

Leukotriene C₄ and histamine in early allergic reaction in the nose

H. BISGAARD,¹ C. ROBINSON,² F. RØMELING,¹ N. MYGIND,¹ M. CHURCH,² & S. T. HOLGATE³

¹Otopathological Laboratory, Department of Otorhinolaryngology, Rigshospitalet, Copenhagen, Denmark, ²Clinical Pharmacology, and ³Medicine I, Southampton General Hospital, Southampton, U.K.

We have examined the measurements of LTC₄ and histamine in nasal lavage fluids and blown secretions as a possible model of the early mediator events during nasal allergy. A nasal challenge with grass pollen extract was undertaken on two separate occasions in 20 patients with a history of seasonal rhinitis and a positive immediate skin test to grass pollen. A 2 ml nasal lavage was performed before allergen challenge, and blown secretion collected separately 15 min after the provocation, followed by a final 2 ml nasal lavage. The dilution of nasal secretion by the lavage fluid was determined using ^{99m}Tc-labelled albumin as an exogenous marker added to the fluid. The amounts of admixture in the nasal lavages did not correlate to the concentrations of LTC₄ and histamine, indicating that the variable amounts of nasal secretion in nasal lavage do not constitute a confounding variable for measurements of LTC₄ and histamine. In the pre-challenge lavages, the median concentrations, of LTC₄ and histamine were 1.7 and 52 nmol/l respectively. Following allergen challenge neither LTC₄ nor histamine measured in nasal lavage showed any significant change from pre-challenge baseline values. However, measurements of both mediators in the blown secretion showed a significantly higher concentration than in the pre- or post-challenge lavage samples, compatible with transitory release during the acute allergic reaction. However, it seems doubtful whether measurements of LTC₄ or histamine can be compared between blown secretion and nasal lavage fluid, even if the dilution factor is disregarded. While allergen provocation of the nasal mucosa releases inflammatory mediators locally, uncontrolled variables confound their measurement in the nasal lavage fluids to an extent which limits the application of this model to study details of mediator release during nasal allergy.

Key words: allergic rhinitis; histamine; LTC₄; nasal lavage.

Accepted for publication 22 September 1987

The sulphidoleukotriene (LT) C₄ and the pre-formed mediator histamine are released from human lung mast cells by an IgE-dependent mechanism after allergen challenge *in vitro*. In molar terms histamine is released in approximately 250-fold higher amounts than LTC₄ (6). There have been several recent reports of the use of nasal lavage in allergic rhinitis as a convenient model to study the release of inflammatory mediators *in vivo*. Sulphidoleukotrienes and histamine have been reported to increase along with other pro-inflammatory mediators in

the nasal lavage fluid after local challenge of the nasal mucosa with allergen (3, 9, 13). The fluid collected by lavage of the nasal cavities will contain a mixture of the introduced fluid and endogenous secretions, the latter being particularly obvious following challenge. Thus, the detection of any substances released from the nasal mucosa and collected by lavage might be influenced by variations in the amounts of admixed nasal secretion. It was the aim of the present study to evaluate this possible confounding variable on the measurements of

LTC₄ and histamine in mucosal lavage before and after allergen challenge. Employing this standardized approach we have proceeded to measure the change in immunoreactive LTC₄ and histamine during early allergic reaction in the nose.

MATERIAL AND METHODS

Reagents

Clinical and laboratory reagents were obtained from the following sources: Timothy grass allergen, the lyophilised alpha-fraction (ALK, Copenhagen). The ^{99m}Tc-labelled albumin was prepared from ^{99m}TcK-6 kit from International CIS, Gif-sur-Yvette, France. All solvents and optiphase-RIA scintillant were purchased from Fisons, Loughborough, U.K. Authentic LTC₄ was a gift from Dr. J. Rokach, Merck Frosst Inc., Canada. Rabbit anti-LTC₄ antibody was a generous gift of Dr. G. White, UpJohn Co. Kalamazoo MI USA. (14,15(n)-³H)-LTC₄ (specific activity 0.8 TBq/mmol) was purchased from Amersham International, U.K.

