Expression of the glucocorticoid receptor alpha and beta isoforms in human nasal mucosa and polyp epithelial cells

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Abstract

The lower sensitivity of the inflamed nasal mucosa to glucocorticoids might be related to an increased expression of the glucocorticoid receptor (GR) beta isoform. We investigated GRα and GRβ mRNA expression in epithelial cells from nasal mucosa and nasal polyps. GRα mRNA was at least 1000 times more expressed than GRβ mRNA in both tissues. GRβ expression (mean ± SEM of 10³ cDNA copies/µg of total RNA) was higher in nasal polyps (1.15 ± 0.19; n = 27; P < 0.01) than in nasal mucosa (0.62 ± 0.10; n = 32). Nasal polyps with > 3% of inflammatory cells had higher GRβ levels (1.40 ± 0.29; n = 16) than both nasal mucosa (P < 0.01) and polyps with ≤ 3% of inflammatory cells (0.80 ± 0.18; n = 11; P < 0.05). No difference in GRβ expression was found between nasal mucosa and polyps with ≤ 3% of inflammatory cells. GRβ expression correlated with the inflammatory cell number, especially with mast cells (r = 0.50, P < 0.0001). There was no difference in GRα mRNA expression between nasal mucosa and nasal polyps. In summary, GRα is far more expressed than GRβ in both tissues. The increased expression of GRβ may be related to the presence of inflammatory cells.

INTRODUCTION

Nasal polyps are evaginations from the sinus mucosa and the middle turbinate that are infiltrated with inflammatory cells, mainly eosinophils and mast cells (1,2). Glucocorticoids are the most frequently administered drugs with a proven effect on reducing polypos size and inflammation. However, not all patients with nasal polyposis respond to corticosteroid treatment, some of them require surgery despite glucocorticoid maintenance therapy (1). These observations suggest that nasal polyposis consist of a tissue relatively resistant to glucocorticoids, but the underlying mechanisms leading to this poor response are as yet unclear.

We previously reported that some cytokines secreted by nasal mucosa and nasal polypl epithelial cells enhance eosinophil survival (2). We also found that glucocorticoids inhibit the survival-promoting effect of the epithelial cell secretions, but this effect was less potent in epithelial secretions from nasal polyps than in those from healthy nasal mucosa (2). This finding suggested a relative resistance of nasal polypl epithelial cells to the effect of glucocorticoids.

Suboptimal response to glucocorticoids has been described in a number of diseases but it has been mainly investigated in bronchial asthma. The asthmatic patients who are at the lower end of the glucocorticoid response range are considered to be glucocorticoid resistant (3). The rationale of our study is based on the fact that nasal and bronchial diseases should be part of the same pathogenic process and that the mechanisms involving glucocorticoid dependence/resistance in asthma may also account for nasal polyposis.

The biological action of glucocorticoids is mediated through activation of intracellular glucocorticoid receptors (GRs) (4). Two human isoforms of GR have
been identified, GRα and GRβ, which originate from the same gene by alternative splicing of the GR primary transcript (5). Upon hormone binding, GRα is phosphorylated, dissociated from heat shock proteins and translocated to the cell nucleus. As a homodimer, GRα binds to specific sequences within the promoter region of target genes, leading to an enhancement or a repression of their transcription. However, most of the antiinflammatory effects of glucocorticoids are mediated through protein–protein interactions between GR and transcription factors, such as AP-1 and NF-κB (6). The GRβ isoform differs from GRα in its carboxy terminus, where the last 50 amino acids of GRα are replaced by a non-homologous 15 amino acid sequence. GRβ neither binds glucocorticoids nor trans-activates target genes (5,7). Transfection studies have revealed the ability of GRβ to act as a dominant negative inhibitor of GRα activity (5,8) through a mechanism that involves the formation of transcriptionally impaired GRα–GRβ heterodimers (8). However, other studies have challenged this concept (7,9,10).

There may be several mechanisms accounting for the resistance to the antiinflammatory effects of glucocorticoids. Certain proinflammatory cytokines may induce activation of the transcription factor AP-1, which would block the activated GRα, thereby leading to an impaired suppression of the inflammatory response (11). Another suggested mechanism is the cytokine-driven reduction of the GRα binding affinity for glucocorticoids (12). An overexpression of GRβ may also be involved in glucocorticoid-resistant diseases. Thus, increased expression of GRβ has been reported in patients with glucocorticoid-insensitive asthma (13–15), ulcerative colitis (16), nasal polyps (17) and in one patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia (18). Downregulation of GRα levels after treatment with glucocorticoids (19–21) has also been postulated to be one of the possible explanations for the secondary glucocorticoid resistance phenomenon (6).

