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Impairment of the β -Adrenergic System of Peripheral Blood Leukocytes in Atopic Patients with Seasonal Allergic Rhinoconjunctivitis

Key Words

β_2 -adrenoceptors
cAMP
Peripheral blood leukocytes
Atopy
Seasonal allergic rhinoconjunctivitis

Abstract

Compared to 7 healthy women, an altered β_2 -adrenoceptor/cyclic adenosine monophosphate (cAMP) system has been observed in a combined study on both expression and function of β_2 -adrenoceptors on peripheral blood leukocytes (PBL) of 7 atopic women suffering from acute seasonal rhinoconjunctivitis. A reduced affinity of the β -adrenergic radioligand ¹²⁵Icyanopindolol to its binding site (equilibrium dissociation constant K_d 6.4 ± 1.0 vs. 3.4 ± 0.6 pmol/l in controls; $p < 0.05$), a reduced sensitivity of the intracellular cAMP formation to isoprenaline (concentration necessary to achieve half-maximal effectiveness EC_{50} $1,088.6 \pm 669.0$ vs. 71.0 ± 51.0 nmol/l in controls; $p < 0.01$), and a reduced intracellular cAMP increase in response to isoprenaline (E_{max} as a percentage of the basal cAMP content E_0 117 ± 3 vs. $145 \pm 8\%$ E_0 in controls; $p < 0.01$) point to a subsensitivity of β_2 -adrenoceptors. A reduced E_0 (4.9 ± 0.8 vs. 8.4 ± 1.3 pmol/ 10^6 cells in controls; $p < 0.05$) suggests an increased activity of the cAMP-degrading enzyme phosphodiesterase. A reduced sympathetic tone in atopy was further confirmed by lower cAMP plasma concentrations (25.7 ± 1.2 vs. 31.3 ± 1.6 nmol/l in controls; $p < 0.05$). The results indicate that more than just one mechanism are involved in the impairment of the PBL β_2 -adrenoceptor/cAMP system in atopy.

Introduction

Activation of β -adrenoceptors may inhibit the release of mediators like histamine [1]; β -sympathomimetic drugs are most effective in relieving acute shortness of breath in bronchial asthma [2]. In agreement with these observations, Szentivanyi [3] suggested that a subsensitivity of β -adrenergic receptors due to a disturbance of their expression and/or function may be related to the pathophysiology of atopic bronchial asthma. Further reports have indicated a subsensitivity of β -adrenoceptors also in other atopic diseases [4, 5].

Usually either the expression of β_2 -adrenoceptors or their function has been investigated in studies on the β -adrenergic system in atopic disease. However, to fully describe receptors pharmacologically, both aspects have to be evaluated. We recently reported a decreased affinity of PBL β -adrenoceptors in atopic bronchial asthma that was

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Ute Bleise is a PhD student; this work is part of her dissertation.

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correlated with the stimulation of the intracellular formation of cyclic adenosine monophosphate (cAMP) by isoprenaline [6]. We have now investigated atopic patients with seasonal allergic rhinoconjunctivitis during the acute phase of their disease.

Materials and Methods

Patients and Controls

20 ml of venous blood was drawn from the antecubital vein of 7 healthy women (21–51 years of age) and 7 atopic women suffering from seasonal allergic rhinoconjunctivitis (22–42 years of age). All samples were taken between 08.30 and 09.30 h in the morning to avoid that the results were influenced by circadian variations [7]. The study was performed in April/May. All subjects had a negative history and showed no signs of pulmonary, cardiac, renal, and hepatic disease, nor of any other disease by clinical inspection. No drugs were taken.

The patients had a history of seasonal rhinoconjunctivitis (April–August) for 4–20 years. They had no personal history of perennial rhinoconjunctivitis and no personal or family history of any other atopic disease (asthma, atopic eczema). Allergy to the relevant allergens (birch or grass pollen) had been adequately demonstrated (prick test, radioallergosorbent test). The investigations started in the pollen season after symptoms had been present for at least 3 days. Antiallergic drugs had not yet been taken.

