Absence of Glucocorticoid Receptor-β in Mice*

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Two human glucocorticoid receptor (GR) isoforms, GRα and GRβ, are derived from the same gene by alternative splicing involving exon 9 of the GR locus. The non-ligand binding isoform GRβ was proposed to act as a transdominant negative inhibitor of GRα, thus modulating glucocorticoid responsiveness of target tissues. To study GRβ in mice we characterized the genomic region around exon 9 of the murine GR gene. Sequence analysis revealed that the presumed exon 9β contained an open reading frame of 59 amino acids. In contrast, human exon 9β encoded only 15 amino acids. Using reverse transcriptase polymerase chain reaction the absence of GRβ mRNA was demonstrated in all adult mouse tissues examined. To exclude the possibility that the polymerase chain reaction conditions employed were not suitable for the amplification of GRβ mRNA, we synthesized an artificial template corresponding to the presumed GRβ mRNA spanning exons 7, 8, and 9β. Various amounts of this template were added to brain cDNA preparations and as little as 25 molecules were detectable under the polymerase chain reaction conditions chosen. Since GRβ is not conserved across species its physiological significance in humans appears questionable.

Glucocorticoids are involved in the regulation of a variety of physiological processes such as development, metabolism, maintenance of homeostasis, and regulation of central nervous system functions (1). Their effects are mediated by the glucocorticoid receptor (GR), a ligand-dependent transcription factor that belongs to the superfamily of nuclear receptors (1–3).

The GR protein is composed of structurally and functionally defined domains. The amino-terminal part of the protein contains a transactivation domain, whereas the central part includes the DNA-binding domain which is crucial for specific interaction of the receptor with DNA sequences containing glucocorticoid receptor responsive elements. The carboxyl terminal minus includes the ligand-binding domain and sequences which are involved in nuclear translocation, receptor dimerization, transactivation, and interaction with heat shock proteins (2–5). Under basal conditions the GR resides in the cytoplasm where it is associated with heat shock proteins and other proteins forming a multiprotein complex (6). Upon hormonal stimulation the GR dissociates from the multiprotein complex and translocates into the nucleus where it can stimulate or suppress transcription of multiple genes (1–3).

Two human glucocorticoid receptor isoforms termed hGRα and hGRβ have been described (7). Both isoforms are derived from the same gene by differential splicing. Whereas hGRα and hGRβ share the first eight exons of the human GR gene, either of the last two exons, i.e. exon 9α or 9β, is spliced into the respective mRNA (8). This leads to the formation of two protein isoforms having the first 727 amino acids in common. Exon 9α encodes 50 amino acids and exon 9β 15 amino acids. Whereas hGRα can bind hormone and is transcriptionally competent, hGRβ is not (8).

Recently, it was demonstrated that hGRβ transiently expressed in COS-7 cells bound specifically to glucocorticoid-responsive elements and acted as a dominant negative inhibitor of hGRα activity (9). Since hGRβ mRNA was expressed in all human tissues examined it was assumed that this isoform might play an important role in modulating tissue sensitivity to glucocorticoids (9). However, the low abundance of hGRβ mRNA is incompatible with such a function. Quantitative RT-PCR experiments revealed that in all human tissues and cell lines analyzed the hGRβ message was expressed 200–500-fold less than the hGRα message (10). In contrast, it was shown by Western blots that the ratio of hGRβ to hGRα varied from 1.0 to 5.0 (11). Moreover, some conflicting results concerning the subcellular localization of the hGRβ protein were obtained. Using hGRβ specific antibodies it was demonstrated that after dexamethasone treatment hGRβ translocated from the cytosol into the nucleus (11). On the other hand, it had been reported that hGRβ resided primarily in the cell nucleus independently of hormonal stimulation (10). To date all these discrepancies have not been conclusively explained.

Since GRβ has been studied so far exclusively in human tissues we were interested to investigate whether this GR isoform would be of physiological importance in other species. Therefore we characterized the genomic region around the presumed exon 9β of the murine GR locus and studied the tissue distribution of GRβ in mice.

EXPERIMENTAL PROCEDURES

Materials

DNA modifying enzymes, Tαq DNA polymerase, deoxynucleotide triphosphates, MgCl₂, and PCR buffer were purchased from Boehringer Mannheim. Reverse transcriptase and first strand buffer were obtained from Life Technologies, Inc., Pfu polymerase from Stratagene, and RNasin from Promega.

Methods

Library Screen and DNA Sequencing—A murine genomic stem cell library (12) was screened with a mouse cDNA probe spanning exons

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The abbreviations used are: GR, glucocorticoid receptor; hGRα, human glucocorticoid receptor-α; hGRβ, human glucocorticoid receptor-β; RT-PCR, reverse transcriptase polymerase chain reaction; bp, base pair(s).
6–9 of the GR gene using high stringency hybridization and washing conditions (13). Three hybridizing genomic phages were purified and characterized by restriction mapping. One of the phages containing exon 9 was subcloned into Bluescript KSII (Stratagene). A 2.8-kilobase HindIII fragment containing the 3′-untranslated region of exon 9α and the putative exon 9β was sequenced on both strands (14).