We hypothesized that the lower sensitivity of the nasal polyp epithelia to glucocorticoids might be due, at least in part, to an increased expression of GRβ in epithelial cells. This study was designed to test this hypothesis.

SUBJECTS AND METHODS

Subjects

Human nasal mucosa was obtained from 32 healthy subjects (20 males) aged 30.3 ± 2.4 years, undergoing nasal corrective surgery for turbinate hypertrophy or septal dismorphism. None of the subjects were treated with corticosteroids. The skin-prick test for common allergens was positive in five subjects (16%) and negative in 27 (84%). Nasal polyps were obtained from 27 patients (19 males) aged 47 ± 2.6 years, undergoing nasal polypectomy. At the time when polypectomy was carried out, eight patients (30%) were receiving intranasal corticosteroids (fluticasone or budesonide, 200–400 μg/day), one (4%) was receiving oral prednisone (40 mg/day), and six (22%) were receiving both intranasal (budesonide or fluticasone, 400 μg/day) and oral corticosteroids (prednisone or deflazacort, 20–40 mg/day). The remaining 12 patients (44%) had not received steroid treatment for at least 1 month prior to surgery. Twelve subjects (44%) had asthma, half of these were aspirin-intolerant. The skin-prick test was positive in five patients (18%), negative in 16 (59%), and it could not be determined in the remaining six patients (22%). The study was approved by the Ethics Committee of our Institution.

Preparation of epithelial cell extracts

Freshly obtained nasal mucosa and nasal polyp specimens were digested with protease and nasal epithelial cell extracts were isolated as previously reported (2). Cell viability was assessed by trypan blue dye exclusion in a haemocytometer. Cell population was histologically characterized using smears obtained by cytocentrifugation (500 rpm, 10 min) and stained with May–Grünwald–Giemsa (MGG). After tissue protease digestion, cell viability was 94 ± 0.8% for nasal mucosa and 95 ± 0.9% for nasal polyps. Compared with healthy mucosa, nasal polyps provided a significantly larger percentage of eosinophils, neutrophils and metachromatic cells (Table 1). Previous studies have shown that most of the metachromatic cells identified in nasal polyps through histochemical staining methods correspond to mast cells, as identified using immunohistochemical staining protocols (22). The percentage of each inflammatory cell type was not significantly different in asthmatics with respect to non-asthmatics. However, there was a tendency for a higher number of eosinophils in patients with aspirin-intolerant asthma (12.3 ± 2.5%; n=6) compared to aspirin-intolerant asthmatics (6.4 ± 1.6%; n=21; P=0.087). There was no difference in the individual inflammatory cell count between the glucocorticoid-treated polyps and the non-treated ones. Nasal polyps with a positive skin-prick test provided a higher number of neutrophils (4 ± 1%; n=5) than those with a negative skin-prick test (1.8 ± 0.3%; n=16; P < 0.05). There was no difference between these two groups of patients with respect to the cell counts of all other inflammatory cell types.

Nasal polyp cell extracts, which had a higher percentage of inflammatory cells than those of nasal mucosa, were processed for panning with an ICAM-3 antibody that efficiently recognizes the ICAM-3 antigen.
of leucocytes and mast cells (23). Thus, cells were incubated for 1 h at 4°C on a Petri dish preabsorbed with the ICAM-3 antibody and were subsequently washed, centrifuged and characterized by MGG staining. After cell panning the percentage of inflammatory cells was reduced to 6.0 ± 1.1%, but it was still significantly higher than in nasal mucosa (2.5 ± 0.3%) (Table I). To normalize the inflammatory cell content of polyps to that of nasal mucosa, polyps were classified into two groups: those which had a percentage of inflammatory cells similar to that of nasal mucosa, i.e. ≤ 3%, and those with an inflammatory cell content > 3% (Table I). Finally, 3–10 × 10⁶ of total cells from either nasal mucosa or nasal polyp epithelial cell extracts were pelleted and frozen at −80°C until RNA analysis.