Patients

Twenty patients with a history of seasonal allergic rhinoconjunctivitis with positive immediate skin tests to timothy grass pollen were studied (7 females, 13 males, aged 19–31). Allergy to timothy grass was confirmed by a positive reaction to nasal challenge with timothy grass extract as defined below. All challenges were undertaken outside the hay fever season and patients were not included in the study if they had ongoing nasal symptoms, upper respiratory tract infections within the previous month, gross anatomical abnormalities in the nose, a history of asthma, positive history or skin test to house dust mite or animal dander, or if they were pregnant or had any acute or chronic disease apart from hay fever. As the study was performed out of season, none of the patients had used any anti-allergic medications for the past 2 months and no one was undergoing immunotherapy. Any other medication

was discontinued at least 48 h before each challenge. The patients gave informed consent before entry, and the study was approved by the Ethical Committee of Rigshospitalet, Copenhagen, According to the standards laid down in the Helsinki II declaration.

Nasal challenge

The allergen threshold concentration was titrated in each subject as the lowest dilution to give a positive reaction as defined by 5 sneezes within the first 2 min and/or a reduction in inspiratory peak flow (insp-PF) of 50% using an adapted mini-Wright peak flow meter. One puff (0.1 ml) of allergen solution was administered into each nostril from a metered-dose, atomized pump spray (Fisons, Loughborough) while the patient inhaled simultaneously through the nose. The provocation was started from an allergen concentration of 100 SQ (standard quantified) units increasing step-wise every 10 min with increments of a factor 10^{0.5} until a positive reaction or a concentration of 100,000 SQ was reached. Nonspecific reactions were ruled out by excluding the patient in the case of a positive reaction to the first challenge.

Nasal lavage

The nose was blown prior to nasal lavage. A 5 cm polyethylene catheter was inserted along the floor of the nasal cavity. The nasal vestibulum was occluded and the head tilted forward. One ml of ^{99m}Tc-albumin was instilled through the catheter and retained in the anterior chamber for 30 s during continued occlusion of the vestibulum. The nose was subsequently blown into a funnel and the collecting vessel frozen to -170°C within 2 min. This procedure was repeated on the opposite side.

Measurement of nasal secretion in the lavage fluid

The amount of nasal secretion in the nasal lavage was calculated by measuring the dilution of a ^{99m}Tc-albumin marker added to the lavage fluid. The specific activity of the whole sample of lavage fluid was determined in a gamma

counter together with the specific activity of an aliquot of the stock solution. The amount of secretion in the nasal washing could then be derived from a simple stoichiometric expression (2).

Study design

Nasal mucosa irrigated by pump spray and different lavage techniques. The nasal mucosa irrigated by the pump spray was visualized in healthy subjects. A ^{133}Tc -pertechnetate solution (1 $\mu\text{Ci/ml}$) was sprayed into the nose with a pump spray as described, followed immediately by a regional scan using a gamma-camera (Maxi camera II, General Electrics, USA). On a subsequent day the nasal mucosa was irrigated by the adopted lavage technique and visualized by a similar ^{133}Tc -pertechnetate solution. On a final day, nasal lavage was performed with a volume of 5 ml into both nostrils and the head bent backward for 5 s. The distribution of activity was then compared by computer imaging after correction for the orientation of the head.

Fate of LTC_4 within nasal secretion. Titrated LTC_4 was added to two samples of nasal secretions obtained after allergen challenge. The samples were centrifuged at $10,000 \times g$ for 5 min and the supernatant decanted from the mucus. The distribution of activity in the sol and gel phases was determined by liquid scintillation spectrometry. Tritiated LTC_4 was spiked into another two samples of nasal secretions obtained after allergen challenge. After 1 and 15 min incubation at room temperature the samples were extracted and subjected to HPLC as described below. Individual fractions were collected and their ^3H content measured by liquid scintillation spectrometry.

Change in LTC_4 and histamine concentration after allergen challenge. The 20 test-persons were challenged on two separate occasions with the allergen threshold concentration. At least 1 week was interposed between the two challenges. Blown secretion was collected for 15 min after challenge. Nasal lavage was performed on each occasion before (pre-challenge) and at 16 min after allergen challenge (post-challenge).