The healthy subjects had no personal or family history of allergic rhinoconjunctivitis and/or any other atopic disease (asthma, atopic eczema) and showed no skin prick reactions to house dust mite, cat epithelia, nor to grass pollen.

Methods

Dextran (Macrodex® 6%; Pfrimmer, Erlangen, FRG) was added to the blood directly after venipuncture, and cells were separated by gravity sedimentation under light protection (room temperature, 90 min). The leukocyte-rich supernatant was separated from the precipitate of erythrocytes and centrifuged at 1,200 rpm for 10 min at 4°C. The resulting cell pellet was washed twice in TCM buffer (pH 7.4) containing 3.0 g/l tris(hydroxymethyl)aminomethane, 0.3 g/l KCl, 7.0 g/l NaCl, 0.147 g/l CaCl₂ × 2H₂O, and 0.2 g/l MgCl₂ × 6H₂O. The cell concentration was finally adjusted to 5 × 10⁶ cells/ml.

The expression of β₂-adrenoceptors was studied in radioreceptor assays with twelve concentrations of (–)-¹²⁵I-cyanopindolol (¹²⁵I-CYP; Amersham Buchler, Braunschweig, FRG) in the range of 1.0–150.0 pmol/l as described elsewhere [8]. The number of high-affinity binding sites (B_{max}) and their equilibrium dissociation constant (K_d) were determined by nonlinear regression analysis.

The function of β₂-adrenoceptors was tested by incubating leukocytes with five concentrations of isoprenaline (10⁻⁸ to 10⁻⁴ mol/l) for 10 min at 37°C. The basal intracellular cAMP content (E₀), the cAMP content maximally achievable by incubation with isoprenaline (E_{max}), and the effective isoprenaline concentration necessary to achieve half-maximal stimulation (EC₅₀) were determined by nonlinear regression analysis. Intracellular cAMP and plasma cAMP were determined by radioimmunoassay (Amersham Buchler).

Mean values are given with their standard errors. To test for differences between patients and healthy controls a Student t test was used. The significance level was α = 0.05

