Pergamon

0960-0760(95)00162-X

J. Steroid Biochem. Molec. Biol. Vol. 55, No. 2, pp. 159–162, 1995

0960-0760/95 $9.50 + 0.00

Identification of a Splice Variant of the Rat and Human Mineralocorticoid Receptor Genes

Laura J. Bloem,* Chunlu Guo and J. Howard Pratt

Department of Medicine, Indiana University School of Medicine and the V.A. Hospital, Indianapolis, IN 46202-5111, U.S.A.

The sequence of a splice variant of the rat mineralocorticoid receptor (MR) gene is presented. A cDNA clone corresponding to rat MR was isolated from a rat brain cDNA library. Sequence analysis of the region corresponding to the DNA binding domain revealed the presence of a 12 base pair (bp) insertion. Analysis of mRNA from several rat tissues suggests that the variant is less abundant than the wild type in most tissues. The insertion variant is also a product of the human MR gene, the identical splice variant was also observed in human white blood cell mRNA. Unlike other splice variants reported for the MR, this variant alters the encoded protein by the addition of four amino acid residues in the DNA binding domain. The altered protein may influence the affinity of the MR for mineralocorticoid or glucocorticoid response elements.

J. Steroid Biochem. Molec. Biol., Vol. 55, No. 2, pp. 159–162, 1995

INTRODUCTION

Mineralocorticoids such as aldosterone increase ion transport in specialized epithelial cells resulting in sodium conservation and potassium excretion [1]. Aldosterone initiates this response by its interaction with the intracellular mineralocorticoid receptor (MR) that regulates the transcription of a specific set of genes [2]. The cDNA for the human kidney MR has been identified by low stringency hybridization to the closely related glucocorticoid receptor (GR) [3]. The DNA binding domain of the MR is nearly identical to that of the GR and both seem to activate similar sequences in nuclear DNA. The ligand binding domains of both receptors also show high sequence homology with each other and the cloned MR has equal affinity for cortisol, which circulates at much higher concentrations than aldosterone [3]. However, in peripheral target tissues the enzyme 11β-hydroxysteroid dehydrogenase converts cortisol to the MR-inactive cortisone and thus protects the MR from activation by glucocorticoids [4–7].

The rat hippocampal brain MR cDNA has also been cloned and shows high homology to the human kidney MR in the coding and 3′ untranslated region, although it diverges considerably in the 5′ untranslated region [8]. It was subsequently shown that there are three MR mRNA subtypes which are identical in the coding regions but differ in the 5′ untranslated regions. One of the subtypes is identical to the human kidney homolog [9]. The MR gene is present as a single copy gene in both rats and humans and analysis of the rat MR gene revealed that all three subtypes result from alternative usage of exons in the 5′ untranslated regions [10]. Because they are in the untranslated regions, none of these alternative splicing variants change the protein sequence of the receptors. We describe here a splice variant found both in rat and human MR mRNA. Unlike previous alternative splice variants described, this new variant exists within a coding region and results in the insertion of four amino acid residues in the DNA binding domain of the encoded protein.

MATERIALS AND METHODS

Cloning and characterization of the MR splice variant

Rat brain RNA was isolated by the guanidinium–HCl precipitation method [11] and then reverse transcribed in a 20 μl reaction using random hexamers and M-MLV reverse transcriptase according to the manufacturers instruction (Gibco-BRL, Gaithersburg, MD, U.S.A.). The cDNA corresponding to rat MR DNA binding domain was then amplified by the polymerase chain reaction (PCR) using the primers 5′-TCCTCAAGACCTTCCAAGATCTGT-3′ and

*Correspondence to L. J. Bloem.
Received 28 Apr. 1995; accepted 29 Jun. 1995.

5′CAGCTTCTTTGACTTTCGAGCTCC-3′. The PCR product was radiolabeled with ^{32}P-dATP using DECAprime II Labeling Kit (Ambion, Austin, TX, U.S.A.) and subsequently used to screen a rat brain cDNA library in the Lambda ZAP vector (Staratagene Cloning Systems, La Jolla, CA, U.S.A.) using standard techniques [12]. A single phage was identified and the plasmid rescued. Alkali-denatured DNA was sequenced using the oligonucleotide primers described above and Sequenase Version 2.0 DNA sequencing kit (US Biochemicals, U.S.A.).

