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Carbohydrates inhibit the potentiating effect of bacteria, endotoxin and virus on basophil histamine release

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Abstract

Histamine release caused by calcium ionophore A23187 and anti-IgE was examined in leukocyte suspensions from 8 healthy individuals. *Staphylococcus aureus*, lipopolysaccharide (LPS) from *Salmonella typhimurium* and influenza A virus were found to enhance the histamine release but did not release histamine *per se*. The potentiation of mediator release depends on a non-transient signal since the potentiating effect was also obtained by preincubation of the cells with LPS followed by wash-out and stimulation of the cells with anti-IgE. The potentiation was abolished or reduced by galactose, N-acetyl-glucosamine, α-methyl-D-glucoside, α-methyl-D-mannoside, N-acetylneuraminic acid and lactose, but not by glucose. These findings indicate that the enhancement of mediator release by bacteria, endotoxin, and virus depends on a sugar-mediated reaction.

Introduction

Previously we have shown that bacteria, endotoxin, and virus enhance histamine release from human basophil leukocytes triggered by specific allergens and bacteria, and in some cases such an effect has also been examined in connection with anti-IgE or calcium ionophore A23187-induced histamine release [1–5]. Since carbohydrates have earlier been shown to inhibit bacteria-induced histamine release [1], in the present study we have examined whether they can inhibit the potentiating effect of bacteria, endotoxin, and virus on basophil histamine release.

Materials and methods

Blood was obtained from 8 healthy individuals. Leukocytes containing ca. 2% basophilocytes were isolated by the Ficoll-Hypaque gradient method, washed twice, and suspended in glucose-free Tris-AMC [6]. Basophil histamine release experiments were performed by incubation of 35 µl leukocyte suspensions for 40 min at 37 °C with 5 µl of the stimulator, i.e. the calcium ionophore A23187 (0.3×10^{-6} *M*, Calbiochem AG, Switzerland) or diluted anti-IgE (rabbit-antihuman IgE, ε-chain, 400 000 IU/ml, Behringwerke AG, FRG) and 10 µl glucose-free Tris-AMC. The release of histamine was assayed spectrofluorometrically by determination of the residual histamine in the washed cell sediment [6]. Only release of more than 10% of the total histamine content in the sample was considered significant, since the spontaneous release of histamine accounted for up to 10%.

A potentiating effect of bacteria, endotoxin or virus (potentiators) on the histamine release was examined by replacing 5 µl glucose-free Tris-AMC by: (1) whole formalin-killed *Staphylococcus aureus* Wood 46 (protein A-deficient) used in final concentrations from 0.03 to 0.3 mg/ml wet weight,

(2) *Salmonella typhimurium* LPS preparation of Westphal type (protein-free LPS) used in final concentrations from 0.01 to 1 μg/ml, or (3) human influenza A virus A/Caen/1/84 (H_3N_2) used in final concentrations from 10^{-5} to 10 ng viral protein/ml.

Abolition of the potentiating effect by carbohydrates was examined by replacing 5 μl glucose-free Tris AMC by D-(+)-galactose, N-acetyl-D-glucosamine, α-methyl-D-glucoside, α-methyl-D-mannoside, lactose or N-acetylneuraminic acid from Sigma, USA or Fluka AG, Switzerland. The carbohydrates were used in final concentrations from 10^{-8} to 10^{-5} *M*.

Statistics

Student's *t*-test was used when comparing samples with and without potentiating agent or carbohydrate.

