Glucocorticoid Receptor β, a Potential Endogenous Inhibitor of Glucocorticoid Action in Humans

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Abstract

Alternative splicing of the human glucocorticoid receptor (hGR) pre-mRNA generates two highly homologous isoforms, termed hGRα and hGRβ. hGRα is a ligand-activated transcription factor which, in the hormone-bound state, modulates the expression of glucocorticoid-responsive genes by binding to specific glucocorticoid response element (GRE) DNA sequences. In contrast, hGRβ does not bind glucocorticoids and is transcriptionally inactive. We demonstrate here that hGRβ is able to inhibit the effects of hormone-activated hGRα on a glucocorticoid-responsive reporter gene in a concentration-dependent manner. [^{3}H]-Dexamethasone binding studies indicate that hGRβ does not alter the affinity of hGRα for its hormonal ligand. The presence of hGRβ in nuclear extracts and its ability to bind to a radiolabeled GRE oligonucleotide suggest that its inhibitory effect may be due to competition for GRE target sites. Reverse transcription-PCR analysis shows expression of hGRβ mRNA in multiple human tissues. These results indicate that hGRβ may be a physiologically and pathophysiologically relevant endogenous inhibitor of glucocorticoid action, which may participate in defining the sensitivity of target tissues to glucocorticoids. They also underline the importance of distinguishing between the two receptor isoforms in all future studies of hGR function and the need to revisit old data. (*J. Clin. Invest.* 1995. 95:2435–2441.) Key words: glucocorticoids • glucocorticoid receptors • reporter genes • polyacrylamide gel electrophoresis • polymerase chain reaction

Introduction

Glucocorticoids are essential for normal development and maintenance of basal and stress-related homeostasis. They are also potent immunosuppressants and regulate a broad range of metabolic processes and central nervous system functions (1). All of their effects are mediated by the glucocorticoid receptor (GR)[1] which, when unliganded, is anchored to heat shock protein (hsp) 90 in the form of a heterohexamer containing the receptor, two molecules of hsp 90, and one molecule each of hsp 70, hsp 56, and hsp 26 (2–5). The receptor is thus kept in a ligand-friendly, but non-DNA binding state. Hormone binding leads to dissociation of the receptor from this complex and binding to glucocorticoid response elements (GREs) in the regulatory regions of glucocorticoid target genes. Usually, two GRE half sites are arranged as inverted palindromes allowing the receptor to bind the DNA as a homodimer. Interaction of the hormone/receptor complex with other DNA elements and/or nuclear proteins then modulate the rate at which these genes are transcribed (6–8).

Cloning of the human glucocorticoid receptor (hGR) cDNA and gene revealed that alternative splicing of the hGR pre-mRNA generates an additional, highly homologous mRNA and, consequently, protein isoform called hGRβ, as opposed to the originally known hGRα (9, 10). Both mRNAs contain the first eight exons of the 10-exon hGR gene, whereas either of the last two exons, i.e., exon 9α or 9β, is spliced into the respective mRNA. The two protein isoforms have the first 727 NH_2-terminal amino acids in common, and, thus, both contain the transactivation and the DNA binding domains. hGRβ differs from hGRα only in its COOH terminus with replacement of the last 50 amino acids of the latter with a unique 15 amino acid sequence. This difference renders hGRβ unable to bind glucocorticoid hormones and to be transcriptionally active (9, 11–14).

The ability of hGRβ to antagonize the effects of hGRα and whether this isoform is physiologically expressed in human tissues have not been studied as yet. We hypothesized that hGRβ could inhibit the effects of the hormone-activated hGRα when both receptor isoforms were present within the same cell. To test this hypothesis, we examined the activity of a glucocorticoid-responsive reporter gene in cells expressing hGRα, hGRβ, or both receptors. Using reverse transcription-PCR analysis, we also determined the expression of hGRα and hGRβ mRNA in multiple human tissues.

Methods

Cell culture. COS-7 monkey kidney tumor cells were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in DME (Biofluids Inc., Rockville, MD) supplemented with 10% FBS, 100 U/ml penicillin and streptomycin, and L-glutamine. The cells were incubated at 37°C in an atmosphere of 5% CO_2. 24 h before transfection, cells were removed from their culture flasks by trypsinization, resuspended in supplemented medium, and plated in 60-mm tissue culture dishes (5×10^5/plate).

