

Alterations of adrenoceptors in the nasal mucosa of allergic patients in comparison with nonallergic individuals

Yvonne J. B. van Megen, PhD,* ** André B. M. Klaassen, PhD,*
Jaap F. Rodrigues de Miranda, PhD,** Cees A. M. van Ginneken, PhD,**
and Bob Th. R. Wentges, MD* Nijmegen, The Netherlands

Nasal hyperreactivity in nasal allergy may be due to changes of the characteristics in adrenergic receptors. Radioligand receptor-binding studies with the antagonists, ³H-prazosin (α_1 -adrenoceptor), ³H-rauwolscine (α_2 -adrenoceptor), and ¹²⁵I-(–)-Cyanopindolol (β -adrenoceptor) were performed in homogenates of nasal mucosa of allergic and nonallergic (NA) patients to investigate this hypothesis. The heterogeneous NA group was subdivided into control individuals and patients with chronic sinusitis and vasomotor rhinitis. No significant differences in affinities or densities of α_1 - and α_2 -adrenoceptors could be demonstrated in allergic patients in comparison with NA and control individuals. The β -adrenoceptor density was significantly reduced in allergic patients in comparison with that of control individuals. Neither changes in agonist binding or in the effect of Gpp(NH)p on the agonist binding to β -adrenoceptors could be observed in allergic patients. The subtype selective antagonist, LK204,090, demonstrated the presence of a homogeneous population of β_2 -adrenoceptors in human nasal mucosa of both NA and allergic patients. In vitro, autoradiography demonstrated specific ¹²⁵I-(–)-Cyanopindolol labeling of the epithelium in NA and allergic patients. In conclusion, no changes in characteristics of α_1 - or α_2 -adrenoceptors in the nasal mucosa could be demonstrated in nasal allergy. However, a decreased number of β -adrenoceptors may reflect a β -adrenergic abnormality in nasal allergy. (J ALLERGY CLIN IMMUNOL 1991;87:530-40.)

Nasal hyperreactivity in AR may originate from an imbalance of the autonomic nerve regulation in the nasal mucosa.¹ Systemic adrenergic abnormalities have been observed in allergic patients. Isoprenaline (β -agonist) infusion resulted in a significant decrease of pulse pressure and adenosine 3'5'-cyclic monophosphate concentration in allergic patients in comparison with that in control individuals.^{2,3} Intranasal administration of terbutaline (β_2 -agonist) or phenylephrine (α_1 -agonist) caused no differences in nasal airway resistance in allergic patients.^{4,5} Until the present time, the influence of β -adrenergic agonists on nasal secretion in allergic patients in comparison with

Abbreviations used

¹²⁵ I-(–)-CYP:	¹²⁵ I-(–)-Cyanopindolol
Gpp(NH)p:	5'Guanylylimidodiphosphate
BSA:	Bovine serum albumin
K _d :	Equilibrium dissociation constant
K _i :	Inhibition constant
B _{max} :	Receptor density
AR:	Allergic rhinitis
NA:	Nonallergic

the influence in control individuals has not been investigated. β -Adrenergic hyporesponsiveness and α -adrenergic hyperresponsiveness in the lower airways have been explained in terms of changes in the characteristics of β - and α -adrenoceptors in patients with chronic airflow obstruction.⁶⁻¹⁰ In contrast with the lower airways, only a few quantitative data (B_{max}) of α_1 - and β -adrenoceptors in the human nasal mucosa are available.^{11,12} In this study, radioligand receptor-binding studies were performed with the antagonists, ³H-prazosin, ³H-rauwolscine, and ¹²⁵I-(–)-CYP, to investigate α_1 -, α_2 -, and β -adrenoceptors, respectively, in NA and allergic patients to elucidate the

From the Departments of *Otorhinolaryngology and **Pharmacology, University of Nijmegen, Nijmegen, The Netherlands.

Supported by Netherlands Astma Fonds Grant 84.09.

Received for publication Sept. 14, 1989.

Revised Oct. 1, 1990.

Accepted for publication Oct. 2, 1990.

Reprint requests: Y. J. B. van Megen, PhD, Duphar B.V., Dept. of Pharmacology, PO Box 900, 1380 DA Weesp, The Netherlands.

1/1/25944

TABLE I. Characteristics of nonallergic subjects and patients with allergic rhinitis

	N	Age (yr)	M/F	Medication	Sm	Skin test	IgE (U/ml)	Eo blood ($\times 10^6$ c/ml)	Eo nose (%)
β-Adrenoceptors									
NA									
c	13	31 $\pm$ 9	5/8	Bi(1)Be(1)L(1)	4	Neg(13)	6-149(8)	45-165(8)	0-5(8)
s	3	40 $\pm$ 25	2/1	Be(1)	0	Neg(3)	28(1)	50/226(2)	0(1)
v	2	26/28	0/2	Vi,Tr(1)Be(1)	0	Neg(2)	110(2)	110/180(2)	0(2)
AR	14	23 $\pm$ 4	9/5	Ve(2)Be(3)Ti(2)	2	HM(10) Po(10)Da(10)	155-4650(12)	60-685(11)	0-9(11)
α_1-Adrenoceptors									
NA									
c	5	29 $\pm$ 12	2/3	Be,Ot(1)	1	Neg(5)	6-49(4)	90-165(4)	0-3(4)
s	1	17	1/0	None	0	Neg(1)	ND	ND	ND
v	1	26	0/1	Vi,Tr(1)	0	Neg(1)	22(1)	110(1)	0(1)
AR	6	22 $\pm$ 6	4/2	Be(1)Ro(1)Ti(1)	0	HM(5) Po(4)Da(4)	226-1110(3)	100-685(3)	0-5(3)
α_2-Adrenoceptors									
NA									
c	8	32 $\pm$ 7	4/4	Be,Ot(1)	3	Neg(8)	10-44(4)	80-120(3)	0-3(3)
s	3	33 $\pm$ 15	2/1	Te(1)	2	Neg(3)	51(1)	70/80(2)	0/70(2)
v	3	30 $\pm$ 6	0/3	Vi,Tr(1)Be(1)L(1)	0	Neg(3)	22-96(3)	110-273(3)	0-25(3)
AR	9	26 $\pm$ 9	6/3	Me(1)Pe(1)Ti(1)	1	HM(7) Po(6)Da(5)	226-1235(5)	286-1320(7)	0-60(7)

