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Detection of Amino Acids in Human Nasal Mucosa Using Microdialysis Technique: Increased Glutamate in Allergic Rhinitis

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SUMMARY Amino acids, the smaller basic biochemical units of neuropeptides, have not been evaluated in the nasal cavity. The purpose of this study was to measure the concentration of neurotransmitting amino acids of the central nervous system, glutamate, aspartate, serine, taurine (2-aminoethane sulfonic acid; a conditionally essential amino acids), and GABA (gamma-amino-butyric acid; an amino acid produced in the brain), in nasal mucosa of allergic rhinitis patients and normal controls using a microdialysis technique. A microdialysis probe appropriate for use on human nasal mucosa was developed using Cuprophan hollow fiber. Glutamate concentration in allergy group was significantly higher (p = 0.004) than in control group, while the concentrations of the other four amino acids showed no significant difference between the two groups. Our findings and review of the literature suggest that glutamate is one of the most potent neurotransmitters associated with the parasympathetic nerve in the nasal cavity, and that the microdialysis technique is useful in studying the pharmacokinetics *in situ* and local organ chemistry of the nasal cavity.

Neuropeptides in the nasal cavity, such as substance P, calcitonin gene-related peptide and neurokinin A in the sensory nerve, vasoactive intestinal polypeptide in the parasympathetic nerve and neuropeptide Y in the sympathetic nerve, are important neurotransmitters contributing not only to the homeostatic control of nasal physiology but also to the pathogenesis of allergic rhinitis.[1] Their effects on vascular permeability, glandular secretion, mucociliary activity, inflammatory and immunological pathways, result in neurogenic inflammation and nonspecific hyperresponsiveness in allergic rhinitis.[1] However, the existence of amino acids that are much smaller biochemical units of neuropeptides acting as neurotransmitters in the central nervous system (CNS) has not been confirmed in the nasal cavity and, moreover, research on any changes of such amino acids in patients with an allergic inflammatory condition has not been executed.

There have been studies of amino acids in bronchoalveolar lavage fluids of asthmatic patients, gamma-amino-butyric acid (GABA) receptors in the

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lung and inhibition of bronchial hyperresponsiveness by GABA agonist, and peripheral glutamate receptors in the bronchus and stimulation of peripheral cholinergic nerves in the rat by glutamate.[2-4] All these studies were carried out only in the lower airway including the lung and bronchus and not in the nasal cavity. Microdialysis is a technique for sampling the chemistry of the extracellular environment of the individual tissues and organs of the body.[5] It was initially developed for use in the CNS and its application has extended peripherally to include adipose tissue, the adrenal gland, and the liver.[6] Thus far, the technique has not been tried in the nasal mucosa in evaluating selected chemical compounds such as amino acids. Therefore, this study aimed to measure the concentration of well known amino acid neurotransmitters of the CNS (glutamate, aspartate, serine, taurine and GABA) in human nasal mucosa by using the microdialysis technique. We evaluated the alteration in concentration of amino acids in normal and allergic mucosa and infer a role for the changed amino acids in allergic rhinitis.

SUBJECTS AND METHODS

Subjects

The normal control group was composed of 10 people (5 females, 5 males) with an age range 23 to 36 years (mean 31.2 years). They had no allergic rhinitis symptoms or any related medical history. They did not have any sinonasal diseases and showed negative skin prick tests utilizing 55 different types of inhalant allergens. None had used any medications for at least 4 weeks before this study. The allergy group included 10 patients (5 females, 5 males) with an age range 21 to 38 years (mean age 33.4 years). They had a positive skin prick test and radioallergosorbent test to house dust mite (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*). They had all visited the Department of Otorhinolaryngology in Pusan National University Hospital because of aggravated rhinitis symptoms at the time of study and none had any history of antihistamine or anti-allergic medication use for at least 2 weeks prior to this study. The study was approved by the Institutional Review Board of Pusan National University Hospital, and informed consent was obtained from all participants in both the normal control and allergy groups.

