

알레르기비염 동물 모델에서 Budesonide, N-Acetyl-Aspartyl-Glutamate 및 Cyclosporin A 국소투여 후 비강내 아미노산 농도의 변화 : 미세투석법을 이용한 측정

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Changes of Amino Acid Concentrations Treated with Budesonide, N-Acetyl-Aspartyl-Glutamate, and Cyclosporin A in Animal Model of Nasal Allergy : Measurement Using Microdialysis

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—ABSTRACT—

Background and Objectives : This study aimed to measure the concentration (conc) of 19 amino acids (AAs) in the nasal cavity, to evaluate the difference of AAs conc among normal, allergic nasal mucosa, and allergic mucosa after intranasal application of budesonise, N-acetyl-aspartyl-glutamate (NAAGA), and cyclosporin (CsA) in guinea pigs. **Materials and Method :** Experimentally induced nasal allergy model was developed with ovalbumin in Dunkin-Hartely guinea pigs. Microdialysis was done in the inferior turbinate submucosa of normal control (C), allergic group (A), allergic group with intranasal application of budesonide (AS), allergic group with intranasal application of NAAGA (AN) and allergic group with intranasal application of CsA (AC). **Results :** All 19 AAs were validated at various conc in the nasal cavity. Glutamate and GABA conc were significantly higher in A than in C group. The conc serine, arginine, tyrosine, isoleucine, leucine, phenylalanine in AS group were significantly lower than those in A and C groups, whereas GABA conc was significantly higher in AS group than in A and C groups. The conc of glutamate, glycine, alanine, methionine, leucine, lysine, phenylalanine in A group were significantly higher than those in AN group, whereas tyrosine conc was reversed. There was no significant changes of AAs conc between A and AC groups. **Conclusion :** These results suggest that glutamate can be a novel amino acid neurotransmitter of parasympathetic nerve and GABA can act as an inhibitor of cholinergic parasympathetic nerve in the nasal cavity. (J Clinical Otolaryngol 2005;16:96-104)

KEY WORDS : Rhinitis · Allergic · Budesonide · N-acetyl-aspartyl-glutamate · Cyclosporin · Amino acid.

서 론

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amino - butyric acid(GABA)

gamma - GABA

glutamate¹⁾
^{2)가}

19

budesonide, N - acetyl - aspartyl - glutamate, cyclosporin A

2001 ARIA(Allergic Rhinitis and Its impact on Asthma)

(common co - morbidities) “ one airway, one disease ”

‘ united airway ’

재료 및 방법

동물과 약물

4 200~250 g Dunkin - Hartely 28

(control group, C , n=8),

(allergic group, A , n=8),

(budesonide : Pulmicort[®]) 4

(allergic group treated with steroid, AS , n=4),

N - acetyl - aspartyl - glutamate(NAAGA : Rhinaaxia[®])

4 NAAGA (allergic group

treated with NAAGA, AN , n=4),

cyclosporin(CsA : Cipol[®]) 3

CsA (allergic group treated with CsA, AC

, n=4) 5 . C

. C⁶⁾

tamate

GABA glu-

organ)

(target

(extracellular fluid)

(microdialysis)

알레르기비염 유발 동물모델의 제작

Kawaguchi⁷⁾ Hong⁸⁾ 20

(Fig. 1).

³⁾⁴⁾

4 mg 가 4 mg%

0.5 ml

20 mg/ml

{Al(OH)₃gel} 0.5 ml

1 ml

⁴⁾

^{5)가}

2 미세투석법을 이용한 비강 점막내 시료 채취

5

200 × 220 × 200 mm
 (ultrasonic nebulizer : NE - U07, OMRON Co., Japan)
 가 가 0.1% 1 2 mm , 2 μ/
 5 4 1 min 24%
 70%

Cuprophan hollow fiber(200 μm , 300 μm , 45 kDa molecular weight cut - off, Fitral, AN 69 - HF, Hospal, Lyon, France) (Fig. 2).
 10% glucose (recovery rate) 20%

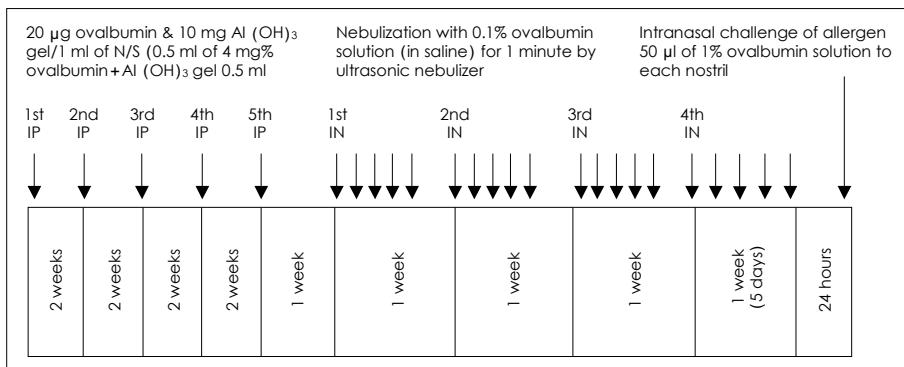


Fig. 1. Schedule for development of the experimentally induced nasal allergy model in guinea pigs. IP : intraperitoneal sensitization, IN : intranasal sensitization.