Au, Augmentin; Beecham Laboratories; Be, Beconase; Glaxo, Inc.; Bi, Bisolvoin; Boehringer Ingelheim; c, control; Da, human or animal dander; Eo, eosinophilia; HM, house-dust mite; La, Lanoxin; Burroughs Wellcome Co.; L, Lomudal; Fisons; Me, Methyrit; Smith Kline & French; ND, not determined; Ot, Otrivin; Geigy Pharmaceuticals; Pe, penicillin; Po, pollen; Ro, Ronnisol; s, chronic sinusitis; Sm, smoker; Te, Tetracycline; Ti, Tinset; Janssen Pharmaceutica; Tr, Triludan; Gist-Brocades; v, vasomotor rhinitis; Ve, Ventolin; Glaxo, Inc., Vi, Vibramycin; Pfizer Laboratories.

Number of patients in parentheses.

supposed alteration in characteristics of neuroreceptors in nasal hyperreactivity. Furthermore, the heterogeneous NA group has been subdivided into control individuals and patients with chronic sinusitis and vasomotor rhinitis. The investigation of the β -adrenoceptors includes not only the receptor density and sensitivity but also the presence of β -adrenoceptor subtypes, agonist affinity states, and the coupling of this receptor to the effector system by the G protein. In vitro autoradiography was performed to localize β -adrenoceptors in the human nasal mucosa of NA and allergic patients.

MATERIAL AND METHODS

^3H -prazosin (specific activity, 18.8 Ci/mmol), ^3H -rauwolscine (specific activity, 73.5 Ci/mmol), and ^{125}I -(-)-CYP (specific activity, 2200 Ci/mmol) were purchased from New England Nuclear, Doorn, The Netherlands; ($\pm$) propranolol was a gift from ICI Ltd., Alderly Park, England; ($\pm$) isoprenaline and LK₂₀₃₋₀₃₀ were gifts from Boehringer Ingelheim, Rheim, Germany, and Sandoz AG, from Basel, Switzerland, respectively. Phentolamine was a gift from Ciba-Geigy, Summit, N.J. Gpp(NH)p and phenylmethylsulfonyl fluoride were obtained from Sigma Chemical Co., St. Louis, Mo.; Nuclear K₂ emulsion was

purchased from Ilford, Amsterdam, The Netherlands. All other chemicals were of analytical grade.

Patients

Biopsy specimens of human nasal mucosa from 2 cm behind the inferior turbinate were obtained from operations, especially during sinus and septum surgeries (general anesthesia with systemic atropine). Patients were classified into an NA and allergic group on the basis of some parameters^{13,14}: nasal symptoms, family history, X-ray films of the sinuses, serum IgE, blood and/or nose eosinophilia, RAST and skin tests. Not all parameters could be obtained from all the patients, especially in the NA group in which RAST and skin tests were rarely performed. The ultimate diagnosis was made by Prof. Dr. R. Th. R. Wentges, who was familiar with the history of the patients. The NA patients were further subdivided into control individuals and patients with chronic sinusitis and vasomotor rhinitis (Table I). The control group consisted of patients with traumas, deviations of the septum, and cosmetic problems and were characterized by low serum IgE values, few eosinophils in the blood or in the nose, and negative RAST and skin tests. The patients with chronic sinusitis had low-grade chronic sinusitis infections, and they either underwent a Caldwell-Luc procedure or an intranasal antrostomy. These patients demonstrated an inflammation of the sinus, but the other

TABLE II. Incubation conditions for receptor assays of α - and β -adrenoceptors

Ligand	R	Buffer*	Incubation (min)	Nonspecific binding (μ mol/L)
^{3} H-Prazosin	α_1	Tris HCl	40 37°C	12 phentolamine
^{3} H-Rauwolscine	α_2	Tris/EDTA	20 25°C	12 phentolamine
^{125} I-(–)-CYP	β	Tyrode plus 0.01% BSA	90 37°C	2 (±) propranolol

R, Adrenoceptor; EDTA, ethylenediaminetetraacetic acid.

*Added buffers (pH 7.4): Tris HCl: 50 mmol/L Tris, 120 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L CaCl₂, 1 mmol/L MgCl₂; Tris/EDTA: 50 mmol/L Tris, 1 mmol/L EDTA; Tyrode: 140 mmol/L NaCl, 3 mmol/L KCl, 2 mmol/L CaCl₂, 1 mmol/L MgCl₂, 12 mmol/L NaHCO₃.

TABLE III. Binding parameters (mean $\pm$ SEM) of ^{3} H-prazosin, ^{3} H-rauwolscine, and ^{125} I-(–)-CYP to α_1 -, α_2 -, and β -adrenoceptors in the nasal mucosa of NA and allergic patients

		B_{max} (t)	B_{max} (p)	N
α_1 -Adrenoceptors				
	K_d (nmol/L)			
NA	0.4 ± 0.1	0.77 ± 0.14	177 ± 33	5
c	0.4 ± 0.2	0.90 ± 0.17	195 ± 40	3
s	0.5	0.35	80	1
v	0.2	0.83	218	1
Allergic	0.3 ± 0.1	0.80 ± 0.11	244 ± 50	4
α_2 -Adrenoceptors				
NA	2.7 ± 0.5	5.53 ± 1.04	1180 ± 127	14
c	2.7 ± 0.6	4.61 ± 0.87	1070 ± 119	8
s	3.7 ± 1.4	9.65 ± 3.80	1630 ± 430	3
v	1.6 ± 0.2	3.86 ± 0.59	1020 ± 198	3
Allergic	2.3 ± 0.4	5.21 ± 0.59	1230 ± 96	9
β -Adrenoceptors				
	K_d (pM)			
NA	2.7 ± 0.2	0.50 ± 0.06	87 ± 11	18
c	2.8 ± 0.3	0.48 ± 0.06	$92 \pm 10^*$	13
s	2.9 ± 0.2	0.67 ± 0.20	100 ± 46	3
v	$2.7/1.6$	$0.32/0.45$	$42/30$	2
Allergic	3.1 ± 0.4	0.41 ± 0.05	$63 \pm 6^*$	14

B_{max} (t), picomoles per gram of tissue; B_{max} (p), femtomoles per milligram of protein; c, control; s, chronic sinusitis; v, vasomotor rhinitis.

