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Enhancement of histamine release from human basophils pretreated with different sialidases

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Abstract

Histamine release from human basophils was investigated *in vitro* after removal of cell membrane sialic acid by three different sialidases. Pretreatment of the cells with sialidases from *Cl. Perfringens*, *V. Cholera* or *Influenza virus* A_2 enhanced histamine release induced by subsequent stimulation of the cells with anti-IgE or the plant lectin Concanavalin A and caused a shift to the left of the dose-response curve for anti-IgE. The enhanced histamine release was reflected in a increased calcium sensitivity, thus suggesting that cell membrane sialic acid might be involved in the calcium fluxes preceeding histamine release. In higher doses the sialidase from *Cl. Perfringens* caused the cells to release histamine by itself, whereas the sialidases from *V. Cholera* and *Influenza virus* A_2 in high doses inhibited the cell response to Concanavalin A.

Introduction

The enzyme sialidase (neuraminidase) cleaves off the ultimate carbohydrate moiety sialic acid (NANA) from adjacent carbohydrate moieties in the cell membrane. It is produced by many bacteria, viruses and mammalian cells [1] and has been widely used in the study of the significance of sialic acid in the cell membrane. In many cell systems cleaving off of NANA modulates the cellular response to stimulation, e.g. lymphocytes, muscle cells, neutrophils, rat mast cells and human basophils [2–6].

We have previously shown that pretreatment with sialidase from C1. Perfringens (CPN) of human basophils *in vitro* enhances histamine release induced by various stimulators [6]. Since the substrate specificity for the various microbial sialdases varies [1], we have in the present study investigated the action of sialidases from *Cl. Perfringens* (CPN), *V. Cholera* (VCN) and influenza A_2 virus (IAN) on histamine release induced by either anti-IgE or the plant lectin concanavalin A (Con A).

Materials and methods

4-methyl-umbelliferone, 4-methyl-umbelliferyl-α-D-N-acetyl-neuraminic acid, and 2-desoxy-2, 3-dehydro-N-acetylneuraminic acid were purchased from Sigma (St. Louis, USA), anti-human-IgE (ε-chain) 400,000 IU/ml was from Behringwerke AG (FRG), and concanavalin A (Con A) from Pharmacia AB (Sweden). Sialidases from *Cl. Perfringens* (CPN), *V. Cholera* (VCN) and Influenza virus A_2 (IAN) were obtained from Boehringer (Mannheim, FRG), Behringwerke AG (FRG) and Hoechst (FRG), respectively.

Eight normal individuals served as donors. They had no previous history of atopy and received no medication.

Preparation of cell suspension

Blood was drawn by venipucture, anticoagulated with EDTA in a final concentration of 0.01 *M* and mixed with double volume of 0.9% NaCl. Monocytes with approx. 2% basophils were isolated by the Ficoll-Hypaque gradient method, as described earlier [7]. In order to prevent histamine release by activation of plasma complement as a source of error, the cells were washed in Tris-AMC (albumin, magnesium, calcium) buffer containing Tris-(hydroxymethyl)-amino-methane 25 m*M* at pH 7.4, $CaCl_2$ 0.6 m*M*, NaCl 0.12 *M*, KCl 5 m*M*, $MgCl_2$ 1.1 m*M*, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml [7]. Thereafter the cells were washed in Tris- AMC (pH 5.6) containing 4 m*M* of $CaCl_2$ and the cells were counted (total count of lymphocytes, monocytes and basophils) and finally resuspended in Tris-AMC (pH 5.6) with 4 m*M* of $CaCl_2$.

Sialidase assay

The activity of the three sialidases was assayed by enzymatic cleavage of 4-methyl-umbelliferyl-α-D-N-acetyl-neuraminic acid to the fluorophor 4-methyl-umbelliferone [8, 9], and 4-methyl-embelliferone served as standard. 1 U of the enzyme was defined as the amount which

cleaved off 1 μmol NANA (sialic acid)/min and NANA was estimated as described earlier [6]. The assay was performed at 37° C for 40 min in Tris-AMC buffer (pH 5.6) including 4 m*M* of $CaCl_2$ corresponding to the experimental conditions used during pretreatment of the cells with sialidase.