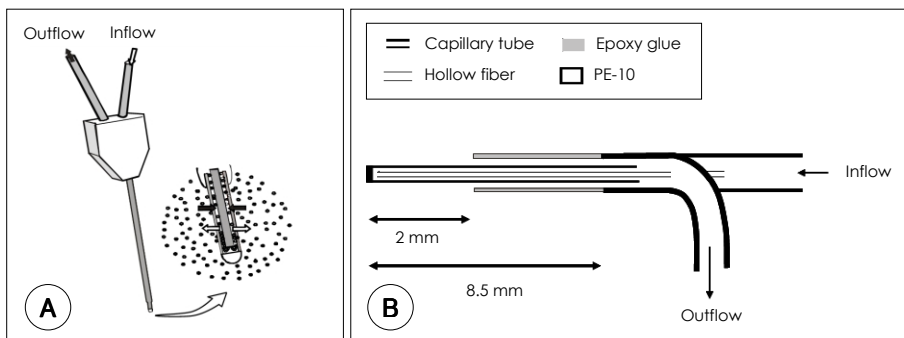


Fig. 2. Schematic drawing of microdialysis catheter for the nasal cavity (A). Catheter was made with Cuprophane hollow fiber (200 μm inner diameter, 300 μm outer diameter) and microdialysis was made through microdialysis membrane (45 kDa weight cut-off, 2 mm in length) which was inserted in submucosa of the inferior turbinate and the remaining portion was sealed with epoxy glue (B).

, microinfusion pump
(Baby Bee syringe pump, BAS, Lafayette, IN)

HPLC
chromatogram

가

chromatogram

Stengard⁹⁾ Lawrence¹⁰⁾

autosampler
excitation wavelength(250 nm)

5% urethane
1500 mg/kg

emission wavelength(395 nm), 37

cut - down tube white

통계학적 검정

silk 4 - 0 tube
(nasal dorsum)

±, C A
student t - test

가
11 2 mm, 5 mm

AS, AN AC A, C Mann -
Whitney p 0.05

가
1 cc Hamilton
microinfusion pump

결 과

, microinfusion pump 2 µl/min

알레르기비염 유발 후 아미노산 농도 변화

60, 30
4

C A alanine, aspartate, GABA, glu-
tamate, leucine, lysine, methionine, phenylalanine,
proline, taurine, tyrosine 가

Ependorf tube

glutamate(p=
0.036) GABA(p<0.001) arginine, cys-
teine, glycine, histidine, isoleucine, serine, threonine

- 70 deep freezer
21

A C
(Table 1).

10

아미노산계 신경전달물질의 측정

스테로이드 투여 후의 아미노산 농도 변화

19 (alanine, arginine, aspartate, cysteine,

AS A serine(p=0.001), arginine(p=
0.045), tyrosine(p=0.024), isoleucine(p=0.036), leu-
cine(p=0.036), phenylalanine(p=0.021) 가

GABA, glutamate, glycine, histidine, isoleucine, leu-
cine, lysine, methionine, phenylalanine, proline, serine,
taurine, threonine, tyrosine, valine) high

, GABA(p<0.001)
가 . AS 가

performance liquid chromatography (HPLC, Waters,
USA) AccQ - Tag

C (Table 1, Fig. 3).

가 2.5 µmol/ml(cystine - 1.25 µmol/ml)

NAAGA 투여 후의 아미노산 농도 변화

AN A glutamate(p=0.04), glycine(p=

Table 1. Mean concentrations of 19 amino acids of control (C), allergy group (A), allergy group treated with budesonide (AS), allergy group treated with NAAGA (AN), and allergy group treated with cyclosporin (AC) pmol/μl (Mean±SD)

