

Bacteria and endotoxin enhance basophil histamine release and potentiation is abolished by carbohydrates

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Histamine release caused by anti-IgE, specific antigens and calcium ionophore A23187 was examined in leukocyte suspensions from healthy individuals and patients allergic to house dust mite and birch pollen. *Staphylococcus aureus* and LPS from *Salmonella typhimurium* were found to cause a synergistic enhancement of the release. The potentiation of mediator release by the bacteria and the endotoxin depends on a binding to the basophilocyte, followed by a non-transient event, since the potentiating effect persists after preincubation of the cells with the LPS followed by washout and leaving the cells for 30 min at 37°C before stimulation with anti-IgE. The potentiation was abolished or reduced by galactose (10^{-7} and 10^{-6} M) and N-acetylglucosamine (10^{-6} and 10^{-5} M), acting by a binding to the basophil cell membrane, demonstrated by the persistence of effect after preincubation and washout of unbound sugar.

Key words: allergic asthma; antigen; anti-IgE; bacteria; basophils; calcium ionophore; endotoxin; histamine release; galactose; inhibition; neuraminidase; potentiation; sugars.

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CLINICAL ASPECTS

Infections seem to play a role in the aggravation of airways diseases, since upper respiratory tract infections frequently precipitate or exacerbate attacks of bronchial asthma. The enhancement by bacteria and endotoxin of the mediator release might be an important mechanism in the aggravation of asthma during infections. That sugars, in very low concentrations, prevent the enhancement of mediator release gives a hint of a new treatment when bacterial infections aggravate bronchial asthma.

Upper respiratory tract infections have frequently been shown to precipitate or exacerbate attacks of bronchial asthma. It is therefore possible that infections play a role in airway hyperreactivity. Bacteria-induced histamine release has been observed in human lung and tonsillar mast cells (2), in nasal mucosa (3) and in superficially lying mast cells in the airways epithelium obtained by bronchoalveolar lavage (4). Further, bacteria and endotoxins were found to release histamine from basophils (5, 13, 14) and also to enhance the

allergic histamine release (10, 11, 12). Since a potentiation of mediator release by bacteria and their components could be involved in the aggravation of bronchial asthma during infections, we examined the potentiation and its abolition in connection with basophil histamine release.

MATERIAL AND METHODS

Eight healthy individuals aged between 30 and 55 years served as donors. None had any previ-

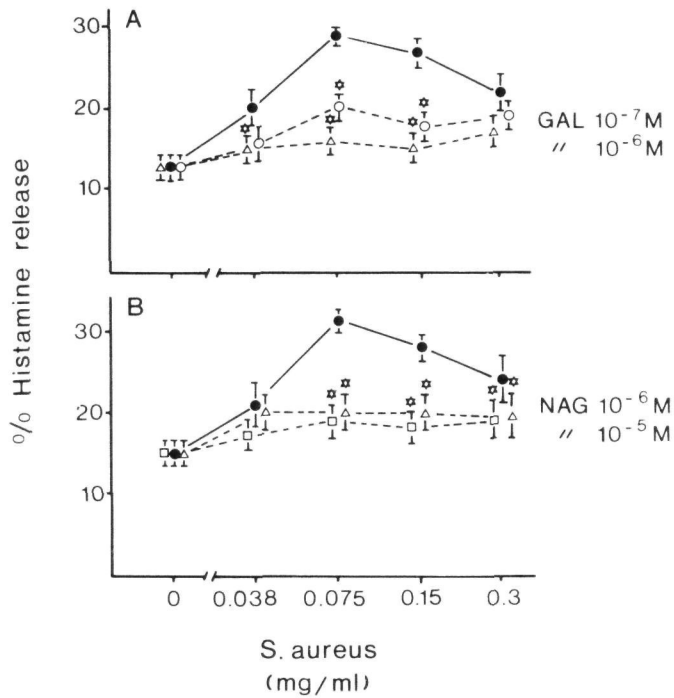


Fig. 1. Potentiation by *S. aureus* of anti-IgE induced histamine release and abolition of the potentiating effect by sugars. Histamine release was induced by anti-IgE in leukocyte suspensions from 6 normal individuals. The mediator release was enhanced by *S. aureus* (●) and the enhancement was inhibited by (A) galactose (GAL) 10^{-7} and 10^{-6} M and (B) N-acetylglucosamine (NAG) 10^{-6} and 10^{-5} M. Mean $\pm$ SEM for the 6 experiments in duplicate; * $P < 0.01$. *S. aureus* did not release histamine *per se*.