Isolation, reverse transcription, and amplification of RNA

Total RNA was isolated from the indicated rat tissues and reverse transcribed using random hexamers and M-MLV reverse transcriptase (Gibco-BRL, Gaithersburg, MD, U.S.A.). The rat cDNA was amplified using the synthetic oligonucleotide primers indicated above and the human cDNA was amplified using the primers 5′CTGGATCTTCAAGACCTTCAA-3′ and 5′-TCCTAAATTCATTCCAGCTTG-3′ in a standard PCR cocktail containing 5.0 μCi ^{32}P-dATP (3000 Ci/mmol). The radiolabeled products were spearated on a 6% denaturing polyacrylamide gel along with a sequence ladder and then visualized by autoradiography.

RESULTS

Isolation of a splice variant of the rat MR mRNA

A probe corresponding to the DNA binding domain of the rat MR was generated by reverse transciption of rat brain mRNA followed by amplification of the cDNA using the PCR. The PCR-generated probe was used to screen a rat brain cDNA library, resulting in the isolation of a single clone (MR260) with a restriction map similar to that of the published rat MR sequence [8]. However, sequencing of the region corresponding to the DNA binding domain revealed that the isolated clone contained a 12 bp insertion between codons for aa residues 634 and 635 of the published sequence (Fig. 1). This insertion results in the translation of an additional four aa residues in the DNA binding domain of the protein encoded by MR260 (Fig. 1).

Distribution of the splice variant in rat tissues

To determine the prevalence and distribution of this variant, RNA was isolated from the rat tissues indicated in Fig. 2. The mRNA was reverse transcribed and then amplified by PCR in the presence of a radiolabeled deoxynucleotide triphosphate. The products were separated by denaturing polyacrylamide gel electrophoresis and visulaized by autoradiography (Fig. 2). Both the shorter form of the MR message which gives the lower 248 bp band and the insertion variant which results in a 260 bp product were present in all the tissues analyzed. The MR260 was less abundant in all tissues with the exception of liver, where the two products seem to be present at similar abundance.

Identification of an identical splice variant of the human MR mRNA

Human white blood cell mRNA was analyzed to determine if this variant is also a product of the human

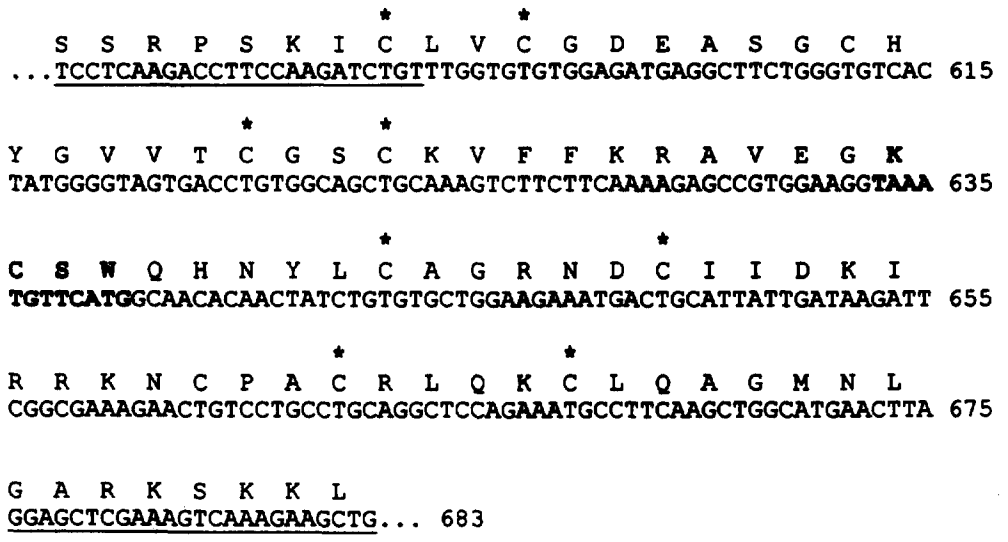

Fig. 1. Partial nt and deduced aa sequence of MR260. The sequence encoding the DNA binding domain of MR260 is shown along with the deduced aa sequence. Regions which vary from the published sequence, including the 12 bp insertion as well as the deduced aa encoded by the insertion are indicated in bold print. The numbers before the insertion correspond to aa residue of the published sequence. After the insertion, the numbers are shifted by 4 relative to the published sequence due to the insertion. The underlined regions indicate binding sites for sequence specific oligonucleotide primers and the starred residues are the cystienes thought to be involved in Zn^{2+} coordination for zinc finger formation.

