Rhinology, 29, 143-149, 1991

The sources of chemical substances in allergic nasal fluid

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SUMMARY

The sources of different chemical substances in the NF of allergic patient, such as albumin, secretory IgA, histamine, leukotrien, kinin and substance P were investigated. To accomplish this, we challenged the inferior turbinate on one side, but separately collected NF from both sides in patients with nasal allergy to house-dust. Provocation was done with paper disc containing dried allergen extract. Collection was done by suction for the first five minutes immediately after the onset of a positive response to nasal provocation. The total amount of the chemical substances on each side was analyzed separately and compared. Significant differences were seen between both sides only for histamine and leukotrien. In consideration with the previous reports, it is suggested that in nasal allergen challenge the major sources are glandular secretion for secretory IgA, and albumin, and secretion for migrating cells for histamine and leukotrien. The major sources responsible for kinin and substance P, however, are not defined.

INTRODUCTION

The study of sources of nasal fluid (NF) is useful to understand the pathophysiology of allergic rhinitis, since NF contains various chemical substances which are produced and transported differently.

It is known that chemical substances such as water, albumin (Al), secretory IgA (sIgA), histamine (Hi), leukotriens (Lt) and kinin (Ki) may be major components in allergic NF (Naclerio et al., 1986). Their sources, however, are still undefined or rather controversial. Al is assumed to be a pure vascular transudate (Mygind, 1978), but is revealed to accompany glandular secretion (Raphael et al., 1988; 1989). sIgA is clarified to be a pure glandular product (Brandtzaeg, 1987), but is

Paper presented at the 13th Congress of the European Rhinologic Society including the IXth I.S.I.A.N., London (United Kingdom), June 1990.

also noted to increase in a condition of increased vascular permeability (Raphael et al., 1988; 1989). Hi and Lt are mainly released from the nasal mast cells (Konno et al., 1983; Brofeldt et al., 1986; Bisgaard et al., 1988; Malmberg et al., 1989), but their amounts in NF greatly varies depending upon experimental conditions (Bisgaard et al., 1988). Kinin may be from multiple-sources (Lichtenstein et al., 1983; Baumgarten et al., 1985).

The studies of sources of chemical substances have been carried out by biochemical analysis of nasal fluid. NF to be examined was often collected after nasal stimulation with both histamine as an inducer of vascular transudation and methacholine as an inducer of glandular secretion, a quantative analysis of the chemical substances in NF was done with both stimulations and a comparison drawn between both (Brofeldt et al., 1986; Raphael et al., 1988, 1989). This method though useful is unsatisfactory since there is no definite evidence that histamine causes only vascular transudation and methacholine only glandular secretion; besides, provocation with these agents is a highly unphysiological procedure in the study of the pathophysiology of allergic rhinitis (Brofeldt et al., 1986). On the other hand, another method has been employed for the same purpose (Konno et al., 1983; Malmberg et al., 1989). After stimulation of only one side, NF from bilateral nasal cavities was separately collected and the quantity of chemical substances on each side was compared. This was in agreement with the fact that NF on the contralateral side is only a reflex-mediated glandular secretion (Konno et al., 1983; Malmberg et al., 1989) although NF on the challenged side is a combination from different sources.

Thus the purpose of the present study is to confirm or reconfirm the previous reports on the major sources of the above mentioned chemical substances together with substances P(SP) using the method of unilateral nasal challenge with allergen.

EXPERIMENTAL METHODS

A total of 40 untreated patients of nasal allergy to house-dust, positive in all of the allergy tests, such as history-taking, rhinoscopy, test for nasal eosinophilia, skin test, nasal provocation test and determination of serum level of specific IgE antibody, were selected for this study. The mean age was 15.9 years, ranging from 6 to 52 years and the sex distribution was 22 males and 18 females.

The methods of provocation and collection of NF have already been described elsewhere (Okuda, 1975, 1989). Briefly, the nasal side to be provoked was randomly selected due to the differences between the two sides in their sensitivity to allergen. We removed as much NF as possible by forceful blowing of the nose and suction. Without delay we provoked the anterior and middle parts of the inferior turbinate on one side by placing three round allergen paper discs (3 mm each in diameter, containing 250 μg of house-dust extract per disc) and

simultaneously applied three discs without allergen as control on the contralateral inferior turbinate. Dried allergen extract in the paper discs slowly elutes in the mucus of the nasal mucosa surface until a threshold level at which it evokes a positive nasal allergic reaction (Okuda, 1989).

