

A preliminary evaluation of the effect of N-acetyl-aspartyl-glutamate on pollen nasal challenge as measured by rhinomanometry and symptomatology

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The preventive effect of a nasally administered single dose of the dipeptide N-acetyl-aspartyl-glutamate (NAAGA) on pollen-induced early nasal reaction as measured by rhinomanometry and symptomatology was investigated in a double-blind cross-over placebo controlled study in 18 patients with nasal sensitization to grass pollen. Mean nasal airways resistance at 20, 40, and 60 min post challenge was, respectively, 33%, 37% ($P = 0.03$) and 27% less after NAAGA than after placebo administration in the same patients. In contrast, no significant effect on sneeze count and rhinorrhoea was found.

Key words: grass pollen; N-acetyl-aspartyl-glutamate; nasal challenge test; rhinomanometry.

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Nasal antigen challenge combined with objective assessment of its effects by rhinomanometry is a valuable method for the aetiological diagnosis of allergic rhinitis. It is also a useful research tool with which to evaluate drugs that may influence the reaction of the sensitized nasal mucosa in response to antigenic challenge.

The dipeptide N-acetyl-aspartyl-glutamic acid (magnesium salt) (NAAGA) is a new anti-allergic agent which has been experimentally shown to prevent anaphylactic and non-anaphylactic mast cell degranulation and to inhibit the cytolytic effect of the activated complement and the release of anaphylatoxins (3, 5, 8).

NAAGA was initially discovered as a naturally occurring dipeptide in the rabbit brain (4). It was subsequently isolated from other mammalian brains (9, 10, 13, 14) including human brain (1) in which its concentration ranges between 100 and 200 µg/g. It is believed to be part of the numerous neurotransmitters and has

been shown to have high affinity for glutamate receptors in brain tissues (17).

Recently, topically administered NAAGA ophthalmic solution has been shown to be effective in relieving ocular inflammatory symptoms in allergic conjunctivitis (16).

In the present study, we report the effects of nasally administered NAAGA on pollen-induced nasal reaction as measured by rhinomanometry and symptomatology in patients with nasal sensitization to grass pollens.

PATIENTS AND METHODS

Eighteen outpatients (8 males and 10 females), mean age 25 years (range 9-38 years), who had been suffering from rhinitis caused by spring pollen for at least 2 consecutive years prior to the experiment, were included in the present trial during the winter and late autumn months of 1985. During that period, the patients nasal

condition was normal. All had a positive skin test to grass pollen extract.

Rhinomanometry technique

Nasal airways resistance (NAR) was measured by posterior rhinomanometry according to a previously described method (6). Briefly, nasal airflow is measured by a Fleisch No. 1 pneumotachograph connected to a mask covering the nose and eyes of the patient.

The air pressures "anterior" and "posterior" to the nasal cavity are respectively determined at the head of the pneumotachograph and at the rhinopharynx by means of an oral probe held in position between the patient's tightened lips. NAR is calculated as the ratio of the antero-posterior air pressure difference to the nasal airflow: $NAR = \Delta P / \dot{V}$. An APPLE II computer equipped with an UTC 06-611 card and loaded with a personal program allows automatic acquisition of pressure and flow data every 10 ms from the beginning of inspiration until the peak inspiratory nasal airflow.

These data are processed in order to produce a plot of the flow/pressure curve and to determine the relationship between the NAR and flow which is expressed in the following equation: $NAR = K_1 + K_2 \dot{V}^{(15)}$. This direct proportional relationship between resistance and flow allows further measurement of the NAR at a given flow. In the present study the NAR were calculated for a nasal airflow of 0.15 l/s.

Nasal antigen challenge

The antigen used was lyophilised purified grass pollen extract (*pollens 4 graminées* purchased from Institut Pasteur, Paris) diluted in saline and applied as one 0.1 ml puff to each nostril.

Initially, nasal reactivity to the antigen was investigated in each subject in order to determine the dose of antigen required to at least double the NAR over the control level, 20 min after nasal challenge.

When this antigen dose had been selected, the effect of pre-challenge single administration of NAAGA nasal spray (6% w/v) and the corresponding placebo (vehicle) on NAR were

compared in each patient according to a randomized double-blind cross-over design.

