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The mechanisms of systemic symptoms following nasal administration of allergens

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SUMMARY

A nasal provocation test was performed by consecutively placing allergen discs on the nasal mucosa of patients with pollinosis. This brought easily the systemic symptoms such as cough, periorbital oedema, and urticaria.

Similarly, the nasal administration of large doses of the allergen induced a change in the respiratory response in sensitized guinea pigs.

These results indicate that nasal mucosa absorbes a high molecular substance such as allergens.

INTRODUCTION

The nasal mucosa absorbs various kinds of chemicals very well. Therefore, nasal administration is attractive as a third route for the application of medicinal products. On the other hand, the nasal mucosa has direct contact with the outer world and is always exposed to the danger of an antigen invasion.

In this experiment, we focused our attention on the nasal absorption of a high molecular substance, such as an allergen, in patients with pollinosis and sensitized guinea pigs.

MATERIALS AND METHODS

1. On 10 patients with allergic rhinitis sensitive to Dactylis gromerata pollen, nasal provocation tests were performed. The tests were performed consecutively 5-6 times at intervals of 30 min using allergen discs. The discs were 3 mm in diameter and each contained 250 μg of Dactylis gromerata pollen extract (made by Hollister-Stier Co., Ltd.). The test was performed by placing the disc on the surface of the inferior turbinate of the nose in the pollen season. The patients were volunteers between 30 and 50 years of age with no distinction of sex.
2. Nasal provocation tests were carried out on the two groups of Hartley-strain guinea pigs sensitized by the following two methods:
   a. Twelve guinea pigs were fed four weeks after they had the nasal application

of 10% (10 mg) of toluene-2, 4-diïsocyanate (TDI) (Nakarai Co., Ltd.) in ethyl acetate once a day for five days.

b. Ten guinea pigs were fed ten days after they had the exposure to an aerosol of 10 mg of α-amylase (extract from Bacillus subtilis, Sigma Co., Ltd.) with an ultra-nebulizer in a glass chamber (27 × 27 × 30 cm) for a period of ten minutes a day for seven days.

The sensitized guinea pigs of both groups were anesthetized with urethane (0.8 g/ kg) given intraperitoneally, and the trachea was exposed. An endotracheal tube was put through the mouth and secured in the upper trachea, then placed in the prone position in an open-type body plethysmograph. The neck seal was then installed. Pleural pressure (Ppl) was measured with a fluid-filled esophageal catheter (0.8 mm × 20 cm) having multiple side holes. The catheter was situated into the stomach, flushed with saline solution, then withdrawn into the esophagus until a maximum negative pressure was observed. Zero pressure was estimated by positioning the transducer at the mid-thoracic level through visual examination. Transpulmonary pressure was obtained by comparing pleural and airway-opening pressures on a differential pressure transducer. The flow was measured with a pneumotachograph. Volume was measured by an electrical integration of the flow signal. Lung flow resistance was measured by means of Amdur et al., 1958. A maximum flow-volume curve (F–V curve) was measured as

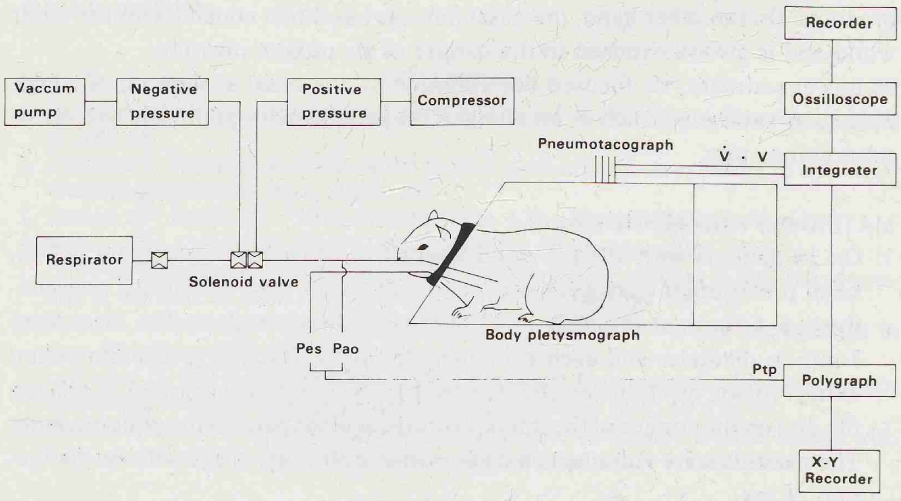

Figure 1. Schematic diagram of experimental set-up for the study of pulmonary function in guinea pig.

follows. After succine (3 mg/kg) was injected, the endotracheal tube was connected to the respiratory pump. Then the respiratory pump was stopped and the endotracheal tube was connected to the air compressor at a pressure of 30 cm H_2O. Immediately after blocking this, the endotracheal tube was connected to the vacuum reservoir at a pressure of -40 cm H_2O. Flow and volume in this forced expiration were recorded on an oscilloscope (Figure 1) (Koo et al., 1976; Lai et al., 1978).