Cell admixture in test samples. Thin films were made from a number of the test samples and stained with May-Grunwald-Giemsa to examine the cellular constituents.

LTC_4 measurements

Protein precipitation. All samples were frozen in liquid nitrogen within 2 min of collection and kept at -80°C until the processing. After thawing, protein was precipitated with acetonitrile at -78°C in a dry ice/acetone bath. After precipitating the pellets by centrifugation ($1500 \times g$, 4°C), the supernatants were decanted into siliconized glass tubes. The pellets were washed with 3×2 ml methanol and again sedimented by centrifugation. The two organic phases were combined and samples reduced to a volume of $< 50 \mu\text{l}$ under a stream of nitrogen prior to injection onto HPLC.

High pressure liquid chromatography (HPLC). The samples were made up to a final volume of $100 \mu\text{l}$ and separated using a 12.5×0.46 cm Techsphere 5 ODS column with guard (5×0.46 cm) and eluted at a flow rate of 1 ml/min with methanol:water:O-phosphoric acid (65:35:0.06) adjusted to pH 5.4 with ammonia solution (specific gravity 0.88 g/ml). The column was calibrated for the retention time of LTC_4 injected on the same solvent system, and was recalibrated after running every fourth sample. Samples eluting with the retention time of authentic LTC_4 were automatically collected on a Gilson Model 202 programmable fraction collector. Collected fractions were dried *in vacuo* in siliconized tubes and redissolved in $200 \mu\text{l}$ 1% sodium bicarbonate solution prior to radioimmunoassay. Recovery of LTC_4 through the extraction procedure was corrected for by inclusion of an internal ^3H - LTC_4 standard and generally ranged from 40–60%.

Radioimmunoassay (RIA) of LTC_4 was performed using an antibody specific for LTC_4 ; 1–3% and 1% cross reactions with LTD_4 and LTE_4 respectively. Samples ($100 \mu\text{l}$) were

incubated in polypropylene microfuge tubes with antibody (diluted 1:500 in pH 7.4 phosphate assay buffer containing gelatine) and 100 μ l of (^{3} H)-LTC₄ in assay buffer (10,000 dpm) for 16 h at 4°C after gentle mixing. Bound and free ligand were separated by dextran-coated charcoal sedimentation at 2000 $\times$ g and 4°C for 15 min. The supernatants were decanted into scintillation vials and the bound radioactivity measured by liquid scintillation counting after addition of 4 ml Optiphase-RIA scintillant. The data were automatically computed on-line using Packard Securia data management system.

The assay was capable of detecting a minimum dose of 31 pg LTC₄ immunoreactivity. The intra-assay coefficient of variation was 8.6%.

Histamine measurements

Histamine in nasal lavage and secretion was assayed using a single isotope radioenzymatic technique (7) with an intra-assay coefficient of variation of 12% and a sensitivity of 0.1 ng/ml.

Statistical methods

Since data did not appear to fulfil an assumption of Gaussian distribution, non-parametric models were utilized throughout the study. Paired comparisons within the same group were based on Pratt's test, a matched pairs signed rank test modified to take zero differences into account (12).

The correlation between two groups of data both having a probability distribution was estimated by Spearman's test. Due to the skew distribution of the mediator measurements, we abstained from condensing the data by descriptive statistics, and chose instead to depict all data. Medians are given as an adjunct within the figures to help locate the central tendency.

RESULTS

Nasal mucosa irrigated by pump spray and different lavage techniques. Fig. 1 shows a typical example

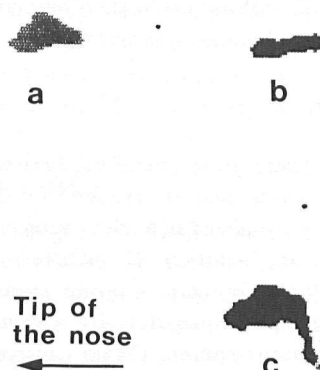


Fig. 1. Activity distribution in one subject (N.M.) after administration of a ^{99m}Tc -pertechnetate solution to the nose via (a) a pump spray; (b) a nasal lavage of 1 ml in both nostrils with the head bent forward; and (c) a nasal lavage of 5 ml in both nostrils with the head bent backwards.