Plasmids. pRShGRα and pRShGRβ contain the full length coding region of hGRα and hGRβ under the control of the constitutively active Rous sarcoma virus promoter (11). The plasmid pRSv-erbA^{-1} that contains a thyroid receptor cDNA in inverse orientation but is otherwise similar to the hGRα and hGRβ plasmids was used as carrier DNA to

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1. *Abbreviations used in this paper:* GR, glucocorticoid receptor; GRE, glucocorticoid response element; hGR, human glucocorticoid receptor; hsp, heat shock protein.

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yield a constant amount of transfected DNA. All three plasmids were kindly donated by Dr. R. Evans (Salk Institute, La Jolla, CA). pMMTV-luc was the kind gift of Dr. G. Hager (National Cancer Institute, National Institutes of Health [NIH] Bethesda, MD), and contains the glucocorticoid-inducible mouse mammary tumor virus promoter linked to the luciferase reporter gene (15). pTK-luc contains the luciferase reporter gene under the control of the glucocorticoid-independent herpes simplex virus thymidine kinase promotor. We are grateful to Dr. J. Segars (National Institute of Child Health and Human Development, NIH, Bethesda, MD) for kindly providing us with this plasmid. pSV-β-gal (Promega Corp., Madison, WI) encodes the β-galactosidase reporter gene linked to the constitutively active, glucocorticoid-independent SV40 promoter, and was used to control for differences in transfection efficiency.

Transfection. Cells were transfected by the lipofection method as previously described (16). 30 μl transfectant (DOTAP, Boehringer Mannheim Corp., Indianapolis, IN) was used per plate. For reporter gene experiments, cells were cotransfected with pRShGRα (1 μg/plate), pMMTV-luc (5 μg/plate), and pSV-β-gal (2 μg/plate). Different amounts of pRShGRβ (0, 1, 5, 10, and 15 μg/plate) were added to the transfection mixture. Appropriate amounts of pRSv-erbA^{-1} were added to yield a constant amount of 23 μg DNA/plate. For hormone binding and gel shift experiments, constant amounts of pRShGRα (1 μg/plate), pRShGRβ (10 μg/plate), and pSV-β-gal (2 μg/plate), but no reporter genes were transfected. 24 h after transfection, the medium was replaced with either normal medium or medium containing 10^{-7} M dexamethasone.

Luciferase assay. Luciferase activity was determined essentially as previously described (17). 48 h after transfection, cells were washed twice with PBS and incubated for 20 min at 4°C with a reporter lysis buffer (Analytical Bioluminescence Laboratory, San Diego, CA). Cell lysates were centrifuged for 3 min at 14,000 rpm, and supernatants were analyzed for luciferase activity in a luminometer (LKB Wallac, Turku, Finland) using a commercially available luciferase assay system (Analytical Luminescence Laboratory, San Diego, CA). β-galactosidase activity was determined in the same samples using a galactosidase assay system (Promega Corp.). Luciferase values were divided by galactosidase values to normalize for variations in transfection efficiency. Statistical analysis (Mann–Whitney test) was carried out using MacIntosh StatView software (Abacus Concepts Inc., Berkeley, CA).

Hormone binding studies. Cells were transfected with pRShGRα (1 μg/plate), pRShGRβ (10 μg/plate), or both plasmids (1 μg/plate and 10 μg/plate, respectively) as described above. To control for transfection efficiency, constant amounts of pSV-β-gal (2 μg/plate) were cotransfected in all cases. 24 h after transfection, cells were washed three times with PBS and incubated with [^{3}H]-labeled dexamethasone (Amersham Corp., Arlington Heights, IL) at five concentrations (1–20 nM) in the absence and presence of 500-fold excess of unlabeled hormone to determine total and nonspecific binding, respectively. After a 1-h incubation at 37°C, cells were washed three times with ice-cold PBS, scraped with a rubber policeman, centrifuged, and resuspended in 500 μl PBS. 250 μl of the cell suspension was set aside for determination of β-galactosidase activity. The other aliquot was transferred to scintillation vials containing 4 ml scintillation fluid, and counted in a β-scintillation counter. The values for nonspecific and total binding were normalized for transfection efficiency by determination of β-galactosidase activity. Specific binding was calculated by substracting nonspecific from total binding. The data was then analyzed by the method of Scatchard. Binding capacities were expressed as fmol/10^6 cells and the dissociation constants in nM.