Results

Potentiation by bacteria, endotoxin, and virus of histamine release

The influence of *S. aureus* on calcium ionophore A23187-induced basophil histamine release was examined in leukocyte suspensions from five normal individuals. The cells were incubated (40 min at 37 °C) with A23187 in a final concentration of 0.3×10^{-6} *M* to obtain a histamine release of less than 25%. When *S. aureus* was included in the samples in final concentrations from 0.03 to 0.3 mg bacterium per ml, the histamine release was enhanced. Maximal increase was obtained by 0.15 mg/ml which enhanced the release from 10% (absence of *S. aureus*) to 27% (Fig. 1 A). A potentiation of the A23187-induced histamine release was also obtained by *S. typhimurium* LPS, where the maximal effect, an increase of 72%, was obtained by 0.1 μg/ml LPS (Fig. 1 B), and by influenza A virus, which had a maximal effect (an increase of 81%) in the range of 10^{-3} to 10^{-1} ng viral protein/ml (Table 1). Table 1 shows that a similar potentiation was found in anti-IgE-induced histamine release which is in accordance with our earlier findings [1–5]. In all the experiments bacteria, endotoxin, and virus enhanced the histamine release significantly ($p < 0.01$), and the potentiation was due to synergism since *S. aureus, S. typhimurium* LPS, and influenza A virus did not release histamine *per se*.

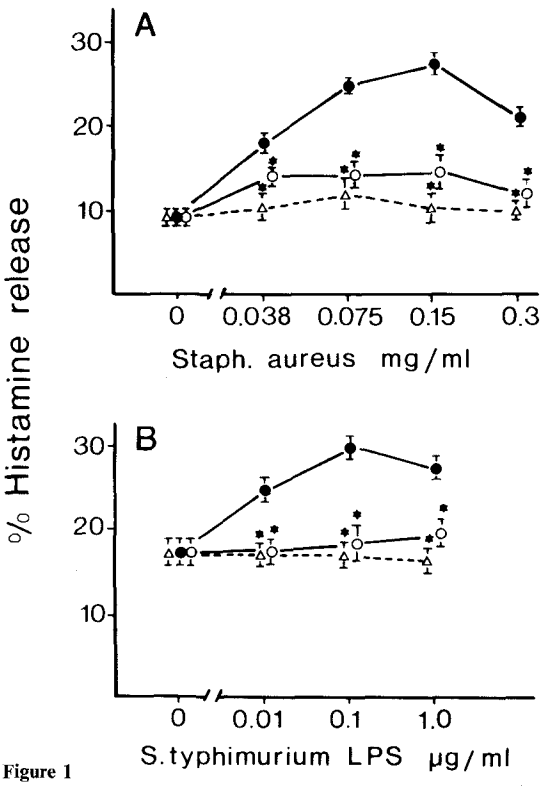

Figure 1
Potentiation of histamine release and abolition of the potentiating effect by carbohydrates. Histamine release is induced by A23187 in leukocyte suspensions from 6 normal individuals. The mediator release is enhanced by *Staph. aureus* (A) and by LPS from *S. typhimurium* (B). Abolition of the potentiating effect by galactose 10^{-6} *M* (△) and by 10^{-7} *M* (○). Mean ± SEM of six experiments in duplicate is given, * $p < 0.01$.

To elucidate whether potentiation depends on a transient or non-transient signal after binding of the potentiator to the cell surface, the cells were preincubated (37 °C, 10 min) with *S. typhimurium* LPS, washed, and kept at 37 °C for 30 min in Tris-AMC, and then stimulated with anti-IgE. This procedure did not change the potentiating effect of the LPS, indicating a non-transient signal in the potentiation of histamine release.

Abolition of the potentiating effect by carbohydrates

The influence of carbohydrates on the potentiating effect of bacteria, endotoxin, and virus was exam-

Table 1
Potentiation by bacteria, endotoxin and virus of basophil histamine release triggered by A23187 or anti-IgE and abolition of the potentiating effect by galactose and N-acetylglucosamine.

