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Analysis of the Human Gene Encoding the Kidney Isozyme of 11β-Hydroxysteroid Dehydrogenase

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11β-Hydroxysteroid dehydrogenase (11β-HSD) catalyzes the conversion of cortisol to cortisone. This activity may be deficient in the syndrome of apparent mineralocorticoid excess (AME). 11β-HSD L (Type I), isolated from liver, is widely expressed and utilizes $NADP^+$ as a cofactor. The gene for 11β-HSD L was found to be normal in patients of AME. A second isoform, 11β-HSD K (Type II), isolated from kidney, is more tissue specific in expression and utilizes NAD^+ as a cofactor. The cDNA clone encoding 11β-HSD K was isolated from sheep kidney. The cDNA is 1.8 kb in length and encodes a protein of 404 amino acid residues with a predicted M_r 43,953. The recombinant enzyme functions as an NAD^+-dependent 11β-dehydrogenase with very high affinity for steroids, but it has no detectable reductase activity. It is 37% identical in amino acid sequence to an NAD^+-dependent isozyme of 17β-hydroxysteroid dehydrogenase. It is expressed at high levels in the kidney, placenta, adrenal and at lower levels in colon, stomach, heart and skin. The human 11β-HSD K gene consists of five exons spread over 6 kb. The nucleotide binding domain lies in the first and the second exon, and the catalytic domain in the fourth exon. The promoter for 11β-HSD K gene lacks a TATA box and has a high GC base content, suggesting that the gene may be transcriptionally regulated by factors that recognize GC-rich sequences. Fluorescent *in situ* hybridization of metaphase chromosomes with a positive bacteriophage P1 genomic 11β-HSD K clone localized the gene to chromosome 16q22. In contrast, the 11β-HSD L gene is located on chromosome 1 and contains 6 exons; the coding sequences of these genes are only 21% identical. Different transcriptional start sites are utilized in kidney and placenta.

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INTRODUCTION

A deficiency of 11β-hydroxysteroid dehydrogenase (11β-HSD) has been implicated in the syndrome of apparent mineralocorticoid excess (AME) [1]. Patients with AME present as children or occasionally young adults with hypertension, hypokalemia, low plasma renin activity, and extended half-life of plasma cortisol.

In vitro, the mineralocorticoid receptor has the same affinities for the glucocorticoids, cortisol and corticosterone, as it does for the mineralocorticoid, aldosterone [2]. However, cortisol and corticosterone are normally very weak mineralocorticoids *in vivo*. It has been hypothesized that the normal specificity of the mineralocorticoid receptor results from the action of 11β-HSD which converts cortisol and corticosterone to cortisone and 11-dehydrocorticosterone, respectively [3, 4]. These latter steroids are not ligands for the receptor. Because aldosterone is not a substrate for the enzyme, it is able to occupy the receptor even though it normally circulates at a level 100–1000 times lower than the levels of cortisol or corticosterone.

Two isoforms of 11β-HSD are described, depending on the co-factor specificity. 11β-HSD L (Type I) was first purified from rat liver microsomes [5]. This isozyme utilizes $NADP^+$ as the cofactor. The enzyme is expressed in several tissues with highest levels of expression in liver [6]. When expressed in mammalian cells as a recombinant protein, this isozyme catalyzes both dehydrogenase and reductase reactions [7] with

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K_m values for steroids of about 1 μM. The second isoform, 11β-HSD K (Type II) is expressed at highest levels in kidney and placenta, utilizes NAD^+ as a cofactor, and has only dehydrogenase activity with an apparent K_m value for steroids of <100 nM [8, 9] (described later).

11β-HSD L (TYPE I) GENE IS UNAFFECTED IN AME PATIENTS

As a step in the delineation of the molecular basis of AME, a human 11β-HSD L gene was isolated [10]. The single gene consists of 6 exons with a total length of over 9 kb. The gene is located on chromosome 1. Results of a Southern blot hybridization suggested that there were no gross deletions or rearrangements in the 11β-HSD L gene of AME patients. Furthermore, no mutations were found in the exons, proximal 5′ or 3′ flanking regions of 11β-HSD L gene in any of the patients. The intron–exon junctions also appeared intact [11]. These negative results, obtained in patients representing a diversity of ethnic groups, suggested a deficiency of an alternate 11β-HSD enzyme present in mineralocorticoid target organs.

