the character of the dose–response curves, latent period, and temperature-dependence of histamine liberation. The liberation of histamine induced by MCD-peptide is an energy-dependent process but is independent of cyclic AMP.

KEY WORDS: *MCD-peptide; mellitin; substance 48/80; mast cells; liberation of histamine.*

One of the most biologically active components of bee (*Apis mellifica*) venom is MCD-peptide, a polypeptide with 22 amino acid residues [5, 11]. On the one hand, this compound has high antiinflammatory activity, and on the other hand, like the principal polypeptide component of bee venom — mellitin — it has a histamine-liberating action. Both polypeptides liberate histamine from rat mast cells, but mellitin also liberates serotonin from rabbit platelets and possesses cytotoxic activity, inducing hemolysis, whereas MCD-peptide has no such action. The surface-active properties of mellitin can be explained on the basis of its structure. The structure of MCD-peptide differs from that of mellitin but is similar to that of the neurotoxin (apamine) of bee venom, which has neither cytotoxic nor histamine-liberating action [9].

To study the relationship between chemical structure and biological action of the physiologically active polypeptides of bee venom, the action of MCD-peptide, mellitin, and substance 48/80, a noncytotoxic histamine liberator, on rat mast cells was compared. Although the structures of MCD-peptide and of substance 48/80 differ, there is a reason to suppose that the mechanism of their action on mast cells is similar. It was therefore interesting to compare some indices of the mechanism of action of MCD-peptide with those of substance 48/80 studied previously [1, 3, 7].

EXPERIMENTAL METHOD

Mellitin and MCD-peptide were isolated by the method described in [8] from bee venom obtained from the Research Institute of Chemistry, Gor'kii University. The results of amino acid analysis, of determination of N-terminal amino acids, and of thin-layer chromatography on silica gel in a system of n-butanol–pyridine–acetic acid–water (30:20:6:24) demonstrated the individuality of the compounds. Mast cells were isolated [2, 7] from a suspension of cells from the peritoneal and thoracic cavities of male Wistar albino rats weighing 300-400 g. The scheme of the experiments on isolated mast cells (purity 90-95%) was described earlier [2]. Histamine was determined spectrofluorometrically [12] and liberation of histamine was expressed as a percentage of its total content in the portion of cells.

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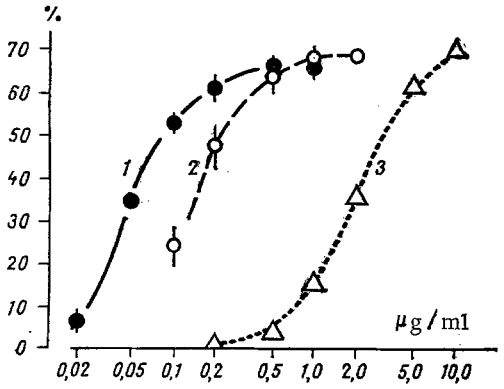

Fig. 1. Dose–response curves for MCD-peptide (1), substance 48/80 (2), and mellitin (3). Cells ($10 \cdot 10^3$–$15 \cdot 10^3$ mast cells per portion) incubated in presence of substance for 5 min at 38°C. Spontaneous liberation of histamine 1.8 ± 0.3%. Abscissa, concentration of substances (in μg/ml); ordinate, histamine liberation (in %).

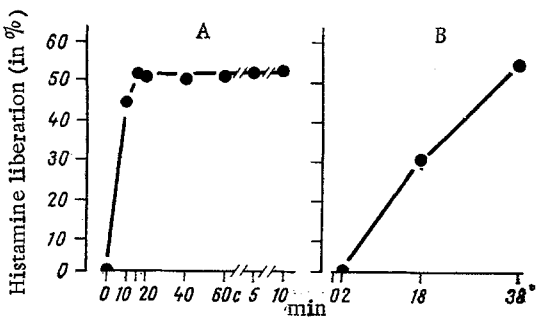

Fig. 2. Dependence of histamine liberation induced by MCD-peptide on time (A) and temperature (B). Cells incubated in presence of MCD-peptide (0.2 μg/ml) at 38°C (for A) and for 5 min (for B).

EXPERIMENTAL RESULTS

The study of the action of MCD-peptide, substance 48/80, and mellitin on mast cells showed (Fig. 1) that histamine liberation reached a maximum in the presence of comparable concentration of the first two compounds. About equal amounts of histamine were liberated in concentrations of MCD-peptide 40 times less than those of mellitin. At 38°C the latent period of the effect of MCD-peptide was under 10 sec (Fig. 2A) and liberation reached a maximum at 15 sec. The action of MCD-peptide was not manifested at a low temperature (2°C), and it increased sharply as the temperature rose to 38°C (Fig. 2B). In the absence of glucose, papaverine inhibited the effect of MCD-peptide to a degree that depended on dose, whereas glucose (10 mM) abolished the action of papaverine (Fig. 3A). In the presence of glucose, a combination of papaverine (0.3 mM) and iodoacetate (0.013 mM) in concentrations in which they themselves had no inhibitory action, considerably inhibited histamine liberation (Fig. 3A, I-III). Neither 1-methyl-3-isobutylxanthine (MIBX), which possesses antiphosphodiesterase activity [1, 7], nor its combination with prostaglandin E_1 (PGE_1) significantly inhibited the effects of MCD-peptide (Fig. 3B).