Table 1
Percentual admixture in nasal lavage fluid.

	Prechallenge %		Postchallenge %	
	Day 1	Day 2	Day 1	Day 2
	18	12	9	43
	45	34	24	17
	24	27	50	33
	13	26	36	53
	63	18	63	56
	21	48	21	31
	30	6	67	20
	71	67	71	77
	23	18	33	77
	10	26	71	63
	25	11	37	36
	28	18	50	36
	21	24	22	23
	37	29	34	16
	13	45	33	71
	20	23	24	6
	5	9	28	32
			28	43
			45	59
Median	23	24	34	36

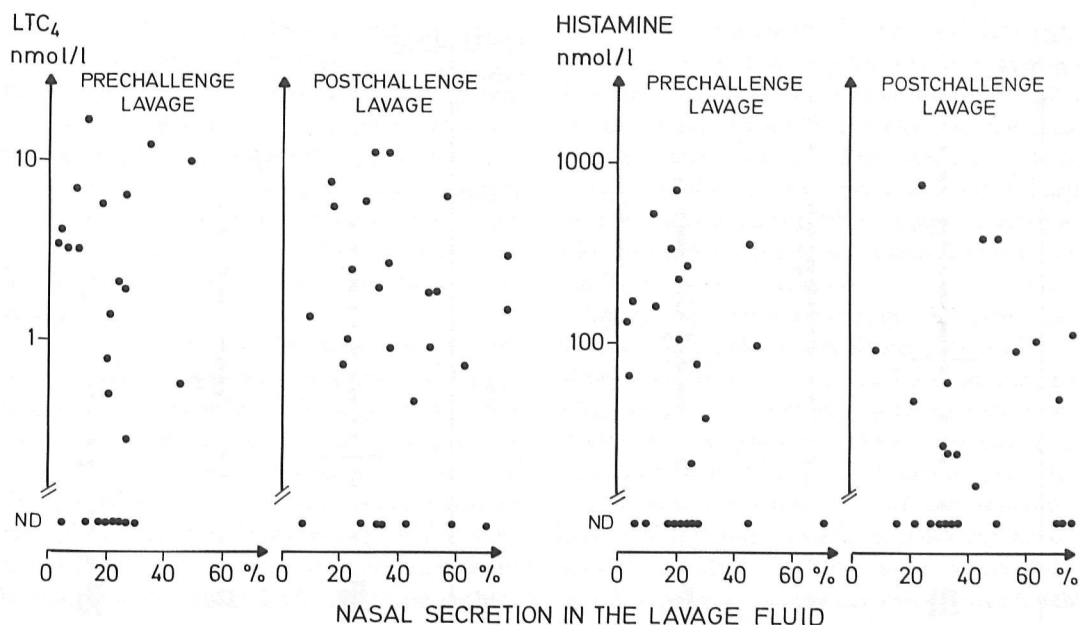


Fig. 2. Relation between percentage admixture of nasal secretion in the lavage fluid (2 ml) and concentrations of LTC₄ and histamine. The amount of nasal admixture did not exhibit any correlation to the concentration of mediators, either before or after allergen challenge.

of the distribution of activity in one of the subjects (N.M.). The nasal lavage technique adopted covered an area approximating that reached by the pump spray, and both applications were restricted to the nasal cavity. However, when lavage was performed with the head bent backward and a 10 ml of lavage volume used, the pharynx was apparently also irrigated and post-nasal loss apparent.

Amount and reproducibility of secretion in the nasal lavage. The amounts of nasal secretion in the lavage fluid are given in Table 1. Nasal lavage on both of the 2 test days was obtained in 17 subjects pre-challenge and in 19 subjects post-challenge. The median percentage nasal secretion in the 2 ml lavage fluids amounted to 23% and 24% in the two pre-challenge lavages and 34% and 36% in the two post-challenge lavages. The nasal secretion in the lavage fluids did not correlate significantly within the two pre-challenge lavages ($P > 0.1$), nor within the two post-challenge lavages ($P > 0.1$).

