Rat peritoneal mast cells release dipeptidyl peptidase II

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Purified rat peritoneal mast cells have a 10–20-fold higher dipeptidyl peptidase II (DPP II) activity as compared with that of macrophages from the same source. Upon stimulation with the secretagogue Compound 48/80, DPP II is released from peritoneal-lavage cells and from purified mast cells, but not from purified macrophages, in a dose-dependent manner. Maximally, about one-third of the DPP II present in peritoneal-lavage cells is released. Substance P and the antigen/IgE system probably produce a similar effect. Both histamine and Zn^{2+} , two ingredients of mast-cell granules, strongly inhibit DPP II at concentrations reported to occur in the granules. A possible role of mast-cell DPP II in the remodelling of connective tissue is discussed.

INTRODUCTION

Dipeptidyl peptidase II (DPP II) has been demonstrated cytohistochemically in rat peritoneal and alveolar mast cells and macrophages [1,2]. The enzyme is exclusively found in granules or lysosomes in both cell types [1–3]. This is not surprising, because DPP II has been observed in lysosomes of many other tissues as well [4,5]. Although the secretory granules of mast cells contain several typically lysosomal hydrolases [6] and have an acidic pH [7], they can easily be distinguished from lysosomes [6]. DPP II seems to be restricted to a subtype of mast-cell granules [2,3] described as ‘heterophagic lysosomes’ [3]. The excretory, histamine-containing, granules give no cytochemical DPP II stain [2,3]. It was concluded from electron-microscopic studies that the DPP-II-containing granules are resistant to exocytosis after stimulation of mast cells with a bivalent-cation ionophore [3]. In contrast with these earlier studies we found that peritoneal-lavage cells seemed to be capable of releasing DPP II after stimulation with the neurohormone substance P. Substance P has been reported to induce the release of histamine from mast cells [8–10]. Therefore we undertook a biochemical study to evaluate the influence of histamine-releasing agents on a possible release of DPP II from mast cells.

MATERIALS AND METHODS

Materials

Bordetella pertussis was a gift from Dr. C. Simon (Universität Kiel, Kiel, Federal Republic of Germany). ϵ -Cbz-lysylproline was given by Dr A. Barth (Universität Halle, Halle, German Democratic Republic). Compound 48/80, theophylline and dibutyl cyclic AMP were purchased from Sigma (München, Germany), lysylalanyl β -naphthylamide from Bachem (Bubendorf, Switzerland) and ovalbumin from Serva (Heidelberg, Germany).

Isolation of cells

Peritoneal-cell suspensions were obtained from male rats (strain Han: WIST; weight about 350 g) by peritoneal lavage with a Tyrode buffer containing 137 mM-NaCl, 2.7 mM-KCl, 0.4 mM- NaH_2PO_4 , 1.8 mM- CaCl_2 , 1 mM- MgCl_2 , 5.6 mM-glucose and 10 mM-Hepes, pH 7.2. The suspension, washed once with this medium, is referred to as ‘peritoneal-lavage cells’. It contained about 5% mast cells as judged from optical microscopy after staining with Toluidine Blue.

For use in some experiments the mast cells were enriched further by a discontinuous-density-gradient centrifugation on a Percoll/sucrose gradient [11]. The cell layer found at the 65%/80%-Percoll interface contained about 90% viable mast cells.

Macrophages were enriched from the peritoneal suspensions by adherence to small plastic vessels. After keeping the cell suspension in these vessels for 1 h at 37 °C, the vessels were washed twice with Tyrode buffer to remove non-adherent cells. For the experiments with Compound 48/80 the vessels (containing about 5×10^5 adherent macrophages) were filled with 200 μl of Tyrode buffer and the desired amounts of Compound 48/80 (dissolved in 50 μl of Tyrode buffer).

Mast-cell-degranulation experiments

Cell suspensions (900 μl) in Tyrode buffer were incubated with 100 μl of a solution of the releasing agent in Tyrode buffer for 10 min at 37 °C. Then the cells were separated by centrifuging at 600 g for 10 min. The supernatant fluids were assayed for histamine and DPP II activity. The sediments were resuspended in 1 ml of water and the cells were destroyed by freezing and thawing before the residual histamine and DPP II were assayed.