Immediately after the onset of nasal response, we removed the discs from both the inferior turbinates and separately collected the induced NF from both the sides into calibrated test-tubes connected with a suction apparatus so as not to stimulate the nasal mucosa. The collection was carried out under rhinoscopic observation for the first five minutes. In our preliminary experiment, during the period of the first five minutes, the amount of NF increased and reached a peak and NF collected contained a few cellular components due to removal of the nasal secretion before provocation and the action of washing-out and dilution of profuse secretion after provocation. The total volume of NF thus collected was measured and mixed with two volumes of cold phosphate buffered saline (PBS) for the determination of Al, sIgA and SP. NF-PBS solutions were divided in parts and one part for the determination of Ki and Lt was further mixed with one volume of cold ethanol. All procedures were carried out without delay and the samples were preserved at −80 °C until use. Due to the small volume of NF collected, it was not possible to measure each of the substances in all the patients under study. The total amount of each substance in NF was determined by the following methods.

The amount of Al was measured by a single radial immunodiffusion using LC-partigen plate (sensitivity: 2.5 mg/100 ml) and LC-V standard serum (Behringwerke AG, West Germany). sIgA was measured by a direct non-competitive enzyme linked immunoassay (ELISA) using purified human sIgA (Cappel, USA), anti-secretory component goat serum (Nordic Immunol Lab, The Netherlands) and peroxidase-conjugated anti-human IgA goat serum (Cappel, USA) (sensitivity; 10 ng/ml) (Raphael et al., 1989). Hi was measured by the use of an autoanalyser and high performance liquid chromatography (HPLC) after extraction with n-butanol-chloroform NaCl mixture from the samples (sensitivity: 0.5 ng/tube) (Hasegawa et al., 1983). Lt was measured using Lt C4/D4/E4 assay system (Amersham, U.K.) after elution of the samples, at an adjusted pH of 3.0, through an Amprep™ minicolumn 1904 (Amersham, U.K.) (sensitivity: 12.5 pg/tube). Ki was measured after NF-PBS was vigorously mixed with four volumes of ethanol (to inactivate intrinsic proteases), deproteinized, extracted and centrifuged. The supernatant was dried and dissolved in 0.1% acetic acid. From this solution, Ki was purified by using Sep Pak column and measured by radioimmunoassay (sensitivity: 6 pg/ml) (Minami et al., 1983). For the extraction of SP, NF-PBS was boiled for ten minutes in 0.5 M acetic acid, cooled, and mixed with three volumes of 75% acetone. The extract was centrifuged and the supernatant was dried and dissolved in 0.5 M acetic acid. After

purification by Sep Pak column, SP was measured by radioimmunoassay (sensitivity: 3 fm/tube) (Minamino et al., 1984).

RESULTS

There was no significant difference in the total quantity of NF, Al, sIgA, Ki, Lt and SP of both nasal sides as shown in Table 1. Statistically significant differences ($p < 0.05$), however, were noted only in Hi and Lt. The total quantity of each substance was greater on the challenged side than on the contralateral side.

Table 1. Total volume of chemical substances in nasal fluid both the sides after unilateral nasal challenge with allergen.

			total volume ($\bar{\chi} \pm$ SE)	
		N	challenged side	contralateral side
NF	(ml)	40	0.7 ± 0.1	0.6 ± 0.1
albumin	(μg)	12	452.8 ± 124.3	404.8 ± 223.5
sIgA	(μg)	12	234.2 ± 83.3	128.4 ± 144.3
histamine	(ng)	21	252.1 ± 76.7	106.6 ± 30.2 *
leukotriene	(ng)	10	7.4 ± 5.2	2.7 ± 1.7 *
kinin	(ng)	23	1.5 ± 0.4	0.8 ± 0.3
substance P	(pg)	20	13.0 ± 2.1	15.5 ± 4.7

* $P < 0.05$

DISCUSSION

The present results suggest that NF, Al, sIgA, Ki and SP originated exclusively from reflex-mediated glandular secretion, if it is true that NF on the contralateral side is purely reflex-mediated. However, Hi and Lt showed increase in levels in the NF by both direct and indirect actions of allergen on the nasal mucosa, if it is true that NF on the challenged side is induced by both direct and indirect stimulation