Gel mobility shift assay. Cells were transfected with pRShGRα (1 μg/plate), pRShGRβ (10 μg/plate), or both plasmids (1 μg/plate and 10 μg/plate, respectively) as described above. 24 h after transfection, the medium was replaced with either normal medium or medium containing 10^{-7} M dexamethasone. 48 h after transfection, cells were washed twice with PBS, and nuclear extracts were prepared essentially as described elsewhere (18). Protein concentrations were determined using a BCA protein assay system (Pierce Chemical Co., Rockford, IL) after the protocol provided by the manufacturer. 8 pmol of a 22-bp double-stranded GRE probe (5′ GATCAGAACACAGT GTTCTCTA 3′; Stratagene Inc., La Jolla, CA) was radioactively labeled by the 5′ end labeling technique using 8 U T4 polynucleotide kinase (Boehringer Mannheim Corp.) and 16 pmol γ[^{32}P]ATP (3,000 Ci/mmol, Amersham Corp.) 5 μg undiluted nuclear extract protein was coincubated with 100 fmol gel-purified ^{32}P-labeled GRE probe in a gel shift incubation buffer (Stratagene Inc.) for 20 min at room temperature. Polyacrylamide gel electrophoresis was performed at 4°C in a glycine buffer (Stratagene Inc.). Radioactive material was visualized by autoradiography.

Reverse transcription-PCR. cDNAs from the following human glucocorticoid target tissues (Clontech, Palo Alto, CA) were analyzed by PCR: total brain, brain cortex, amygdala, hippocampus, hypothalamus, pituitary, bone marrow, thymus, spleen, peripheral blood leukocytes, liver, kidney, lung, abdominal fat, skeletal muscle, placenta (term), and fetal lung. Two successive amplification reactions were carried out using the nested primer technique. The following primers were used for amplification of hGRα-specific cDNA (positive control): 5′ outer primer, 5′ position at 2135 bp (exon 7): 5′ TT TCTTATGGCATTTGCTCTGG 3′; 3′ outer primer, 5′ position at 2858 bp (exon 9 α, 3′ untranslated region): 5′ GATGACGACTCAACTGCTTCTG 3′; 5′ inner primer, 5′ position at 2188 bp (exon 7): 5′ CCTGCTGTGTTTTGCTCCT GAT 3′; 3′ inner primer, 5′ position at 2693 bp (exon 9α, 3′ untranslated region): 5′ TTTAAGGCAACCATTCTTATTA 3′. hGRβ-specific PCR primer sequences were as follows: 5′ outer primer, 5′ position at 2135 bp (exon 7): 5′ TTTCTTATGGCATTTGCTCTGG 3′; 3′ outer primer, 5′ position at 2644 bp (exon 9β, 3′ untranslated region): 5′ CTTATTATTGACAACGAAGTGC 3′; 5′ inner primer, 5′ position at 2269 bp (exon 8): 5′ GCTGTATGTTTCCTCTGAG TTA 3′; 3′ inner primer, 5′ position at 2591 bp (exon 9β, 3′ untranslated region): 5′ TTTTTGAGCGCCAAGATTG TTG 3′. 30 cycles of PCR were carried out in a thermal cycler (Perkin-Elmer Cetus Instruments, Norwalk, CT). Each cycle consisted of incubations at 94°C for denaturation (1 min), 54°C for annealing (1.5 min), and 72°C for primer extension (2 min). The initial denaturation period was 5 min, the final extension time was 10 min. PCR products were electrophoresed in a 2.5% agarose gel and visualized by ethidium bromide staining.

Southern hybridization. PCR products were further analyzed by Southern hybridization using the following γ[^{32}P]ATP-labeled oligonucleotide probes: hGRα-specific probe, 5′ position at 2618 bp (exon 9α, 3′ coding region): 5′ TTGGTATCTGATTGGTGATGATTTCAG-CTAACATCTC GGG 3′. hGRβ-specific probe, 5′ position at 2552 bp (exon 9β, 3′ coding region): 5′ ATGTGTGAGATGTGCTTTCTG-GTTTTAACCACATAACATT 3′. Southern blotting and hybridization were performed as described elsewhere (19). In addition, the hGRβ-specific sequence of the amplification product obtained from hippocampus cDNA was confirmed by direct DNA sequencing using the dideoxy chain termination method after standard protocols (19).