ous history of atopy and they received no medication. Six patients suffering from bronchial asthma and hay fever, and with a well-documented history of allergy to either house dust mite or birch pollen, were also included.

The bacteria used were whole formalin-killed *Staphylococcus aureus* Wood 46 (protein A-deficient), prepared according to (5) and as endotoxin was used *Salmonella typhimurium* LPS preparation of Westphal type (protein-free lipopolysaccharide) from Difco Laboratories, Detroit, USA. D-(+)-galactose, D-(+)-glucose and N-acetyl-D-glucosamine were purchased from Sigma, St. Louis, USA. Calcium ionophore A 23187 was purchased from Calbiochem AG and anti-IgE (rabbit-antihuman IgE, ϵ -chain, 400,000 IU/ml) was from Behringwerke AG, FRG. House dust mite (*Dermatophagoides pteronyssinus*) and birch pollen (*Betula verrucosa*), both Soluprick-SQ 3HEP, were from ALK, Denmark.

Preparation of cell suspension

Blood was drawn by venipuncture, 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 (18). The leukocytes were washed twice and suspended in a glucose-free Tris-AMC containing 25 mM Tris at pH 7.6, 0.12 M NaCl, 5 mM KCl, 0.6 mM CaCl_2 , 1.1 mM MgCl_2 , and 0.3 mg of human serum albumin/ml (18).

Histamine release

Histamine release experiments were performed by incubation of 35 μl leukocyte suspension for 40 min at 37°C with 5 μl of diluted anti-IgE, allergen or calcium ionophore A23187 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 (18). Only release of $> 10\%$ of the total histamine content in the sample was considered significant, since the spontaneous release of histamine amounted to up to 10%. A potentiating effect of *S. aureus* or *S. typhimurium* LPS on the histamine release was examined by replacing 5 μl glucose-free Tris-AMC by dilutions of the bacterium or the endotoxin. To examine whether carbohydrates were able to inhibit the potentiating effect, 5 μl glucose-free Tris-AMC was replaced by 5 μl dilutions of galactose, N-acetyl-D-glucosamine or glucose.

In some experiments the potentiating effect of the endotoxin was examined by preincubating the cells for 10 min at 37°C with *S. typhimurium* LPS and after washout the cells were left at 37°C for 30 min before incubation (37°C , 40 min) with anti-IgE. In other experiments the cells were preincubated with galactose (37°C , 10 min), washed and incubated for 40 min at 37°C with anti-IgE plus endotoxin, to study the effect of galactose.

Table 1

Basophil histamine release induced by <i>S. aureus</i> and <i>S. typhimurium</i> LPS in cell suspensions from normal individuals				
<i>S. aureus</i> (mg/ml)	0.038	0.075	0.15	0.3
% Histamine release (Mean ±SEM of 8 exp.)	0	2.3 ± 0.9	2.4 ± 0.9	3.4 ± 1.2
<i>S. typhimurium</i> LPS (µg/ml)	0.01	0.1	1.0	
% Histamine release (Mean ±SEM of 12 exp.)	2.3 ± 0.5	1.7 ± 0.3	2.0 ± 0.4	

Statistics

Student's t-test was used when comparing samples with and without bacteria, endotoxin or carbohydrate.