MR gene. The mRNA was reverse transcribed and amplified by PCR using the primers indicated in Fig. 3. Again, two products were identified, one with a size of 234 bp as predicted from the published human MR cDNA [3] and the 12 bp longer 246 bp product. The larger fragment was extracted from the gel, reamplified by PCR, subcloned, and the nucleotide sequence determined. The sequence was identical to the published cDNA with the exception of the insertion of the same 12 bases as identified in the rat MR260. The insertion was due to the use of an alternative splice site between exon 3 and intron C of the MR gene having the sequence .....GTGGAAG ∧ gtaaatgttcat ∧ gtggg..... The two splice sites are indicated by the arrows and demonstrate that the consensus acceptor site is conserved for both splice variants (Dr Maria-Christina Zennaro, personal communication). Use of the second splice site results in the in-frame insertion described here. The ratio of the two products in this tissue appears to be of similar abundance, with the insertion variant having a slightly higher abundance. However, a systematic quantitation of the two forms has not been attempted, and the PCR conditions used here may not adequately reflect the abundance ratio at the mRNA level.

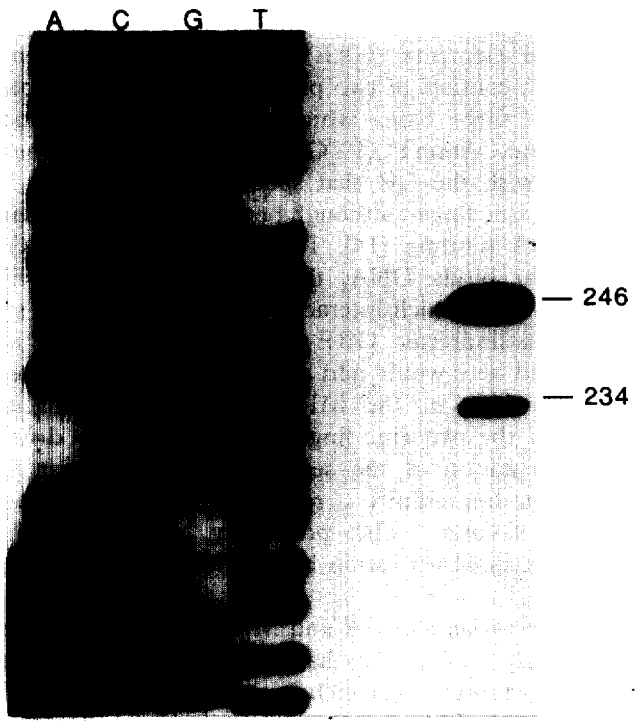

Fig. 3. Amplification of human MR mRNA. Whole blood was collected in EDTA containing vacutainers. Samples were centrifuged, nucleated cells collected and RNA was extracted [11]. Total RNA was reverse transcribed and then amplified by PCR using the primers flanking the human MR DNA binding domain in a standard mixture along with 5 μCi ^{32}P-dATP (3000 Ci/mmol). The radiolabeled products along with a sequence ladder were separated on a 6% denaturing polyacrylamide gel and visualized by autoradiography. The size of the products are indicated, 234 bp for the product corresponding to the published sequence and 246 bp for the splice variant.

