Expression of glucocorticoid receptor α- and β-isoforms in human cells and tissues

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Pujols, Laura, Joaquin Mullol, Jordi Rocã-Ferrer, Alfons Torrego, Antoni Xaubet, John A. Cidlowski, and César Picado. Expression of glucocorticoid receptor α- and β-isoforms in human cells and tissues. Am J Physiol Cell Physiol 283: C1324–C1331, 2002. First published June 20, 2002; 10.1152/ajpcell.00363.2001.—Alternative splicing of the human glucocorticoid receptor (GR) primary transcript generates two protein isoforms: GR-α and GR-β. We investigated the expression of both GR isoforms in healthy human cells and tissues. GR-α mRNA abundance (×10^6 cDNA copies/μg total RNA) was as follows: brain (3.83 ± 0.80) > skeletal muscle > macrophages > lung > kidney > liver > heart > eosinophils > peripheral blood mononuclear cells (PBMCs) > nasal mucosa > neutrophils > colon (0.33 ± 0.04). GR-β mRNA was much less expressed than GR-α mRNA. Its abundance (×10^6 cDNA copies/μg total RNA) was as follows: eosinophils (1.55 ± 0.58) > PBMCs > liver ≥ skeletal muscle > kidney > macrophages > lung > neutrophils > brain ≥ nasal mucosa > heart (0.15 ± 0.08). GR-β mRNA was not found in colon. While GR-α protein was detected in all cells and tissues, GR-β was not detected in any specimen. Our results suggest that, in physiological conditions, the default splicing pathway is the one leading to GR-α. The alternative splicing event leading to GR-β is minimally activated.

reverse transcriptase-competitive polymerase chain reaction; Western blotting; healthy human tissues; inflammatory cells;

GLUCOCORTICOIDS MODULATE a large number of metabolic, cardiovascular, immune, and behavioral functions. The biological action of glucocorticoids is mediated through the activation of intracellular glucocorticoid receptors (GR). The GR belongs to the superfamily of steroid/thyroid/retinoic acid receptor proteins that function as ligand-dependent transcription factors (3, 4, 17, 25). Two human isoforms of GR have been identified, termed GR-α and GR-β, which originate from the same gene by alternative splicing of the GR primary transcript (16, 23, 33). GR-α is the predominant isoform of the receptor and the one that shows steroid binding activity (23). However, most of the studies analyzing GR expression did not distinguish between GR-α and GR-β isoforms. With the development of GR-α-specific antibodies, the expression of GR-α has been reported in different cell and tissue types (12, 24, 34). In the absence of ligand, GR-α resides primarily in the cytoplasm of cells and is held inactive by its binding to heat shock proteins. Upon hormone binding, GR-α is phosphorylated, dissociated from heat shock proteins, and subsequently translocated to the cell nucleus, where it mediates either transactivation or transrepression of target genes. The mechanisms for gene activation are mediated through binding of a GR homodimer to specific glucocorticoid response elements on the promoter region of target genes. The mechanisms for gene repression, which account for most of the immunosuppressive and anti-inflammatory responses of glucocorticoids, mostly involve protein-protein interactions between the GR and transcription factors, such as activator protein-1 and nuclear factor-κB (3, 4, 11, 15, 25, 41).

Within the last few years, a number of studies have centered their attention on the GR-β isoform. GR-β differs from GR-α in its carboxy terminus, where the last 50 amino acids of GR-α are replaced by a nonhomologous 15-amino acid sequence. GR-β does not bind glucocorticoids or transactivate target genes (22, 23, 33). Transfection studies revealed the ability of GR-β to act as a dominant negative inhibitor of GR-α activity (2, 32, 33) through a mechanism that involves the formation of transcriptionally impaired GR-α-GR-β heterodimers (32). However, other investigators have challenged this concept (5, 14, 22). The expression of GR-β, both at the mRNA and protein level, seems to be much lower than that of GR-α (10, 22, 24, 33, 35). Immunohistochemical studies have reported expression of GR-β in specific cell types, mostly inflammatory cells (9, 20, 21, 28, 35, 47, 48). Although the physiolog-

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GLUCOCORTICOID RECEPTORS IN HUMAN TISSUES

A549 cells, an epithelial cell line from human lung carcinoma, BEAS-2B cells, a human bronchial epithelial cell line, and COS-7 cells, a fibroblast-like cell line from kidney simian cells, were cultured as previously reported (31, 40). COS-7 cells were transfected with the GR-β expression vector pCMVhGRβ, which contains the GR-β-specific coding sequences and the GR-β 3′-untranslated region, using the calcium phosphate coprecipitation technique as previously described (33, 40).