Results

Inhibition of hGRα-mediated gene transcription by hGRβ. The results of the reporter gene experiments are shown in Fig. 1. Transfection of COS-7 cells with the pMMTV-luc reporter plasmid alone did not result in an increase in luciferase activity in response to dexamethasone, confirming that these cells do not contain significant amounts of functional GR (11). In cells transfected with hGRα, luciferase activity increased 56-fold upon stimulation with 10^{-7} M dexamethasone, whereas hGRβ alone did not influence luciferase activity. When coexpressed with hGRα, however, hGRβ inhibited transcription in a dose-dependent manner, with 15-fold overexpression of hGRβ leading to 90% reduction of luciferase activity (Fig. 1 *A*). In contrast, transfection of high amounts of pRShGRα (16 μg/plate) did not result in significant reduction of luciferase activity (Fig. 1 *A*). The inhibitory effect of hGRβ on hGRα-mediated trans-

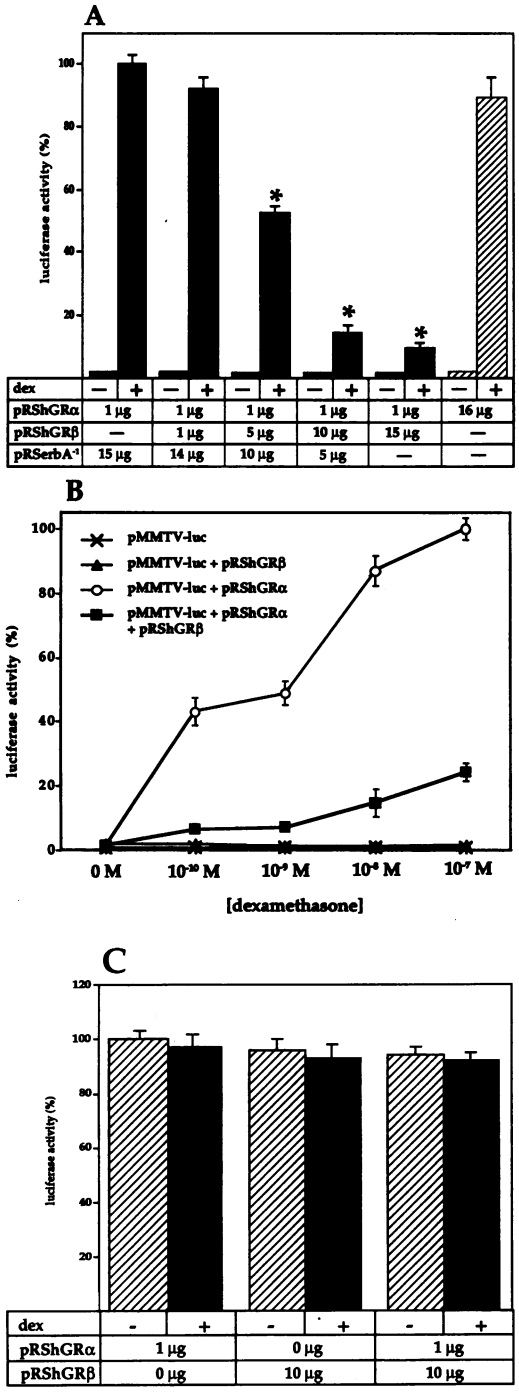


Figure 1. (*A*) Inhibition of hGRα-mediated gene transcription by hGRβ. Each column represents the mean±SEM of three different experiments performed in quadruplicates. * Significant changes between groups ($P < 0.005$). (*B*) hGRβ represses hGRα-mediated transcription at different dexamethasone concentrations. (*C*) hGRβ does not inhibit transcription of the glucocorticoid-independent TK-luc reporter gene. dex, dexamethasone.

activation could also be demonstrated with lower levels of dexamethasone in a concentration-dependent manner (Fig. 1 *B*). hGRβ did not influence transcription of a nonglucocorticoid-responsive TK-luc reporter plasmid, even when 10-fold overexpressed (Fig. 1 *C*).