Verification of microdialysis probe

A Cuprophan hollow fiber (200 μm inner diameter, 300 μm outer diameter, 45 kDa molecular weight cutoff, Fitral AN 69-HF, Hospal Co., Lyon, France) was used to design the probe for microdialysis and the length of the active microdialysis membrane was set at 2 mm for use in sampling the subepithelial lamina propria of the inferior turbinate (Fig. 1). The designed microdialysis probe was sterilized overnight with UV light and before microdialysis it was dipped into 70% alcohol and washed by perfusion with sterilized normal saline. Sterilizing was confirmed by bacterial culture of the active membrane and perfusate. In addition, the insertion site of the nasal mucosa, attachment site of the microinfusion pump (baby Bee syringe pump, BAS, Lafayette, IN) and collecting Eppendorf tube used to carry the perfusate were carefully washed and sterilized. The designed probes were perfused with 10% glucose solution and only those with a recovery rate more than 20% were considered optimal and used for this experiment (Fig. 2).

Microdialysis of the inferior turbinate

Subjects were placed in a supine position so that the anterior surface of right inferior turbinate, that would be the insertion site of microdialysis probe, could be anesthesized with a 2% lidocaine soaked cotton applicator. The probe was inserted under endoscopic guidance so that the probe tip, where the active membrane exists, was placed in the subepithelial lamina propria of the right side of the inferior turbinate (Fig. 1, Fig. 3). The microinfusion pump was then started and perfusion was begun at a rate of 2 μl/minute with sterilized normal saline. The perfusate obtained during the first 60 minutes was discarded. Then, at 30 minute intervals, the samples of perfusate were collected 4 times. In order to prevent changes of amino acid concentration caused by the ambient temperature, the collection tube was immersed in an ice filled-container during perfusate sampling. The extracted samples were immediately stored in a -70ºC deep freezer.

Measurement of amino acid concentration

The perfusate concentration of five amino acids (glutamate, aspartate, serine, taurine and

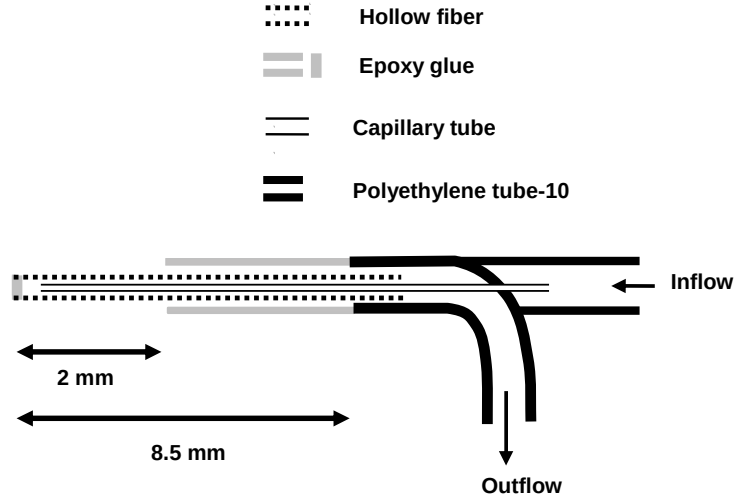

Fig. 1 Schematic drawing of microdialysis probe for human nasal mucosa. The probe was made with Cuprophan hollow fiber (200 µm inner diameter, 300 µm outer diameter). Microdialysis was made through active membrane (45 kDa weight cutoff, 2 mm in length) which was inserted into right side inferior turbinate mucosa and the remaining portion was sealed with epoxy glue and polyethylene.