Administration: 10 mg of TDI or 1 mg of α-amylase was administered intra-nasally to the sensitized guinea pigs. A respiratory tracing or flow-volume curve was recorded for a period of 30 min after administration. If no change was shown, re-administration was performed 60 min after the first trial.

RESULTS

The nasal provocation test using Dactylis gromerata pollen extract disc produced sneezing, watery rhinorrhea, and nasal obstruction in all patients. Repeated provocation tests provoked the following symptoms.

Case 1. M.A. (F) - Following the fifth provocation, massive periorbital oedema appeared accompanied by wheezing.

Case 2. N.S. (M) - Following the fifth provocation, burning and itching appeared at the left eyelid.

Case 3. S.S. (F) - Following the second provocation, itching of both eyes and periorbital oedema appeared. Following the third provocation, wheezing and dyspnea gradually appeared.

Case 4. H.K. (M) - Following the second provocation, periorbital oedema appeared. Following the fourth provocation, itching of the chin and posterior neck appeared.

Case 5. I.K. (F) - No symptoms except nasal ones appeared during the five provocations.

Case 6. A. K. (F) - Following the second provocation, itching of the eye appeared. Following the fourth provocation, itching of the pharynx appeared. Following the fifth provocation, itching of the head and urticaria appeared in the whole body.

Case 7. O.I. (M) - Following the third provocation, periorbital oedema and itching of the eyes appeared.

Case 8. Y.O. (F) - No symptoms except nasal ones appeared during five provocations.

Case 9. O.S. (M) - Following the second provocation, itching appeared. Following the third provocation, left periorbital oedema appeared.

Case 10. O.K. (M) - Following the second provocation, itching of both eyes and pharynx appeared.

These symptoms were summarized in Table 1. Case 3 showed massive periorbital oedema (Figure 2). The F-V curve of case 3, before and after the provocation is shown in Figure 3. After the first nasal administration of allergen to the sensitized guinea pigs, one out of six sensitized by α-amylase and none out of four sensitized

Table 1. The patients showed allergic symptoms after repeated nasal provocation test with pollen extract.

Cases	Itching of eye	Periorbital edema	Erythema of face	Wheezing	Urticaria	Itching of pharynx
1. M.A. ♀	+	+	+	+		
2. N.S. ♀	+					
3. S.S. ♀	+	+	+	+		
4. H.K. ♂	+	+				+
5. I.K. ♀						
6. A.K. ♂	+		+		+	+
7. O.I. ♂	+	+				
8. Y.O. ♀						
9. O.S. ♂		+				+
10. O.K. ♂						+

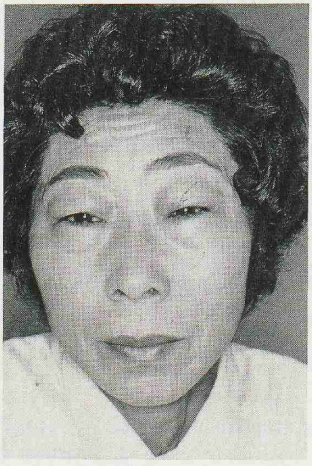

Figure 2. The case 3 showed massive periorbital oedema.

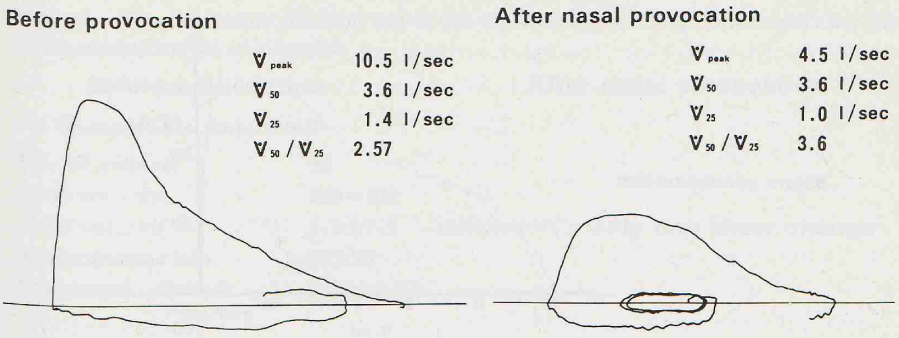

Figure 3. F-V curve for case 3, before and after the provocation.