EVIDENCE FOR ANOTHER ISOZYME OF 11β-HSD

Several recent biochemical studies have demonstrated the existence of an additional 11β-HSD isozyme (or possibly several related isozymes) in rabbit kidney cortical collecting duct cells [9], sheep kidney [12], human placenta [8] and human fetal tissues [13]. This isozyme utilizes NAD^+ instead of $NADP^+$ as a cofactor, catalyzes dehydrogenase but not reductase reactions, and has apparent K_m values for steroids of <100 nM.

CLONING OF CDNA ENCODING NAD^+ DEPENDENT 11β-HSD

A sheep cDNA encoding the 11β-HSD K (Type II) activity was isolated (14). Sequence analysis revealed that the cDNA insert of this clone is 1840 bp long, not including the poly(A) tail. It consists of a 5′-untranslated region of 94 bp, an open reading frame of 1212 bp, and a 3′-untranslated region of 534 bp. The 5′ end of the cDNA is notably GC-rich (77% over the first 300 bp) with a high proportion of CpG dinucleotides. This is reflected in the presence of restriction endonuclease recognition sites containing CpG that are rarely seen in cDNAs, including one EagI and two BssHII sites. Such clusters of sites ("CpG islands") are often seen at the 5′ ends of genes and are associated with transcriptional regulatory sequences [15]. Their functional significance in this gene has not yet been defined.

Predicted structural features of the enzyme

The ATG at the beginning of the open reading frame is in good context for initiation of translation with G nucleotides at the -3 and $+4$ positions [16]. The protein initiating at this ATG is predicted to contain 404 amino acid residues with a total M_r of 43,953 (these figures differ from those previously reported in Ref. [14] due to correction of two frameshift errors near the 3′ end of the coding sequence). Two potential sites for N-linked glycosylation occur at residues 96–98 and 245–247; it is not known if this enzyme is actually glycosylated.

A search of sequence data bases revealed sequence similarity to members of the short chain alcohol dehydrogenase super-family (Fig. 1). The 11β-HSD K isozyme was most similar (37% sequence identity) to the Type II (placental, NAD^+-dependent, microsomal) isozyme of 17β-hydroxysteroid dehydrogenase (17β-HSD) [17]. It was only 20–26% identical (depending on whether a two-way or a multiple alignment was used) to the L isozyme of 11β-HSD. The relatively high degree of similarity between the 11β-HSD K isozyme and placental 17β-HSD (comparable to the similarity between cytochrome *P*450 gene family members) suggests that these two enzymes may be in the same gene family within the short chain dehydrogenase superfamily.

Short chain dehydrogenases share two strongly conserved regions. The functions of these regions have been elucidated by X-ray crystallographic analysis of 3α,20β-hydroxysteroid dehydrogenase from *S. hydrogenans* [18] and by site-directed mutagenesis of a number of enzymes, including the L isozyme of 11β-HSD [19, 20]. The first region, which is located near the amino terminus (residues 85–95 in the 11β-HSD K isozyme), constitutes part of the binding site for the nucleotide cofactor. The other region is always located about 140 residues further toward the carboxyl terminus (residues 232–236 in the K isozyme). It contains absolutely conserved tyrosine and lysine residues (Tyr-232 and Lys-236 in this enzyme) that function in catalysis. The region immediately to the NH_2-terminal side of the catalytic residues forms part of a putative steroid binding pocket in 3α,20β-HSD. This region is notably well conserved (10/18 identical residues) between the two isozymes of 11β-HSD, consistent with a role in binding the substrate.

A hydropathicity plot shows three successive hydrophobic segments of approx. 20 amino acids each in the NH_2-terminal region prior to the cofactor binding domain. The three segments resemble each other in amino acid sequence, and each is bounded by helix-breaking residues (glycine or proline). These could function as trans-membrane segments anchoring the K isozyme to the membrane of the endoplasmic reticulum, although it is also possible that the entire NH_2-terminal region functions as a signal peptide that