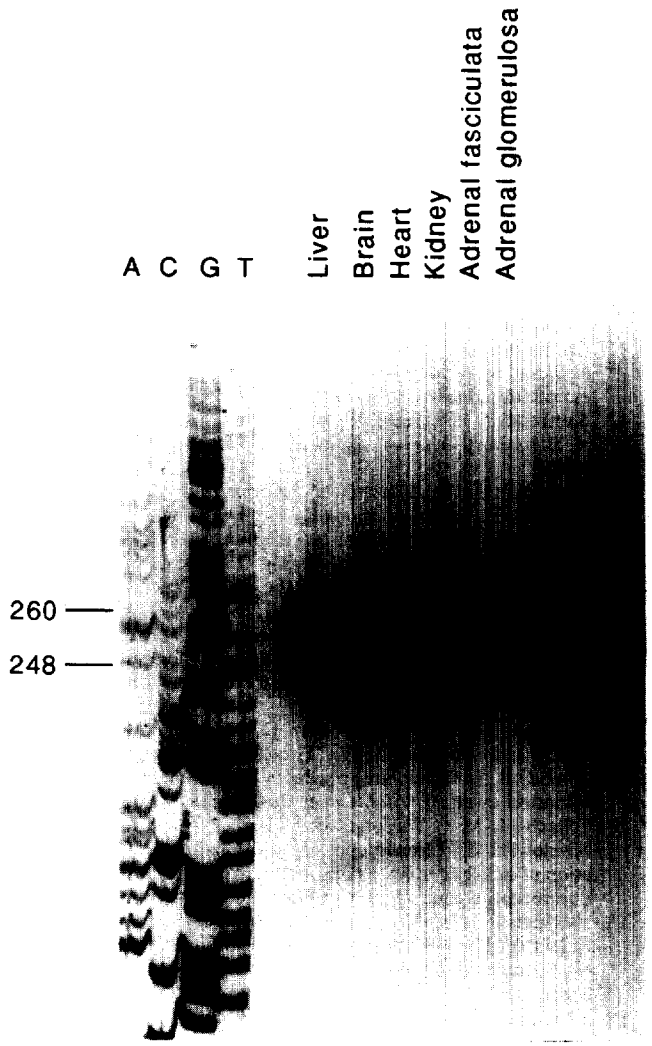

Fig. 2. Amplification of MR248 and MR260 in rat tissues. RNA isolated from the rat tissues indicated was reverse transcribed and then PCR amplified in the presence of ^{32}P-dCTP using the primers flanking the DNA binding domain. The radiolabeled products along with a sequence ladder were separated on a 6% denaturing polyacrylamide gel and visualized by autoradiography. The size of the products are indicated, 234 bp for the product corresponding to the published sequence and 246 bp for the splice variant. The size of the products in bp is indicated to the left of the figure.

DISCUSSION

The target DNA specificity of nuclear hormone receptors has been shown to be entirely encoded in the DNA binding domain, a region that displays considerable homology between members of the steriod hormone receptor super-family. Several residues within the DNA binding domain are highly conserved throughout the steriod receptor family, including two sets of four cysteine residues that in the case of the GR, have been shown to be tetrahedrally coordinated to Zn^{2+} ions to form two "zinc fingers" [13, 14]. MR

has been shown to bind efficiently to glucocorticoid response elements (GRE), the consensus sequence of which consists of two palindromic half sites separated by three bases which can be any nucleotide (5′-AGAACAnnnTGTTCT-3′) [14]. It has been shown with the GR that specificity is conferred by the first zinc finger, although both fingers are necessary for DNA binding [14]. In addition, when the GR monomer binds DNA, the domain dimerizes with a second GR which interacts with the second half site of the palindromic GRE [15]. The 12 bp insertion identified here would add 4 aa residues between these two domains in the MR. Increasing the spacing between the two zinc fingers may sufficiently distort the orientation of the second finger such that the DNA binding affinity is reduced and/or dimerization is not favored. The possibility also exists that transcriptional activation by the variant MR may be altered.

Splice variants of GR and MR have been described previously [9, 10]. This has been shown to be due to multiple promoters which control both the rat MR gene and the mouse GR gene resulting in alternative splicing of three 5′ untranslated exons to one common second exon which encodes the translation initiation site [9, 10]. The retinoic acid receptor and progesterone receptor genes, both members of the steroid receptor superfamily, also demonstrate alternative usage of promoter and 5′ untranslated exons [16–20]. In contrast to the GR and MR genes, specific receptor isoforms are encoded by each splice variant through alternative use of initiation codons. The different isoforms of the retinoic and progesterone receptors are thought to be functionally distinct and each may play a specific role during different developmental stages. Whether or not the splice variant described here for MR is associated with a specific 5′ untranslated sequence is not clear. It also remains to be determined if this product is developmentally regulated and perhaps is the more predominant transcript at specific stages of development.

The presence of the additional four aa residues in the DNA binding domain of the MR could appreciably alter the binding for a GRE. It remains to be determined if the K_D for the GRE is the same for the two forms and if they activate transcription in an equivalent manner. In addition, the affinity of the two forms for a putative mineralocorticoid response element (MRE) may not be the same, although to date, no specific MRE has been identified.

Acknowledgements—Supported by grants from the Veterans Administration, the American Heart Association, Indiana Affiliate (IN-94-013-GIA), The National Institutes of Health (RO1-HL-35795), and the Indiana University Biomedical Research Support Program. The authors are grateful to MaryAnne Wagner for technical assistance.