Amino acids	C (n=8)	A (n=8)	AS (n=4)	AN (n=4)	AC (n=4)
Alanine	12.01 ± 5.21	13.32 ± 7.91	9.10 ± 3.01	7.30 ± 3.56	11.13 ± 8.62
Arginine	7.00 ± 4.46	6.44 ± 4.28	1.60 ± 0.40	5.95 ± 3.35	7.18 ± 5.79
Aspartate	2.35 ± 1.76	2.66 ± 1.86	1.76 ± 0.41	2.38 ± 1.26	2.56 ± 1.58
Cysteine	2.89 ± 1.72	2.36 ± 1.45	2.98 ± 1.09	2.46 ± 0.51	1.97 ± 0.84
GABA	0.03 ± 0.06	0.29 ± 0.28	0.82 ± 0.13	1.08 ± 1.97	0.17 ± 0.07
Glutamate	9.20 ± 4.50	12.71 ± 7.22	8.36 ± 3.27	7.83 ± 3.33	9.34 ± 5.66
Glycine	34.52 ± 18.80	26.75 ± 12.07	21.88 ± 6.85	13.46 ± 7.77	15.39 ± 14.67
Histidine	12.13 ± 6.34	11.90 ± 7.54	6.32 ± 1.68	6.81 ± 4.92	18.45 ± 6.41
Isoleucine	3.15 ± 1.61	3.00 ± 1.59	1.66 ± 0.29	1.81 ± 0.61	2.49 ± 1.37
Leucine	5.52 ± 2.80	5.96 ± 2.98	3.43 ± 0.60	3.28 ± 1.08	5.87 ± 4.10
Lysine	5.71 ± 3.57	7.74 ± 5.14	5.64 ± 2.66	2.87 ± 1.01	5.93 ± 6.05
Methionine	1.06 ± 0.47	1.36 ± 0.91	0.70 ± 0.14	0.49 ± 0.14	1.00 ± 0.51
Phenylalanine	2.23 ± 0.92	2.53 ± 1.45	1.38 ± 0.22	1.39 ± 0.50	2.21 ± 1.38
Proline	7.37 ± 3.49	9.37 ± 7.54	7.49 ± 2.66	6.15 ± 3.40	8.88 ± 7.52
Serine	10.38 ± 5.28	10.06 ± 5.70	4.40 ± 1.49	8.09 ± 4.47	12.46 ± 9.01
Taurine	8.65 ± 7.41	9.87 ± 9.36	14.38 ± 7.38	5.00 ± 4.84	16.07 ± 5.29
Threonine	8.54 ± 4.24	8.27 ± 5.40	4.99 ± 1.22	5.27 ± 2.63	8.78 ± 6.70
Tyrosine	2.55 ± 1.40	2.81 ± 1.54	1.53 ± 0.44	5.27 ± 2.63	16.07 ± 5.29
Valine	8.24 ± 4.06	7.80 ± 3.96	5.00 ± 0.82	4.90 ± 1.56	6.38 ± 3.41

n means the number of animal and the expressed concentration is mean ± SD from four times measurement in each animal

0.003), alanine(p=0.04), lysine(p=0.002), methionine (p=0.002), leucine(p=0.013), phenylalanine(p=0.027) 가 , tyrosine(p=0.036) 가 . glutamate 가 tyrosine C 가 (Table 1, Fig. 4).

CsA 투여 후의 아미노산 농도 변화

AC C A

고 찰

(homeostasis)

. Arginine nitric oxide (basal tone)

15)

. 11) glutamate aspartate , taurine

(osmoregulator), (antioxidant), (inotropic)

12)

serotonin, tryptophan, GABA, glutamate, aspartate, glycine, histamine, histidine, tyrosine, adenosine, arginine , glutamate, aspartate, tyrosine, tryp-

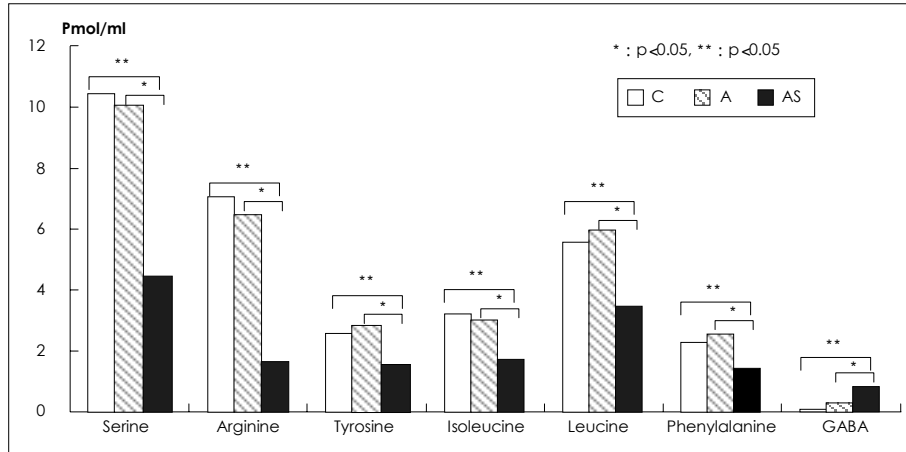


Fig. 3. Significantly changed amino acids after topical application of budesonide. The concentrations of serine, arginine, tyrosine, isoleucine, leucine, phenylalanine in AS group were significantly lower than those in A and C groups, whereas GABA concentration was significantly higher in AS group than in A and C groups.