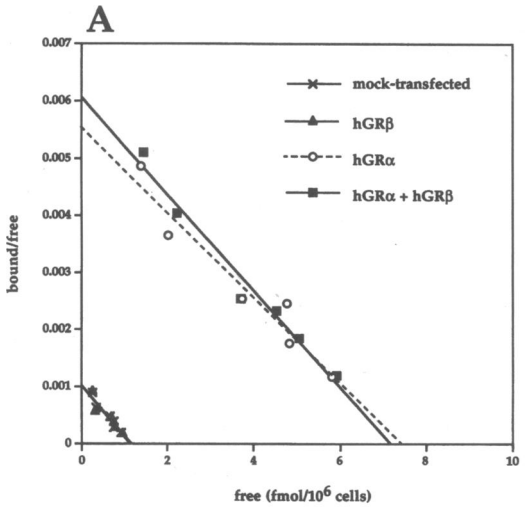
[^{3}H]Dexamethasone binding parameters. The results of three [^{3}H]dexamethasone radioligand binding assays performed with transfected COS-7 cells are shown in Fig. 2. Fig. 2 *A* shows one representative Scatchard plot, while the B(max) and K_d values (expressed as mean±SEM) are shown in Fig. 2, *B* and *C*, respectively. In mock-transfected cells, a low amount of specific glucocorticoid binding sites (1.13 fmol/10^6 cells) with a mean K_d of 7.32 nM was demonstrated. In cells transfected with 10 μg pRShGRβ/plate, similar specific [^{3}H]dexamethasone binding and affinity were observed to those of mock-transfected cells [B(max) = 1.16 fmol/10^6 cells, K_d = 7.27 nM]. In cells transfected with 1 μg pRShGRα/plate, [^{3}H]-dexamethasone binding rose to 7.59 fmol/10^6 cells, while the K_d of the transfected receptor was 8.67 nM. When pRShGRα (1 μg/plate) and pRShGRβ (10 μg/plate) were coexpressed, the amount of [^{3}H]dexamethasone binding sites (7.18 fmol/10^6 cells) and their affinity (K_d = 7.85 nM) were similar those transfected with pRShGRα (1 μg/plate) alone.

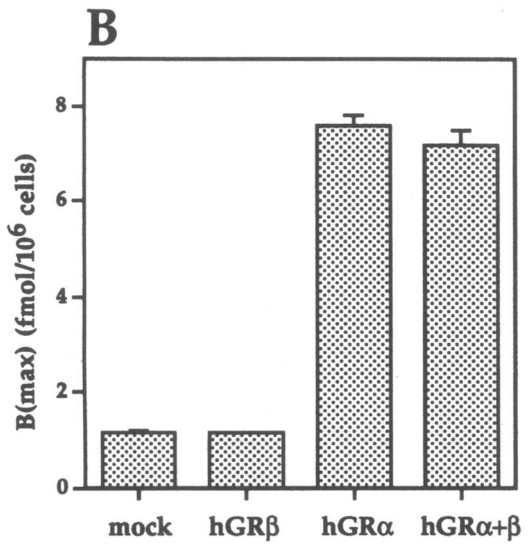
Gel mobility shift assay. To examine whether hGRβ homodimers and/or heterodimers with hGRα bind specifically to GREs, nuclear extracts of dexamethasone-stimulated COS-7 cells transfected with either pRShGRα, pRShGRβ, or both plasmids, were coincubated with a radioactively labeled probe containing a palindromic GRE sequence. GR/GRE binding was then determined in a gel mobility shift system. Fig. 3 shows that nuclear extracts from cells transfected with 1 μg/plate pRShGRα (lane *3*), 10 μg/plate pRShGRβ (lane *5*), and both vectors (1 and 10 μg/plate, respectively, lane *7*) bind to the GRE, whereas extracts from untransfected cells show only minimal binding activity (lane *2*). Binding of both receptor isoforms to the labeled oligonucleotide could be specifically inhibited by the addition of excess cold GRE probe (lanes *4, 6,* and *8*).

Expression of hGRβ mRNA in multiple human tissues. To study the tissue expression of hGRβ, we performed reverse transcription-PCR analysis of 17 known human glucocorticoid target tissues using hGRα- and hGRβ-specific primers. As would be expected, we demonstrated hGRα mRNA in all tissues. A hGRβ-specific product of the predicted size (321 bp) was also amplified in all tissues analyzed in this study, i.e., total brain, brain cortex, amygdala, hippocampus, hypothalamus, pituitary, bone marrow, thymus, spleen, peripheral blood leukocytes, liver, kidney, lung, abdominal fat, skeletal muscle, placenta (term), and fetal lung (Fig. 4, *top*). The hGRβ-specific sequence of the amplification product was confirmed by Southern hybridization in all cases (Fig. 4, *bottom*). In addition, direct DNA sequencing confirmed the hGRβ-specific sequence in the hippocampus. In blood leukocytes, however, the hGRβ-specific probe hybridized with a smaller, barely visible band (~ 200 bp) suggesting that a shorter hGRβ isoform is present in these cells. The exact sequence and the functional consequences of this variant remain to be elucidated.

