

Influenza A virus enhances basophil histamine release and the enhancement is abolished by carbohydrates

P. CLEMENTSEN, S. NORN, K. S. KRISTENSEN & C. HANNOUN¹

Dept. of Pharmacology, University of Copenhagen, Denmark, ¹Institut Pasteur, Unit of Viral Ecology, Paris, France

Basophil histamine release was studied in leukocyte suspensions from normal individuals and from patients allergic to house dust mite or birch pollen. Mediator release caused by IgE-mediated reactions was examined by stimulating the cells with anti-IgE or specific antigens, and the calcium ionophore A23187 was used for a non-immunological histamine release. In all experiments influenza A virus caused a synergistic enhancement of the mediator release and the potentiation was abolished by galactose (10^{-7} to 10^{-6} M) and by 10^{-6} to 10^{-5} M of N-acetylglucosamine, alpha-methyl-D-glucoside, alpha-methyl-D-mannoside, N-acetylneuraminic acid and lactose, but not by glucose. Wash-out experiments show that the sugars prevent the aggravation of mediator release by a binding of sugar to the basophil cell membrane, thereby causing a blockade of binding sites responsible for the potentiating effect of virus.

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

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CLINICAL ASPECTS

Viral infections appear to play a role in the aggravation of airways diseases since upper respiratory tract infections frequently precipitate or exacerbate attacks of bronchial asthma. Enhancement of the mediator release by viruses 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 viral 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 viral infections play a role in airway hyperreactivity. Influenza A virus aggravates both the allergic histamine release (2) and mediator release caused by bacteria (4) and by activation of the complement cascade (3). Since a potentiation of mediator release by viruses could be involved in the aggravation of asthma during infections, we examined the potentiation and its abolition in connection with basophil histamine release.

MATERIAL AND METHODS

Twelve healthy individuals aged between 20 and 55 years served as donors. None had any previous history of atopy and they received no medication. Further, three patients with bronchial asthma and hay fever, and a well-documented history of allergy supported by a positive skin prick test and positive basophil histamine release to either house dust mite or birch pollen were included.