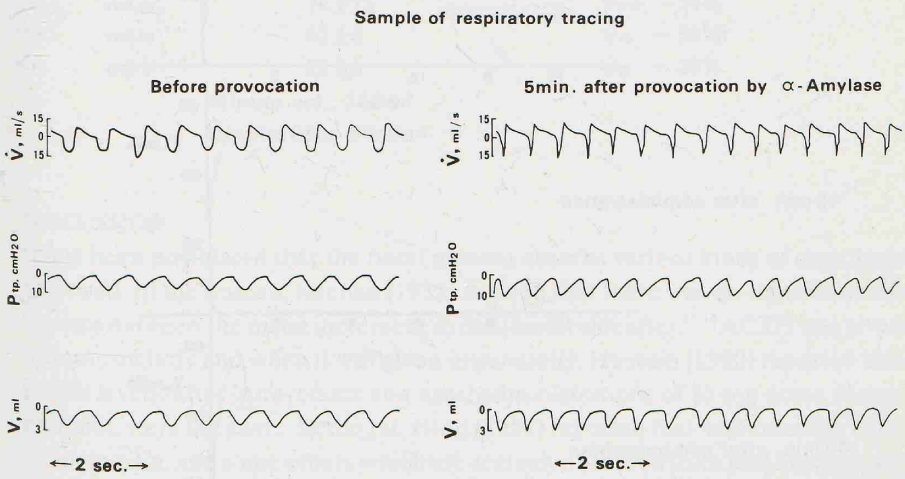

Figure 4. Respiratory tracing of the sensitized guinea pig before and after the nasal administration of α-amylase.

by TDI had increased transpulmonary pressure and flow resistance (Figure 4). In the F-V curve, three out of eight sensitized by TDI, and six out of ten sensitized α-amylase showed significant decrease of expiratory flow rates (Figure 5).
In the case of a negative response after the first provocation, the second administration was made. After the second challenge, in five out of eight and seven out of ten, positive responses were observed. Both results, sensitized with TDI and α-amylase, were combined and showed in Table 2.