RESULTS

Potentialiation of basophil histamine release and inhibition of the potentiating effect was exam-

ined in leukocyte suspensions from six normal individuals. The histamine release was triggered by incubating the cells with anti-IgE for 40 min at 37°C. Final dilutions of 10⁻⁵ to 10⁻⁴ were used to obtain a low release of histamine (< 25 %). When *S. aureus* was included the mediator release was enhanced and maximal enhancement (110 and 123 %) was obtained in a final concentration of 0.075 mg/ml wet weight of the whole bacterium (Fig. 1). The potentiating effect of the bacterium was due to synergism since *S. aureus* in concentrations from

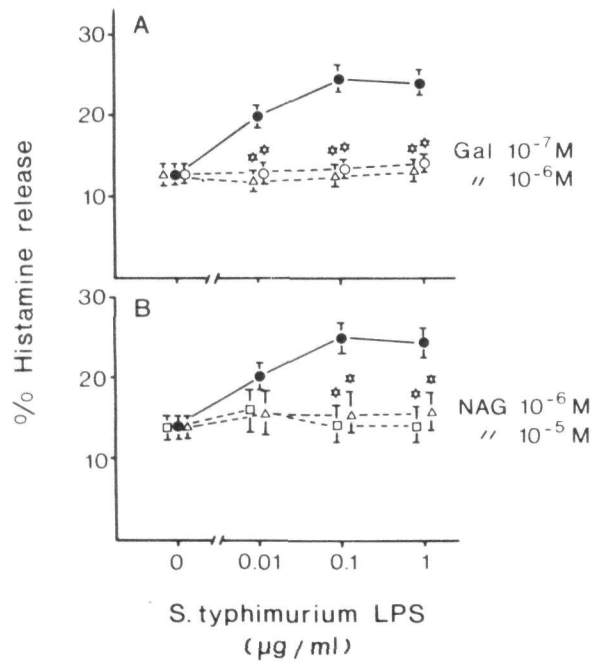


Fig. 2. Potentiation by *S. typhimurium* LPS of anti-IgE induced histamine release and abolition of the potentiating effect by sugars. Potentiation (●) and its abolition by (A) galactose (Gal) (10⁻⁷ and 10⁻⁶ M) and (B) N-acetylglucosamine (NAG) (10⁻⁶ and 10⁻⁵ M). Mean ±SEM for 6 experiments in duplicate: *P < 0.01. *S. typhimurium* LPS did not release histamine *per se*.

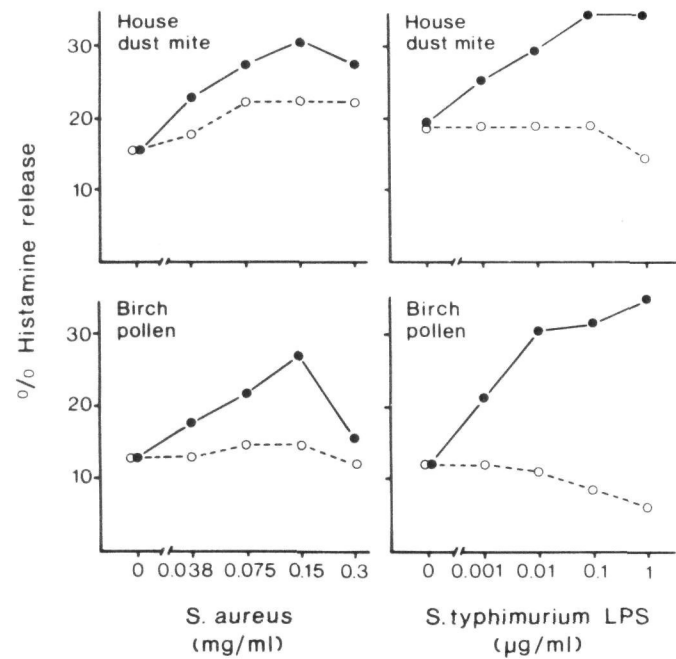


Fig. 3. Potentiation by *S. aureus* and *S. typhimurium* LPS of histamine release induced by specific allergens in leukocyte suspensions from patients sensitized to house dust mite or birch pollen. Potentiation (●) and its abolition (○) by galactose 10⁻⁶ M. Duplicate determinations. *S. aureus* and *S. typhimurium* LPS did not release histamine *per se*.