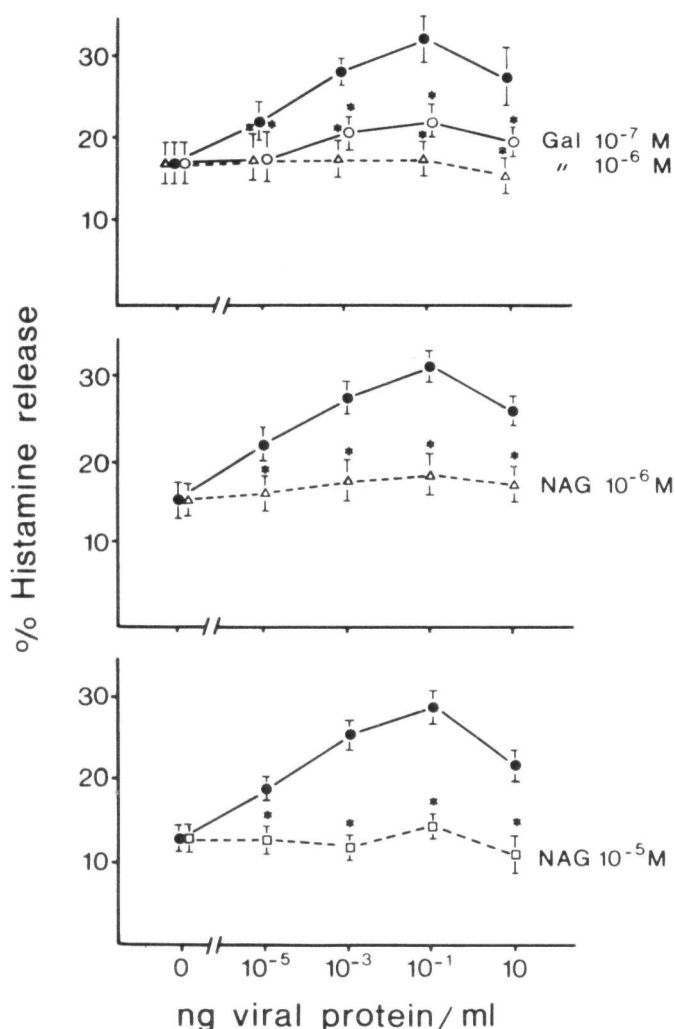


Fig. 1. Potentiation by influenza A virus of anti-IgE induced histamine release and abolition of the potentiating effect by sugars. Histamine release was induced by anti-IgE in leukocyte suspensions from 6 normal individuals. The mediator release was enhanced by influenza A virus (●) and the enhancement was abolished by galactose (Gal) 10^{-6} M and N-acetylglucosamine (NAG) 10^{-6} and 10^{-5} M (open symbols). Mean $\pm$ SEM for the 6 experiments in duplicate; * $P < 0.01$.

Human influenza A virus A/Caen/1/84 (H3N2) was obtained as previously described (3). 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. D-(+)-glucose, D-(+)-galactose, alpha-methyl-D-mannoside, N-acetyl-D-glucosamine and N-acetylneuraminic acid type IV were purchased from Sigma, St. Louis,

USA and alpha-methyl-D-glucoside was from Calbiochem AG, Switzerland, and lactose from Merck, Darmstadt, FRG.

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 (13). 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 (13).

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 (13). Only release of more than 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 influenza A virus on the histamine release was examined by replacing 5 μl glucose-free Tris-AMC by the virus used in 10-fold dilutions. 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, alpha-methyl-D-glucoside, alpha-methyl-D-mannoside, N-acetylneuraminic acid, lactose and glucose. In some experiments the cells were preincubated with galactose (37°C , 10 min), washed and incubated for 40 min at 37°C with anti-IgE plus virus.

Statistics

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

RESULTS

Potentiation of basophil histamine release and its abolition was examined in leukocyte suspen-

sions from 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 influenza A virus was included the mediator release was enhanced and maximal enhancement (approx. 100%) was obtained by 0.1 ng viral protein/ml (Fig. 1). The potentiating effect of the virus was due to synergism, since it did not release histamine *per se* (Table 1). Galactose inhibited the potentiating effect and a complete abolition was obtained in a final concentration of 10⁻⁶ M, while 10⁻⁷ M caused a significant inhibition of 66% (P < 0.01). When galactose was replaced by N-acetylglucosamine a complete inhibition of the potentiating effect was obtained by 10⁻⁶ to 10⁻⁵ M N-acetylglucosamine (Fig. 1).

Similar experiments were performed with alpha-methyl-D-glucoside, alpha-methyl-D-mannoside, N-acetylneuraminic acid, lactose and glucose. Glucose, 10⁻⁶ to 10⁻⁴ M, did not change the potentiating effect of influenza A virus on anti-IgE induced histamine release (results not shown). However, the other sugars caused a complete inhibition of the potentiation when used in final concentrations of 10⁻⁶ to 10⁻⁵ M. Representative examples of three to four experiments are given in Fig. 2.

To examine the effect of galactose on the potentiation of allergen-induced histamine release, similar experiments were performed with cells from patients allergic to house dust mite or birch pollen. Fig. 3 shows that influenza A virus enhanced the histamine release caused by the

specific antigens and that the potentiation was abolished by galactose 10⁻⁷ M.

Potentiation and its abolishment was also examined in connection with non-immunological histamine release caused by the calcium ionophore A23187 in cells from normal individuals. A23187, 3 × 10⁻⁷ M, caused a histamine release of 15% which was enhanced to approx. 30% by influenza A virus. The potentiation was completely abolished by galactose 10⁻⁶ and 10⁻⁷ M and by N-acetylglucosamine 10⁻⁵ M while 10⁻⁶ M caused significant inhibition of 63% (Fig. 4).