Similarity of the "dose–response" curves for MCD-peptide and substance 48/80 was thus found, in agreement with the observations of other workers [6, 10]. Weight for weight, MCD-peptide proved to be a more active histamine liberator than the cytotoxic component of bee venom (mellitin), and this also confirms the existing information [10]. MCD-peptide is a rapidly acting agent and the latent period of the response to it is similar to that of substance 48/80, inducing histamine liberation during the first few seconds after addition to the mast cells.

Activity of MCD-peptide, like that of other noncytotoxic histamine liberators, is completely inhibited at a low temperature and rises sharply as the temperature rises to 18 and 38°C.

The writers showed previously [1, 7] that papaverine, besides its antiphosphodiesterase activity, also has the ability to exhaust ATP reserves in mast cells in the absence of glucose (through inhibition of respiration) and so to depress the energy-dependent stage of noncytotoxic histamine liberation induced by substance 48/80 or by specific antigens [1, 2]. Addition of glucose to the medium, restoring the ATP reserves through the glycolytic metabolic pathway, abolished the inhibitory action of papaverine [1, 3, 7]. In the present investigation papaverine was found to have the same action on histamine liberation induced by MCD-peptide. In the presence of glucose the simultaneous administration of papaverine, inhibiting respiration, and of iodoacetate, inhibiting glycolysis, depressed histamine liberation induced by MCD-peptide. These results are thus evidence of the similarity between histamine liberation induced by the noncytotoxic agents and by MCP-peptide and of the dependence of both these reactions on the availability of energy.

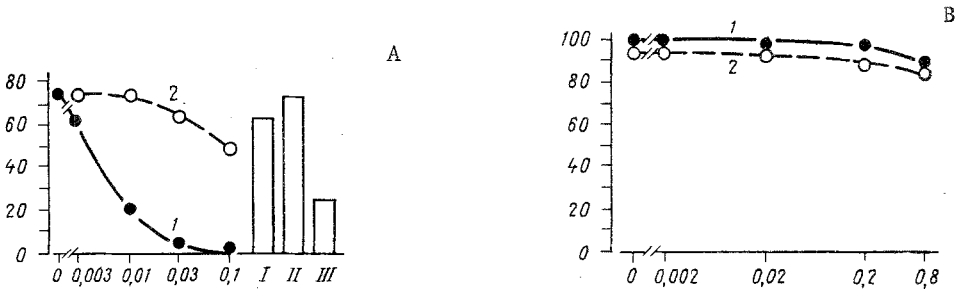

Fig. 3. Action of papaverine, MIBX, and MIBX + PGE_1 on histamine liberation induced by MCD-peptide. A) Action of papaverine: 1) in absence, 2) in presence of glucose (10 mM). In presence of glucose: I) papaverine (0.033 mM); II) iodoacetate (0.013 mM); III) papaverine (0.033 mM) + iodoacetate (0.013 mM). B) Action of MIBX (1) and MIBX + PGE_1 (1.4 mM) (2). Cells preincubated at 38°C in presence of inhibitors for 10 min. MCD-peptide (0.5 µg/ml) then added and incubation continued for a further 5 min. Abscissa, concentration (in mM) of papaverine (A) and MIBX (B); ordinate, histamine liberation in % (A) and in % of control (B). Histamine liberation in control (without MIBX and PGE_1) 64.5%.

The writers showed previously that the antiphosphodiesterase compound MIBX and the adenylate cyclase stimulator PGE_1 can increase the content of cyclic AMP in mast cells [1, 7]. MIBX, in concentrations up to 0.8 mM, caused a 20-fold increase in cyclic AMP, whereas PGE_1 (1.4 mM) increased the cyclic AMP content fivefold and potentiated the action of phosphodiesterase inhibitors. It was also shown that histamine liberation induced by substance 48/80 is independent (unlike anaphylactic liberation) of cyclic AMP [3, 7]. The present experiments showed that neither MIBX (in concentrations producing a marked increase in cyclic AMP) nor additional activation of adenylate cyclase by PGE_1 inhibited histamine liberation induced by MCD-peptide.

MCD-peptide is known also to have an antiinflammatory action which is unconnected with its ability to liberate histamine [4, 5]. In connection with the results indicating the noncytotoxic nature of the histamine-liberating action of MCD-peptide it is interesting to study analogs of this peptide obtained by chemical modification of the natural molecule, and to discover such differences as may exist between the histamine-liberating and antiinflammatory action of compounds of this series.

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