Stimulator	Potentiator	% increase by potentiator	% inhibition of this increase by:			
			galactose		N-acetyl-glucosamine	
			$10^{-6}M$	$10^{-7}M$	$10^{-5}M$	$10^{-6}M$
A23187	*S. aureus*	270	95	70	100	98
	LPS	72	104	92	73	71
	Influenza A virus	81	99	97	104	63
Anti-IgE	*S. aureus*	120	83	55	77	69
	LPS	96	100	92	100	87
	Influenza A virus	85	100	69	93	84

Values are means of 5–6 experiments using cells from normal individuals. Duplicate determinations.
Note that the stimulators were used in concentrations causing <25% histamine release and that the potentiators *per se* caused no release of histamine. In all experiments the potentiating effect and its abolition by the carbohydrates was significant ($p < 0.01$).

ined in parallel with the experiments mentioned above. When galactose was added to the cells together with A23187 and *S. aureus* the potentiating effect of the bacterium was abolished by the carbohydrate (Fig. 1 A and Table 1). A complete abolition was obtained with galactose in a final concentration of 10^{-6} *M*, while 10^{-7} *M* caused a significant inhibition of 70% ($p<0.01$). In these concentrations galactose also abolished the potentiating effect of both *S. typhimurium* LPS and influenza A virus on A23187-induced histamine release (Fig. 1 B and Table 1). When similar experiments were performed in connection with anti-IgE-induced histamine release the potentiating effect of *S. aureus*, *S. typhimurium* LPS, and influenza A virus was also abolished or reduced by the carbohydrate (Table 1). In other experiments galactose was replaced by N-acetylglucosamine (10^{-5} *M*) which caused a similar inhibition of the potentiating effect (Table 1). This was also the case when α-methyl-D-glucoside, α-methyl-D-mannoside, N-acetylneuraminic acid and lactose was examined in concentrations of 10^{-6} to 10^{-5} *M* in connection with anti-IgE and influenza A virus, but glucose (10^{-6} to 10^{-4} *M*) did not influence the potentiation (results not shown). The abolition of the potentiation was still maintained when the cells were preincubated (37 °C, 10 min) with galactose and then washed and exposed to anti-IgE and virus indicating a binding of carbohydrate to the cell.

Discussion

We have found previously that allergic (IgE-mediated) histamine release is enhanced by bacteria, endotoxins, and virus [1–3]. It is tempting to speculate that this potentiation of mediator release might play a role in airway diseases since upper respiratory tract infections have frequently been shown to precipitate or exacerbate attacks of bronchial asthma. In the present study the potentiation of both IgE-mediated- and non-immunological histamine release was investigated and it was established that bacteria, endotoxin and virus enhanced basophil histamine release triggered by anti-IgE and the calcium ionophore A23187. The potentiation was due to synergism, since the microorganisms and LPS in the concentrations used caused no release of histamine *per se*. It is dependent upon a non-transient signal or event since the potentiating effect was still maintained when the cells previously had been exposed to the potentiator and then to the stimulator after wash out.
Influenza A virus causes potentiation by its neuraminidase [3, 5] which contains carbohydrates such as galactose and N-acetylglucosamine [7]. Endotoxins as *S. typhimurium* LPS also contain these sugars [8], and N-acetylglucosamine is found in the peptidoglycan structure of bacteria [9]. We, therefore, investigated whether the potentiation could be due to a carbohydrate-dependent step in which microbial sugars bind to and interact with binding

sites on the cell membrane leading to potentiation. An indirect proof was the findings that galactose and N-acetylglucosamine abolished the potentiation by bacteria, endotoxin, and virus of histamine release triggered by anti-IgE and A23187. Also lectin-binding sugars as α-methyl-D-glucoside, α-methyl-D-mannoside and, furthermore, N-acetylneuraminic acid and lactose, but not glucose, were able to inhibit the potentiation. It is, therefore, possible that the potentiation depends on a sugar-mediated reaction. If a potentiation of mediator release plays a crucial role in the aggravation of asthma, carbohydrates might be a new treatment when infections aggravate asthmatic attack. A clinical trial with galactose is now being performed at the Rigshospitalet, Copenhagen.

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