To investigate whether the carbohydrate inhibits the potentiation by binding to the cell membrane, the cells were preincubated with galactose 10⁻⁶ M for 10 min at 37°C and then washed free of unbound sugar and incubated for 40 min at 37°C with anti-IgE + influenza A virus. In analogy with the usual procedure a complete inhibition of the potentiation was obtained (Fig. 5), indicating a binding of galactose to the cell membrane.

A possible effect of the sugars on the histamine release induced by anti-IgE, allergens or A23187 was examined by incubating the cells with these stimulators in the presence of sugar in the concentrations mentioned above, and none of the sugars caused a change in the mediator release. Nor did the combination of virus and sugar cause release of histamine.

DISCUSSION

Previously we have found that viruses aggravate allergic histamine release from human basophil

Table 1
Basophil histamine release (%) induced by influenza A virus in cell suspensions from normal individuals and allergic patients.
Mean ± SEM is given

Subjects	No. of experiments	Influenza A virus (ng viral protein/ml)			
		10 ⁻⁵	10 ⁻³	10 ⁻¹	10
Normal individuals	20	2.2	3.0	2.6	2.0
		±0.6	±0.8	±0.8	±0.6
Allergic patients	10	4.8	5.0	4.6	5.0
		0.67	±0.8	±0.5	±0.9

Note that the histamine release was not significant (< 10%)

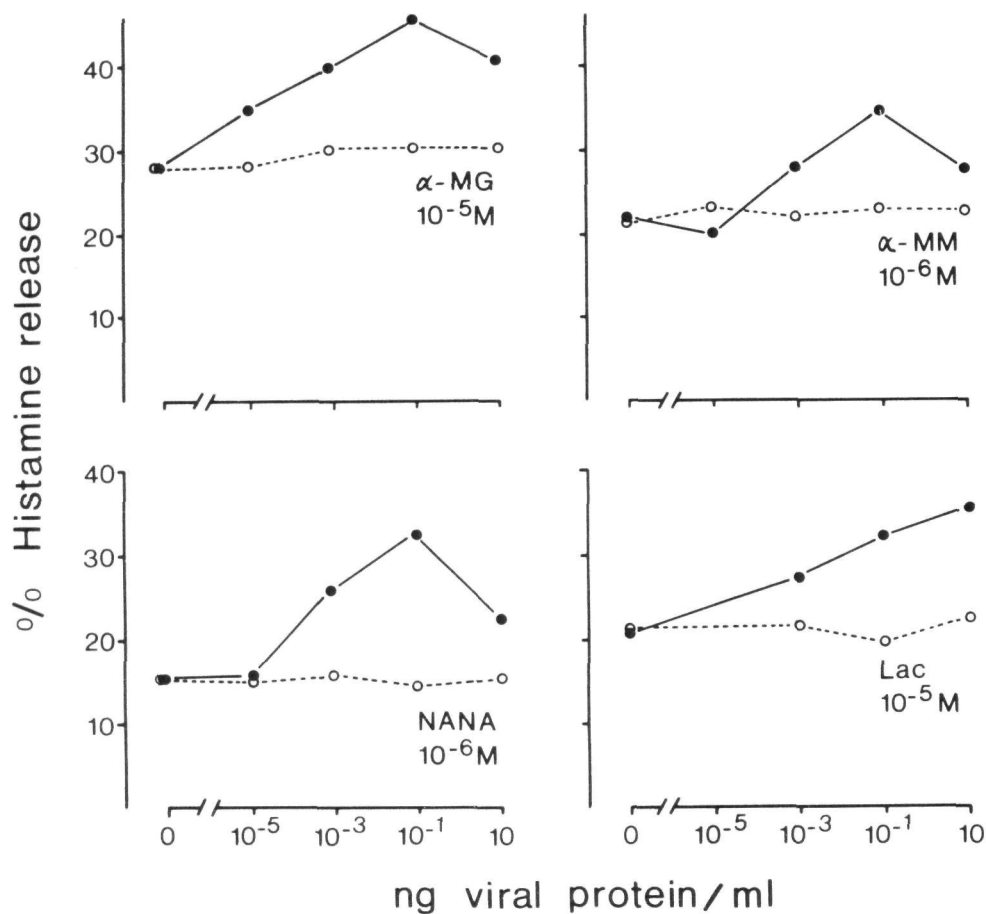


Fig. 2. Potentiation by influenza A virus of anti-IgE induced histamine release and abolition of the potentiating effect by sugars. Cells from normal individuals. Potentiation (●) and its abolition by sugars (○), i.e. alpha-methyl-D-glucoside (α-MG), alpha-methyl-D-mannoside (α-MM), N-acetylneuraminic acid (NANA) and lactose (Lac). Duplicate determinations.