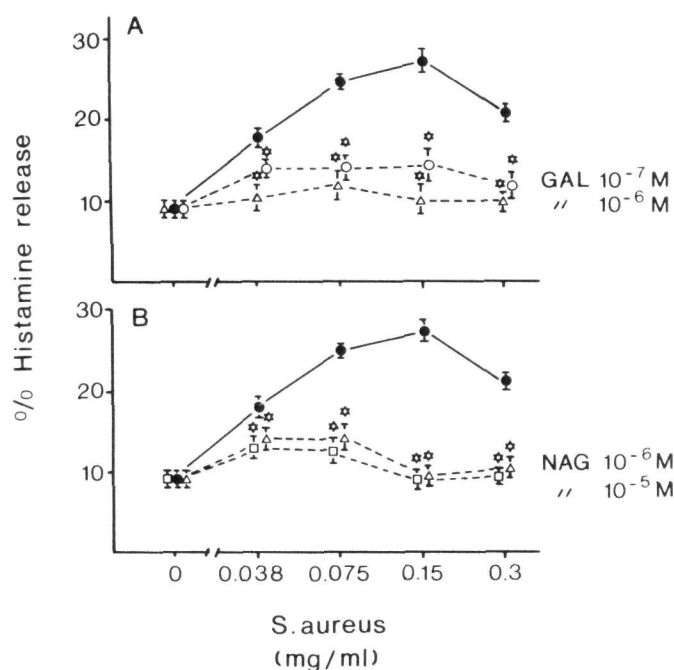


Fig. 4. Potentiation by *S. aureus* of A23187-induced histamine release and inhibition of the potentiating effect by galactose and N-acetylglucosamine. Mean $\pm$ SEM of 6 experiments in duplicate; * $P < 0.01$.

0.038 to 0.30 mg/ml did not release histamine *per se* (Table 1). Fig. 1 shows that galactose inhibited the potentiating effect. A significant inhibition ($P < 0.01$) of 53 and 81% was obtained with the sugar in final concentrations of 10^{-7} and 10^{-6} M, respectively. Also N-acetylglucosamine, 10^{-6} and 10^{-5} M, caused a significant inhibition ($P < 0.01$) with a reduction of 69 and 75%, respectively. When *S. aureus* was replaced by *S. typhimurium* LPS, used in final concentrations from 0.01 to 1 μ g/ml, the endotoxin caused a similar synergistic enhancement in anti-IgE induced histamine release and the potentiation was inhibited significantly ($P < 0.01$) by galactose and N-acetylglucosamine used in the concentrations mentioned before (Fig. 2.). Similar experiments were performed with cells from patients allergic to house dust mite or birch pollen. Fig. 3 shows that the histamine release induced by the specific allergens was enhanced by the bacterium and the endotoxin, and that the potentiating effect was inhibited by galactose 10^{-6} M.

Potentiation and its inhibition were also examined in connection with non-immunological histamine release caused by the calcium iono-