The authorship institutional review board and ethics committee approved the study, and patients gave informed consent.

RT-competitive PCR. Total RNA from the tissue specimens was isolated using a rapid extraction method (TRI-Reagent, as described elsewhere (40). Total RNA from inflammatory cells was isolated using the RNAqueous-4PCR kit according to the manufacturer’s instructions. Total RNA (2–4 μg) was reverse transcribed to cDNA using random hexanucleotide primers and SuperScript II RNase H− reverse transcriptase, and the RT-PCR reaction conditions have been described extensively elsewhere (40). 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.

Western blotting. Human tissues from brain cortex, heart, lung parenchyma, kidney cortex, skeletal muscle, colonic mucosa, nasal mucosa, and liver, as well as human neutrophils, PBMCs, A549, BEAS-2B, and COS-7 cells were resuspended in lysis buffer containing one protease inhibitor cocktail tablet (Complete), 50 mM HEPES buffer, 0.05% Triton X-100, 0.62 mM phenylmethylsulfonyl fluoride, and 20 mM sodium molybdate. Total proteins from tissues and cells were isolated as described elsewhere (40) and were resolved by electrophoresis through 5% SDS-polyacrylamide Tris-glycine gels. Protein electrophoresis (100 μg) was routinely performed on a Bio-Rad Mini-Protean II cell (Hercules, CA). To achieve optimal separa-

Statistical data analysis. Expression of GR-α or GR-β mRNA is expressed as the arithmetic mean ± SE of 106 copies of GR-α cDNA or 103 copies of GR-β cDNA per microgram of total RNA. Statistical comparisons were performed

MATERIALS AND METHODS

Materials. Random hexanucleotide primers, SuperScript II RNase H− reverse transcriptase, and the RT-PCR buffers were obtained from Life Technologies (Barcelona, Spain). Dextran and Ficoll-Hypaque were purchased from Amer-
using the nonparametric Mann-Whitney U-test. \( P < 0.05 \) was regarded as statistically significant.

**RESULTS**

Expression of GR-\( \alpha \) and GR-\( \beta \) mRNAs in human cells and tissues. GR-\( \alpha \) mRNA was expressed in all analyzed cells and tissues. The abundance of GR-\( \alpha \) mRNA (\( \times 10^6 \) GR-\( \alpha \) cDNA copies/\( \mu \)g total RNA) in tissues (Fig. 1A) was as follows: brain (3.83 ± 0.80; \( n = 4 \)) > skeletal muscle (3.11 ± 0.07; \( n = 3 \)) > lung (2.16 ± 0.98; \( n = 5 \)) > kidney (1.35 ± 0.32; \( n = 4 \)) > liver (0.99 ± 0.31; \( n = 3 \)) > heart (0.89 ± 0.51; \( n = 3 \)) > nasal mucosa (0.59 ± 0.15; \( n = 5 \)) > colon (0.33 ± 0.04; \( n = 4 \)). GR-\( \alpha \) mRNA abundance in inflammatory cells (Fig. 1B) was as follows: macrophages (2.29 ± 0.12; \( n = 3 \)) > eosinophils (0.83 ± 0.25; \( n = 3 \)) > PBMCs (0.74 ± 0.13; \( n = 4 \)) > neutrophils (0.53 ± 0.10; \( n = 6 \)).