Protocol of rhinomanometric study

Dose selection challenge and challenges performed during NAAGA and placebo treatments were carried-out at 8-day intervals, always at the same time of day in the individual subject in order to eliminate possible circadian variations. According to a randomization list, nine subjects underwent the NAAGA test first and then the placebo test and the other nine the reverse, in order to eliminate the influence of possible carry-over effects on data analysis.

In both NAAGA and placebo test sessions, the following three consecutive NAR determinations were made prior to the antigen challenge:

- basal NAR (B)
- control NAR (C): 15 min after nasal administration of one 0.1 ml saline spray into each nostril,
- drug NAR (D): 15 min after nasal administration of 2×0.075 ml sprays of NAAGA or placebo solutions (NAAGA solution vehicle) into each nostril.

Three additional NAR determinations were then made 20, 40 and 60 min after the nasal antigen challenge with the previously selected dose of pollen 30 min after treatment with NAAGA or placebo.

Symptomatology

The symptoms assessed were sneezing and nasal secretion. The number of sneezes in the 60 min following antigen challenge was recorded in each patient.

Nasal discharge was objectively assessed in nine patients by determining the total weight of secretions eliminated by spontaneous and pre-NAR called-for nose blowings, during the 60 min following antigen challenge. The nasal secretions were collected in standardized paper handkerchiefs and immediately placed in standardized sealed plastic bags to prevent drying.

The nasal secretions were weighed on a PE360 Mettler electronic balance after correcting for the weights of handkerchiefs and plastic bags.

Drugs

NAAGA nasal spray (Rhinaaxia®, ZYMA, France) had the following composition: NAAGA (6 g) benzalkonium chloride (0.01 g), sodium chloride (0.20 g), hydrochloride acid (ad. pH 6.8) purified water (ad. 100 ml) contained in 15 ml glass vials equipped with a mechanical pump delivering 0.075 ml at each puff. The placebo sprays contained the same ingredients, with the exception of NAAGA, and had the same appearance as NAAGA sprays.

Statistical analysis

NAR data were processed by analysis of variance. Sneezing counts and nasal secretion weights were analysed by Student's *t*-test. The *P* value ≤ 0.05 was regarded as significant.

RESULTS

The selected pollen doses were 20 µg in five patients, 10 µg in four, 7 µg in six and 3.5 µg in three. In the pollen dose-finding test, NAR increased from 2.71 ± 0.14 , (mean $\pm$ SEM) HPa/l.s (control value) to 6.34 ± 0.49 , 20 min after antigen challenge, thus representing a 2.34-fold increase (range: 1.97–3.77) over the control NAR level.

The mean time courses of NAR in the placebo and NAAGA tests are presented in Fig. 1. Before antigen challenge, basal NAR (B), control NAR (C) and drug NAR (D) did not differ significantly from each other within and between the placebo and NAAGA tests. However, a trend toward a lesser increase of NAR (D) after NAAGA (+ 4% vs. control) than after placebo (+ 17%) was observed.

Nasal challenge with the pre-selected pollen dose induced a marked increase of NAR for 40 min, followed by a progressive return to control values which was almost completed 60 min after challenge in both series. At 20, 40 and 60 min post challenge, the NAR in the NAAGA series are, on average, respectively 33%, 37% and