Discussion

In the present study, we have addressed the functional properties of the human glucocorticoid receptor β as well the expression of its mRNA throughout the human body. We demonstrate here that overexpression of hGRβ can disrupt the enhancing effects of hGRα on gene transcription. The inhibitory effect of hGRβ was not due to differences in transfection efficiency, which was controlled for by determination of galactosidase activity in all experiments. As opposed to hGRβ, overexpression of hGRα





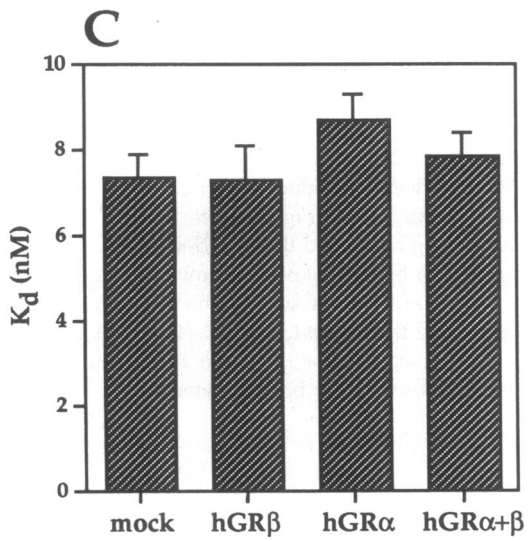


Figure 2. Overexpression of hGRβ does not reduce the number or the affinity of hGRα for its hormonal ligand. (*A*) Representative Scatchard plot of [^{3}H]dexamethasone binding to COS-7 cells transfected with pRSv-erbA^{-1} (mock-transfection), pRShGRβ, pRShGRα, or both expression vectors. (*B*) [^{3}H]dexamethasone binding capacity [*B(max)*] of three transfection experiments expressed as mean±SEM. No significant differences between mock- and hGRβ-transfected or between hGRα- and hGRα/hGRβ-transfected cells were observed ($P > 0.05$). (*C*) [^{3}H]dexamethasone binding affinity (K_d) of three transfection experiments expressed as mean±SEM. No significant differences between any of the groups were observed ($P > 0.05$).

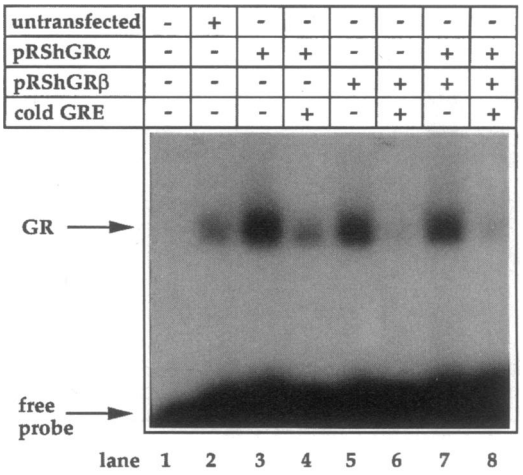


Figure 3. Binding of hGRβ to a GRE consensus sequence. Nuclear extracts of COS-7 cells transfected with pRShGRα, pRShGRβ, or both plasmids were analyzed for their ability to bind to a radiolabeled 22-bp GRE oligonucleotide. Both hGRα and hGRβ bind to the consensus element. No reduction of GRE binding activity is observed when both receptor isoforms are coexpressed.

did not result in significant repression of reporter gene activity, indicating that overexpression of receptor was not by itself inhibitory. Neither was the suppressive effect of hGRβ due to nonspecific sequestration of basic transcription factors, since hGRβ did not influence transcription of a nonglucocorticoid-responsive TK-luc reporter plasmid. Finally, hGRβ did not reduce the expression of hGRα as revealed by similar [^{3}H]-dexamethasone binding parameters in hGRα-transfected and in cotransfected cells. The previously shown inability of hGRβ to bind glucocorticoids and to mediate their enhancing effects on gene transcription was confirmed in this study (9, 11–14). In summary, the hGRβ isoform specifically inhibits the effects of its transcriptionally active counterpart, and, therefore, fits the definition of a dominant negative inhibitor of hGRα (20).