GR-\( \beta \) mRNA was detected in all cells and tissues except the colon (\( n = 4 \)), although in concentrations at least 400 times lower than GR-\( \alpha \) mRNA. The abundance of GR-\( \beta \) mRNA (\( \times 10^6 \) GR-\( \beta \) cDNA copies/\( \mu \)g total RNA) in tissues (Fig. 2A) was as follows: liver (0.94 ± 0.11; \( n = 3 \)) > skeletal muscle (0.90 ± 0.25; \( n = 3 \)) > kidney (0.74 ± 0.17; \( n = 4 \)) > lung (0.44 ± 0.16; \( n = 5 \)) > brain (0.19 ± 0.07; \( n = 4 \)) > nasal mucosa (0.18 ± 0.04; \( n = 5 \)) > heart (0.15 ± 0.08; \( n = 3 \)). GR-\( \beta \) mRNA abundance in inflammatory cells (Fig. 2B) was as follows: eosinophils (1.55 ± 0.58; \( n = 3 \)) > PBMCs (1.36 ± 0.44; \( n = 4 \)) > macrophages (0.67 ± 0.23; \( n = 3 \)) > neutrophils (0.39 ± 0.11; \( n = 6 \)).

Expression of GR-\( \alpha \) and GR-\( \beta \) proteins in human cells and tissues. In an attempt to quantify the relative abundance of GR-\( \alpha \) and GR-\( \beta \) proteins in human tissues, tissue protein extracts were subjected to electrophoresis until achieving separation between GR-\( \alpha \) and
GR-β bands. Proteins were then immunoblotted with the anti-GR antibody 57, which is raised against epitopes common to both GR-α and GR-β. COS-7 cells (15 µg) and BEAS-2B cells (30 µg) were loaded alone or as a mixture of both protein extracts (BEAS-2B + COS-7).

Because antibody 57 only detected GR-α in our tissues (Fig. 3), we sought to analyze the expression of GR-α in all of the specimens by immunoblotting with both antibody 57 and AShGR. Antibody 57 revealed expression of GR-α in all analyzed samples (Fig. 4A). GR-α expression, compared with that of 50 µg total proteins from A549 cells, was high in nasal mucosa, liver, lung, skeletal muscle, brain, kidney, and PBMCs and low in heart, colon, and neutrophils. GR-α was detected in neutrophils only after long film exposure, i.e., when GR-α signal in A549 cells was fully saturated.

The pattern of expression identified with AShGR (Fig. 4B) was similar to that detected with antibody 57 (Fig. 4A). Thus, with the exception of skeletal muscle, those cells and tissues expressing high GR-α content with antibody 57 also displayed strong band intensities for GR-α with AShGR. As for antibody 57, GR-α protein expression was low in heart and was not even detected in colon and neutrophils.

BShGR detected GR-β in COS-7 cells transfected with pCMVhGRβ (Fig. 5A). As previously reported (35), no GR-β was detected in GR-β-transfected COS-7 cells incubated with BShGR preabsorbed with the peptide antigen. No protein band was found to fully comigrate with recombinant GR-β in any of the analyzed cells and tissues. BShGR identified one band above and another one below recombinant GR-β in liver, kidney (Fig. 5B), nasal mucosa, and lung. Immunoreactivity for these two bands was still detected after incubation with BShGR preabsorbed with the peptide antigen, which suggests that these two bands account for non-specific interactions between the antibody and proteins expressed in these tissues.

DISCUSSION

The multiple actions of glucocorticoids are mediated through activation of a unique receptor. A number of studies have analyzed GR expression in individual human tissues, using different approaches. However, the variations in the expression levels of the receptor in different tissues has barely been investigated. In addition, the earliest reports on the analysis of GR expres-
expression did not distinguish between GR-α- and GR-β-specific isoforms. A few years ago, both GR-α and GR-β mRNAs (2, 33), as well as their protein products (12, 35), were detected in human tissues and cell lines. Since then, GR-β expression has been reported in a number of cell types. However, its relative abundance compared with GR-α is as yet unknown and is still a matter of controversy. In the present study, we report the mRNA expression of both receptor isoforms in a variety of human inflammatory cells and tissues and further quantify their mRNA expression levels by means of RT-competitive PCR. In addition, we have analyzed the expression of GR-α and GR-β proteins by Western blotting using isoform-specific antibodies, as well as an antibody that recognizes both receptor isoforms.