Vector for LTC₄ and histamine. Neither at pre-challenge nor post-challenge did the concentrations of LTC₄ and histamine show any correlation with the amount of nasal secretion (Fig. 2).

LTC₄ and histamine concentrations in nasal secretion. Of 120 possible samples 13 were lost during processing. Seven samples of blown secretion could not be collected. A total of 28 tests were complete with respect to LTC₄ measurements in pre-challenge lavage, blown secretion and post-challenge lavage, while 26 were complete with respect to histamine measurements. The concentrations of LTC₄ and histamine are depicted for the complete samplings in Figs. 3 and 4. Median pre-challenge concentration in the lavage fluid was 1.7 nmol/l for LTC₄ and 52 nmol/l for histamine. The mediator concentrations in the post-challenge nasal lavage did not differ from that of the pre-challenge lavage ($P > 0.1$). The concentrations of LTC₄ and histamine were significantly higher in the blown secretion than in the pre-challenge nasal lavage

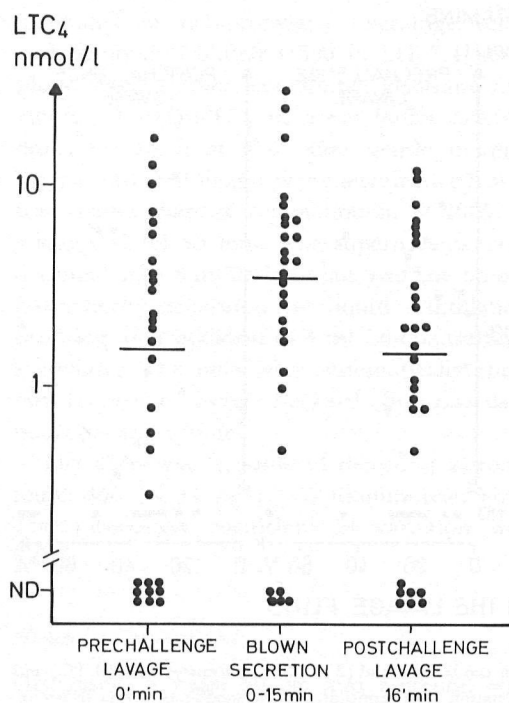


Fig. 3. LTC_4 concentration in nasal lavage fluids before and 16 min after allergen challenge, as well as in the blown secretion accumulated during the 15 min from the challenge to the final lavage. LTC_4 was significantly increased in the blown secretion ($n = 28$; $P < 0.01$), yet not in the post-challenge lavage fluid as compared to the pre-challenge lavage.

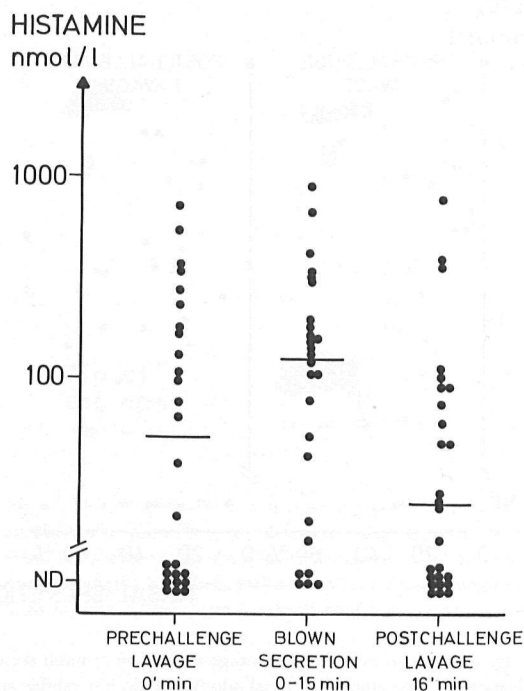


Fig. 4. Histamine concentration in nasal lavage fluids before and 16 min after the allergen challenge, as well as in the blown secretion accumulated during the 15 min from the challenge to the final lavage. Histamine was significantly increased in the blown secretion ($n = 26$; $P < 0.05$), yet not in the post-challenge lavage fluid as compared to the pre-challenge lavage.