**Reverse transcription—competitive PCR**

Total RNA from nasal mucosa and nasal polyp epithelial cells was isolated using a rapid extraction method (TRI-Reagent) and reverse transcribed to complementary DNA (cDNA), as previously described (20). GRα and GRβ cDNAs were analyzed by competitive PCR, which is an accurate and sensitive method to quantify gene expression (24,25). This technique is based on the coamplification of the target cDNA and known amounts of an exogenous DNA, called competitor or internal standard, in the same test tube (20). Since the initial amount of internal standard was known, the initial amount of target cDNA was determined.

GRα and GRβ PCR reactions were performed before reaching the plateau phase. Primers were designed to span introns, so that any genomic DNA product would be distinguished from the target cDNA by size difference. GRα and GRβ cDNAs were amplified using specific antisense primers that shared the same sense primer, whose sequences were as follows: 5′-GGCAATACCCAGTTTCAAGCATC-3′ (GRα antisense), and 5′-ATTATCCAGCCTTCAAGCATC-3′ (GRβ antisense), corresponding to nucleotide start positions 1869, 2692, and 2870, respectively. The PCR reaction and cycling conditions have been extensively described elsewhere (20). Both, GRα- and GRβ-specific primers amplified their respective target cDNA and competitor with the same efficiency. In addition, this competitive PCR assay was sensitive enough to detect two-fold differences in GR expression.

To ensure that the RNA was effectively reverse transcribed to cDNA, the PCR for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was routinely performed for each sample.

### Statistical data analysis

The expression of GRα or GRβ mRNA is expressed as the arithmetic mean ± standard error of the mean (SEM) of 10³ copies of GRα or GRβ cDNA per μg of total RNA. Statistical comparisons were performed using analysis of variance (ANOVA) with the Dunnett t-test. P < 0.05 was regarded as statistically significant.

### RESULTS

The expression of GRα mRNA was much higher than GRβ mRNA expression in all specimens. Thus, the GRα/GRβ ratio was 3224 ± 402 for nasal mucosa (n=32) and 1722 ± 289 for the nasal polyp epithelial cell extracts (n=27; P < 0.01).

However, there was a higher expression of GRβ mRNA (× 10³ cDNA copies/μg of total RNA) in the epithelial cell extracts from nasal polyps (1.15 ± 0.19; P < 0.01) than in those from nasal mucosa (0.62 ± 0.10). After classifying the nasal polyps into two groups, according to their inflammatory cell content, we found an even more increased expression of GRβ mRNA in those polyps that had more than 3% of inflammatory cells.

### Table I. Cell populations obtained after protease digestion and cell panning of nasal mucosa and nasal polyps

<table>
<thead>
<tr>
<th></th>
<th>After protease digestion</th>
<th>After panning</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NM</td>
<td>NP</td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Total inflammatory cells (%)</td>
<td>2.5 ± 0.3</td>
<td>13.5 ± 1.6*</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.0 ± 0.0</td>
<td>77 ± 14*</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Metachromatic cells (%)</td>
<td>0.3 ± 0.1</td>
<td>2.4 ± 0.3*</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>1.6 ± 0.2</td>
<td>2.5 ± 0.4*</td>
</tr>
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NM, NP and IC denote nasal mucosa, nasal polyp and inflammatory cells, respectively.

aP < 0.05, compared with NM.
bP < 0.05, compared with NP with ≤ 3% IC.
Fig. 1. Expression of GRα and GRβ mRNA in nasal mucosa and nasal polyp epithelial cell extracts with either ≤ 3% or > 3% of inflammatory cells. Total cellular RNA from nasal mucosa and nasal polyp epithelial cell extracts was isolated and reverse transcribed to cDNA. GRα and GRβ cDNAs were amplified by competitive PCR using GRα- and GRβ-specific primers. Four serial dilutions of the competitors (internal standard, GRα-S or GRβ-S) were added to a constant amount of target cDNA. After amplification, the PCR products were resolved by agarose gel electrophoresis and ethidium bromide staining. The relative amounts of target and GR-S products, which differed in size, were analyzed and compared in each sample by densitometric analysis. Because the amount of GR-S added to each PCR reaction was known, the absolute initial amount of target could be determined. Insets show representative gels of the GRα and GRβ PCR products, with four increasing dilutions of GRα-S (1091 bp) or GRβ-S (1118 bp), which compete with a constant amount of the target cDNA (GRα, 824 bp; GRβ, 1002 bp).