* $p < 0.05$.

characteristics were similar to those of the control group. The pathophysiology of the group with vasomotor rhinitis resembled the pathophysiology of the group with AR, but an allergic component could be excluded. All these patients demonstrated nasal obstruction, and the other characteristics of this group were similar to characteristics of the control group. The patients with vasomotor rhinitis were admitted for a concomitant septal deviation and underwent a sub-mucous septoplasty. AR was diagnosed on high IgE values, many eosinophils in the blood and/or the nasal mucosa, and a positive RAST and/or skin test for house dust mites, pollen, and/or dander. None of the allergic patients demonstrated an acute clinical manifestation of the AR at the time they had surgery.

Some NA and allergic patients received medication (Table I). The different medications can be classified into six groups:

1. antibiotics (amoxilline [Augmentin; Beecham Laboratories, Bristol, Tenn.], penicillin, tetracycline, and doxycycline hyclate [Vibramycin; Pfizer Laboratories, Parsippany, N.J.]
2. antiallergics (beclomethasone dipropionate [Beconase; Glaxo, Inc., Research Triangle Park, N.C.]
3. antihistamines (sodium chromoglycate [Lomudal; Fisons, Loughborough, U.K.], chloofenaminemaleate [Methyrit; Smith Kline & French, Rijswijk, The Netherlands], oxatamide [Tinset; Janssen Pharmaceutica,

Piscataway, N.J.), and terfenadine [Triludan; Gist-Brocades, Rijswijk, The Netherlands])

4. decongestant (oxymetazoline [Otrivin; Geigy Pharmaceuticals, Summit, N.J.] and salbutamol [Ventolin; Glaxo Inc.]
5. cough mixture (bromohexine [Bisolvon; Boehringer Ingelheim, Alkmaar, The Netherlands])
6. heart medication (digoxin [Lanoxin; Burroughs Wellcome Co., Research Triangle Park, N.C.] and β -pyridyl carbinol [Ronicol; Roche Laboratories, Nutley, N.J.]

Radioligand receptor binding to homogenates of nasal mucosa

Nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was stored at -80°C for maximal 43 months (NA, 18 ± 12 months; allergic, 17 ± 7 months). In rat nasal mucosa, no differences in binding parameters could be observed in fresh tissue in comparison with frozen mucosa. Similar binding parameters were obtained with human nasal mucosa stored for 36 months in comparison with 7 months.

The binding procedures to human nasal mucosa were analogous to the procedures as described for rat nasal mucosa.¹⁵⁻¹⁷ In summary, the tissue was homogenized in ice-cold buffer with an Ultraturrax for 2×10 seconds. The homogenate was centrifuged at 1000 *g* for 5 minutes, the supernatant was centrifuged at 100,000 *g* for 1 hour (4°C), and the pellet was resuspended by Potter homogenization. Protein determination was performed according to the method of Bradford¹⁸ with BSA as a standard.

Radioligand receptor-binding studies were performed in glass tubes containing 40 μl of radioligand, 40 μl of buffer, and 320 μl of tissue homogenate. The different incubation conditions for the various receptors are listed in Table II. Instead of the buffer, unlabeled ligand was added for the determination of the non-specific binding. Incubations were terminated by rapid dilution with 2×2 ml of ice-cold buffer and filtration through Whatman GF/C filters (Whatman, Inc., Clifton, N.J.) under vacuum, followed by 3×5 ml rinses of the filter with buffer. Radioactivity collected on each filter was measured in a gamma counter (NE1612, New England Nuclear) at 78% counting efficiency in case of the ^{125}I -labeled ligand, whereas ^3H -radioactivity was counted in a liquid scintillation counter. The binding parameters were calculated by subjecting these data to a non-linear least-square curve-fitting procedure with the Gauss-Newton algorithm.¹⁹ Inhibition curves were analyzed according to a one- or two-binding sites model.

Histology

Specimens of human nasal mucosae were washed in 0.9% NaCl and immediately fixed in 2.5% glutardialdehyde in 0.1 mol/L of phosphate buffer (pH 7.0), dehydrated in ethanol, and embedded in glycol methacrylate (JB4 embedding kit, Polysciences, Inc., Warrington, Pa.). Sections (2 μm) were stained with 2.5% toluidine blue in 2.5% anhydrous sodium dicarbonate.

In vitro labeling and autoradiography of β -adrenoceptors in cryostat sections

Nasal mucosae were immediately washed in 0.9% NaCl after removal. The tissue was rapidly frozen with isopentane cooled in liquid nitrogen and stored for maximal 2 months before in vitro autoradiography.

Binding characteristics were examined before autoradiographic experiments were performed, as described for rat nasal mucosa.²⁰ In summary, cryostat sections (10 μm) were thaw mounted on glass slides coated with gelatin/chrome-alum. The sections were air-dried and stored at -80°C . Before incubation, the sections were dried again at room temperature and fixed at 4°C in 0.5% glutardialdehyde in Tyrode's buffer for 30 minutes. The sections were rinsed in buffer at 4°C for 30 minutes and additionally processed for either radioreceptor assays or autoradiography. Several nonmounted parallel sections were collected for protein determination according to the method of Lowry et al.²¹ (1951) with BSA as a standard. Protein determination was performed after solubilization of the sections in 0.1 N NaOH at 100°C (boiling water bath) for 15 minutes. Parallel sections were stained with methyl green pyronin (1%) for histologic verification of tissue quality.