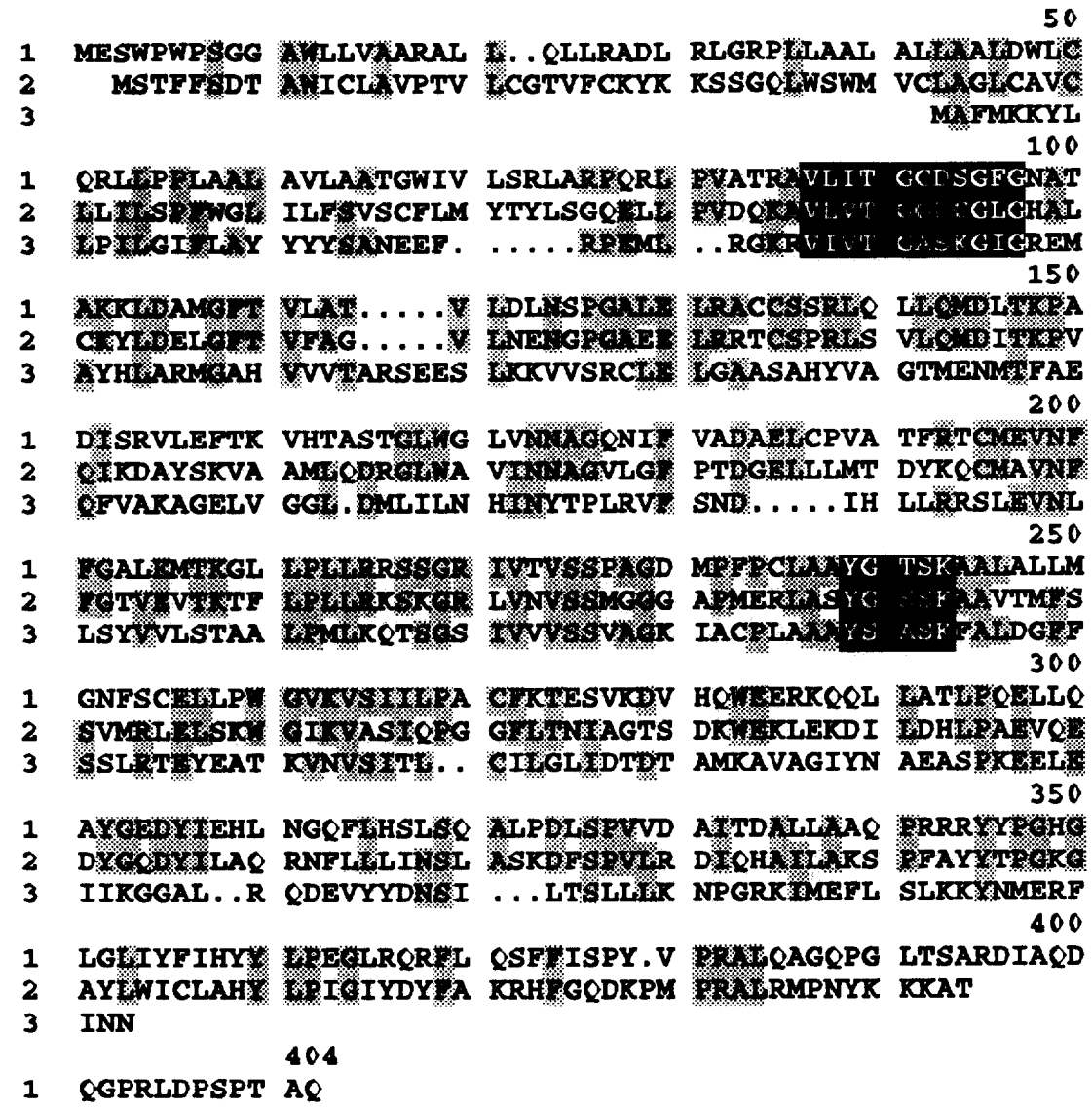

Fig. 1. Alignment of the predicted amino acid sequence of the K isozyme of 11β-HSD [1] with the human placental (Type 2) isozyme of 17β-HSD [2] and the sheep L isozyme of 11β-HSD [3]. Identical residues are shaded, and the highly conserved cofactor binding region and catalytic site are denoted by dark boxes with white lettering. The numbers above each line are alignment positions and do not correspond exactly to the numbering of residues in 11β-HSD K. A space is printed every 10 positions in the alignment; actual gaps introduced to optimize the alignments are marked by dots.

is cleaved when the newly synthesized enzyme is inserted into the endoplasmic reticulum.