Pretreatment of cells with sialidase

The cell suspension was incubated for 40 min at 37° C with sialidase. If not otherwise stated, CPN, VCN and IAN were used in amounts of 10 μU, 1 mU and 1 mU/10^6 cells, respectively. Thereafter 9 ml of ice-cold Tris-AMC (pH 7.4) containing 0.6 m*M* $CaCl_2$ was added and the cells were washed twice and resuspended in the buffer. The cell suspension was kept at 4° C before challenge with anti-IgE or Con A. Cell suspension incubated without sialidase served as control.

For the calcium experiments (Fig. 3) the cells were washed (150 g for 6 min at 4°C) free of calcium using Tris-A (pH 7.4) without $MgCl_2$ and $CaCl_2$, but with EDTA 5 m*M* and thereafter by Tris-AM (pH 7.4) without $CaCl_2$, and the cells were finally resuspended in Tris-AM (pH 7.4) with 0, 0.06, 0.6 or 1.8 m*M* $CaCl_2$.

To exclude that the effect of pretreatment of the cells with the sialidase preparations was caused by contaminating impurities such as proteases or phospholipase C, the synthetic sialidase inhibitor 2-desoxy-2,3-dehydro-N-acetyl-neuraminic acid (1 m*M*) was added to the enzyme before addition of the cells. This compound blocks the action of all known microbial sialidases and will thus reveal an effect produced by contaminants.

Histamine release

Challenge of the sialidase-pretreated cells with anti-IgE or Con A was performed by incubating 90 μl of the cell suspension with 10 μl of anti-IgE (400 IU/ml) or Con A (300 μg/ml) for 40 min at 37° C. In some experiments (Fig. 2), however, anti-IgE was used in different concentrations. The reaction was terminated by addition of 3 ml ice-cold Tris-A (pH 7.4) containing 1 m*M* EDTA, and histamine release was assayed by estimation of the residual histamine in the cell sediment, as previously described [7]. The stimulators were omitted for spontaneous histamine release and for determination of the total histamine content; in the latter case, the samples were not incubated at 37° C but left at 4° C. The release of histamine was expressed as a percentage of the total histamine content of the sample. Since spontaneous histamine release amounted to up to 10%, only histamine release > 10% was considered significant.

Statistics

Wilcoxon's ranking test for paired data was used.

Results

The influence of pretreatment of human basophils with sialidases was examined on histamine release *in vitro*. The actual enzyme activities were examined under our experimental conditions, using the fluorogenic substrate 4-methyl-umbelliferyl-α-D-N-acetyl-neuraminic acid. CPN and VCN showed an activity of approximately 1 U/ml which is in accordance with the data given by the manufacturer, but IAN showed only 1.2–1.6 U/ml in contrast to 315 U/ml stated by the manufacturer. Our determinations were used as the actual activities in this investigation.

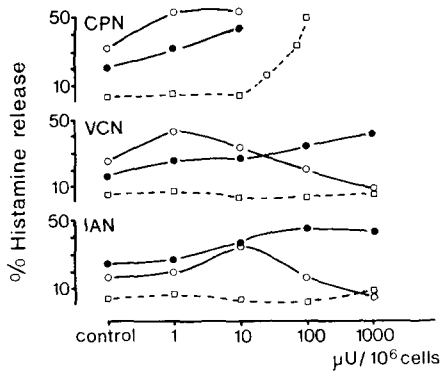

Figure 1
Modulation of histamine release by pretreatment of the cells with sialidase from Cl. Perfringens CPN, V. Cholera (VCN) and Influenza virus (IAN). Sialidase was used in increasing concentrations from 1–1000 μU/10^6 cells. After pretreatment the cells were washed and challenged with anti-IgE (●—●) or Con A (○—○). Spontaneous histamine release (□ .... □). Representative examples of 6 experiments.

By pretreatment of the cells with sialidase, CPN was found to release histamine when the amount of CPN exceeded 10 μU/10^6 cells (Fig. 1). No histamine release was found by VCN and IAN in amounts up to 1 mU/10^6 cells. Therefore, pretreatment with the sialidase preparations was designed using concentrations up to 10 μU and 1 mU, respectively.

Fig. 1 shows sialidase-pretreated cells challenged with anti-IgE or Con A. Pretreatment with any of the three sialidases significantly enhanced the release of histamine by anti-IgE ($p < 0.01$). The enhancing ability of the three sialidases proved to be similar, CPN showing perhaps a slightly greater potency.