For the radioreceptor assay, the tissue sections were dried and overlaid with 150 μl of ^{125}I -(-)-CYP in Tyrode's buffer (with 0.01% BSA) at various concentrations of radioligand. Subsequently, the sections were incubated at 37°C for 2 hours in a humid atmosphere. For the measurements of the nonspecific binding, parallel incubations were performed in the presence of 2 $\mu\text{mol/L}$ ($\pm$) propranolol at the same conditions. After the incubation, 75 μl of the incubation buffer was counted to determine the free-concentration radioligand; the remaining incubation buffer was removed by rinsing the sections in fresh buffer (room temperature) during 4×15 minutes. The radioactivity in the sections was counted by gamma counting (NE1612 gamma counter, New England Nuclear). The binding parameters were calculated as described above.

For the autoradiography, sections were incubated with 0.02 nmol/L of ^{125}I -(-)-CYP according to the method described above. After slides were washed in buffer they were rinsed in distilled water to remove salts. The specimens were coated with 0.5% gelatin/chrome-alum, air-dried, dipped in liquid emulsion (nuclear K₂ emulsion, Ilford), and diluted with distilled water (1:1). After 2 months of exposure (4°C), the autoradiographs were developed in amidol (18°C for 8 minutes), rinsed in distilled water, fixed in sodium thiosulfate (30%) at 18°C for 1 to 3 minutes, thoroughly washed in distilled water (15 minutes), and stained with 1% methylgreen pyronin.

Statistical analysis

Agonist inhibition curves were fitted to a one-binding or two-binding sites model. Preference was given to a two-binding sites model when a significant diminution in the residual sum of squares was reached (*F* test). Differences in affinity values (negative logarithm of K_d , K_H , and K_L), which are known to be normally distributed,^{22, 23} were com-

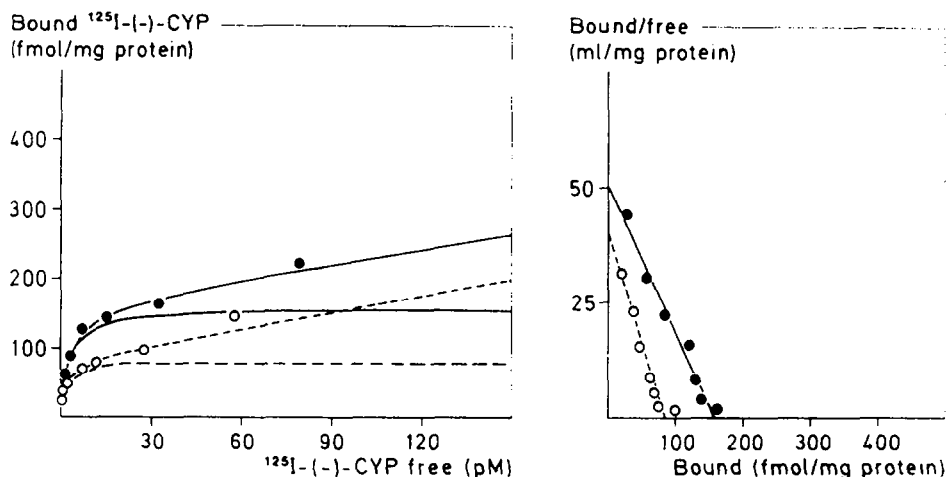


FIG. 1. Left, Total (●, ○) and specific ¹²⁵I-(+)-CYP binding to β-adrenoceptors in human nasal mucosa membranes of NA (—) and allergic (---) patients. Nonspecific binding was determined in the presence of 2 μmol/L of (±) propranolol. Right, Scatchard plot of the specific part of the binding to nasal mucosa membranes. Data illustrated are the means of triplicates from representative experiments.

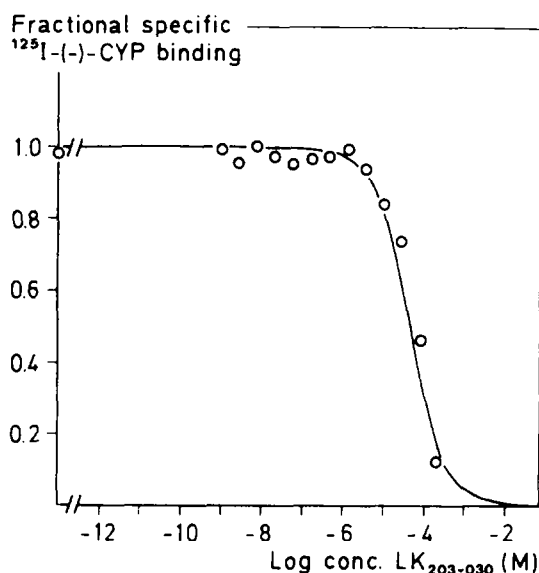


FIG. 2. Inhibition of specific ¹²⁵I-(+)-CYP binding by the antagonist, LK₂₀₃₋₀₃₀. Homogenates of nasal mucosa of NA patients were incubated at 37° C for 120 minutes at 16 pM ¹²⁵I-(+)-CYP in the presence or absence of inhibitor. The inhibition curve could adequately be fitted by a one-binding site model. Data illustrated are the means of triplicates from a representative experiment.