Possible mechanisms through which hGRβ could exert its dominant negative effect include formation of non-DNA–binding heterodimers with hGRα, squelching, i.e., titration of limiting amounts of accessory proteins or coactivators of hGRα, and occupation of DNA target sequences by nontransactivating α/β-heterodimers and/or β/β-homodimers (8, 21, 22).

Formation of non-GRE/DNA–binding complexes with the glucocorticoid receptor is believed to be the predominant mechanism by which previously described inhibitors of glucocorticoid action, such as c-*jun* (23–25), calreticulin (26, 27), and the p65 subunit of the transcription factor NF-κB (28), exert their dominant negative effects. It was demonstrated that these proteins bind directly to the activated GR and prevent it from binding to GREs on the target DNA. The results of the gel mobility shift assays in this study make it seem unlikely that

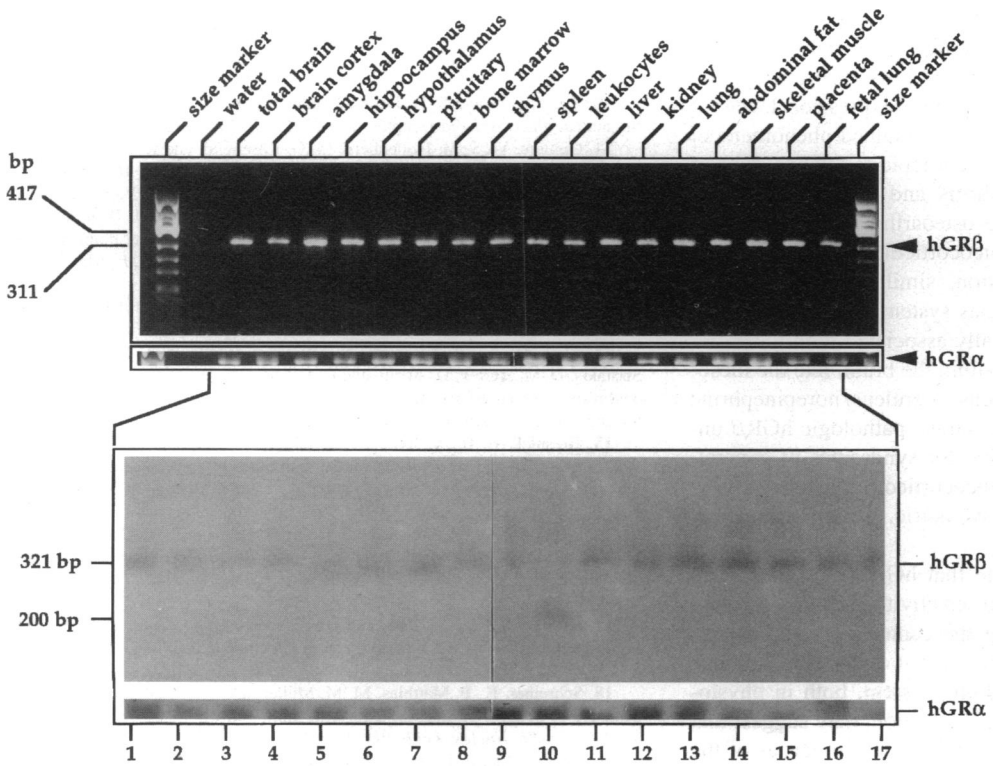


Figure 4. hGRβ mRNA is expressed in multiple human tissues. (*Top*) Agarose gel electrophoresis of amplification products obtained by RT-PCR using hGRα- and hGRβ-specific primers. (*Bottom*) Southern hybridization of the same gel with ^{32}P-labeled hGRα- and hGRβ-specific oligonucleotide probes.

formation of non-DNA–binding hGRα/hGRβ heterodimers accounts for the inhibitory effect of hGRβ. If this were the case, one would have expected GRE binding of extracts obtained from hGRα/hGRβ–cotransfected cells to have been minimal.