When Con A was used as a stimulator, a diphasic response was obtained by VCN and IAN causing increased histamine release ($p < 0.01$) in the area of 1 or 10 μU sialidase/10^6 cells, respectively, and a decreased response by higher amounts of enzyme. CPN only increased the response ($p < 0.01$). A diphasic response was not observed in connection with anti-IgE.

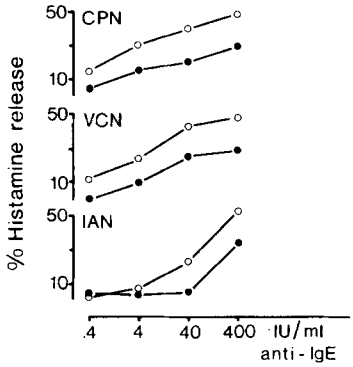

Figure 2
Effect of pretreatment of the cells with sialidase (CPN 10 μU; VCN and IAN 1 mU per 10^6 cells) on histamine release induced by anti-IgE. ●: control and ○: sialidase-treated. Histamine release was significantly enhanced ($p < 0.01$) in all points exceeding 10% release. A representative example of 6 experiments is given.

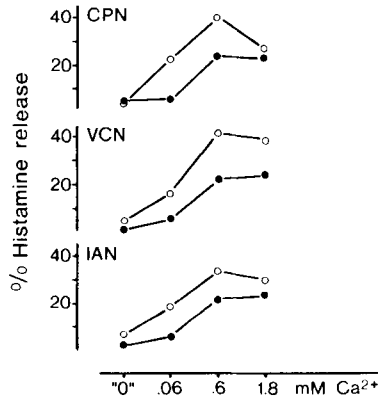

Figure 3
Effect of pretreatment of the cells with sialidase on histamine release as a function of extracellular calcium concentration. ●: control, ○: sialidase-treated. The cells were challenged with anti-IgE (40 IU/ml). Histamine release was significantly enhanced in all points exceeding 10% release ($p < 0.01$). A representative example of 6 experiments is given.

Fig. 2 shows that all three sialidases enhanced the maximal response to anti-IgE and caused a shift to the left of the dose-response curve. The amounts of enzyme used in these experiments were those causing maximal enhancement of the response to anti-IgE.

Histamine secretion from human basophils requires the presence of extracellular calcium [10]. Since sialic acid (NANA) is known to constitute a major calcium binding site in the cell membrane of muscle cells [11–13], we examined the effect of enzymatic removal of NANA from our cells on calcium-dependent histamine release. After pretreatment of the cells with sialidase, both free and membrane-associated calcium was removed by washing of the cells in a buffer containing 5 m*M* EDTA.

Fig. 3 shows that pretreatment of the cells with any of the sialidases markedly increased the sensitivity of the cells to extracellular calcium. At 0.06 m*M* of calcium, where untreated cells were unable to respond to anti-IgE, the sialidase-treated cells released histamine, thus showing a shift to the left of the calcium dependence curve. In order to ensure that the histamine release from the enzyme-treated cells were still calcium-dependent, experiments were included where the cells were resuspended in a buffer without calcium. None of the sialidase-treated cells (CPN, VCN or IAN) released histamine upon stimulation with anti-IgE under these low-calcium conditions.

Experiments were performed to exclude that contaminating impurities in the enzyme preparation such as proteases or phospholipase C were responsible for the changed cell response in the sialidase-pretreated cells. This was done by addition of the specific sialidase inhibitor 2-desoxy- 2,3-dehydro-N-acetyl-neuraminic acid to the enzyme before addition of the cells. The compound, which inhibits the action of all known microbial sialidases [14], completely abolished the effect of pretreatment of the cells with the three sialidase preparations (Table 1)

Table 1
Modulation of histamine release by pretreatment of the cells with sialidase is abolished by the specific sialidase inhibitor 2-desoxy-2, 3-dehydro-N-acetyl-neuraminic acid.

		% Histamine release			
		No inhibitor		Inhibitor	
		Control	Sialidase	Control	Sialidase
CPN 10 μU	anti-IgE	38 ± 3	49 ± 2*	37 ± 3	35 ± 3
	Con A	29 ± 1	40 ± 1*	31 ± 1	28 ± 1
VCN 1 mU	anti-IgE	38 ± 3	50 ± 4*	37 ± 3	34 ± 4
	Con A	29 ± 1	17 ± 2*	31 ± 1	27 ± 1
IAN 1 mU	anti-IgE	27 ± 3	39 ± 2*	27 ± 2	29 ± 2
	Con A	28 ± 1	14 ± 1*	29 ± 2	25 ± 2

Mean of 6 experiments ± SEM is given. *$p < 0.05$.

without influencing the histamine release in controls.