leukocytes (2). It is tempting to speculate that enhancement of mediator release by viruses could play a role in airway diseases since upper respiratory tract infections have frequently been shown to precipitate or exacerbate attacks of bronchial asthma (6, 9, 10, 15). 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. Influenza A virus caused a synergistic enhancement of the IgE-mediated histamine release triggered by anti-IgE and the specific antigens, which is in accordance with our earlier findings (2). Also non-immunological histamine release triggered by the calcium ionophore A23187 was found to be enhanced by the virus.

The mechanism of the potentiating effect of the virus is not known. However, the neuraminidase portion of the virus seems to be important, since the potentiating effect is mimicked

by isolated neuraminidase and abolished by the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (2). The viral neuraminidase is a glycoprotein which contains carbohydrates, such as galactose and N-acetylglucosamine (5). In the present study these sugars were found to abolish the potentiating effect of the virus on both IgE-mediated and non-immunological histamine release. Also such lectin-binding sugars as alpha-methyl-D-glucoside and alpha-methyl-D-mannoside and, furthermore, N-acetylneuraminic acid and lactose, but not glucose, were able to inhibit the potentiation. The inhibitory effect appears to depend on sugar binding to the basophil cell membrane since it persists after preincubation of the cells with galactose followed by wash-out of unbound sugar. This also applied to the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid in our preliminary experiments (unpublished results), indicating that at least part of its inhibitory effect on the potentiation is

caused by a binding of the neuraminidase inhibitor to the basophilocyte. Thus, the mechanism of action of the potentiating effect of influenza A virus on the histamine release appears to depend on interaction of the neuraminidase portion of the virus with basophil binding sites which are blocked by the sugars. It is therefore suggested that the neuraminidase-sugars are responsible for this interaction, but it is not known whether its aminoacids might also be involved.

Our results show that the sugars abolish the potentiation by virus in very low sugar concentrations, i.e. 10^{-6} to 10^{-7} M for galactose and the other sugars in the range of 10^{-5} to 10^{-6} M. To prevent triggering of histamine release (7, 8, 12), phagocytosis (11), or bacterial adherence (1, 14), higher sugar concentrations (10^{-4} to 10^{-1} M) are needed. Therefore, if a potentiation of mediator release by virus plays a crucial role in the aggravation of asthma, carbohydrates might be a new treatment when infections aggravate

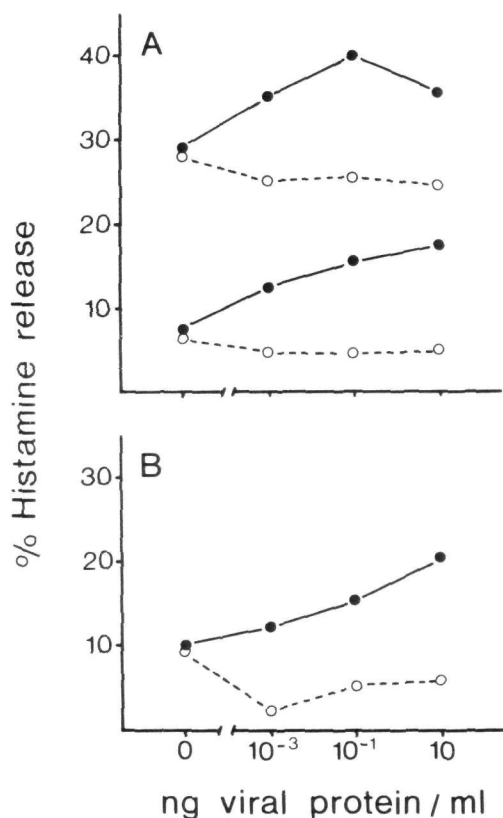


Fig. 3. Potentiation by influenza A virus of histamine release induced by specific allergens in leukocyte suspensions from patients sensitized to house dust mite (A) or birch pollen (B). Potentiation (●) and its abolition by galactose 10^{-7} M (○). Duplicate determinations.