If inhibitors of the mast-cell degranulation were used, 800 μl of the cell suspension in Tyrode were preincubated with 100 μl of the inhibitor (in Tyrode buffer) for 10 min at 37 °C before the releasing-agent solution (100 μl in Tyrode buffer) was added.

Abbreviations used: Cbz-, benzyloxycarbonyl; DPP II, dipeptidyl peptidase II (EC 3.4.14.2).

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Assay procedures

Histamine was determined by the fluorimetric procedure of Shore *et al.* [12], modified by omitting the extraction procedure [13] and by using citrate buffer [14].

DPP II activity was monitored with a photometric procedure originally described for dipeptidyl peptidase IV [15]. This assay was modified as follows. Lysylalanyl β -naphthylamide was used as substrate. The enzyme solutions (1 ml) were diluted with 0.2 M-cacodylate buffer, pH 5.5, containing 0.2 mM-substrate, and were incubated for 1 h at 37 °C. Care was taken that all individual assays of a series contained identical concentrations of salts, because of their inhibitory action on DPP II.

The amounts (*A*) of released histamine and DPP II are given as percentages, if not stated otherwise, and are calculated by the equation:

$$A = S \times 100 / (S + P)$$

where *S* means the amount of histamine or DPP II in the supernatant and *P* means the amount found in the sediment of the degranulation experiments described above.

Experiments with sensitized mast cells

To produce IgE-sensitized mast cells, male Wistar rats were immunized by intraperitoneal injection of *Bordetella pertussis* in Freund's adjuvant (1 ml) and simultaneous intramuscular injection of ovalbumin (1 mg) in 0.9% NaCl solution (200 μ l) as described by Mota [16]. At 3 weeks after these injections the rats obtained a booster dose of 100 μ g of ovalbumin in 100 μ l of 0.9% NaCl. The animals were killed 2 weeks after this last injection and the peritoneal-lavage cells were collected as described above.

RESULTS

Experiments with purified mast cells and macrophages

Rat peritoneal lavage is reported to contain more than 80% macrophages and about 5% mast cells [17]. Both types of cells contain DPP II [1–3]. From a rough quantitative study with purified mast cells and macrophages (Table 1) it can be concluded that both cell types contribute about one half to the total DPP II activity of the peritoneal lavage, i.e. mast cells have a 10–20-fold higher DPP II activity as compared with macrophages.

The release of DPP II from both purified mast cells and purified macrophages (Fig. 1) and the histamine release

Fig. 1. Release of DPP II by Compound 48/80 from mast cells purified by density-gradient centrifugation ($\square$, about 90% mast cells), from peritoneal-lavage cells ($\bullet$, about 5% mast cells) and (inset, same axis labels) from peritoneal macrophages purified by glass adherence ($\bullet$)

Results are means $\pm$ S.D. obtained with cells from four rats.

from purified mast cells is already high without stimulation by secretagogues. Since the basal percentage releases are much lower with peritoneal lavage cells, we conclude that the cells are partly damaged by the isolation procedure. Therefore most of the quantitative experiments have been performed with peritoneal-lavage cells (see below). However, in spite of this limitation, the results shown in Fig. 1 clearly indicate that Compound 48/80 exerts a DPP II-releasing effect on purified mast cells and not on purified macrophages.

Release of DPP II from peritoneal-lavage cells by Compound 48/80

Compound 48/80 is a polyamine that very effectively releases histamine and other ingredients of the secretory

Table 1. DPP II activity in mast cells and macrophages

The cells were destroyed by freezing and thawing before DPP II was assayed by the standard procedure at 37 °C.