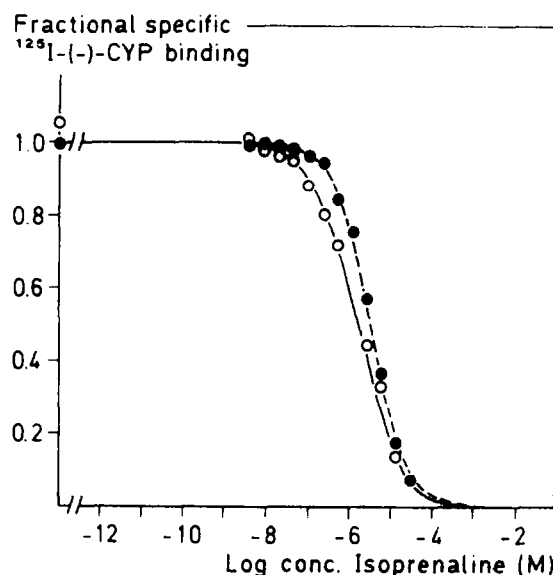


FIG. 3. Inhibition of ¹²⁵I-(+)-CYP binding by the agonist, isoprenaline, in absence (○) or presence (●) of 5.10⁻⁴ mol/L of Gpp(NH)p. The curve in the absence of Gpp(NH)p could significantly be better fitted according to a two-binding sites model, whereas the curve in the presence of Gpp(NH)p could adequately be fitted according to a one-binding site model.

pared with the Student's *t* test. Significance was accepted at *p* < 0.05.

RESULTS

Histology

Various tissue components were present in biopsy specimens of human nasal mucosa: epithelium and the

lamina propria with glands, blood vessels, and inflammatory cells. Nerve fibers could not be observed in the specimens. Biopsy specimens of patients with AR demonstrated basophilic cells in the epithelium and an increased number of basophilic cells in the lamina propria in comparison with that of NA patients. No other histologic changes have been observed.

TABLE IV. Inhibition of ¹²⁵I-(–)-CYP binding by the agonist, isoprenaline, in absence or presence of Gpp(NH)p in control subjects and allergic patients

– Gpp(NH)p				+ Gpp(NH)p			
Ki ₁ (μmol/L)	Ki ₂ (μmol/L)	RH (%)	N	Ki ₁ (μmol/L)	Ki ₂ (μmol/L)	RH* (%)	N
Control							
0.66	—	—	1	→ 1.0	—	—	1
0.016 ± 0.024	0.48 ± 0.23	15 ± 6	4	→ 0.16 ± 0.11	1.93 ± 0.81	42 ± 28	3
				→ 0.36	—	—	1
Allergic							
0.19/0.17	—	—	2	→ 0.22/0.44	—	—	2
0.027 ± 0.003	0.79 ± 0.29	21 ± 10	3	→ 0.68/0.47	1.23/1.74	65/46	2
				→ 0.60	—	—	1

RH, High-affinity agonist binding sites.
*A different set of high-affinity binding sites with a lower agonist affinity than the high-affinity state determined without Gpp(NH)p. Inhibition curves were analyzed according to a one- or two-binding sites model. These data of the significant best fit (*F* test) are presented.

α-Adrenoceptors in nasal mucosa membranes

Specific binding of ³H-prazosin and ³H-rauwolscine to α₁-adrenoceptors and α₂-adrenoceptors, respectively, was saturable and of high affinity in NA as well as allergic patients. Scatchard plots point to homogeneous classes of binding sites. The binding parameters, K_d, and B_{max}, are listed in Table III. No significant differences in binding parameters could be observed in allergic patients in comparison with NA individuals. The heterogeneous NA group was subdivided into control individuals and patients with chronic sinusitis and vasomotor rhinitis. The binding parameters of ³H-prazosin and ³H-rauwolscine binding were not significantly different between these subgroups or between allergic patients and the subgroups.

β-Adrenoceptors in nasal mucosa membranes

Specific ¹²⁵I-(–)-CYP binding to β-adrenoceptors was saturable and of high affinity in nasal mucosa membranes of NA and allergic patients (Fig. 1). The Scatchard plot points to a homogeneous class of binding sites. Similar binding parameters were obtained in allergic patients and NA individuals. The NA group was divided in subgroups as described above. The β-adrenoceptor density, expressed in femtomoles per milligram of protein, was significantly decreased in allergic patients in comparison with that of control individuals (Table III). No significant differences in binding parameters could be demonstrated in allergic patients in comparison with patients with chronic sinusitis and vasomotor rhinitis (Table III). Elimination of the medicated patients in this study

demonstrated that the β-adrenoceptor density was still significantly reduced in nonmedicated allergic patients (64 ± 9 fmol/mg of protein; N = 6) in comparison with nonmedicated control individuals (95 ± 12 fmol/mg of protein; N = 10) (Student *t* test, *p* < 0.05). Differences in binding parameters could not be demonstrated between smokers in comparison with nonsmokers in these groups.

The subtype selective β₁-adrenergic antagonist, LK₂₀₃₋₀₃₀, was used to define the relative proportions of β₁- and β₂-adrenoceptors in human nasal mucosa. The inhibition curve was monophasic and could adequately be fitted according to a one-binding site model in control and allergic patients (Fig. 2). The low Ki values (8.9 ± 2.8 and 7.4 ± 3.3 μmol/L, respectively) indicated the presence of a homogeneous population of β₂-adrenoceptors in control individuals and allergic patients.

Inhibition studies of ¹²⁵I-(–)-CYP with the agonist, isoprenaline, in the absence or presence of Gpp(NH)p were performed to investigate the agonist affinity states and the coupling of the β-adrenoceptors to the G proteins in human nasal mucosa (Table IV). The inhibition curve appeared to be biphasic in four of five control individuals and three of five allergic patients and could be significantly better fitted according to a two-binding sites model, indicating the presence of 15% ± 6% and 21% ± 10% high-affinity sites, respectively. Monophasic inhibition curves were demonstrated in one control individual and two allergic patients. In the presence of 5 · 10^{–4} mol/L of Gpp(NH)p, a shift of the monophasic and a steepening of the biphasic curves was observed (Table IV). In two biphasic curves (one control individual and one allergic patient), Gpp(NH)p caused