GR-α mRNA was expressed to varying degrees in all analyzed cells and tissues. GR-α protein expression was characterized by immunoblotting with antibody 57, which recognizes both GR-α and GR-β, and ASGR. It is of significant interest that, although ASGR and antibody 57 are raised against different epitopes of the GR protein, both antibodies displayed similar patterns of expression. The main discrepancy was found in skeletal muscle. Thus, while antibody 57 detected relatively high GR levels, ASGR antibody did not appear to detect much GR-α in this tissue. One explanation for this discrepancy could be that posttranslational modifications of the GR-α protein taking place in skeletal muscle might lead to a GR-α variant that would not be efficiently recognized by the ASGR antibody.

The expression of GR-α protein in most cells and tissues matched up quite well with GR-α mRNA levels. One exception to this was found in nasal mucosa, where the relatively high GR-α protein levels, as revealed by both ASGR and antibody 57, contrasted with the low expression of its transcript. The actual mechanisms accounting for this discrepancy are unknown. Compared with other tissues, the nasal mucosa might have a differential regulation of GR gene expression, such as an increased translational efficiency or a decreased protein degradation.

The fact that GR-α, both message and protein, was detected in all cells and tissues is consistent with the numerous and widespread physiological effects of glucocorticoids in humans (42). Corticosteroids have effects in the brain on memory, the aging process, the stress response, and the maintenance of homeostasis. Glucocorticoids also influence the normal function of skeletal muscle, stimulate liver gluconeogenesis, control the renal fluid and electrolyte balance, affect the cardiovascular system, regulate lung maturation, and have profound anti-inflammatory and immunosuppressive effects. In keeping with their relevant physiological functions, significant expression of GR-α was found in brain, skeletal muscle, lung, liver, kidney, and PBMCs. The lowest GR-α expression was found in heart, colon, mucosa, and neutrophils. Several studies have reported high expression levels of non-isoform-specific GR in rat and human brain (13, 38, 50, 51) as well as in human liver and kidney (50, 51). Total GR has also been found in skeletal muscle (29, 49), heart (26), nasal mucosa (27), lung (1), colon (44), neutrophils (30), PBMCs (18, 30), alveolar macrophages (37), and eosinophils (39), but, because of the use of different techniques, data obtained from these studies are not always comparable. It is important to point out that the low GR-α levels we have found in neutrophils concur with the results published by Miller and coworkers (30) in which the authors detected low expression of GR-α protein by both Western blotting with antibody 57 and radioligand binding techniques. Both our findings and those of Miller are consistent with the low sensitivity of neutrophils to glucocorticoids (43).

GR-β mRNA was detected in all inflammatory cells, i.e., PBMCs, eosinophils, macrophages, neutrophils, and tissues, except the colonic mucosa. However, its concentration was at least 400 times lower than the GR-α message. Our results are in line with RT-PCR and Northern blot analysis performed on whole human tissues and cell lines (10, 18, 24, 33, 40, 52) and suggest that the default splicing pathway is the one leading to GR-α mRNA, as has already been pointed out by Oakley and coworkers (33). Thus the alternative splicing event leading to GR-β mRNA would be a minor pathway. Alternative splicing is tightly regulated in a cell-type- or developmental-stage-specific manner (46). We report significant tissue-specific differences in the primary GR mRNA pattern of splicing. For instance, eosinophils and PBMCs expressed higher levels of GR-β mRNA than the brain cortex. Conversely, GR-α mRNA expression levels in eosinophils and PBMCs were lower than in brain cortex.

The fact that we did not detect GR-β protein, either with ASGR or with antibody 57, in any of the examined cells and tissues is in line with the low expression...
of its transcript. Similarly, using Western blots, several researchers have detected little (22, 24, 35) or no GR-β protein (18, 40) in various human cell types and tissues. In agreement with us, Gagliardo and coworkers (18) did not detect GR-β in PBMCs, and Honda and coworkers (24) only detected GR-β in PBMCs from certain patients with ulcerative colitis. In contrast, as revealed by immunohistochemistry, GR-β has been reported in specific cell types, mostly inflammatory cells (9, 20, 21, 28, 35, 47, 48). However, we have not found GR-β protein in inflammatory cells claimed to contain GR-β, such as PBMCs (20, 28) and neutrophils (48).