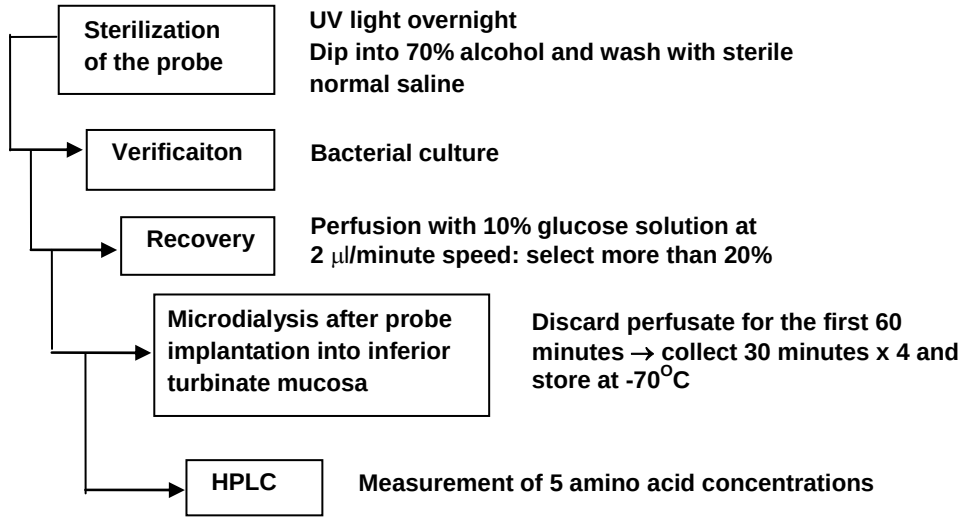

Fig. 2 Procedure for measurement of amino acids concentration in human nasal mucosa by microdialysis technique.

GABA) was measured by an AccQ-Tag method. A standard solution was obtained by diluting a standard form step by step where the amino acid concentration was 2.5 µmol/ml (cystine-1.25 µmol/ml). After induction, high performance liquid chromatography (HPLC) was carried out on the solution and the obtained chromatogram was used to confirm the maintenance and delay time. The gauging line was determined by the area ratio of each chromatogram and amino acid concentrations of the sample were calculated using the established gauging line. An autosampler was used for solution injection. The analytical temperature was maintained at 37ºC.

Statistical analysis

All data are expressed as the mean ± SEM. The difference in amino acid concentrations between

the normal and allergy group was analyzed for significance using the Student t-test. Differences were considered significant with *p*-values < 0.05.

RESULTS

The amino acid concentrations when arranged by time interval

When 4 measurements at an interval of 30 minutes were made, the concentration of glutamate, aspartate, serine and taurine was elevated in the allergy group at all times when compared to the control group. In the case of GABA, the concentration was not consistently higher in the allergy group (Fig. 4).

The average amino acid concentration

The average concentrations of glutamate, aspartate, serine, taurine and GABA were higher in the allergy group than in the controls. However, only glutamate reached significant concentration difference ($p = 0.04$) (Table 1).

DISCUSSION

Until now, amino acids have been known to contribute to the physiological and biochemical reactions of lower respiratory tract disorders. For example, arginine, a nitric oxide precursor, acts as a basal tone regulator of the bronchial smooth muscle and contributes to the decrease of nitric oxide during glucocorticoid inhalation in asthmatic patients.[7] Glutamate and aspartate have been shown to modulate the contractile response of the bronchial muscle.[8] As components of the pulmonary surfactant complex, phosphoethanolamine and ethanolamine play a role in the pathophysiology of acute respiratory distress syndrome.[9] The purpose of this study was to measure, by using microdialysis method, the concentration of neuroexcitatory neurotransmitters of the CNS such as glutamate, aspartate, serine and neuroinhibitors such as GABA and taurine in human nasal mucosa, and how the concentration of these amino acids responds to an inflammatory allergic condition. All 5 amino acids that were studied reached various concentrations in human nasal mucosa and all were elevated when the allergy group was compared to the control group. However, only the increase in glutamate reached significance. The possibility of glutamate acting as a neurotransmitter not only in the CNS but also in the nasal cavity cannot be clearly confirmed by this study. However, we suggest this possibility and further research will be required to confirm this hypothesis.

Our suggestion that glutamate plays a role in

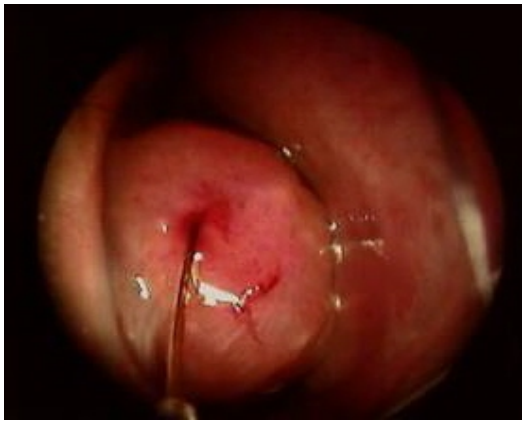

Fig. 3 Microdialysis of nasal mucosa. The tip of probe, where active microdialysis membrane exists, is implanted into the right inferior turbinate mucosa.