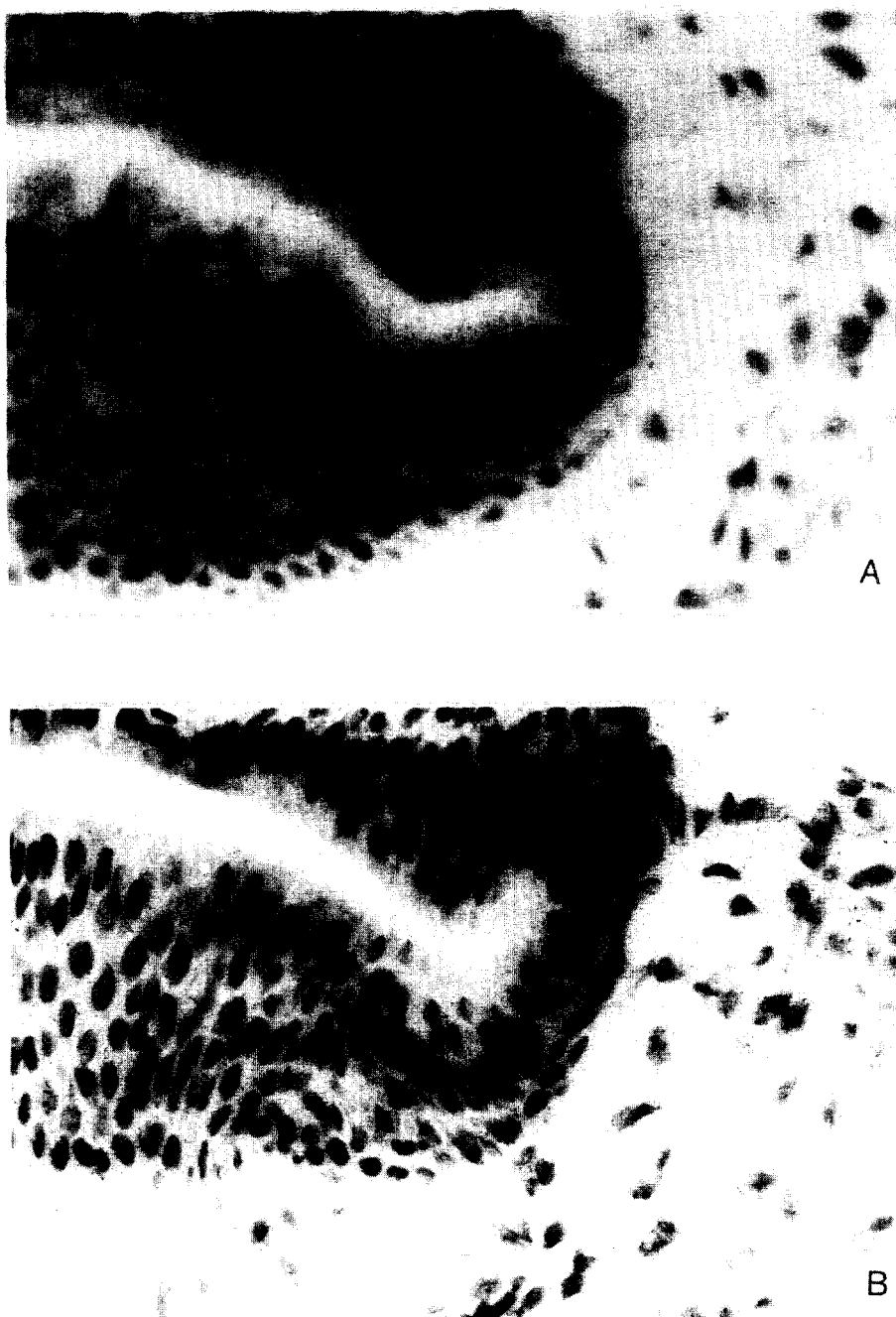


FIG. 4. Autoradiograms of 0.5% glutaraldehyde-fixed cryostat sections (10 μ m) of the nasal mucosa of an NA patient after incubation with **A**, 16 pM of ^{125} I-(+)-CYP or with **B**, 2 μ mol/L of ($\pm$) propranolol in addition to the radioligand, followed by dipping in the nuclear emulsion. The autoradiograms illustrate specific labeling of the nasal epithelium. (Staining with methyl green-pyronin [1%]; original magnification $\times 800$.)

a complete conversion of the high- into the low-affinity sites (Fig. 3). No remarkable changes in agonist inhibition or coupling to G proteins could be demonstrated in allergic patients.

Localization of β -adrenoceptors in human nasal mucosa

Before the autoradiography, radioligand receptor-binding experiments to cryostat sections of human

nasal mucosa were performed. The saturation curves were used to determine the optimal free concentration radioligand for the *in vitro* autoradiography. Specific $^{125}\text{I}(-)-\text{CYP}$ binding to β -adrenoceptors was saturable and of high affinity in cryostat sections of NA (K_d , 4.3 ± 2.4 pM; $B_{\max}$, 3.7 ± 2.8 fmol/mg of protein; $N = 4$) and allergic patients (K_d , $6.3/5.8$ pM; $B_{\max}$, $6.3/3.5$ fmol/mg of protein; $N = 2$). After 2 months of exposure, the autoradiographs demonstrated $^{125}\text{I}(-)-\text{CYP}$ labeling of the nasal epithelium of NA as well as allergic patients (Fig. 4). Autoradiographs made from sections incubated in the presence of $2 \mu\text{mol/L}$ ($\pm$) propranolol, in addition to the radioligand, demonstrated only a faint homogeneous labeling of the nasal mucosa. Nasal glands, blood vessels, and connective tissue were devoid of silver grains.