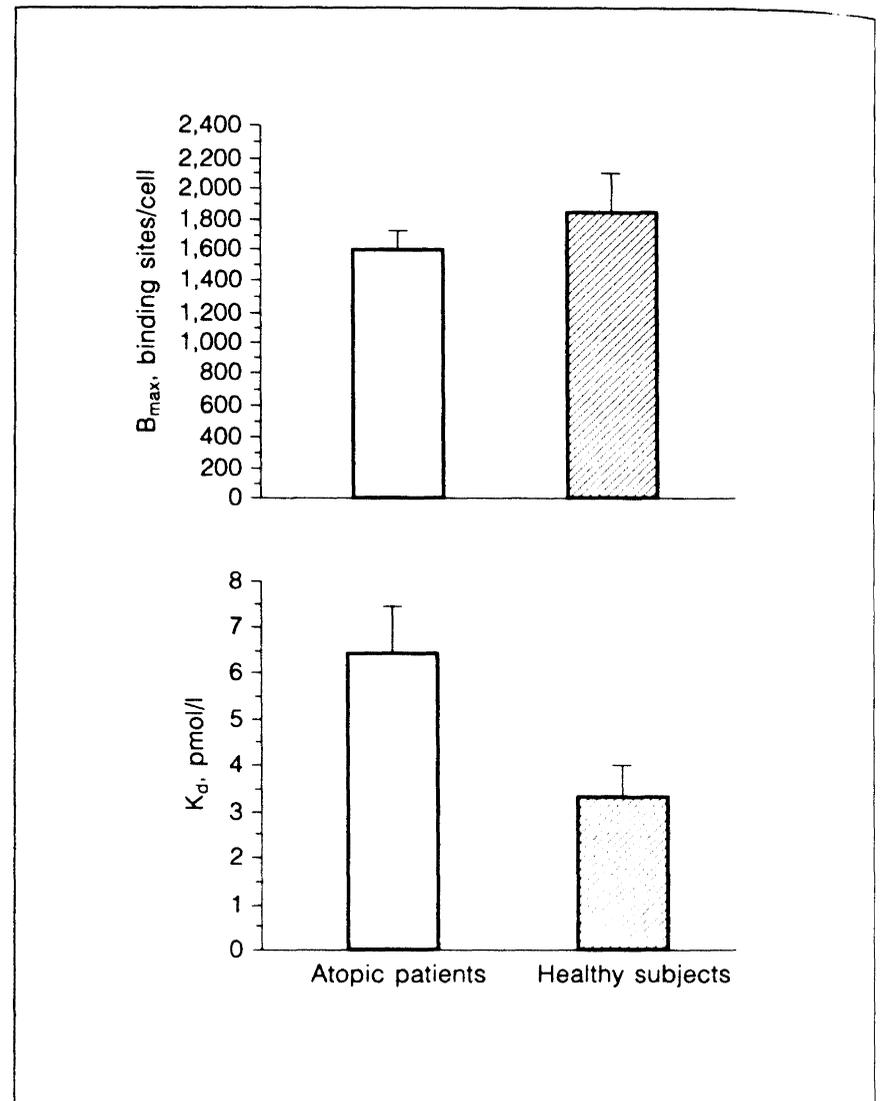


Fig. 1. Expression of β₂-adrenoceptors on peripheral blood leukocytes of 7 atopic women suffering from seasonal allergic rhinoconjunctivitis compared to 7 healthy women. β₂-Adrenoceptor density (top panel) and the equilibrium dissociation constant (a parameter representing inversely the affinity of adrenergic ligands to β₂-adrenoceptors, lower panel) were determined in radioreceptor assays with the adrenergic radioligand ¹²⁵I-CYP. Mean values ± SE are shown.

Results

No significant differences were observed in the B_{max} values of patients (1,607 ± 94 ¹²⁵I-CYP-binding sites/cell) and controls (1,863 ± 248 ¹²⁵I-CYP-binding sites/cell; fig. 1, top panel). K_d, however, was increased in the atopic patients (6.4 ± 1.0 vs. 3.4 ± 0.6 pmol/l in controls; p < 0.05), indicating a decreased affinity of the adrenergic radioligand for its binding site (fig. 1, bottom panel).

E₀ of leukocytes obtained from the atopic patients (4.9 ± 0.8 pmol/10⁶ cells) was significantly lower than in the healthy subjects (8.4 ± 1.3 pmol/10⁶ cells; p < 0.05; fig. 2, top panel). E_{max} (expressed as a percentage of E₀) was significantly decreased in the atopic patients (117 ± 3 vs.