Table 1 Mean concentrations (μmol/ml) of glutamate, aspartate, serine, taurine and GABA between control and allergy groups

Group	glutamate	aspartate	serine	taurine	GABA
Control	5.41±2.63	0.69±0.60	10.55±3.68	3.78±1.29	3.54±1.13
Allergy	7.30±2.88	0.85±0.78	11.57±4.95	4.32±2.43	3.58±1.58
***p*-value**	0.004*	0.338	0.333	0.246	0.907

The concentration is mean ± SEM of four time measurements at 30 minute interval in each 10 subjects
*$p = 0.004$.

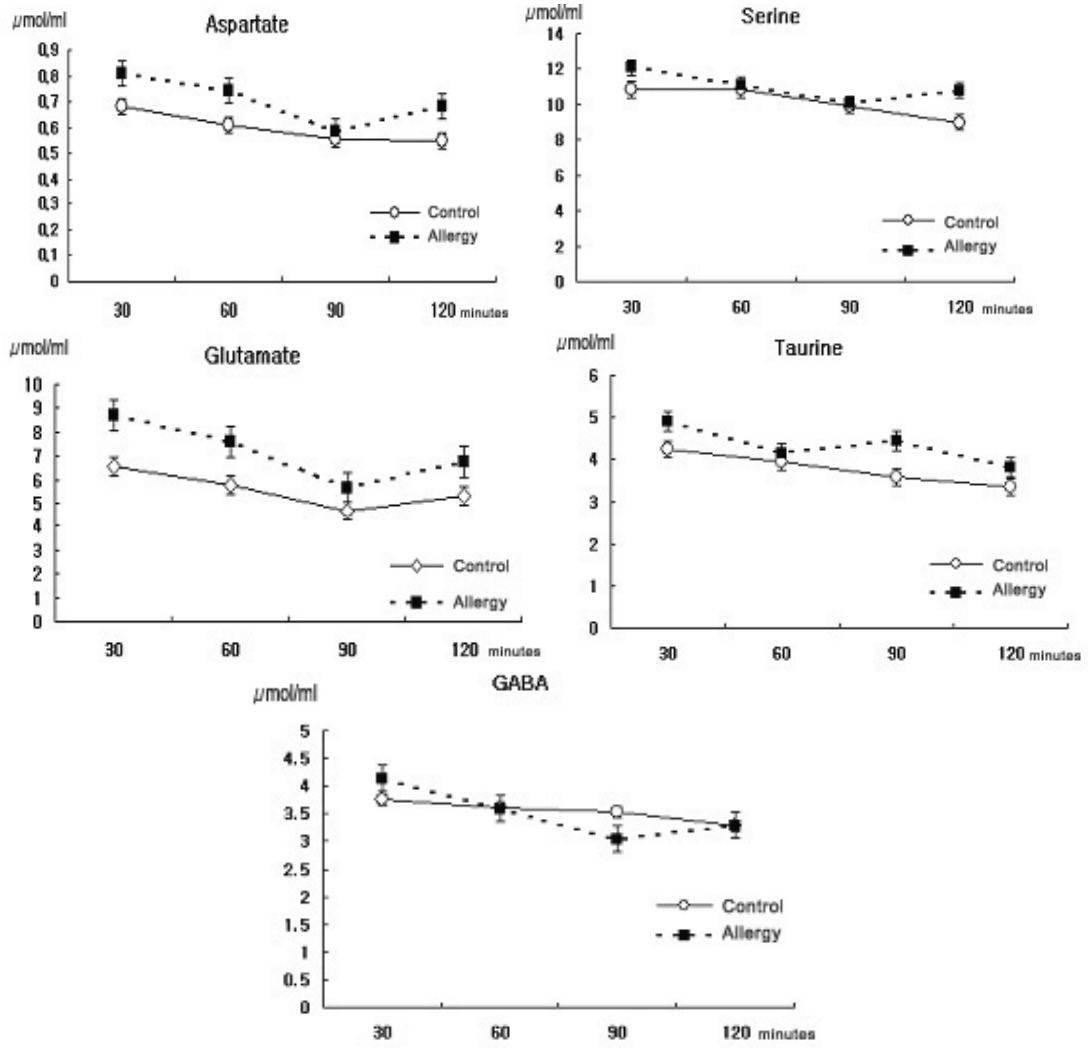

Fig. 4 Amino acids concentrations followed by a lapse of time in the control and allergy group.