(LTC_4 $P < 0.01$; histamine $P < 0.05$). The molar ratio between the median histamine and LTC_4 concentrations was 1 to 31 in the pre-challenge nasal lavage, and 1 to 17 in the post-challenge lavage. Addition of exogenous (^3H)- LTC_4 to nasal secretion showed a concentration in the gel phase of 88–98% of the concentration in the sol phase. No evidence was found of metabolism of (^3H)- LTC_4 in the nasal secretion within 15 min. Serial dilutions of test samples produced an RIA displacement curve that paralleled the LTC_4 standard curve.

The LTC_4 and histamine measurements exhibited a pronounced variability between as well as within subjects (Table 2).

Cell admixture. Overall cellularity was sparse, and only very few granulocytes were observed.

DISCUSSION

Lavage of the nasal mucosa would appear to be a convenient model for measuring mediator release during an allergic reaction *in vivo*. It contains the advantage of being close to the site of the inflammatory response and being relatively non-traumatic. However, several methodological questions arise as to the interpretation of the results from this model. In this study we have scrutinized the possible confounding effects from variations in the amount of nasal secretion obtained by the lavage, and the area washed by the lavage technique. Any substance measured in a nasal lavage fluid might be confounded by variable degrees of admixture from the nose, such as secretion, exudation, and tears, which will further dilute any released

mediator and may contribute to their degradation. In agreement with previous reports we have found that LTC₄ was distributed equally between the gel and the sol phase of nasal discharge (3, 9). Moreover, LTC₄ was not metabolized to any appreciable degree within the nasal secretion. It was therefore reasonable to suggest that the two mediators accumulate in the nasal secretion following their endogenous release.

The admixture with secretions in the returned nasal lavage fluids was quantified by a dilution principle using ^{99m}Tc-labelled albumin as an exogenous marker. This technique was simple and safe and allowed for the precise determination of small amounts of admixture in the lavage fluid (2). However, by taking dilution effects into account we were unable to demonstrate that either LTC₄ or histamine concentrations appeared in the lavage fluid in any proportion to the amount of nasal secretion admixed either before or after challenge. Cells were only found sparsely in the lavage fluid, and were constituted by granulocytes. The concentrations of LTC₄ and histamine measured in the return fluid after a 30-s nasal lavage therefore seemed to be the results of equilibration in the nose between mediators in nasal secretion and nasal lavage fluid. Measurements of these mediators in lavage fluid would consequently not be confounded by variable amounts of admixture from nasal discharge, provided the area of nasal mucosa irrigated during the lavage is standardized as well as the time of intranasal exposure of the lavage fluid.

The area washed by the adopted lavage technique seemed to cover an area comparable to that reached by the allergen challenge delivered by pump spray. The nasal mucosa, but not the pharynx, was washed by the lavage fluid when the lavage volume was standardized to 1 ml in both nostrils, the head bent forward, and the time of exposure of the lavage in the nose fixed at 30 s. When larger volumes were used for lavage and the head bent backwards, as described in previous studies (3, 9, 13), the pharynx was irrigated and postnasal loss was apparent. Ninety-nine percent of the instilled volume could be recovered from non-allergic noses by

our lavage technique (2). The time of exposure of the lavage fluid in the nose was fixed at 30 s. The technique was well standardized, optimizing the chance of washing a reproducible area of the nasal mucosa. Furthermore, it was always possible to perform the nasal lavage despite nasal blockage after allergen challenge and therefore the use of a vasoconstrictor spray could be avoided.

Employing the nasal lavage technique described, the median pre-challenge concentration of immunoreactive LTC₄ was 1.7 nmol/l in the present study. Shaw et al. reported a mean pre-challenge concentration of 0.1–1.2 nmol/l (13), and Creticos et al. approx. 1 nmol/l (3). The two studies employed a radioimmunoassay using an antibody with a pronounced cross-reactivity to LTD₄, and to a lesser extent LTE₄ (8). The results would therefore overestimate the amount of sulphidoleukotriene existing as LTC₄. Yet, 2–5 times larger lavage volumes were used compared to the present study. We purified all samples through extraction on HPLC prior to subsequent assay where a monospecific LTC₄ antibody was employed. It is noteworthy that the baseline reported in the study of Creticos et al. (3) was measured after