DISCUSSION

Nasal hyperreactivity in allergic patients may be due to an imbalance of the autonomic regulation.¹ In this study, the hypothesis of changes in adrenergic, α_1 -, α_2 -, and β -adrenergic characteristics has been investigated. Changes of agonist binding, coupling to the G protein, and localization were only investigated for the β -adrenoceptors because of the unfavorable ratio specific/nonspecific binding in the α -adrenergic assays. Because of the limited amount of human tissue, the rat nasal mucosa has been used as a model to investigate the biochemical characteristics of adrenoceptors and the localization of β -adrenoceptors.^{15-17, 19}

^3H -Prazosin and ^3H -rauwolscine binding to α_1 - and α_2 -adrenoceptors in nasal mucosa membranes of NA patients was saturable and of high affinity. The K_d values were in agreement with values observed in rat nasal mucosa^{15, 16} or other human tissues.^{24, 25} The heterogeneous NA group was subdivided into control individuals and patients with chronic sinusitis and vasomotor rhinitis. Similar binding parameters of the ^3H -prazosin and ^3H -rauwolscine binding were obtained in these subgroups. Because high tissue concentrations (25 mg/ml) were required to observe specific ^3H -prazosin binding, only a limited number of patients could be investigated. No large differences in affinities or densities of α_1 - and α_2 -adrenoceptors could be demonstrated in allergic patients in comparison with NA and control individuals. However, the number of binding studies to α -adrenoceptors must be extended for definite conclusions. As far as the α_1 -adrenoceptors, these findings are in agreement with similar reactivity to α_1 -agonists in allergic and NA patients.⁵ In the lower airways, α -adrenergic hyper-

responsiveness has been explained by an increased density of α_1 -adrenoceptors.⁷ ^3H -Prazosin, but not ^3H -rauwolscine binding, has been demonstrated in human nasal mucosa (K_d , 0.31 nmol/L¹¹ and K_d , 1.10 nmol/L¹²). These studies by Japanese authors reported a decreased number of α_1 -adrenoceptors in the nasal mucosa of allergic patients in comparison with patients with chronic sinusitis. The discrepancy between both studies may be due to the use of patients with chronic sinusitis as a control group or to differences in biopsy removal and/or in medication. Regarding the unchanged affinities and densities of α -adrenoceptors demonstrated in this study, α -agonists do not appear to be the appropriate antiallergic drugs. Bende and Laurin²⁶ also suggested that α -agonists are adequate decongestants but no specific antiallergic drugs.

The K_d values of the $^{125}\text{I}(-)-\text{CYP}$ binding to β -adrenoceptors in nasal mucosa membranes of NA and allergic patients were in agreement with values observed in rat nasal mucosa¹⁷ and other human tissues.²⁷ No significant differences of the binding parameters could be demonstrated in allergic patients in comparison with NA individuals. However, if the NA group was further subdivided, a significant decrease of the β -adrenoceptor density, expressed per milligrams of protein, could be demonstrated in allergic patients in comparison with control individuals. These findings emphasize the importance of an accurate characterization of the patients. The control individuals are the most "normal" individuals, whereas other factors may play a role in the other NA patients. The $B_{\max}$ expressed per milligram of protein is probably more accurate than the expression per gram of tissue, since the protein content of the tissue is determined after ultracentrifugation of the tissue (losing watery secretion), whereas the tissue weight is determined before ultracentrifugation. The decreased number of β -adrenoceptors may reflect a β -adrenergic abnormality in nasal allergy.^{2, 3} However, intranasal administration of terbutaline caused no changes in the nasal airway resistance in allergic patients in comparison with changes in NA patients.⁴ Until the present time, the influence of β -adrenergic agonists on the nasal secretion in allergic patients in comparison with that in NA patients has not been investigated. In the lower airways, an infusion of terbutaline resulted in an increased mucociliary transport in patients with asthma. The small shift in $B_{\max}$ may reflect a possible β -adrenergic abnormality in nasal allergy but is probably too small to explain the complex allergic reaction. Large differences in binding parameters can probably not be demonstrated because the allergic patients did

not demonstrate an acute allergic manifestation. A decreased β -adrenoceptor density has been reported in allergic patients in comparison with that found in patients with chronic sinusitis with the antagonist, ^3H -dihydroalprenolol.^{11, 12} In the present study, no significant differences in B_{max} could be observed in allergic patients in comparison with patients with chronic sinusitis, probably because of the limited number of patients investigated. The discrepancy between both studies may also be explained by differences in biopsy removal and/or in medication. However, further analysis of these data revealed similar binding parameters in individuals with medication and individuals without medication of the NA group, control group, or group with AR.

The agonist inhibition curves of ^{125}I -(-)-CYP binding to β -adrenoceptors in nasal mucosa membranes of four of five control subjects and three of five allergic patients demonstrated $15\% \pm 6\%$ and $21\% \pm 10\%$ high-affinity sites, respectively. The K_i values of the high- and low-affinity sites were in agreement with values observed in rat nasal mucosa¹⁷ and in human smooth muscle.²⁷ Generally, a heterogeneity of agonist binding has been a predominant feature of β -adrenoceptors in a variety of tissues.^{28, 29} However, the agonist binding of one control individual and two allergic patients demonstrated only low-affinity sites. A small proportion ($<10\%$) of high-affinity sites, admixed with low-affinity sites, might be undetectable in such experiments. It has been accepted that the high-affinity complex consists of agonist, receptor, and G protein. Guanine nucleotides appeared to convert high-affinity states into low-affinity states of the receptor.^{28, 30} In the presence of 5.10^{-4} mol/L of Gpp(NH)p, a shift of the monophasic and a steepening of the biphasic agonist curves have been demonstrated. A complete conversion of the high- into the low-affinity sites has been observed in one control individual and one allergic patient. Since the protease inhibitor, phenylmethylsulfonyl fluoride, had no effect on the inhibition curve, proteolytic activity is probably not responsible for the incomplete conversion. Complete conversion has not been demonstrated in rat nasal mucosa,¹⁷ bovine trachea smooth muscle,²⁷ and rat heart.³¹ A β -adrenergic abnormality of allergic patients may be reflected in the agonist binding. However, no differences in agonist binding or in effect of Gpp(NH)p on the agonist binding between allergic and control individuals could be observed. The number of agonist inhibition curves must be extended for definite conclusions. In the lower airways, differences in agonist binding could not be observed in patients with chronic airflow obstruction in comparison with control subjects.³² However, allergen challenge of pa-

tients with asthma resulted in a reduced β -adrenergic agonist binding in lymphocytes.⁹ The antagonist and agonist binding to in vitro antigen-challenged nasal mucosa of allergic patients will elicit more information on an autonomic disbalance in the more acute manifestation of AR.