Cell type	No. of expts.	DPP II activity (munits/ 10^6 cells) (mean $\pm$ S.D. or single result)
Peritoneal-lavage cells	7 (with seven rats)	2.4 $\pm$ 0.6
Purified mast cells	Single	
	No. 1	17.4
	No. 2	91.4
	No. 3	24.5
Purified macrophages	15 (with cells from three rats)	2.3 $\pm$ 0.12

Fig. 2. Release of histamine and DPP II from rat peritoneal mast cells by Compound 48/80

Results are means $\pm$ S.D. from three series of experiments with peritoneal-lavage cells from three rats. ●, Histamine release; ■, DPP II release. A volume of lavage containing about $(10-13) \times 10^3$ mast cells was used for a single histamine-release measurement; a 5-fold amount was used for a single DPP II-release measurement.

granules from mast cells [6]. If peritoneal-lavage cells containing about 5% mast cells are treated with Compound 48/80 over the usual concentration range, histamine is half-maximally released at about $0.3 \mu\text{M}$ (Fig. 2). This confirms observations published by others [8,9]. In the experiments depicted in Fig. 2 we simultaneously measured DPP II activity in the cell-free, histamine-containing, supernatants after stimulation with the releasing agent. Compound 48/80 significantly releases DPP II in a dose-dependent manner (cf. also Figs. 3, 4 and 7). However, there is no linear correlation between DPP II and histamine release (Fig. 3). A dose of $5 \mu\text{M}$ -Compound 48/80 releases a maximal amount of histamine (about 80%), whereas at the same concentration of Compound 48/80 only about one-third of the DPP II present in the lavage cells is released. (In contrast with Fig. 3, all other experimental data shown in the Figures are not corrected for the basic histamine and DPP II release.)

The possibility that DPP II activity in the cell-free supernatants is mimicked by some other released compound which could give a blue azo dye in the standard assay procedure is excluded by the results of kinetic assays: the production of the blue dye is linear with time and can be inhibited by ϵ -Cbz-lysylproline, a competitive inhibitor of both DPP II (G. Struckhoff, unpublished work) and dipeptidyl peptidase IV [18].

Theophylline [6] and dibutyryl cyclic AMP [13] are known as inhibitors of the degranulation of mast cells. Both compounds also decrease the amount of DPP II that

Fig. 3. Correlation between histamine release and DPP II release

The results presented here are derived from the experiments shown in Figs. 2 and 4. The basic release without secretagogue has been subtracted.

can be released from peritoneal-lavage cells by Compound 48/80 (Fig. 4).

Two of the low- M_r ingredients of mast-cell granules, namely histamine and Zn^{2+} , could be inhibitors of DPP II, because small cations generally inhibit this peptidase [4,5]. In the standard assay procedure, 0.1 M-histamine inhibits solubilized DPP II by 55%, and 10 mM- Zn^{2+} inhibits by 83%. A combined application of 0.4 M-histamine and 4 mM- Zn^{2+} inhibits by 85%, and 0.4 M-histamine plus 20 mM- Zn^{2+} inhibits by 94%.

Release of DPP II from peritoneal-lavage cells by natural mast-cell secretagogues

The most common natural mast-cell-degranulating event is believed to be the stimulation by the antigen/IgE system [6]. To evaluate whether, in such an event, DPP II is released too, we sensitized rats with *Bordetella pertussis* and ovalbumin. The peritoneal lavage from these rats, which should contain IgE-sensitized mast cells, was treated with various concentrations of ovalbumin which had been used to immunize the rats. One of three experiments of this type is shown in Fig. 5. Clearly DPP II is released by the antigen in a dose-dependent manner. However, the released percentage of DPP II is lower than that released by Compound 48/80.

A similar effect can be observed with Substance P (Fig. 6), which has frequently been discussed as a natural mast-cell secretagogue [8-10]: again, the DPP II release is dose-dependent and the maximal percentage of secreted enzyme is lower with substance P than with Compound 48/80.

DISCUSSION

DPP II has frequently been discussed as a typical lysosomal enzyme involved in the ingestion of proteins

Fig. 4. Inhibition of histamine release and DPP II release

(a) Inhibition by 1 mM-dibutyryl cyclic AMP; (b) inhibition by 3 mM-theophylline. The cells (peritoneal lavage) for the four series shown in (a) and (b) were each obtained from a single male rat. $\circ, \bullet$, histamine release; $\square, \blacksquare$, DPP II release; $\circ, \square$, values measured in the presence of inhibitor; $\bullet, \blacksquare$, no inhibitor. See the legend of Fig. 2 for further details.