Previously we had revealed for the first time that the major part of NF is produced by indirect nervous reflex mechanism in allergic rhinitis (Okuda, 1977). We said so, because, on provocation of unilateral turbinate with an allergen the amount of NF increased in both the challenged and unchallenged side to the same extent and simultaneously (Okuda, 1977), but did not increase on both the sides if the challenged side was pre-treated with topical anaesthetic (Okuda and Mygind, 1980). NF on the challenged side may be of multiple-sources. However, Konno and we have clearly demonstrated that allergen induced NF on the challenged side is also mostly reflex-mediated since it is inhibited by pre-treatment with topical anaesthetic, Vidian neurectomy (Konno et al., 1983) and topical anti-cholinergic agent (Okuda, 1977). This previous conclusion has been confirmed by the present study as well as by Konno et al. (1983) and Malmberg et al. (1989),

although there was only a slight difference in the total amount of NF from both sides after unilateral provocation with allergen.

No significant difference was noted in the total amount of Al between the challenged and unchallenged side in the present study unlike Petersson et al. (1989), suggesting glandular origin of Al as well as NF although there was slightly more difference with Al as compared with NF. This result was quite contradictory to the general concept that Al increases in NF as a result of increased vascular transudation (Mygind, 1978), thus passing through the mucosal epithelium.

This contradiction, however, may be explained by the following facts:

1. Al in NF may accompany glandular secretion (Raphael et al., 1988; 1989);
2. Al passively diffuses into secretory gland from the plasma and is transported into secretion (Arglebe, 1981);
3. Al was found in the secretory gland cells by immunohistological technique (our unpublished data);
4. histamine provokes marked transudation resulting in marked increase of Al in NF while methacholine and allergen do not (Brofeldt et al., 1986).

In other words, beside from the nasal epithelium, Al in the lamina propria also passes through the secretory gland and appears in the NF. The ratio of glandular secretion to the epithelial transudation may vary depending upon experimental methods, but glandular secretion may be predominant in allergen challenge. In the near future we could present further evidences that Al is concentrated in the glandular cells as well as the intercellular space after nasal challenge with allergen in immunohistological study.

Generally sIgA is assumed to be a pure glandular product (Brandtzaeg, 1987). In the present study, there being a minimal difference in the quantities of sIgA from both sides, it suggests that glandular secretion may be the major source, with secretion from epithelial goblet cells having secretory activity, being the minor source.

Greater difference in quantity between both sides were noted in Hi like Malmberg et al. (1989) and unlike Petersson et al. (1989) and in Lt like Volowitz et al. (1988) and Bisgaard et al. (1988). Since it is known that both the substances are released from the mast cells on contact with allergen which accumulate on the nasal mucosal surface in allergy (Bisgaard et al., 1988; Petersson et al., 1989), these were possibly produced exclusively from the mast cells and other target cells on the challenged side. On the other hand, a considerable amount of both substances were also determined on the contralateral side. Therefore, as a minor source, the glandular secretion should also be considered.

The amount of immunoreactive kinin was greater in NF on the challenged side as compared to the contralateral side although the difference was statistically

insignificant. It is assumed that tissue kallikrein-like enzyme is released from the mast cells (Lichtenstein et al., 1983), plasma kallikrein is transudated from plasma and glandular kallikrein is secreted from the secretory gland (Baumgarten et al., 1985). All this could contribute to local generation of kinin. Baumgarten et al. (1985) suggested that there is an increase in vascular permeability and a transudation of kininogens from plasma into nasal secretion where they can provide substrate for kinin forming enzymes during allergic reaction. Immunoreactive bradykinin and related substances determined in NF in the present study may be originating from different sources, i.e., mast cell and/or vascular transudation on the challenged side and glandular secretion on the contralateral side.

There was only a minimal difference in the total of immunoreactive SP like Petersson et al. (1989) and Walker et al. (1988). This seems to suggest that SP in NF came from glandular secretion contrary to the general assumption that SP is released from nerve ending of C fiber (Lundblad et al., 1983). However, we should consider that SP released in NF might be degraded, since it is reported that NF rapidly degrades SP (Petersson et al., 1989), or the amount of SP released might be too minute to determined. On the other hand Tønnessen et al. (1988) suggested that SP in NF originates from vascular transudation, since the level of SP in NF is very similar to that of plasma and did not differ on challenge with allergen or methacholine.

Summarizing, there may be four major different sources for the chemical components in NF: glandular secretion, epithelial transudation, goblet cell secretion and secretion from the migrating cells in nasal allergy. The present results suggested as major source, glandular secretion for sIgA, for Al and NF, and cellular secretion for Hi and Lt in allergen changes. The major source, however, was not defined for kinin and SP.

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