REFERENCES

1. Marver D.: Aldosterone. *Meth. Enzymol.* 191 (1990) 520–550.
2. Rossier B. C., Verrey F. and Kraehenbuhl J.: Transepithelial sodium transport and its control by aldosterone: a molecular approach. *Curr. Topics Membr. Transp* 34 (1989) 167–183.
3. Arriza J. L., Weinberger C., Cerelli G., Glaser T. M., Handelin B. L., Housman D. E. and Evans R. M.: Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 237 (1987) 268–275.
4. Funder J. W., Pearce P. T., Smith R. and Smith A. I.: Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 242 (1988) 583–585.
5. Naray-Fejes-Toth N., Watlington C. O. and Fejes-Toth G.: 11β-hydroxysteroid dehydrogenase activity in the renal target cells of aldosterone. *Endocrinology* 129 (1991) 17–21.
6. Ulick S., Wang J. Z., Blumenfeld J. D. and Pickering T. G.: Cortisol inactivation overload: a mechanism of mineralocorticoid hypertension in the ectopic adrenocorticotropin syndrome. *J. Clin. Endocr. Metab.* 74 (1992) 963–967.
7. Williams G. H.: Editorial: guardian of the gate: receptors, enzymes, and mineralocorticoid function. *J. Clin. Endocr. Metab.* 74 (1992) 961–962.
8. Patel P. D., Sherman T. G., Goldman D. J. and Watson S. J.: Molecular cloning of a mineralocorticoid (Type I) receptor complementary DNA from rat hippocampus. *Molec. Endocr.* 3 1877–1885.
9. Kwak S. P., Patel P. D., Thompson R. C., Akil H. and Watson S. J.: 5′-Heterogeneity of the mineralocorticoid receptor messenger ribonucleic acid: Differential expression and regulation of splice variants within the rat hippocampus. *Endocrinology* 133 (1993) 2344–2350.
10. Castren M. and Damm K.: A functional promoter directing expression of a novel type of rat mineralocorticoid receptor mRNA in brain. *J. Neuroendocr.* 5 (1993) 461–466.
11. Chomczynski P. and Sacchi N.: Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analyt. Biochem.* 162 (1987) 156–159.
12. Sambrook J., Fritsch E. F. and Maniatis T.: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989).
13. Freedman L. P., Luisi B. F., Korszun Z. R., Bazavappa R., Sigler P. B. and Yamamoto K. R.: The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. *Nature* 334 (1988) 543–546.
14. Freedman L. P.: Anatomy of the steroid receptor zinc finger region. *Endocrine Rev.* 13 (1992) 129–145.
15. Luisi B. F., Xu W. X., Otwinowski Z., Freedman L. P., Yamamoto K. R. and Sigler P. B.: Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* 352 (1991) 497–505.
16. Conneely O. M., Kettelberger D. M., Tsai M.-J., Schrader W. T. and O'Malley B. W.: The chicken progesterone receptor A and B isoforms are products of an alternate translation initiation event. *J. Biol. Chem.* 264 (1989) 14,062–14,064.
17. Jeltsch J.-M., Turcotte B., Garnier J.-M., Lerouge T., Krozowski Z., Gronemeyer H. and Chambon P.: Characterization of multiple mRNAs originating from the chicken progesterone receptor gene. *J. Biol. Chem.* 265 (1994) 3967–3974.
18. Kastner P. H., Krust A., Mendelsohn C., Garnier J. M., Zelent A., Leroy P., Staub A. and Chambon P.: Murine isoforms of retinoic acid receptor gamma with specific patterns of expression. *Proc. Natn. Acad. Sci. U.S.A.* 87 (1990) 2700–2704.
19. Zelent A., Mendelsohn C., Kastner P., Krust A., Garnier J.-M., Ruffenach F., Leroy P. and Chambon P.: Differentially expressed isoforms of the mouse retinoic acid receptor b are generated by usage of two promoters and alternative splicing. *EMBO J.* 10 (1991) 71–81.
20. Leroy P., Krust A., Zelent A., Mendelsohn C., Garnier J.-M., Kastner P., Dierich A. and Chambon P.: Multiple isoforms of the mouse retinoic acid receptor a are generated by alternative splicing and differential induction by retinoic acid. *EMBO J.* 10 (1991) 59–69.