The K_d values of the ^{125}I -(-)-CYP binding to cryostat sections of human nasal mucosa are similar to K_d values observed in homogenates. The β -adrenoceptor density in homogenates is higher than in sections, probably because of differences in protein contents; homogenates consist of a relatively pure membrane fraction, whereas sections contain all tissue constituents. The ^{125}I -(-)-CYP saturation curve was used for the determination of the optimal free concentration radioligand in autoradiography. No reliable quantitative data on grain densities in the autoradiographs can be obtained because the dipping technique does not guarantee a uniform thickness of the emulsion layer.³³

After 2 months of exposure, the autoradiographs of both NA and allergic patients demonstrated specific ^{125}I -(-)-CYP labeling of the epithelium. No differences in the labeling of the different epithelial cell types could be observed, suggesting that no single cell type account for the localization. The presence of adrenoceptors in the epithelium of human and rat nasal mucosa has been demonstrated before by histochemical and autoradiographic experiments.^{17, 34} No data of the influence of β -adrenergic agonists on the nasal mucosa are available. It has been suggested for the lower airways that β -adrenoceptors may play a role in the mucociliary transport, active ion transport, or the production of epithelium-derived factors.^{35, 37} No specific ^{125}I -(-)-CYP labeling could be observed in the nasal blood vessels, which is in agreement with other studies.³⁸ However, a small population of β -adrenoceptors might be present.³⁹

Inhibition studies with the subtype selective antagonist, $\text{LK}_{203-030}$, in homogenates and in vitro autoradiography indicated that the ^{125}I -(-)-CYP labeling in the autoradiographs occurs to a homogeneous β_2 -adrenoceptor population in epithelium of the human nasal mucosa. The combination of radioligand receptor binding and in vitro autoradiography suggests a decreased β -adrenoceptor density in the epithelium of allergic patients in comparison with that of control subjects. This finding is in agreement with quantitative autoradiographic results of the lower airways in chronic airflow obstruction.⁸

Histologic examination of the biopsy specimens pointed to mast cells in the epithelium of allergic patients, whereas no mast cells could be observed in NA patients. Clinical pharmacologic studies refer to

the antiallergic effect of β -agonists by inhibiting histamine release from mast cells.⁴⁰ No specific ¹²⁵I-(−)-CYP labeling of mast cells could be observed in NA and allergic patients in this study.

In summary, no changes in affinities or densities of α_1 - or α_2 -adrenoceptors in the nasal mucosa of allergic patients in comparison with that of control individuals could be demonstrated. The decreased number of β -adrenoceptors may reflect a β -adrenergic abnormality in allergic patients. No differences in agonist binding, coupling to G proteins, or localization of β -adrenoceptors could be demonstrated in nasal allergy.

We thank Mrs. I. Moelans, Mr. M. Dooper, and Mr. S. Marras for technical assistance, Dr. P. Huygen for statistical advice and Dr. W. Kuijpers for photographic advice. We are indebted to the surgeons of the ear, nose, and throat department, St. Radboud Hospital, Nijmegen, for the supply of nasal biopsy specimens.

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Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: Relationship to the development of late asthmatic responses

Sally E. Wenzel, MD,* Jay Y. Westcott, PhD,** and Gary L. Larsen, MD*
Denver, Colo.

Inflammatory mediators have been implicated in the pathogenesis of human asthma and have been demonstrated to increase in bronchoalveolar lavage fluid during the time of the immediate asthmatic response (IAR) after allergen instillation in the lungs. However, the relationship of these mediators, measured early to the late asthmatic response (LAR), airway reactivity, and clinical asthma, is unknown. In the present study, we evaluated mediator levels in bronchoalveolar lavage fluid before and 5 minutes after allergen challenge from three subject groups: atopic subjects without asthma (N = 7), atopic subjects with asthma and without LAR ((-) LAR) (N = 6), and atopic subjects with asthma and with LAR ((+) LAR) (N = 6). Subjects with asthma were differentiated into subjects with and without LARs based on at least a 15% decrease in FEV₁ between 3 to 8 hours postallergen inhalation. The mediators, prostaglandin D₂, thromboxane B₂, leukotriene C₄ (LTC₄), and histamine, were measured both before and after allergen instillation. Baseline prechallenge levels were similar, except in the case of LTC₄. LTC₄ was detectable at baseline significantly more frequently in the atopic subjects with asthma with and without LAR when these subjects were compared to the atopic subjects without asthma (nine of 12 detectable versus one of seven detectable). In all groups, significant increases in mediator levels were observed in the groups with asthma postallergen challenge, compared to the atopic subjects without asthma. Atopic subjects with asthma and without LAR had significantly higher levels of all four mediators after challenge than atopic subjects with asthma and with LAR and atopic subjects without asthma. No correlation was found between the mediator levels and airway physiology among any of the three groups. Although atopic subjects with asthma and with LAR were more reactive to methacholine than the other two groups, the difference did not reach significance. In addition, no correlation was observed with cell counts, differentials, or proteins. It is hypothesized from these findings that multiple factors are involved in the production of an LAR. These factors most likely include mediator levels and underlying airway reactivity, with the more reactive airways requiring less mediators for bronchoconstriction to occur. This finding implies that therapeutic options in asthma should focus not only on blockage of mediator activity but also on reversal of underlying airway hyperreactivity. (J ALLERGY CLIN IMMUNOL 1991;87:540-8.)

From the *Departments of Medicine and Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., and **Webb-Waring Lung Institute and Cardiovascular Pulmonary Research Department, University of Colorado School of Medicine, Denver, Colo.

Supported by National Institutes of Health Grants NRSA HL-07433, HL-36577, and HL-34303.

Received for publication June 19, 1990.

Revised Oct. 2, 1990.

Accepted for publication Oct. 2, 1990.

Reprint requests: Sally E. Wenzel, MD, Assistant Professor of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

1/1/25934