Table 2
Between and within subject variation of LTC₄ and histamine measurements in blown secretions

LTC ₄ , nmol/l		Histamine, nmol/l	
Day 1	Day 2	Day 1	Day 2
5.49	6.38	500	67
2.28	8.02	710	5
2.94	2.38	337	534
1.73	4.34	357	218
3.30	6.78	ND	76
ND	2.62	163	21
ND	4.70	73	1061
4.17	1.93	226	97
6.79	5.49	38	6
ND	28.02	130	ND
29.73	23.85	ND	ND
ND	8.48	ND	ND
3.72	9.05	105	> 276
17.33	1.60	73	ND
1.22	1.01	ND	ND

ND = not detectable

repeated nasal washings in order to decrease the pre-challenge concentration, though it seems comparable to the concentrations of LTC_4 reported without pre-washings. The pre-challenge histamine concentration of 52 nmol/l measured in the present study by a micro-enzymatic radiotransfer assay corresponds well with previous reports. Okuda et al. (11) reported a decrease in histamine concentration after allergen challenge from a baseline concentration of 161 nmol/l in nasal secretion obtained by microsuction from allergic subjects. Eggelston et al. (5) measured histamine by a fluorometric technique in nasal lavage fluid from hay fever patients and reported a concentration of 0–3754 nmol/l without any relation to symptoms. Using a radioenzymatic technique, Davies et al. (4) reported a concentration of approximately 200 nmol/l in full secretion from six patients after allergen challenge and the same concentration after challenge with methacholine. Naclerio et al. (9) showed a maximal mean increase in histamine of 11 nmol/l (fluorometry) after allergen challenge, recognizing that vigorous prewashing of the nasal mucosa was necessary to reduce the high baseline levels of the mediator.

Despite extensive precautions over the lavage technique we were unable to show that either LTC_4 or histamine increased significantly from the pre-challenge to the post-challenge nasal lavage performed 16 min after challenge. However, measurements of both mediators in the blown secretion collected separately over 15 min after allergen challenge showed a significantly higher level than in the pre- as well as in the post-challenge lavage. This could be compatible with a spill-over of mediators in the initial minutes after the reaction no longer being detectable 16 min later when the post-challenge lavage was undertaken. The peak release of histamine (10) and LTC_4 (13) has been reported to occur within 7 min of challenge. It seems doubtful, however, whether mediator measurements made in blown secretions can be compared to nasal lavage fluid, even though our data do not support dilution as a confounding factor. It can be argued that the blown secretion is collected from another area

of the nose than that washed by the nasal lavage technique. More appropriate controls would be blown secretion obtained with different forms of mucosal stimulation. Davies et al. (4) have reported increased concentrations of histamine in blown secretion after allergen challenge as compared to pre-challenge lavage, yet not when compared to blown secretions following challenge with methacholine. LTC_4 was found in appreciable amounts in the nasal lavage following allergen challenge but not following methacholine challenge (13). Other studies have reduced the pre-challenge level of mediators in the lavage fluid by repeated pre-washings and thereafter demonstrated a rise in histamine back to a level comparable to the baseline level we and others have found (9). In that case it is not clear whether a post-challenge increase in mediator represents a return to normal physiological conditions or is an abnormal pathophysiological event. In either case the process may be accelerated by the increased secretion after allergen challenge.

Our failure to find increased mediator concentration in nasal lavage after allergen challenge could be due to a very pronounced variability of the measurements. To investigate this possibility the same 20 subjects were challenged on two separate occasions and mediator measurements made. These results revealed wide variation in mediator measurements within as well as between subjects. Considering the poor reproducibility only major increases in mediator concentrations in lavage would be revealed. We titrated the lowest possible allergen concentration to give a symptomatic allergic reaction as indicated by a reduction in the inspiratory peak flow by 50 % and/or 5 sneezes within the first 2 min of allergen challenge. More vigorous challenge would probably increase the chances of recovery of released mediators but would be of less physiological interest and would be ethically questionable.