Discussion

Removal by sialidase of the ultimate, calcium-binding carbohydrate moiety N-acetyl-neuraminic acid (sialic acid, NANA) from the cell membrane has been widely used in the study of the significance of this negatively charged aminosugar in the membrane (for references see 1). The removal of NANA has been shown to enhance cell responses *in vitro* such as lymphocyte proliferation, neutrophil cytotoxicity, smooth muscle contraction and mediator release from rat mast cells and human basophils [1–6].

The enzyme sialidase, which cleaves off NANA from adjacent carbohydrate moieties, is produced by bacteria, viruses and mammalian cells [1]. The substrate specificity of the various microbial sialidases varies, depending on the nature of substrate and the type of linkage between NANA and the neighbouring carbohydrate [1, 15]. Thus, BACH & BRASHLER [5] have shown both similarities and differences in the action of various sialidases on rat mast cells. We have previously shown that pretreatment of human basophils with CPN enhances histamine release induced by subsequent stimulation with various stimulators [6]. In this study we compared the action of three different sialidases, two bacterial (CPN and VCN) and one viral (IAN). In order to compare the activity of the sialidases we determined the enzyme activity under our experimental conditions using 4 - methyl - umbelliferyl - α - D - N - acetyl - neuraminic acid, which has been shown to be a suitable substrate for all three sialidases [8, 9].

The histamine releasing capability of the sialidases proved to be different, since only CPN caused release of histamine by itself. We have previously shown that this release was caused by the enzyme and not by contaminants in the preparation [6]. Furthermore, it does not depend on extracellular calcium (data not shown) and may thus be due to a cytotoxic effect. The difference between the sialidases might be due to differences in substrate specificity, but the nature and conformation of the substrate involved in the cell membrane is as yet unknown.

The reason for the diphasic pattern in histamine release induced by Con A in cells pretreated with VCN or IAN also remains obscure. We have previously shown that Con A binds to carbohydrate moieties in the cell membrane irrespective of the presence of IgE molecules on the cell surface [16]. Furthermore, immunofluorescence microscopy using FITC-conjugated Con A revealed no difference in relative fluorescence on the cell surface between untreated cells and cells pretreated with either 10 μU or 1 mU per 10^6 cells of VCN or IAN (data not shown), indicating that the cellular binding of Con A was not impaired by treatment of the cells with sialidase.

Pretreatment of the cells with any of the sialidases enhanced the maximal response to subsequent stimulation with anti-IgE and also caused a shift to the left of the dose-response curve. NANA is present in the IgE receptor on rat mast cells and rat basophilic leukaemia cells [17, 18] and here plays a role in IgE binding and activation. However, no differences between untreated cells and cells pretreated with sialidase (CPN, VCN or IAN) were found in relative immunofluorescence using FITC-conjugated anti-IgE, indicating no change in cell-bound IgE by the enzymatic treatment (data not shown). It might therefore be speculated whether the enzymes act on NANA in the basophil IgE receptor thereby rendering the cell more alert to stimuli.

Another possibility is that NANA in the membrane is involved in the events taking place between antigen stimulation and histamine release. NANA together with membrane phospholipids provide a low affinity, high capacity reservoir for calcium [11, 13] and partial removal of NANA has been shown to increase the exchangeability for calcium in cultured heart cells and in guinea pig taenia coli [11–13]. It might therefore be possible that NANA in the membrane is involved in the transmembraneous calcium fluxes preceeding histamine release [10]. All three sialidases markedly increased the cell sensitivity for extra-cellular calcium, although there remained a need for calcium since cells in a calcium-free buffer were unable to respond to stimulation. Alterations in transmembraneous calcium fluxes might thus be a possible explanation of the enhanced response to stimulation with anti-IgE.

The action of the three sialidases on

histamine release seems to be similar, the only exception is that CPN in high doses releases histamine by itself. This may be due to an action on a substrate where VCN and IAN are inactive, but the nature of this substrate and the nature of the substrates where the three sialidases act by enhancing histamine release cannot be deducted from the present experiments.

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