Heterogeneity in the polyglutamine tract of the glucocorticoid receptor from different rat strains

K.L.Gearing, J.-Å.Gustafsson and S.Okret*

Department of Medical Nutrition, Karolinska Institute, Novum, S-141 86 Huddinge, Sweden

Received January 14, 1993; Revised and Accepted March 15, 1993

EMBL accession nos X69666 – X69670 (incl.)

The glucocorticoid receptor (GR) is a well characterised sequence specific DNA binding protein. Functional mapping of rat GR has shown that it consists of a C-terminal ligand binding domain, a central DNA binding domain and an N-terminal transactivation domain (for a review, see 1).

Comparison of the amino acid sequence of the N-terminal domains of the human, mouse and rat GR reveals a polyglutamine tract, whose function is unknown, which varies in length between these species (see Figure). The rat GR cDNA cloned from both a hepatoma cell line, J.2.17.2 (2) and from prostate (3) was shown to contain a stretch of 21 glutamines whereas the mouse and human receptors contain 9 and 2 glutamines respectively (4, 5). We have isolated a genomic clone from a λ library of Sprague–Dawley rat genomic DNA (Clontech) which contains the first coding exon of GR. Sequence analysis of this clone showed that the polyglutamine tract was only 7 amino acids long.

human GR	KGSVSNAQQ-----PDLSKAVS
mouse GR	KGSA S NAQQQQQQQ-----PQPDLSKAVS
rat GR J2.17.2 cDNA	KGSTSNVQQR Q QQQQQQQQQQQQQQQQQPGLSKAVS
rat GR J2.17.2 PCR product	KGSTSNVQQR Q QQQQQQQQQQQQQQQQQPDLSKAVS
rat GR prostate cDNA	KGSTSNVQQR Q QQQQQQQQQQQQQQQQQPDLSKAVS
rat GR Sprague Dawley genomic DNA λ clone	KGSTSNVQQR Q QQQQ-----PDLSKAVS
rat GR Sprague Dawley PCR product a)	KGSTSNVQQR Q QQQQ-----PDLSKAVS
PCR product b)	KGSTSNVQQR Q QQQQQQQQQQQQQQ-----PDLSKAVS
rat GR Wistar PCR product	KGSTSNVQQR Q QQQQ-----PDLSKAVS

Figure 1. Comparison of the amino acid sequences of the polyglutamine containing region of the glucocorticoid receptor. PCR reactions were carried out using 2 μ g genomic DNA in 10 mM Tris, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 0.2 mM dNTP, 2.5 mM MgCl₂ using the GR specific primers 5' AGGATTCTCCTTGATTCTCGAAA and 5' CATATACAGCCCCATGGACAGTGA. The DNAs were denatured at 94°C for 5 min. before addition of 2.5 units Taq DNA polymerase and PCR for 40 cycles of 94°C (1 min), 55°C (1 min) 72°C (2 min). The PCR products were purified from GTG agarose (FMC BioProducts) and ligated with M13. Recombinant clones (at least 6 per PCR reaction) were sequenced. The predicted amino acid sequences are shown above. The first and last amino acid positions shown are derived from the PCR primers and the first amino acid corresponds to position 68, 68 and 67 in the mouse, rat and human receptors respectively. The amino acids which differ between clones are shown in bold type.

PCR analysis of genomic DNA prepared from a Sprague–Dawley rat (Clontech), from the liver of a Wistar rat and from the rat hepatoma cell line J.2.17.2 confirmed the variation in length of this region between different rat strains (see Figure). The DNA sequence of the PCR product from J.2.17 genomic DNA predicts a polyglutamine tract of 21 amino acids whereas the PCR product from the Wistar rat genomic DNA predicts a polyglutamine stretch of 7 amino acids. Sprague–Dawley rat genomic DNA gave 2 PCR products which encode either 7 or 17 glutamines at this position. A similar degree of heterogeneity was also seen in several cell lines tested (data not shown).

Many transcription factors have been found to contain glutamine rich regions in their activation domains (6) although the function of this region in GR has not yet been determined. Other structurally related DNA binding proteins from the nuclear receptor superfamily also contain polyglutamine tracts in the same region and in the case of the androgen receptor (AR) this has been shown to vary in length (7). Enlargement of the polyglutamine tract in AR has been associated with X-linked spinal and bulbar muscular atrophy in humans (8), indicating that the polyglutamine region is important in receptor function. No gross differences in transactivation function have been reported between the human and rat GR clones which contain 2 and 21 glutamines respectively and it remains to be seen whether differences in the polyglutamine tract can affect the function or stability of the GR *in vivo*.

ACKNOWLEDGEMENTS

We would like to thank M.McPhaul for discussion and K.Yamamoto for providing the cell line J.2.17.2. This work was supported by a Fellowship from the Karolinska Institute (to K.G.) and by grants from the Swedish Cancer Society (to K.G. and S.O.) and the Swedish Medical Research Council (no. 13X-2819).

REFERENCES

- Evans, R.M. (1988) *Science* **240**, 889–895.
- Miesfield, R. *et al.* (1986) *Cell* **46**, 389–399.
- Chang, C. *et al.* (1987) *Nucleic Acids Res.* **15**, 9603.
- Danielsen, M. *et al.* (1986) *EMBO J.* **5**, 2513–2522.
- Hollenberg, S.M. *et al.* (1985) *Nature* **318**, 635–641.
- Mitchel, P.J. and Tjian, R. (1989) *Science* **245**, 371–378.
- Tilley, W.D. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 327–331.
- La Spada, A.R. *et al.* (1991) *Nature* **352**, 77–79.

* To whom correspondence should be addressed