Enzymatic analysis

When expressed in Xenopus oocytes, the K isozyme functioned exclusively as a dehydrogenase; no reductase activity was detectable with either NADH or NADPH as a cofactor. When cortisol was the substrate, the enzyme used NAD^+ exclusively as the cofactor (43.4% conversion to cortisone after 2 h, mean of two determinations). However, the enzyme could use $NADP^+$ to dehydrogenate corticosterone at a rate one-fourth as great (9.7 vs 42.6% conversion after 2 h) as that observed when NAD^+ was used as a cofactor. Qualitatively similar results were obtained when the cDNA was transfected into TK-143 human osteosarcoma cells that had been transfected with recombinant vaccinia virus containing the T7 RNA polymerase gene (data not shown).

Apparent K_m and V_{max} values were determined in two independent experiments, each with duplicate determinations at each of five concentrations for each substrate. Apparent K_m values were 14–16 nM for cortisol and 0.7 nM for corticosterone. Apparent V_{max} values were 1.2–2.6 and 1.1–1.2 nmol/h/g protein for cortisol and corticosterone, respectively. First order rate constants V_{max}/K_m were 0.08–0.19 for cortisol and 1.6–1.7 for corticosterone even in an animal, the sheep, in which cortisol is the main glucocorticoid. These data are qualitatively similar to those obtained in sheep

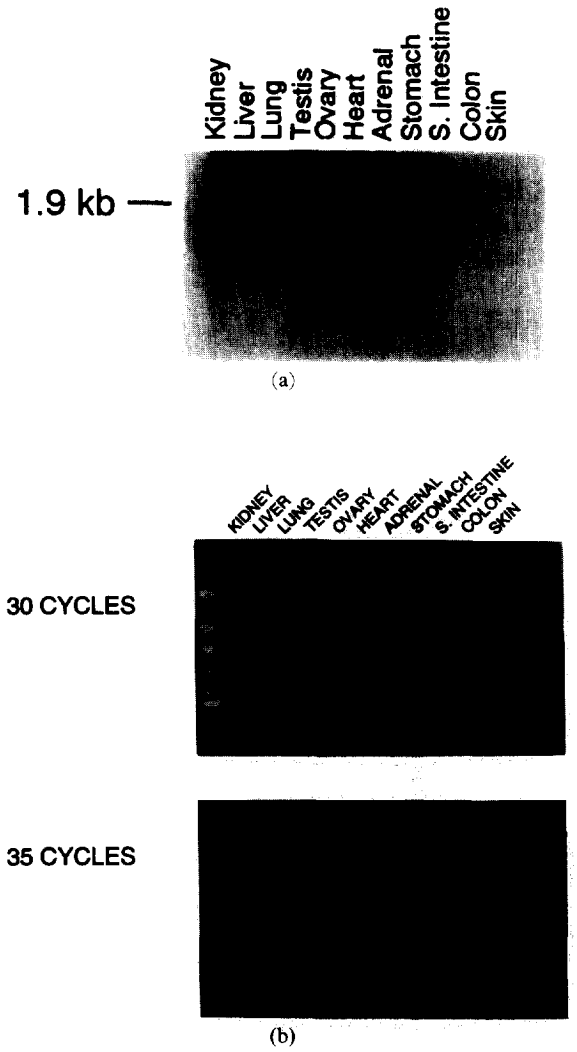

Fig. 2. (a) Hybridization of 11β-HSD K cDNA to RNA from various ovine tissues. (b) Reverse transcriptase polymerase chain reactions (RT–PCR) of ovine tissues as in (a).

kidney microsomes. The native 11β-HSD activity in microsomes is exclusively in the dehydrogenase direction, has about 20-fold higher activity with NAD^+ instead of $NADP^+$ as a cofactor, and has an apparent K_m for cortisol of 69 nM [12].

Tissue expression

The labeled sheep cDNA hybridized strongly to a single 1.9 kb species in kidney and adrenal RNA, and detectably to colon RNA. There was no detectable hybridization under these conditions to RNA from liver, lung, testis, ovary, heart, stomach, small intestine, or skin [Fig. 2(a)]. A RT–PCR amplification of the sheep RNA using nested primers corresponding to sheep sequences (nucleotides 341–357, sense and 659–677, antisense) amplified detectable levels of message in colon, heart, stomach and skin [Fig. 2(b)]. The additional DNA product amplified in stomach did not hybridize to the 11β-HSD K probe (data not shown).