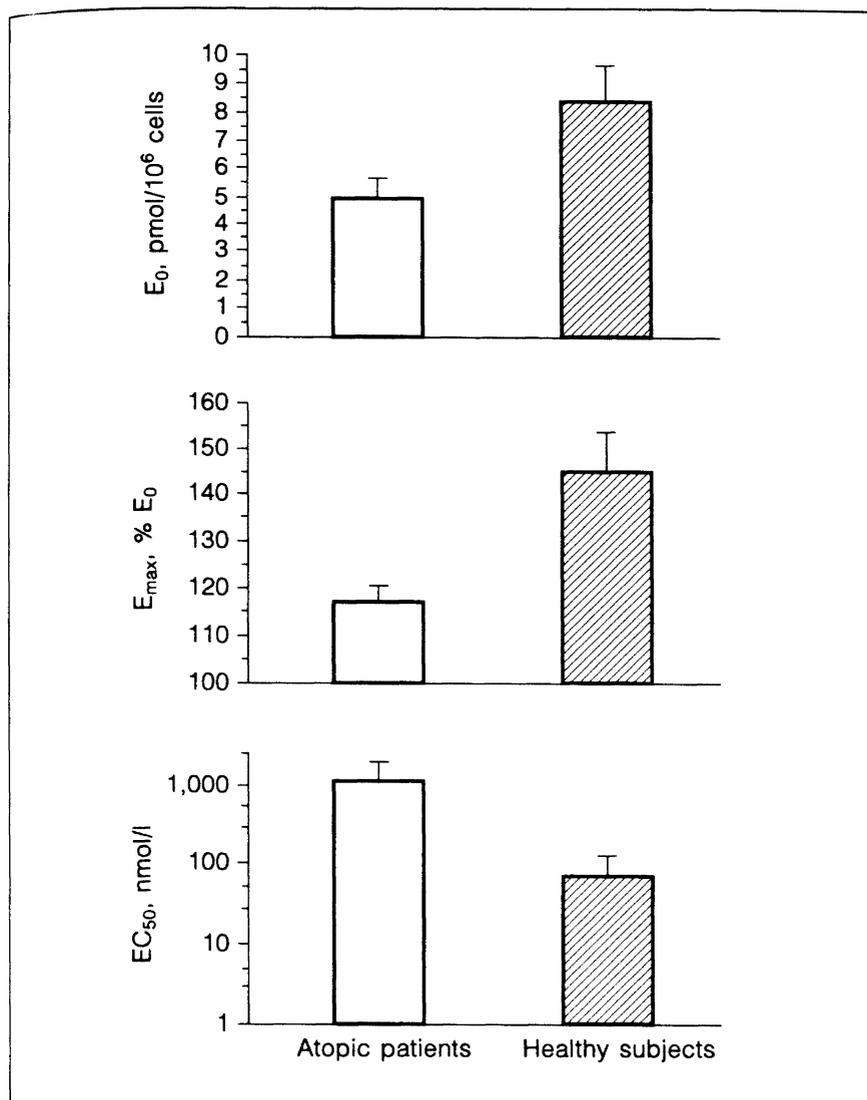


Fig. 2. Function of β_2 -adrenoceptors on peripheral blood leukocytes of 7 atopic women suffering from seasonal allergic rhinoconjunctivitis compared to 7 healthy women. Basal cAMP content (top panel), its increase maximally achievable by incubating the cells with isoprenaline (middle panel), and the isoprenaline concentration necessary to achieve the half-maximal effect (a parameter representing inversely the sensitivity of β_2 -adrenoceptors to adrenergic ligands, lower panel) were determined from dose-response curves for isoprenaline. Data represent mean values \pm SE.

$145 \pm 8\%$ E_0 in controls; $p < 0.01$; fig. 2, middle panel). EC_{50} was higher in the atopic patients than in the healthy subjects ($1,088.6 \pm 669.0$ vs. 71.0 ± 51.0 nmol/l; $p < 0.01$; fig. 2, bottom panel), indicating a reduced sensitivity of β_2 -adrenoceptors to β -adrenergic stimuli.

The cAMP plasma concentrations were decreased in the atopic patients, too (25.7 ± 1.2 nmol/l in patients vs. 31.3 ± 1.6 nmol/l in controls; $p < 0.05$; fig. 3).