Although positive immunoreactivity for GR-β has been reported in a variety of cells, there is still a lot of controversy concerning the relative abundance of GR-β compared with GR-α protein. For instance, Strickland and coworkers (48) have recently reported high constitutive expression of GR-β in neutrophils from healthy individuals. The authors also reported higher expression of GR-β than GR-α in these cells. Our findings do not agree with the results of Strickland et al. Thus, although we report low expression of GR-α mRNA and protein in neutrophils, the expression of the GR-β transcript was still much lower than that of GR-α, and GR-β protein was not detected. A similar discrepancy has been reported in HeLa cells as follows: de Castro and coworkers (12) reported five times more GR-β than GR-α in these cells, whereas two different groups (22, 52), using an antibody that recognized both GR isoforms, demonstrated that GR-α was more abundant than GR-β. In our opinion, conflicting results can mainly be explained by the different methodological approaches used. First, immunohistochemical studies based on the use of different antibodies for the detection of each GR isoform do not reflect the GR-α-to-GR-β ratio of the cell with accuracy because the antibodies may have different affinities to the epitopes. In addition, absolute quantification of GR-α and GR-β proteins by Western blotting (12, 48) may not be technically accurate enough to determine the actual proportion of each receptor isoform. Because of this, various investigators have pointed out that the best way to compare the relative levels of GR-α and GR-β proteins would be by using a single antibody that recognized a common epitope in both isoforms of the receptor (22, 52). In keeping with this, we attempted to quantify the relative expression of GR-α and GR-β proteins in our tissues by immunoblotting with antibody 57. Only GR-α was detected in all samples. As with BShGR antibody, no GR-β protein was detected in any specimen. Although we cannot ultimately rule out the possibility that our Western blotting conditions were not sensitive enough to detect small amounts of GR-β protein, our results demonstrate that GR-α is clearly predominant over GR-β in all the cells and tissues analyzed so far.

The possible physiological role of GR-β is currently a matter of debate. In cotransfection studies, it has been shown that, when GR-β is more abundant than GR-α, GR-β acts as a dominant negative inhibitor of GR-α activity (2, 33) through a mechanism that mostly involves the formation of transcriptionally impaired GR-α-GR-β heterodimers (32). However, other investigators (5, 14, 22) found no evidence for a specific dominant negative effect of GR-β on GR-α activity. It has been argued that the ability of GR-β to regulate GR-α activity in vivo would depend on its expression level relative to that of GR-α and the strength of its association with heat shock protein (hsp) 90 (32). With reference to the latter, GR-β, as well as GR-α, binds to hsp90, but GR-β-hsp90 complexes are less stable than those of GR-α-hsp90 (32). An overexpression of GR-β in pathological conditions, together with a GR-β-hsp90 unstable binding, might increase the dimerization of GR-β with GR-α and therefore inhibit GR-α activity. Increased expression of GR-β has been reported in patients with glucocorticoid-insensitive asthma (20, 28), ulcerative colitis (24), chronic lymphocytic leukemia (45), and nasal polyposis (21). The low levels of GR-β, compared with GR-α, reported herein suggest that, at least in physiological conditions, GR-β is not expressed at levels sufficient to inhibit GR-α function. Nevertheless, further studies analyzing the relative amounts of GR-α and GR-β proteins, particularly in those cell types claimed to contain high levels of GR-β (9, 20, 35, 47, 48), are needed.

In summary, we report the mRNA and protein expression of GR-α- and GR-β-specific isoforms in a variety of human inflammatory cells and tissues. The expression of GR-α mRNA was at least 400 times in excess over GR-β mRNA expression. Characterization of GR-α and GR-β proteins by using isoform-specific antibodies and an antibody that recognizes both receptor isoforms revealed that GR-α was expressed to varying degrees in all cells and tissues, whereas GR-β protein was not detected in any specimen. Our results suggest that the alternative splicing event leading to GR-β is minimally activated in most cells and tissues. Because of the low expression of GR-β, compared with GR-α, GR-β is unlikely to have any inhibitory effect on GR-α function.

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