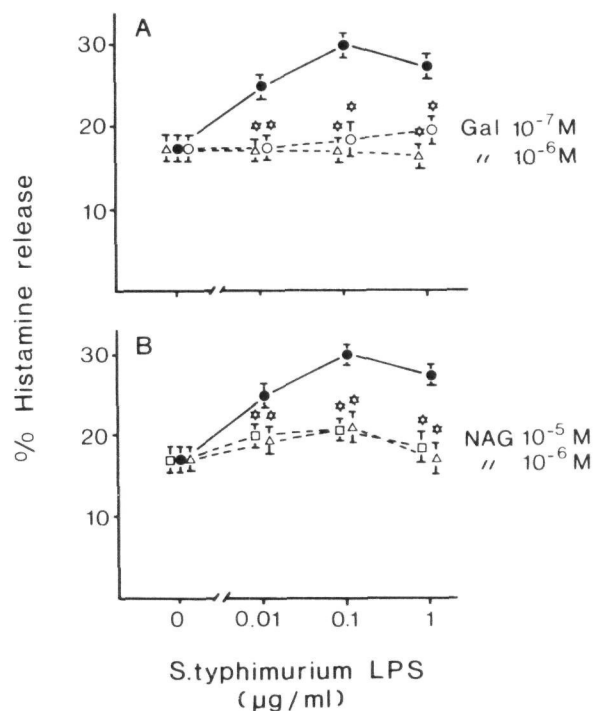


Fig. 5. Potentiation by *S. typhimurium* LPS of A23187-induced histamine release and inhibition of the potentiating effect by galactose and N-acetylglucosamine. Mean $\pm$ SEM of 6 experiments in duplicate; * $P < 0.01$.

phore A23187. Cell suspensions from six normal individuals were incubated with A23187, 3×10^{-7} M, to obtain a histamine release below

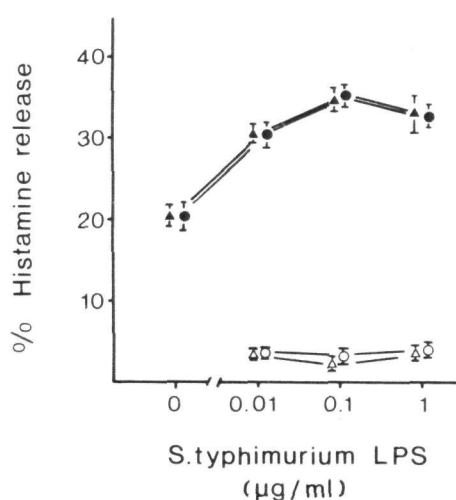


Fig. 6. Potentiation by *S. typhimurium* LPS of anti-IgE induced histamine release. The cells were preincubated with LPS for 10 min, then washed and left for 30 min at 37°C before incubation with anti-IgE ($\blacktriangle$), or the cells were incubated with a mixture of LPS and anti-IgE ($\bullet$). No significant release of histamine ($< 10\%$) was obtained when cells treated by similar procedures were incubated with LPS alone (open symbols).

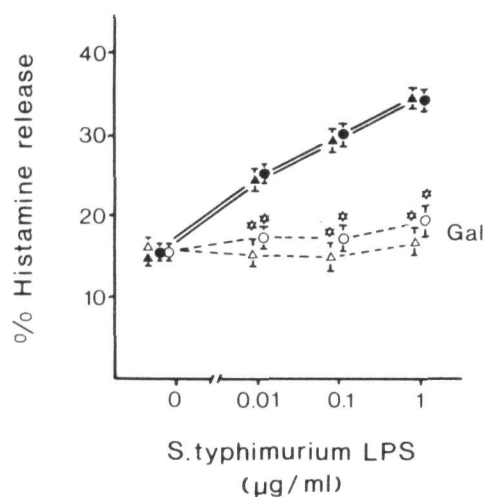


Fig. 7. Potentiation (closed symbols) by *S. typhimurium* LPS of anti-IgE induced histamine release and abolition (open symbols) of the potentiating effect by galactose (GAL) 10^{-6} M. The cells were preincubated with galactose (Δ) or Tris-AMC ($\blacktriangle$) and then washed and incubated with anti-IgE and LPS, or the cells were incubated with anti-IgE and LPS in the presence ($\circ$) and absence ($\bullet$) of galactose. Mean $\pm$ SEM of 5 experiments in duplicate: * $P < 0.01$.

25%. *S. aureus* (0.15 mg/ml) enhanced the histamine release from 10 to 28%, and abolition or significant inhibition of the potentiating effect was obtained by galactose (10^{-6} and 10^{-7} M) and N-acetylglucosamine (10^{-5} and 10^{-6} M) (Fig. 4). The A23187-induced histamine release was also enhanced by *S. typhimurium* LPS and the potentiating effect of the endotoxin was abolished by galactose (10^{-6} and 10^{-7} M), while N-acetylglucosamine (10^{-5} and 10^{-6} M) caused a significant inhibition of 72% (Fig. 5).