[1–5, 19–21]. There are no reports on the secretion of DPP II save a preliminary observation: the epithelial cells of epididymis and ductus deferens seem to secrete DPP II into the duct lumen [21].

The results described here show that Compound 48/80 releases a certain amount of the intracellular DPP II from rat peritoneal mast cells. The secretion can be suppressed by typical inhibitors of mast-cell degranulation (Fig. 4).

Fig. 5. DPP II release and histamine release from peritoneal lavage cells by ovalbumin

The cells were obtained from a single rat sensitized with chicken ovalbumin. $\bullet$, histamine; $\square$, DPP II.

Fig. 6. Release of DPP II and histamine from peritoneal-lavage cells by substance P

Results are duplicates for cells from a single rat. $\bullet$, histamine release; $\blacksquare$, DPP II release.

After enzyme-histochemical staining of mast cells, DPP II does not show up in the normal excretory granules, but rather in perinuclear granules that may represent typical lysosomes [2]. If this is so, it may explain why the percentage of DPP II releasable with Compound 48/80 is relatively low: mast cells might accidentally lose some lysosomes during the excretion process.

On the other hand, the histochemical staining of mast cells for DPP II may be misleading because of the high contents of univalent cations and of Zn^{2+} in the secretory granules. Small cations are known as strong inhibitors of

DPP II [4,19]. It has been reported that a single mast cell contains about 25 pg of histamine and 2 [22]–10 fmol [23] of Zn^{2+} . The volume of a single cell is about $1000 \mu\text{m}^3$, 50% of which is represented by the granules [6]. Thus the intragranular concentration of histamine is about 0.4 M, and that of Zn^{2+} , 4–20 mM. As is shown above, these concentrations should inhibit the intragranular DPP II almost completely.

The proteins of mast-cell granules have previously been analysed by SDS/polyacrylamide-gel electrophoresis [24]. Two of the seven major protein bands, designated 'a₂' and 'a₃' [24], have a subunit M_r near that of rat lysosomal DPP II, namely about 65000 [25]. These proteins are less readily released from the granules as compared with other inclusions, including the smaller proteins [24]. This fact would provide another explanation for the low percentage of solubilized DPP II found in the experiments described here. A decision between the two possibilities described is not yet possible.

As shown in Fig. 3, there is no linear correlation between the releases of DPP II and histamine. This could mean that either the two compounds are secreted from different types of cells, or they are released from differing cell compartments, e.g. differing granules. The latter possibility would agree with the observation of Randell & Sannes [2]. However, the inhibition experiments (Fig. 4) point to similar, if not identical, mechanisms for the release of both mast-cell ingredients.

There remains the question of whether the secretion of DPP II is of any importance in the biochemistry of inflammation. The preliminary experiments shown in Figs. 5 and 6 indicate that natural mast-cell secretagogues similarly influence the release of DPP II, as is shown for Compound 48/80. We speculate that the secreted peptidase may be involved in an extracellular degradation of collagen *in vivo*. Several authors have proposed an involvement in collagen digestion as a natural function of DPP II [2,4,20]; however, this was believed to be an ingestive, intralysosomal, process. There exist numerous reports on the involvement of mast cells in the regulation of connective-tissue components. It has been reported that mast-cell proteinases degrade collagen, elastin, and other protein components of connective tissue [26]. Electron-microscopic studies suggest that exocytosed mast-cell granules are directly involved in the degradation of extracellular fibre structures [27]. DPP II appears to be an essential supplement to the machinery necessary for the extracellular degradation of proline-rich structural proteins by mast cells. The proline-rich peptides that are produced by the mast-cell proteinases from collagen should be especially good substrates for DPP II [4,20,25]. Mast cells and fibroblasts seem to interact in the remodelling of collagen structures [26,28].

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