After allergen challenge the median molar ratio between LTC_4 and histamine was 1 to 32 in the secretion and 1 to 17 in the nasal lavage fluid after allergen challenge. However, it may be unwarranted to extrapolate from measurements of the spill-over of mediators measured

outside the tissue to concentrations that may exist adjacent and within the target tissue. Leukotriene C_4 is known to be unstable *in vivo* in humans (14) while histamine is probably less vulnerable to degradation. The demonstration of LTC_4 in concentrations approaching those of histamine could point to a role of LTC_4 in nasal allergy. One of the bioconversion products, LTD_4 , has previously been demonstrated to cause nasal obstruction when administered to the nasal mucosa in humans (1). In conclusion, our studies cast serious doubt on the validity of mediator measurements in nasal lavage in relation to symptoms following local nasal challenge with allergen. By employing a method to correct for secretion admixture we have been unable to improve the sensitivity or precision of nasal lavage as a source of preformed and newly generated mediators for physiological and pharmacological studies. However, our findings do suggest that following challenge, nasal secretions contain both histamine and LTC_4 that is likely to be of pathophysiological significance in the pathogenesis of acute rhinitis symptoms.

REFERENCES

1. Bisgaard, H., Olsson, P. & Bende, M.: The effect of leukotriene D_4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. *Clin. Allergy* 16, 289-297, 1986.
2. Bisgaard, H., Korsgaard, O. W. & Mygind, N.: Measurement of nasal secretion by ^{99m}Tc -albumin. *Clin. Sci.* 73, 217-222, 1987.
3. Creticos, P. S., Peters, S. P., Adkinson, N. F., et al.: Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N. Engl. J. Med.* 310, 1626-1630, 1984.
4. Davies, R. J., Corrado, O. J., Änggård, A. & Borum, P.: Rhinitis and asthma. In Mygind, N. & Weeke, B. (eds.): *Allergic and vasomotor rhinitis: clinical aspects*, pp. 65-79. Munksgaard, Copenhagen, 1985.
5. Eggleston, P. A., Hendley, J. O., Gwaltney, J. M. Jr., Eggleston, A. W. & Leawell, B. S. Jr.: Histamine in nasal secretions. *Int. Arch. Allergy Appl. Immunol.* 57, 193-200, 1978.
6. Fox, C. C., Kagey-Sobotka, A., Schleimer, R. P., Peters, S. P., MacGlashan, D. W. Jr. & Lichtenstein, L. M.: Mediator release from human basophils and mast cells from lung and intestinal mucosa. *Int. Arch. Allergy Appl. Immunol.* 77, 130-136, 1985.
7. Gilloux, L., Hartmann, D. & Ville, G.: Enzymatic isotopic assay for human plasma histamine. *Clin. Chim. Acta* 116, 269-275, 1981.
8. Hayes, E., Lombardo, D., Girard, Y., et al.: Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radioimmunoassay. *J. Immunol.* 131, 429-433, 1983.
9. Naclerio, R. M., Meier, H. L., Kagey-Sobotka, A., et al.: Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.* 128, 597-602, 1983.
10. Norman, P. S., Naclerio, R. M., Creticos, P. S., Torgias, A. & Lichtenstein, L. M.: Mediator release after allergic and physical nasal challenges. *Int. Arch. Allergy Appl. Immunol.* 77, 57-63, 1985.
11. Okuda, M.: Mechanisms in nasal allergy. Parts I & II. *ORL Digest* (2), 26-34 + (3) 22-31, 1977.
12. Rahe, A. J.: Tables of critical values for the Pratt matched pair signed rank statistic. *J. Am. Stat. Assoc.* 69, 368-373, 1974.
13. Shaw, R. J., Fitzharris, P., Cromwell, O., Wardlaw, A. J. & Kay, A. B.: Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB_4 in allergic rhinitis. *Allergy* 40, 1-6, 1985.
14. Ørning, L., Kaijser, L. & Hammerstrøm, S.: In vivo metabolism of leukotriene C in man: urinary excretion of leukotriene E. *Biochem. Biophys. Res. Commun.* 130, 214-220, 1985.

Address:

Hans Bisgaard

Otopathological Laboratorium

Dept. of Otorhinolaryngology

Rigshospitalet, Copenhagen

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.