DNA and RT-PCR Analysis—Total RNA from a variety of adult mouse tissues was isolated after homogenization in guanidinium thiocyanate (15). The quality of the RNA preparations was controlled by phoretic separation in denaturing agarose gels. 2′-Ethidium bromide staining of the 18S and 28S rRNA after electrophoretic separation (16)). The primers used for the amplification of the 194-bp fragment (amplified from the genomic 2.8-kilobase HindIII fragment) containing the putative exon 9β having a 5′-overhang homologous to the 5′ end of exon 8. Primers used for the amplification of the 290-bp fragment were as follows: 5′-AGCAAGAGACTCTCCTAC-3′ (upstream, outer primer 1) and 5′-GGGTTTGACACCATAAACATTATCATGATCGAGTACACAA-3′ (downstream, inner primer 1). Primers used for the amplification of the 194-bp fragment were as follows: 5′-TTGGACTCTCCTAGATGATAATGTTATATGGTTA-3′ (upstream, inner primer 2) and 5′-AGCTTTTATACATGATCTTACTCATGATTATTACAAA-3′ (downstream, outer primer 2). Both PCR fragments were gel purified and fused in a second PCR experiment using the two outer primers. PCR conditions were the same as described above. The 484-bp fusion product spanning exons 7, 8, and 9β was purified by gel electrophoresis, subcloned into Bluescript KSII, and sequenced on both strands (14).

RESULTS

To investigate GRβ in mice we isolated and characterized a genomic phage containing exons 8 and 9 of the murine GR locus. A 2.8-kb genomic HindIII fragment including exons 9β was sequenced on both strands to locate exons 9α and 9β. The nucleotide sequence of the presumed exon 9β and its preceding intron is shown in Fig. 1A. This sequence shows strong similarity to the sequence of human exon 9β. However, as depicted in Fig. 1B the murine and rat sequences (19) homologous to human exon 9β are not preceded by a splice site conforming to the GT/AG rule (18). The putative murine exon 9β would contain an open reading frame of 59 amino acids (Fig. 1A) since the stop codon of human exon 9β is not conserved in mice and rats (Fig. 1B). The first 15 amino acids of the presumed murine exon 9β are highly conserved, the remaining contain a remarkable stretch of basic amino acids (Fig. 1A). These sequence data make the existence of GRβ in mice unlikely.

To obtain evidence for or against the existence of GRβ in mice we performed RT-PCR analysis of GRα and GRβ mRNA in...
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In vivo experiments revealed that GRβ was only a weak transdominant negative inhibitor of GRα since 5-fold overexpression was required to get a 50% reduction of GRα activity (9). Moreover, there was a large discrepancy observed concerning the mRNA and protein levels of GRβ in humans. While the GRβ mRNA was 200–500-fold less expressed than the GRα mRNA (10) the ratio of GRβ to GRα protein varied between 1 and 5 (11). These discrepancies which have never been explained conclusively, as well as conflicting results about the subcellular localization of GRβ protein (10, 11), raised some doubts concerning the existence and hence the physiological importance of this GR isoform in humans. We therefore decided to study as an additional approach GRβ in mice and to examine whether it was conserved across species. Using quantitative RT-PCR experiments we could not find any evidence for the existence of GRβ mRNA in all adult mouse tissues examined, among them liver, brain, pituitary, and kidney which have been reported to contain considerable amounts of GRβ protein in humans (11). We conclude from these experiments that GRβ mRNA structurally comparable to that in humans (10) is not synthesized in mice. Whereas GRβ mRNA was ubiquitously expressed in humans and was amplified under comparable PCR conditions after 45 cycles (10) we failed to demonstrate GRβ in mice even after 2 × 35 cycles of PCR. To control the suitability of our PCR conditions we synthesized an artificial GRβ template structurally corresponding to the human GRβ mRNA. The amplification product of this template was easily detectable even if very low amounts of template were added to the cDNA preparation. In addition, the obvious absence of GRβ in mice is supported by two other findings. First, splicing of the putative murine exon 9β would give rise to a protein distinctly different from human GRβ. Apart from the first 15 amino acid residues of the presumptive exon 9β which indeed show high homology to the 15 amino acids encoded by human exon 9β, the additional 44 amino acids of the open reading frame have no counterpart in human GRβ. They contain a remarkable stretch of basic residues. Therefore one can conclude that, if GRβ would exist in mice it might have properties distinctly different from its human variant. In rat, a putative exon 9β would encode 48 amino acids and only the first 7 amino acids would show high homology to human exon 9β.

Second and even more important, the splice site in front of the putative exon 9β is not in accordance with the consensus splice sequences at all (18). In sharp contrast, however, all other splice junctions in the murine GR gene (20) and the intron preceeding human exon 9β (8) conformed to the GT/AG rule (18). Therefore it seems reasonable to assume that exon 9β is not spliced and belongs to the 3’-untranslated region of the murine GR gene.

From these results we conclude that GRβ is not conserved across species. It is tempting to speculate that during evolution a point mutation in the splice acceptor site is responsible for the absence of GRβ mRNA in rodents. The obvious lack of GRβ protein in mice argues against an important role of this isoform in humans. This assumption is supported by recent findings (21) demonstrating that the expression level of GRβ protein in humans is much lower than previously reported (11) and providing strong evidence against a transdominant negative activity of GRβ.

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