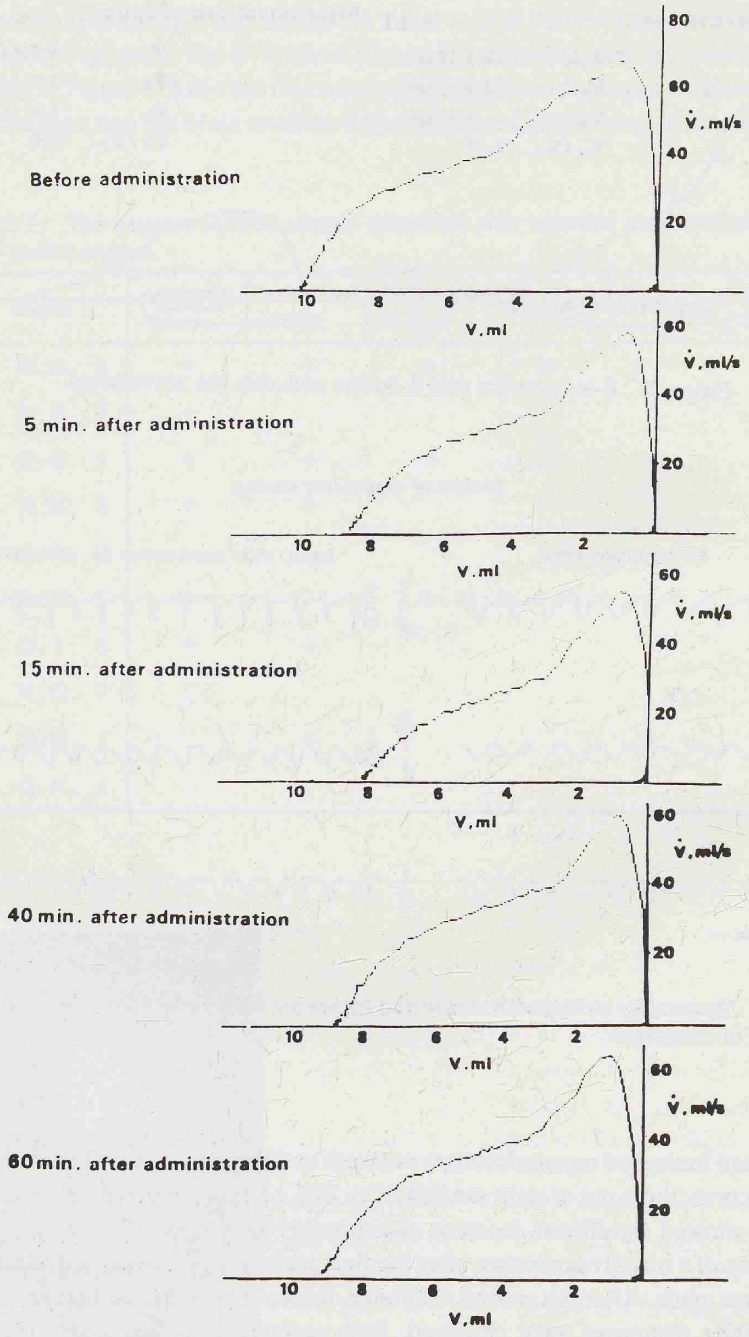

Figure 5. A sample of changing pattern of F–V curve after nasal administration of the allergen in the sensitized guinea pigs.

Table 2. The respiratory function test of the sensitized guinea pigs before and after the nasal administration of allergens.

Before provocation		After nasal provocation
Spontaneous respiration		
No. of animals	10	
Body wt., gm	280~380	
Tidal vol., ml	2.9±1.9	⟹ Only one show change
Respirations/min.	80±25	
Resistance, CnH_2O /ml/sec	0.17±0.07	
Artifical ventilation※		
No. of animals	18	12 show change
Body wt., gm	280~380	Maximum rate of change
$\dot{V}_{peak}$ ml/s	70±15	⟹ $\dot{V}_{peak}$ −25%
$\dot{V}_{50}$ ml/s	43±6	$\dot{V}_{50}$ −50%
$\dot{V}_{25}$ ml/s	28±5	$\dot{V}_{25}$ −30%

※ Minute vol., 150ml
Respirations 80/min.

DISCUSSION

It has been postulated that the nasal mucosa absorbs various kinds of chemicals very well. In the human, Keenan (1971) showed that there was no significant difference between the mean increment in corticosteroids after $^{1-18}$ACTH was given intramuscularly and when it was given intranasally. Hussain (1980) reported that serum levels, after intravenous and nasal administrations of 10 mg doses of propranolol, were the same. In the rat, Hirai (1981) reported that sulbenicillin, cefazolin, insulin, and some others which are scarcely absorbed from oral mucosa, are well absorbed into the systemic circulation from the nasal mucosa. Because of this good absorption, nasal administration is attractive as a third route for the application of medicinal products.

On the other hand, pathogenic organisms and other antigens often invade the host by the way of the mucosa of the respiratory or the digestive organ. The nasal mucosa most often comes directly in contact with the outer world and is always exposed to the danger of an invasion by antigens. Antigens are generally high molecular substances composed of protein, polysaccharide, and others. There are few reports about absorption of high molecular substances from nasal mucosa. The purpose of this investigation was to study the nasal absorption of a high molecular substance using the allergens which induced allergic reaction to sensitized humans or guinea pigs. As allergens, in human, Dactylis gromerata pollen was used because of its strong allergic activities. In guinea pigs, TDI and α-amylase

were used. α-amylase has a molecular weight of about 50.000. The use of α-amylase to induce respiratory sensitivity in guinea pigs has been established as an animal model of allergic asthma, but nasal symptoms were not evoked (Yagura, 1973). TDI evokes nasal symptoms such as sneezing, watery rhinorrhea in guinea pigs. But there is much argument whether TDI behaves as an allergen or not (Pepys et al., 1978). The molecular weight of TDI is 174 and it is too small as an antigen in the general sense. TDI is an important component in the manufacture of polyurethane, which has many uses in the paint, varnish, plastic industries, and the like. In the industrial environment, workers probably become sensitized by inhalation of TDI. Respiratory hypersensitivity is thought to develop in approximately 5% of those who are regularly exposed to TDI (Brugsch et al., 1963), but no evidence of an immunologic basis for the clinical manifestations produced by exposure to TDI has been found.