allergic rhinitis can be supported by 3 lines of evidence. First, it is well established that monosodium glutamate (MSG) causes the Chinese restaurant syndrome. This is a syndrome where MSG causes parasympathomimetic stimulation that leads to symptoms such as facial flushing, sweating, nausea, headache, a burning sensation along the back of the neck and dyspnea.[10,11] It has also been proven that MSG may induce bronchial asthma.[12] Second, L-glutamate receptor sites exist in the cholinergic nerve of the airway and play a role in the contraction of bronchial smooth muscle when stimulated, and in addition these receptors may mediate MSG-induced asthma.[4,8] Third, Rhinaaxia® (Zy 15106, Leclerc & Co, Schaffhausen, Switzerland), a drug used for allergic rhinitis in Europe, precludes the action of glutamate as a neurotransmitter in the nasal cavity. Rhinaaxia® is a magnesium salt form of N-acetyl-aspartyl-glutamic acid (NAAGA). NAAGA is an endogenous dipeptide with a high affinity to glutamate receptors in the brain.[13] The success of NAAGA when used to treat allergic rhinitis has been attributed to: 1) modulation of histamine release from mast cells,[14] 2) inhibition of complement activation of C3a and C5a,[15] 3) decreased leukotriene B4 secretion,[16] 4) inhibition of inflammatory cell recruitment and 5) releasing eosinophil cationic protein and myeloperioxidase.[17] However, the results of this study when combined

with the three lines of evidence cited above, lead us to conclude that glutamate could be a novel neurotransmitter of the parasympathetic nervous system in the nasal cavity and be involved in the neurogenic inflammation of allergic rhinitis. One possible mechanism of NAAGA efficacy when used in treating allergic rhinitis may be that NAAGA is a competitive antagonist of glutamate at the glutamate receptors sites in the nasal cavity much like its CNS action.

The basic principle of microdialysis is to mimic the passive function of a capillary blood vessel by perfusing physiologic solution into a microdialysis probe implanted in nasal mucosa.[5] Given sufficient time, the perfusate and extracellular fluid (ECF) of the target tissue equilibrate by passing through the active semipermeable dialysis membrane as a result of osmotic pressure. The chemically analyzed perfusate reflects the composition of ECF of the target tissue. Factors that affect the recovery rate of metabolites from the ECF are properties of the active membrane such as length and permitted molecular weight cutoff, flow speed of the perfusion liquid, diameter of the probe, and perfusion liquid.[5] In order to measure amino acid concentrations in nasal mucosa, the target tissue in this study, a dialysis probe with an inner diameter of 200 μm and an outer diameter of 300 μm was specially designed using a Cuprophan hollow fiber. The length of the active membrane that does not allow permeation of elements with a molecular weight over 45 kDa was set at 2 mm and a dialysis probe with a recovery rate over 20% at a perfusion rate of 2 μl/minute with 10% glucose solution was selected. With these conditions, the amino acid concentrations in the subepithelial lamina propria were successfully measured. The glucose metabolism in the target tissue decreases at the early stage, when inserting a dialysis probe causes microtrauma-inducing stress to the target tissue, while the release of amino acids may increase. It has been reported that such a reaction continues for the first several minutes after probe insertion but then the abrupt increase in amino acid concentrations slowly decreases and returns to normal by 20-30 minutes after insertion.[18] Therefore, to avoid such errors in this study, the collected perfusate for the first 60 minutes of microdialysis was discarded and then, at 30 minute intervals, the perfusate was collected and analyzed at 4 different times.

In summary, we demonstrate the existence of five kinds of amino acids, previously known as neurotransmitters of the CNS, in human nasal mucosa and show a significant increase of glutamate concentration in allergic mucosa when compared to control mucosa in the nasal cavity. Our results and literature review suggest the possibility of glutamate serving as a novel neurotransmitter of the parasympathetic nerve in the nasal cavity and that glutamate is involved in the pathophysiology of allergic rhinitis. A microdialysis technique that accurately reflects the ECF environment of the target tissue could provide a big advantage in the research of the pharmacokinetics of nasal mucosa, and be employed in analyzing the delivery rate of therapeutic medications such as antibiotics and steroids when treating inflammatory diseases such as allergic rhinitis and sinusitis.

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