(1.40 ± 0.29: GRα/GRβ ratio = 1372 ± 390; n = 16), when compared to both nasal mucosa (P < 0.01) and polyps with ≤ 3% of inflammatory cells (0.80 ± 0.18: GRα/GRβ ratio = 2230 ± 395; n = 11; P < 0.05) (Fig. 1). However, no statistical difference in GRβ mRNA expression was found between nasal mucosa and nasal polyps with ≤ 3% of inflammatory cells. In addition, a linear correlation was found between GRβ mRNA expression and the percentage of inflammatory cells in all studied specimens (r = 0.38; n = 59; P < 0.01) (Fig. 2). More specifically, there was strong correlation between GRβ expression and the number of metachromatic cells in all specimens (r = 0.50; n = 59; P < 0.0001) (Fig. 2), as well as in all nasal polyps (r = 0.49; n = 27; P < 0.01) and nasal polyps with more than 3% of inflammatory cells (r = 0.60; n = 10; P < 0.05). No correlation was found between GRβ mRNA expression and other inflammatory cells (neutrophils, eosinophils and lymphocytes) (Fig. 2).

In contrast, no differences in GRα mRNA expression were found between nasal mucosa (1690 ± 240; n = 32) and all nasal polyps (1370 ± 190; n = 27; NS) or nasal mucosa and polyps with either less (1670 ± 410: n = 11; NS) or more (1170 ± 160; n = 16; NS) than 3% of inflammatory cells (Fig. 1). There was no correlation between GRα mRNA expression and the inflammatory cell count.

No differences in the expression of GRα or GRβ mRNA were found between asthmatics (GRα = 1560 ± 310; GRβ = 1.29 ± 0.26; n = 12) and non-asthmatic subjects (GRα = 1230 ± 250; GRβ = 1.03 ± 0.28; n = 15), nor between aspirin-tolerant (GRα = 1470 ± 240; GRβ = 1.13 ± 0.23; n = 21) and aspirin-intolerant asthmatics (GRα = 1050 ± 200; GRβ = 1.20 ± 0.35; n = 6). No significant differences in either GRα or GRβ mRNA expression were found between glucocorticoid-treated polyps (GRα = 1190 ± 160; GRβ = 1.24 ± 0.28; n = 15) and non-treated polyps (GRα = 1600 ± 390; GRβ = 1.03 ± 0.26; n = 12). In nasal mucosa specimens, no differences in the expression of GRα or GRβ were found between atopic (GRα = 1800 ± 730; GRβ = 0.63 ± 0.18; n = 5) and non-atopic subjects (GRα = 1660 ± 260; GRβ = 0.62 ± 0.12; n = 27). Similarly, the expression of both GR isoforms in nasal polyps was similar between atopic (GRα = 1210 ± 540; GRβ = 0.85 ± 0.40; n = 5) and non-atopic patients (GRα = 1530 ± 270; GRβ = 1.35 ± 0.25; n = 16).

**DISCUSSION**

It is well established that some patients with nasal polyps fail to respond to glucocorticoid therapy (I). On the basis of our previous studies suggesting a relative resistance of
nasal polyp epithelial cells to the antiinflammatory effect of glucocorticoids (2), we hypothesized that nasal polyp epithelial cells might mediate part of this resistance through overexpression of the GRβ isoform. The aim of this study was to evaluate the expression of GRα and GRβ isoforms in epithelial cell extracts from nasal mucosa and nasal polyps. The main findings of our study were: (1) GRα mRNA expression was more than 1000 times higher than that of GRβ in both nasal mucosa and polyps, (2) GRβ expression was higher in nasal polyps than in nasal mucosa, (3) the increased expression of GRβ in nasal polyps correlated with the inflammatory cell number.

The expression of GRβ mRNA was found to be much less abundant than that of GRα in both nasal mucosa (GRα/GRβ ratio=3224 ± 402) and nasal polyps (GRα/GRβ ratio=1722 ± 289). These findings are in line with RT-PCR and Northern blot analysis performed on whole human tissues and cell lines (5,16,20,26–28). Oakley and coworkers (5), using external standards to quantify the GR transcripts, reported about 200–500 times more GRα mRNA than GRβ mRNA in two adult tissues and two human cell lines. Using a semi-quantitative RT-PCR approach, Honda and coworkers (16) detected GRβ mRNA in only 10% of the PBMCs from healthy subjects. The authors detected GRβ mRNA in 83% of the PBMCs from patients with glucocorticoid-resistant ulcerative colitis, but its concentration was still 605 times lower than the GRα message. Cell-type-specific differences or differences in the RT-PCR technique used may account for the variability in the GRα/GRβ ratio reported among different investigators. Other researchers have even required the use of nested PCR to detect the GRβ transcript (27).