The human serum dialized against glycin buffer, after addition of TDI showed the characteristic benzyl ring in UV absorption spectrum (Figure 6), and an increase

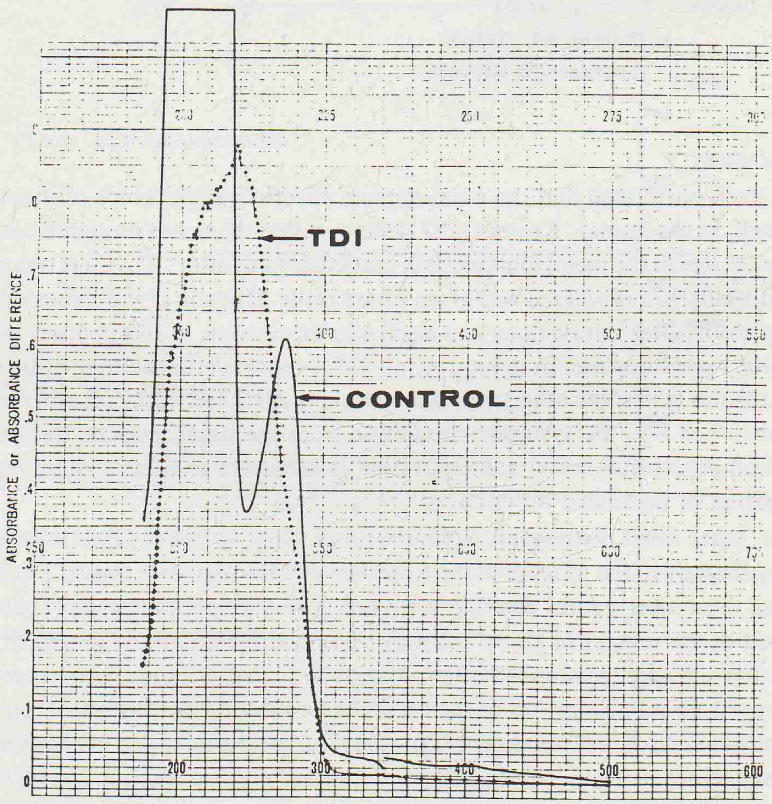

Figure 6. Human sera dialized against glycin buffer, after addition of TDI showed characteristic of benzyl ring in UV absorption spectrum.

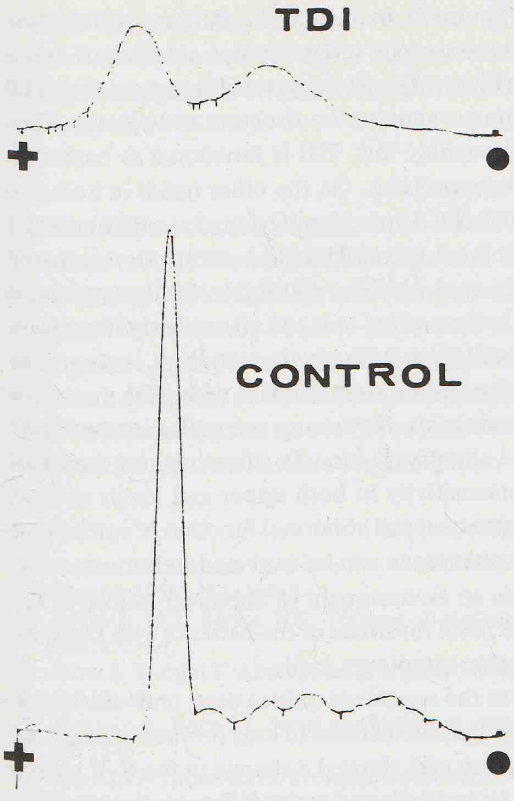

Figure 7.
Electrophoretic pattern of human sera, normal and TDI reacted. TDI-reacted sera showed increased mobility and change of profile.