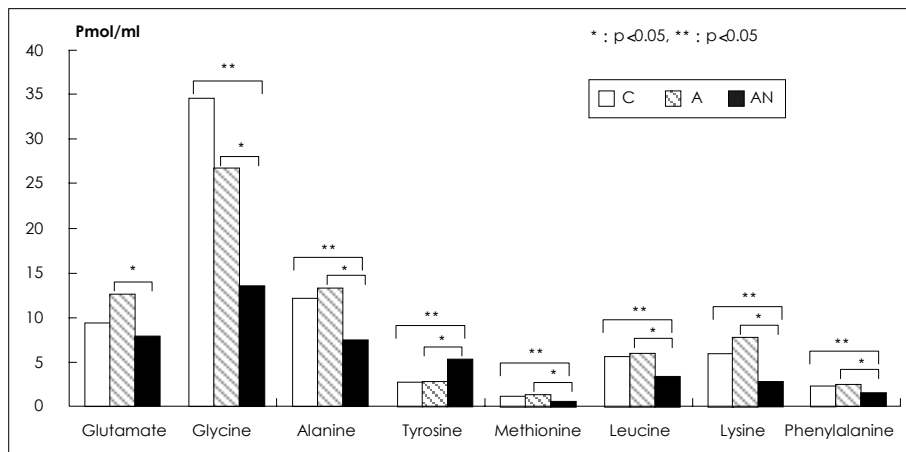


Fig. 4. Significantly changed amino acids after topical application of N-acetyl-aspartyl-glutamate. The concentrations of glutamate, glycine, alanine, methionine, leucine, lysine, phenylalanine in A group were significantly higher than those in AN group, whereas tyrosine concentration was reversed.

tophan (neuroexcitatory) , 가 가 ,
 glycine, taurine, GABA (neuro-inhibitor) 가
 13) (bron-choalveolar lavage fluid) 16 19
 asparagine, phospho-ethanolamine, taurine 가 1.0 nmol/ml glutamate GABA
 14) 6)

glutamate가 NO 가
²⁾¹⁵⁾ NO 가
 glutamate가 NO . A
 , NAAGA AS arginine 가
 가 glutamate 가 NO arginine
 가 NAAGA arginine NO가 가
 4 NAAGA glutamate ¹⁷⁾ cortisol
 glutamate NAAGA가 (diurnal rhythm)가 (status
 glutamate glutamate asthmaticus) cortisol 가 serine, isoleu-
 cine, arginine (gluconeogenic) 가
¹⁸⁾
 GABA AS A
¹⁾¹⁶⁾ GABA Phenylalanine tyrosine
 C A GABA catecholamine norepinephrine epinephrine
 0.03 ± 0.06 pmol 0.29 ± 0.28
 pmol A 가 C A AS phenylalanine tyrosine 가
 GABA가 가
 , AN A phenylalanine
 tyrosine 가 NAAGA
 GABA
 , C A CsA 가
 AS GABA 가 CsA
 가 가 CsA가
 IL - 1, IL - 5, tumor necrosis factor,
 granulocyte macrophagecolony - stimulating factor
 cytokine T
 , GABA
 가
 AS C A 가
 serine, arginine, tyrosine, isoleucine, leucine, pheny-
 lalanine (immunomodulation) ¹⁹⁾
 , CsA his-
 가
 가 arginine nitric ²⁰⁾ CsA가
 oxide(NO) , NO C, A

AC, CsA, glutamate, GABA, glutamate, GABA, NAAGA, serine, arginine, tyrosine, isoleucine, leucine, phenylalanine, glycine, alanine, methionine

가, 가, GABA(p<0.001) 가, glutamate(p=0.04), glycine(p=0.003), alanine(p=0.04), lysine(p=0.002), methionine(p=0.002), leucine(p=0.013), phenylalanine(p=0.027) 가, tyrosine(p=0.036) 가, AC, A, 19, glutamate, GABA, 3가, serine, arginine, tyrosine, isoleucine, leucine, phenylalanine, glycine, alanine, methionine

결론

(C), (A), (AS), N-acetyl - aspartyl - glutamate(NAAGA : Rhinaaxia®) (AN), cyclosporin (AC) 19, 1) C 19, 2) glutamate(p=0.036) GABA(p<0.001), 3) AS, A, C serine(p=0.001), arginine(p=0.045), tyrosine(p=0.024) isoleucine(p=0.036), leucine(p=0.036), phenylalanine(p=0.021)

중심 단어 : Budesonide · N - acetyl - aspartyl - glutamate · Cyclosporin

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