At the protein level, Western blotting studies have revealed little (7,16,29) or any GRβ (20,27) in a variety of cells and tissues. Immunohistochemical studies have
reported expression of GRβ protein in inflammatory cells (13–15,17,30–32). Thus, GRβ has been detected in human neutrophils from healthy subjects (31), in airway T-cells (14,15) and macrophages (15) from glucocorticoid-insensitive asthma, and in inflammatory cells from patients with nasal polyposis (17). The expression of GRβ in cell types of non-inflammatory origin has been hardly investigated. Oakley and coworkers (29) detected GRβ only in a few cell types within the whole tissue, namely, the epithelial cells lining the terminal bronchiol of the lung, forming the outer layer of Hassall’s corpuscle in the thymus, and lining the bile duct in the liver.

An increased expression of GRβ mRNA was found in epithelial cell extracts from nasal polyps compared to nasal mucosa, which was more evident in those polyp samples containing more than 3% of inflammatory cells. The higher expression of GRβ in nasal polyps apparently accounts for the inflammatory cells that remained after panning, for a correlation was found between GRβ expression and the number of inflammatory cells, especially mast cells, present in the epithelial cell extracts. We are tempted to speculate that if we had not removed the inflammatory cell content from nasal polyps, we would probably have found a more marked difference in the expression of GRβ mRNA in nasal polyps with respect to nasal mucosa. Indeed, Hamilos and coworkers (17) have recently reported that GRβ expression in nasal polyps is confined to inflammatory cells, particularly T lymphocytes, eosinophils, and macrophages. The authors also reported increased number of GRβ-positive inflammatory cells in nasal polyps compared to control middle turbinates. Our results showing increased GRβ expression in epithelial cell extracts from nasal polyps are not in contradiction with those of Hamilos (17). Our findings reveal that epithelial cells, as opposed to inflammatory cells, from either nasal mucosa or polyps do not significantly express GRβ, and further reinforce the concept that the expression of GRβ is mainly restricted to inflammatory cells. Contradicting our hypothesis, the relative resistance of nasal polyp epithelial cells to the anti-inflammatory effect of glucocorticoids (2) does not account for an overexpression of GRβ in these cells.

The relationship between insensitivity to glucocorticoid treatment and increased expression of GRβ has also been reported in asthma (13–15,30), ulcerative colitis (16), as well as in chronic lymphocytic leukemia associated with systemic glucocorticoid resistance (18). Increased GRβ has also been found in animal models of systemic glucocorticoid resistance (33). However, other investigators have not observed any correlation between GRβ expression and glucocorticoid insensitivity. For instance, Gagliardo and coworkers (27) were unable to find increased GRβ expression in PBMCs from patients with glucocorticoid-dependent asthma. Similarly, no significant differences in GRβ expression were observed in both putitary and ectopic ACTH-secreting tumors (26). In addition, the possible physiological role of GRβ is still a matter of debate (33,34). Cotransfection studies have shown that when GRβ is more abundant than GRα, GRβ acts as a dominant negative inhibitor of GRα activity (5) through a mechanism which mostly involves the formation of transcriptionally impaired GRα–GRβ heterodimers (8). However, other researches found no evidence of a specific dominant negative effect of GRβ on GRα activity (79,10). Although certain cell types are known to contain GRβ, further studies analyzing the GRα/GRβ ratio are needed. In addition, the possible contribution of a few cells overexpressing GRβ to the disease condition is as yet unknown.

In summary, we report that GRα mRNA expression is far more abundant than the expression of GRβ in both nasal mucosa and nasal polyp epithelial cells. The increased expression of GRβ mRNA in nasal polyps correlates with the number of inflammatory cells present in the nasal polyp extracts. Our results suggest that epithelial cells from either nasal mucosa or nasal polyps are not a primary target for GRβ expression.

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GLUCOCORTICOID RECEPTORS IN NASAL MUCOSA AND POLYPS 95