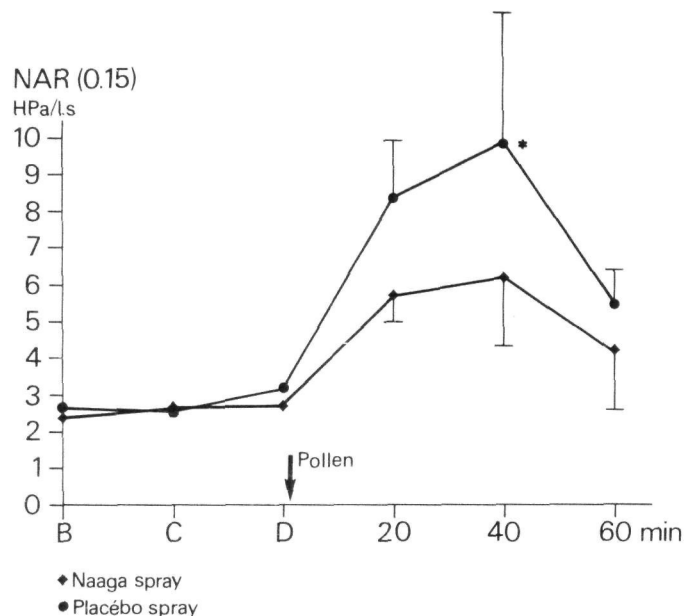


Fig. 1. Effect of NAAGA and single intranasal placebo administration on nasal airways resistance (NAR) before and after nasal pollen challenge in 18 patients with nasal sensitization to grass pollen.

NAR was calculated for a nasal airflow of 0.15 l/s (NAR 0.15).

B = basal NAR; C = control; D = drug NAR. Bars represent standard errors of the means. *Significantly different from NAAGA ($P = 0.03$).

27% less than the NAR measured at the same times during placebo testings in the same patients. Differences between mean post challenge NAR during NAAGA and placebo are statistically significant at 40 min ($P = 0.03$).

The number of sneezes recorded during the 60-min period post challenge was the same during both treatments: 8.11 ± 1.64 (mean $\pm$ SEM) on placebo and 8.11 ± 1.29 on NAAGA. The weight of nasal secretions eliminated during the same period was 3.17 ± 0.65 g (mean $\pm$ SEM) on placebo and 4.06 ± 1.04 g on NAAGA. The difference is not statistically significant. No statistically significant correlation was found between sneezing count and rhinorrhea ($r = -0.24$), sneezing count and NAR increase ($r = 0.09$), rhinorrhea and NAR increase ($r = -0.217$) in the control series.

DISCUSSION

The ability of NAAGA to block allergic responses at nasal allergen challenge was invest-

igated in two ways: (i) by rhinomanometric measurement of nasal obstruction, and (ii) by quantitative assessment of two clinical symptoms: sneezes and rhinorrhea.

The results obtained in the present study with single-dose NAAGA given intranasally 30 min prior to allergen show that the only change attributable to a drug effect is a reduction in allergen-induced nasal obstruction, which is statistically significant at the time of peak post-challenge NAR increase (40 min) over NAR baseline. On the other hand, the drug showed no effect on sneeze counts and rhinorrhea weight.

Nasal blockage and such symptoms as sneezing and rhinorrhea differ in terms of physiological mechanisms, timer course pattern after allergen challenge and allergen dose-response relationship. Sneezing and rhinorrhea are reflex mediated (12). They occur within the first minutes of exposure to allergen and are relatively short-lasting (11). Nasal obstruction due to vascular changes and possible formation of edema in the mucosa is thought to be the result of a direct histamine effect on vascular receptors (12). Nasal obstruction develops more slowly and is more long-lasting than sneezing and rhinorrhea.

In a large series of nasal challenge tests (7), we could determine that the NAR increase became statistically significant 20–25 min after allergen administration, that is when sneezes and rhinorrhea had ceased. Moreover, in the same study, we could show that the increase in NAR correlated well with the dose of allergen administered, whereas no correlation was found between the number of sneezes and the NAR increase.

In the present study we again confirmed the absence of correlation between sneezes and NAR increase and found that this was also the case for rhinorrhea. Interestingly, there is also no correlation between sneeze count and the weight of secretions.

We consider that since symptoms and increased NAR differ in their mechanism and are not correlated it is to be expected that a drug may reduce NAR increase in response to allergen challenge without influencing nasal

symptoms, or the reverse. An example is the known ineffectiveness or slight effect of ordinary H_1 antihistamines on nasal blockage in allergic rhinitis in contrast to their marked effects on nasal sneezing and discharge (12).

In our opinion the lack of effect of NAAGA on sneezes and rhinorrhea in the present study does not weaken our finding that this drug reduces the obstructive response of sensitized nasal mucosa to allergen. It could be that the dose (single dose) of NAAGA used in our study is insufficient to control the symptoms although clearly effective in reducing nasal obstruction. Further studies should be done to determine the effects of repeated nasal doses of NAAGA on nasal symptoms and obstruction induced by allergen challenge.

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