ISOLATION AND STRUCTURE OF THE HUMAN 11β-HSD K (TYPE II) GENE

A human genomic library in bacteriophage P1 [21] was screened with a partial sheep cDNA and two nearly identical human genomic P1 clones were isolated. All sequences on each clone that hybridized with the sheep kidney 11β-HSD cDNA were located on a 12 kb XbaI fragment, which was subcloned and analyzed further. This fragment was highly enriched in CpG dinucleotides, containing sites for restriction enzymes with 8 bp recognition sequences such as AscI and SfiI, as well as multiple sites for other enzymes that cut human DNA rarely, including BssHII & EagI.

The 11β-HSD K gene consists of 5 exons spanning approx. 6.2 kb [22]. Exon 1 is located approx. 3.5 kb upstream from the other exons, which are separated from each other by short (117,106 and 201 bp) introns. The putative binding site for the NAD^+ cofactor (including the core sequence, GxxxGxG) is split between exons 1 and 2, whereas the putative catalytic residues, Tyr-232 and Lys-236, are encoded by exon 4.

The exonic sequences differ from the previously reported human cDNA sequence [23] in the 5′ untranslated region, 5 nt upstream from the initial AUG (−5 nt), where two additional nucleotides were detected, and in codon 148, which is GTG, encoding valine, rather than TTG, leucine. These changes were confirmed by analysis of uncloned PCR products from normal human DNA. The predicted peptide sequence is 83% identical to the predicted sequence of sheep 11β-HSD K cDNA [14].

COMPARISON OF 11β-HSD L AND K GENES

The predicted amino acid sequence of 11β-HSD K is only 21% identical to the predicted sequence of the human liver (Type I) isozyme of 11β-HSD L. When these sequences are aligned, the introns do not correspond in number or location (Fig. 3). These data indicate that these two isozymes belong to different gene families. For this reason, we feel that nomenclatures for these genes such as 11β-HSD1 and 11β-HSD2 [23] are misleading and should be avoided. As a contrasting example CYP11B1 and CYP11B2 encode isozymes of steroid 11β-hydroxylase that are 93% identical [24].

EXPRESSION OF 11β-HSD K IN HUMAN TISSUES

An RNA blot of human mid-gestation fetal tissues was hybridized with a radiolabeled PCR fragment corresponding to nucleotides 355–683 of human kidney cDNA, washed in high strigency (65°C in 15 mM NaCl, 1.5 mM sodium citrate, 0.1% SDS). 11β-HSD K transcripts were detectable in placenta and fetal kidney, lung and testis (Fig. 4). This pattern of

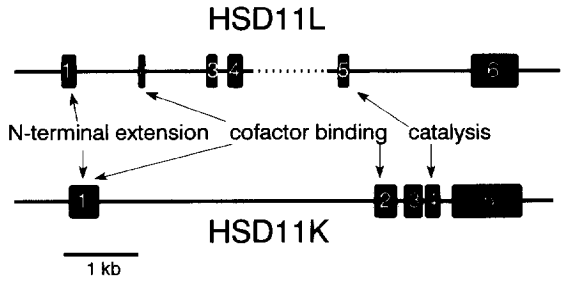

Fig. 3. Comparative structures of human 11β-HSD L and 11β-HSD K genes. Exons are denoted by filled boxes numbered 1–6. Dotted line indicates the intronic sequence not determined.

expression is similar to that observed in adult humans [23]. Whereas human fetal and adult tissues contain transcripts of 2.0 kb, fetal tissues also express transcripts of approx. 5 and 7 kb, based on comparisons of mobilities to 28S and 18S rRNA. These may represent utilization of alternative polyadenylation sites or partially processed transcripts.

Ribonuclease protection analysis showed that transcripts in the adult kidney begin at −116 nt. This site is utilized to a minor extent in the placenta, in which transcription begins predominantly at −74 nt (Fig. 5).