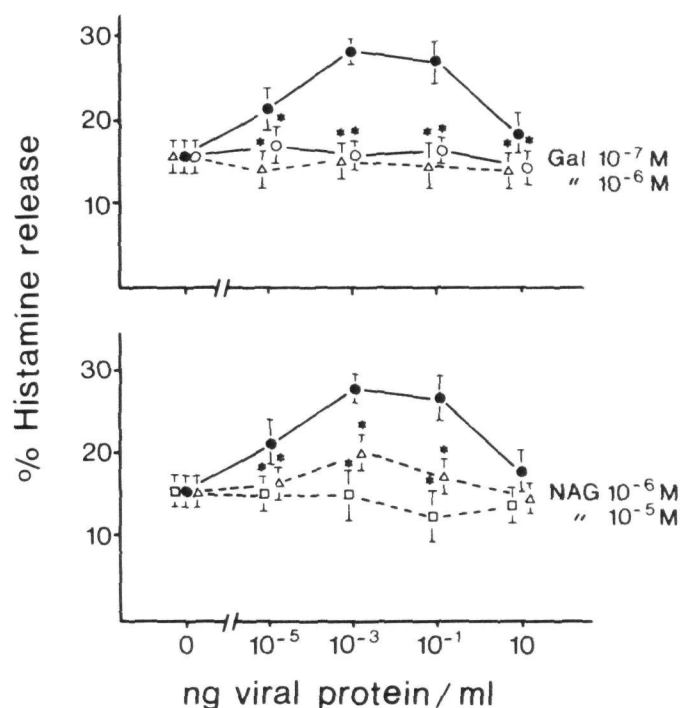


Fig. 4. Potentiation by influenza A virus of A23187-induced histamine release (●) and abolition of the potentiating effect by galactose (Gal) and N-acetylglucosamine (NAG) (open symbols) in cells from normal individuals. Mean $\pm$ SEM of 6 experiments in duplicate; * $P < 0.01$.

asthmatic attacks. A clinical trial with galactose is in progress at the University Hospital (Rigshospitalet), Copenhagen.

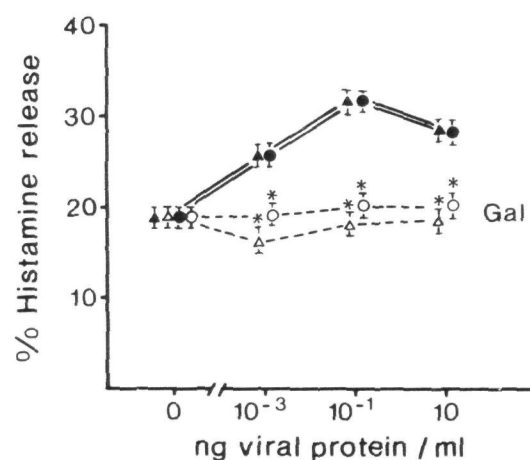


Fig. 5. Potentiation (closed symbols) by influenza A virus of anti-IgE induced histamine release and abolition (open symbols) of the potentiating effect by galactose (Gal) 10^{-6} M. Cells from normal individuals were preincubated with galactose (Δ) or buffer ($\blacktriangle$) and then washed and incubated with anti-IgE and virus, or the cells were incubated with anti-IgE and virus in the presence ($\circ$) and absence ($\bullet$) of galactose. Mean $\pm$ SEM of 6 experiments in duplicate; * $P < 0.01$.

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Address:

P. Clementsen

Department of Pharmacology

University of Copenhagen

Juliane Maries Vej 20

DK-2100 Copenhagen Ø

Denmark

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