Competition for functionally limiting cofactors (squelching) has previously been proposed as a mechanism by which the action of different classes of nuclear hormone receptors may be interfered with at the transcriptional level (29). Repression of GR function by the hormone-activated estrogen receptor may represent an example for this type of inhibition (29). To our knowledge, however, direct evidence that squelching of a known accessory factor might lead to inhibition of GR function has not been presented so far. Our results make competition for GR-specific cofactors seem unlikely, since 'self-squelching' and, thus, self-inhibition could not be demonstrated when hGRα was overexpressed alone. If hGRβ interfered with hGRα in a nonspecific fashion by using general cofactors we would have expected reduction of transcription of a glucocorticoid-independent reporter gene, which did not occur in our study. Strong GRE binding of the overexpressed hGRβ protein in vitro suggests that occupation of GRE target sites by transcriptionally inactive hGRβ molecules may be the predominant mechanism of its inhibitory effect. It remains to be determined to which extent hGRα/hGRβ-heterodimers and/or hGRβ/hGRβ-homodimers participate in the occupation of GRE sequences. Our binding studies indicate that if heterodimer formation does occur, it clearly does not interfere with the capacity of hGRα to bind its hormonal ligand. A pattern of inhibition similar to that exerted by hGRβ was previously described for the thyroid receptor isoform c-erbAα2 (30, 31), which blocked thyroid hormone-induced gene activation by binding to and competing for thyroid hormone response elements in target genes (21).

Interestingly, c-erbAα2 also needed to be overexpressed to cause inhibition (21, 30–32). This was shown to be due to a lower affinity of c-erbAα2 than c-erbAβ and α1 for thyroid response elements in the DNA (32). Whether low affinity binding to hormone response elements also accounts for the high levels of hGRβ required to inhibit hGRα function remains to be shown. Lower binding activity of extracts from hGRβ- as compared to hGRα-transfected cells suggests that this may be the case. Alternatively, hGRβ may not be able to translocate to the cell nucleus as efficiently as hGRα. It could also be that high levels of hGRβ are required to neutralize an abundant hGRα-specific cofactor (if squelching was indeed a contributing mechanism of hGRβ-mediated inhibition). Finally, binding of hGRβ to the heat shock protein complex could silence its inhibitory function. High levels of hGRβ would then be neccessary to maintain a high enough fraction of non-hsp–bound hGRβ molecules. Dissociation from the hsp complex is thought to be the critical step in hormone-induced activation of hGRα (21, 30–32), regardless of whether it is localized in the cytosol as in most cell types (33) or in the nucleus as in this and in previously described cell culture transfection systems (34, 35). It is tempting to speculate that as yet undefined ligands of hGRβ may also be able to free it from the hsp complex, thus allowing it to act at much lower cellular levels than used in this study. However, the existence of a ligand for the β receptor is strictly hypothetical at the present time, since no such molecule has been described as yet.

The fact that hGRβ mRNA is widely expressed throughout the human body indicates that it may play a role in regulating a tissue's response to glucocorticoids both in physiologic and pathophysiologic conditions. Further experiments will have to be performed to allow quantitative analysis of both isoforms in each tissue and to determine whether their expression is subject to hormonal or other regulation, as shown previously for the

different forms of the thyroid receptor (36). This will lead to further understanding of the physiologic role of hGRβ as an intracellular antagonist of glucocorticoid activity. From a pathophysiologic perspective, abnormally high expression levels of hGRβ may participate in the as yet unexplained phenomena of tissue-specific and/or aquired glucocorticoid resistance, such as that observed in rheumatoid arthritis and other autoimmune disorders (13, 37), degenerative osteoarthritis (38), steroid-resistant asthma (39), and the glucocorticoid resistance associated with AIDS (40). In addition, similar hyperexpression might be present in central nervous system disorders, such as addiction and depression, potentially associated with local glucocorticoid resistance of targets within the brain, like the mesocorticolimbic system and the locus coeruleus/norepinephrine system, respectively (1, 13). In contrast, pathologic hGRβ underexpression might be responsible for syndromes of generalized (41) or localized (1, 13) glucocorticoid hypersensitivity, which could result in endocrine, psychiatric, or immunosuppressive states.

In summary, our data indicate that hGRβ may be an important regulator of glucocorticoid sensitivity in multiple glucocorticoid target tissues, including the central nervous system, the limbic-hypothalamic-pituitary-adrenal axis, the immune system, and the endocrine–metabolic system, both in physiologic and pathophysiologic states. In addition, they suggest that it will be important to redefine the functional properties of the hGR at a cellular and molecular level, since previous studies of hGR function did not distinguish between the two receptor isoforms and might have led to erroneous conclusions. In this regard, the as yet unknown interactions of hGRβ with the c-*jun*/c-*fos* heterodimer, calreticulin, the p65 subunit of NF-κB, and with "negative" glucocorticoid response elements will be of special importance.

Acknowledgments

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