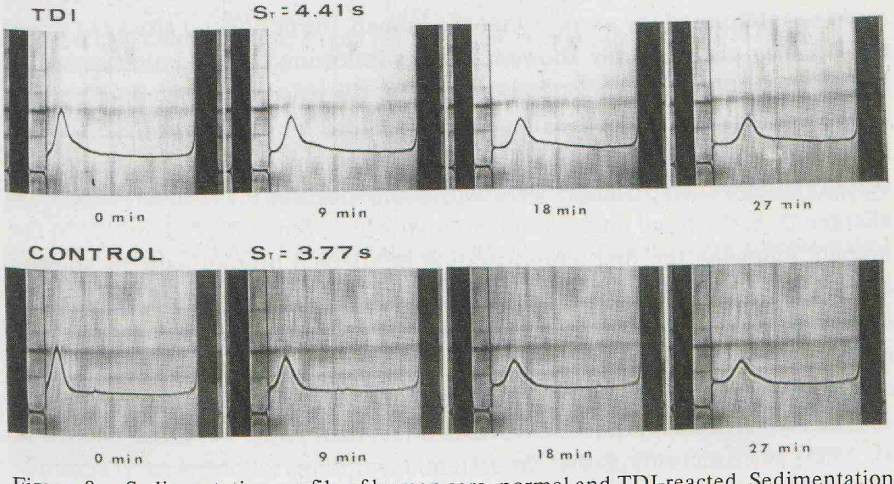

Figure 8. Sedimentation profile of human sera, normal and TDI-reacted. Sedimentation analysis were performed under the following condition. 60.000 rpm, photographic interval: 9 min, temp: 20 °C.

of mobility in an electrophoresis (Figure 7). In an analytical ultracentrifugation, TDI-reacted serum showed an increment in sedimentation coefficient and a change of sedimentation pattern (Figure 8). All these results suggest that TDI easily binds to serum proteins by contact and that the structure and polarity of the bound protein are altered. So it is possibly that TDI is functional as hapten or allergen as a result of their binding to proteins. On the other hand, in homogeneous passive cutaneous anaphylaxis (PCA) reaction (Ovary et al., 1976) using 1 ml of 0.5% Evans blue dye containing 2 mg of TDI-HSA conjugates prepared according to the method of Ted Tse et al. (1979), γ_1 antibody was recognized in seven out of ten and IgE antibody in four out of ten, and all antibody titers were lower than $\times 4$ to $\times 12$. Considering the low antibody titers in PCA, it can not be neglected that the hypersensitivity resulting from contact with TDI may have pharmacologic as well as immunologic basis. However, even such a low antibody titer could be good enough to cause allergic reaction. In this study, we used TDI as a substance which evokes hypersensitivity in both upper and lower airways easily. Using TDI, loss of cilia was observed and abnormal function of epithelium can be expected. However, similar phenomen can be expected in human cases, even with low dose of TDI, such as an environment of chemical industry. Repeated application of allergen to the nasal turbinate of the patients with Dactylis gromerata pollinosis induced systemic symptoms easily.

In nasal administration of allergen to the sensitized guinea pigs, only one out of six which was sensitized by α-amylase had an increase in lung flow resistance, but about two thirds of all sensitized guinea pigs showed a change in the F–V curve. The difference in results is thought to be attributed to the difference in sensitivities of those examined. Though it is uncertain that the F–V curve of guinea pigs could be interpreted the same as that of a human, the peripheral airway of sensitized guinea pigs certainly showed changes following nasal administration of large doses of allergen. The endotracheal tube was situated in the upper trachea and prevented an inflow of the allergen into the lung. Furthermore, the electrical irritation to the nasal mucosa of these guinea pigs showed no change in the F–V curve. Therefore these changes were considered to be due to the absorption of the allergen from the nasal mucosa, and not to the nose-lung reflex. Similarly in the human, following the first administration of allergen disc all patients showed excessive swelling of the nasal mucosa so that the allergen contained in the disc could not be inhalated to the lower airway. Considering the evidence that the patients who have Vidian neurectomy for allergic rhinitis do not show systemic symptoms following nasal administration of histamine up to 0.5 mg, and that the content of histamine in the nasal mucosa is less than 10 μg/wet tissue (Konno et al., 1983), the conclusion is that the systemic symptoms following nasal administration of large doses of allergen are due to the chemical mediators released not only in the nasal mucosa but also in the blood flow by reaction of the antibody

with the allergen absorbed from the nasal mucosa. These results mean that the nasal mucosa also could absorb a high molecular substance as an allergen, though it remains unclear whether sensitization makes the absorption of the allergen easier or not.

RÉSUMÉ

Un test de provocation de muqueuse nasale a été effectué par des applications consécutives de disques d'allergènes sur la muqueuse nasale de patients souffrant de pollinose. Cela n'a pas manqué de provoquer les symptômes systémiques, tels que toux, oedème periorbital et urticaires.
De la même façon l'administration nasale de grandes doses de l'allergène a produit chez des cobayes sensibilisés un changement dans la réponse respiratoire. Ces résultats indiquent que la muqueuse nasale absorbe des substances à poids moléculaire élevé, telles que les allergènes.

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