The influence of glucose was examined using anti-IgE or A23187 as stimulator and the bacterium or LPS as potentiator. Glucose 10^{-6} and 10^{-4} M caused no change in the potentiation.

To examine whether the potentiation by endotoxin depends on a binding of LPS to the cell surface, the cells were preincubated with *S. typhimurium* LPS, and after washing away unbound LPS, the cells were left at 37°C for 30 min before stimulation with anti-IgE. The potentiation did not differ from that obtained by the usual procedure, by which the cells were stimulated with a mixture of anti-IgE and endotoxin (Fig. 6), indicating a cellular binding of LPS. When the cells were preincubated with galactose 10^{-6} M, washed and incubated with

anti-IgE plus *S. typhimurium* LPS, the sugar caused a similar inhibition of the potentiation as by the usual procedure (Fig. 7), indicating a binding of galactose to the cell surface.

DISCUSSION

We have previously shown that bacteria, endotoxins and other bacterial products enhance allergic (IgE-mediated) histamine release (10, 11, 12). An enhancement of mediator release by bacteria or their components might play a role in the aggravation of bronchial asthma during infections. In the present study potentiation of mediator release and its abolition was investigated in connection with basophil histamine release in leukocyte suspensions from normal individuals and patients allergic to house dust mite or birch pollen. *S. aureus* bacteria and endotoxin derived from *S. typhimurium* caused a significant enhancement of the IgE-mediated histamine release triggered by anti-IgE and specific antigens, which is in accordance with our earlier findings (11, 12). Also non-immunological histamine release triggered by the calcium ionophore A23187 was found to be enhanced by the bacterium and the endotoxin. Overall, the potentiation of mediator release was due to synergism, since *S. aureus* and *S. typhimurium* LPS, in the concentrations used, caused no release of histamine *per se*.

The mechanism of action of the potentiating effect is not known, but it has been elucidated in connection with the endotoxin (Fig. 6). It appears to depend on a binding of the LPS to the basophil cell membrane followed by a non-transient signal or event, since the potentiation persists after preincubation of the cell with the LPS followed by washout and leaving the cells for 30 min at 37°C before the stimulation with anti-IgE.

Both gram-positive and gram-negative bacteria contain peptidoglycan (6, 15), and this bacterial component could be responsible for the potentiation by *S. aureus*, since peptidoglycan isolated from *S. aureus* enhances allergic histamine release (12). Peptidoglycan contains N-acetylglucosamine, and this sugar is also found in *S. typhimurium* LPS together with galactose (9,

15). If sugar residues are responsible for the potentiation by interacting with binding sites on the basophil cell membrane, the potentiation should be inhibited by the free sugars blocking the binding sites by competition. Our experiments showed that both galactose and N-acetylglucosamine, but not glucose, inhibited or abolished the potentiation caused by the bacterium and the endotoxin. This was the case in all experiments, whether the release of histamine was triggered by anti-IgE, specific antigens or A23187. The inhibition of the potentiation depends on a binding of the sugar to the cell membrane, since it persists after preincubation and washout of galactose. Although these findings point to the sugar residues being responsible for the interaction with the binding sites, the aminoacids of bacterial surface components and the lipid backbone (lipid A) of the LPS cannot be excluded as possible candidates.

Our results show that the sugars abolish the potentiation by bacteria and endotoxin in very low concentrations, i.e. galactose 10^{-6} to 10^{-7} M and N-acetylglucosamine 10^{-5} to 10^{-6} M. To prevent triggering of histamine release (7, 8, 17), phagocytosis (16) or bacterial adherence (1, 19), higher concentrations (10^{-4} to 10^{-1} M) of the sugars are needed. Therefore, if a potentiation of mediator release by bacteria and their products 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 in progress at the University Hospital (Rigshospitalet), Copenhagen.

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