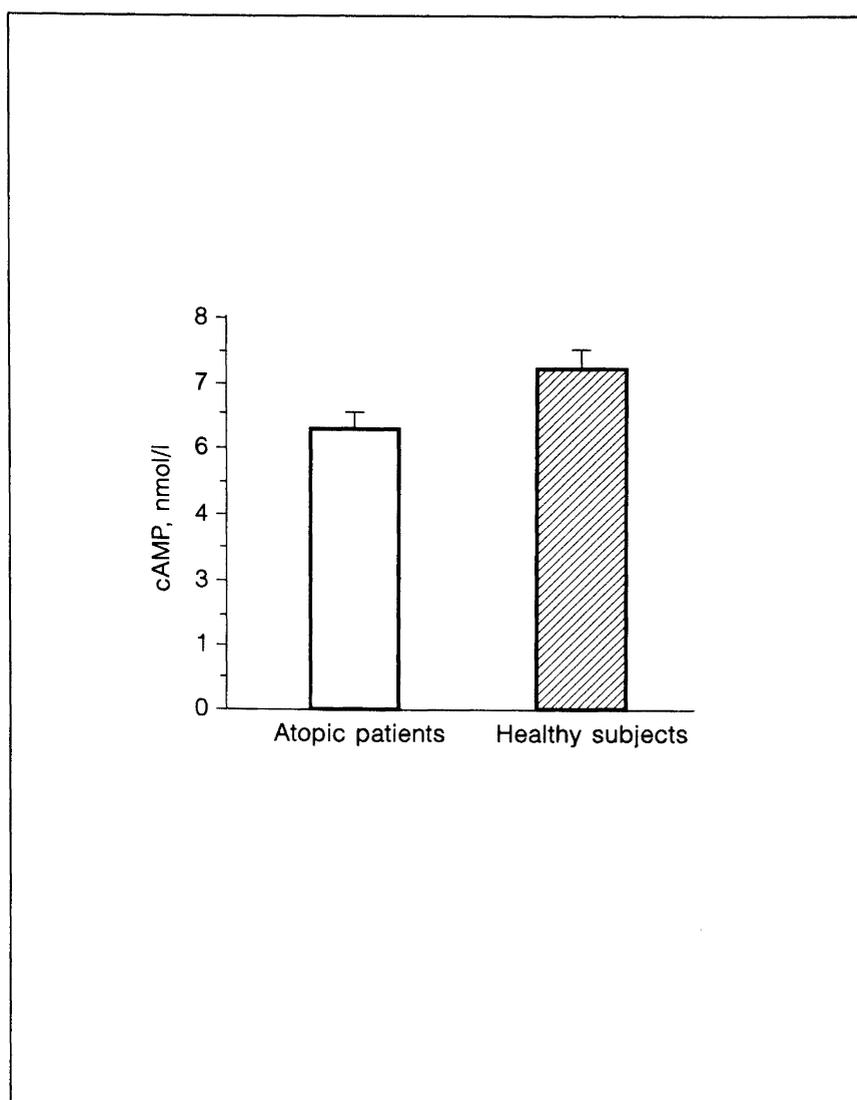


Fig. 3. cAMP plasma concentration, a general marker for the sympathetic tone, of 7 atopic women suffering from seasonal allergic rhinoconjunctivitis compared to 7 healthy women. Mean values \pm SE are shown.

Discussion

The results point to an altered expression and function of β_2 -adrenoceptors on PBL in patients with seasonal rhinoconjunctivitis. Compared to controls, the results demonstrate three features of the PBL β_2 -adrenoceptor/cAMP system that are compatible with 'subsensitivity of β_2 -adrenoceptors PBL in atopic disease: (1) a reduced affinity of the β -adrenergic radioligand to its binding site (K_d); (2) a reduced sensitivity of the intracellular cAMP formation to β -adrenergic stimuli (EC_{50}), and (3) a reduced intracellular cAMP increase in response to isoprenaline (E_{max} expressed as a percentage of E_0). Correspondingly, cAMP plasma concentrations, a general

marker for the sympathetic tone, were found reduced as well.

However, subsensitivity of β_2 -adrenoceptors does not sufficiently explain the reduced basal cAMP content of the cells. The basal cAMP content reflects the steady state between intracellular formation and degradation of cAMP. Besides decreased formation of cAMP, an increased activity of the cAMP-degrading enzyme phosphodiesterase would, therefore, result in a reduced basal cAMP content, too. Such an increased phosphodiesterase

activity had been suggested by Grewe et al. [9]. More than just one mechanism seems to be involved in the impairment of the PBL β_2 -adrenoceptor/cAMP system in atopy.

Regardless whether the impairment of the PBL β_2 -adrenoceptor/cAMP system in atopic seasonal rhinoconjunctivitis is primary or secondary due to disease, it may be pathophysiologically relevant. Further work is under way in order to elucidate possible changes in the reported variables during and out of the symptomatic season.

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