PROXIMAL PROMOTER REGION OF 11β-HSD K GENE

The proximal promoter region of the gene lacks TATA and CAAT boxes and has a high GC base content [22]. There are numerous putative binding sites for Sp1, AP-2 and other transcription factors that recognize GC-rich sequences [25, 26]. Whereas the Sp1 transcription factor is expressed ubiquitously, AP-2 is more restricted in its tissue distribution of expression and may influence tissue specific expression of this gene. AP-2 is also regulated by cAMP [27], which is consistent with regulation of 11β-HSD K by cAMP and the protein kinase A signaling pathway [28].

CHROMOSOMAL LOCALIZATION

Fluorescent *in situ* hybridization of human metaphase chromosomes localized 11β-HSD K to the long arm of chromosome 16 [22]. The chromosomal assignment was confirmed by simultaneous hybridization with a chromosome 16-specific centromeric probe (D16Z2) [29, 30]. Measurements of 10 specifically hybridized chromosomes 16 showed that the gene is located 55% of the distance from the centromere to the telomere of 16q, consistent with a location within band 16q22. This location is distant from that of 11β-HSD L, which is located on chromosome 1 [10]. It is, however, relatively near the gene encoding the Type 2 (placental, NAD^+-dependent) isozyme of 17β-HSD, which has been localized to 16q24.1–q24.2 [31]. This is of interest because this latter enzyme is 37% identical to 11β-HSD K in its predicted amino acid sequence, suggesting that these two enzymes may be in the same gene family and may have arisen from an ancestral duplication. Nevertheless, the corresponding genes are clearly in distinct cytogenetic locations, and the gene for 17β-HSD Type 2 is not located on the P1 clones carrying 11β-HSD K. A human plasma membrane Na^+/H^+ Exchanger (NHE5) gene is also located at

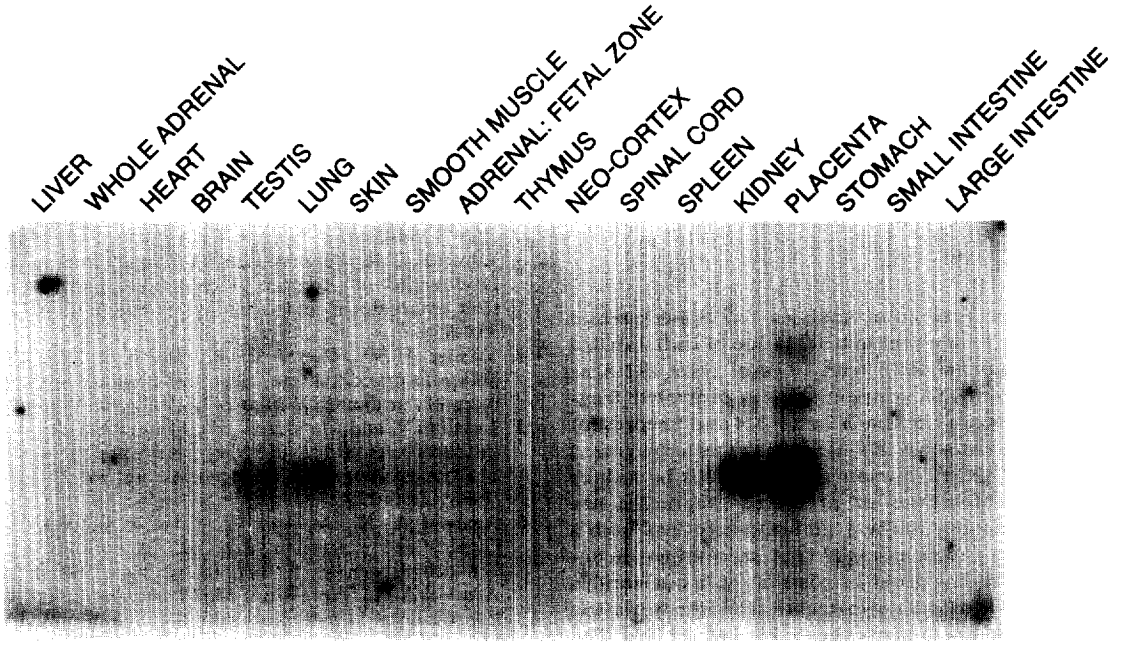

Fig. 4. Hybridization of blots of human midgestation fetal RNA from various tissues with human 11β-HSD K cDNA. The major transcript is 2.0 kb, and minor transcripts are approx. 5 and 7 kb.

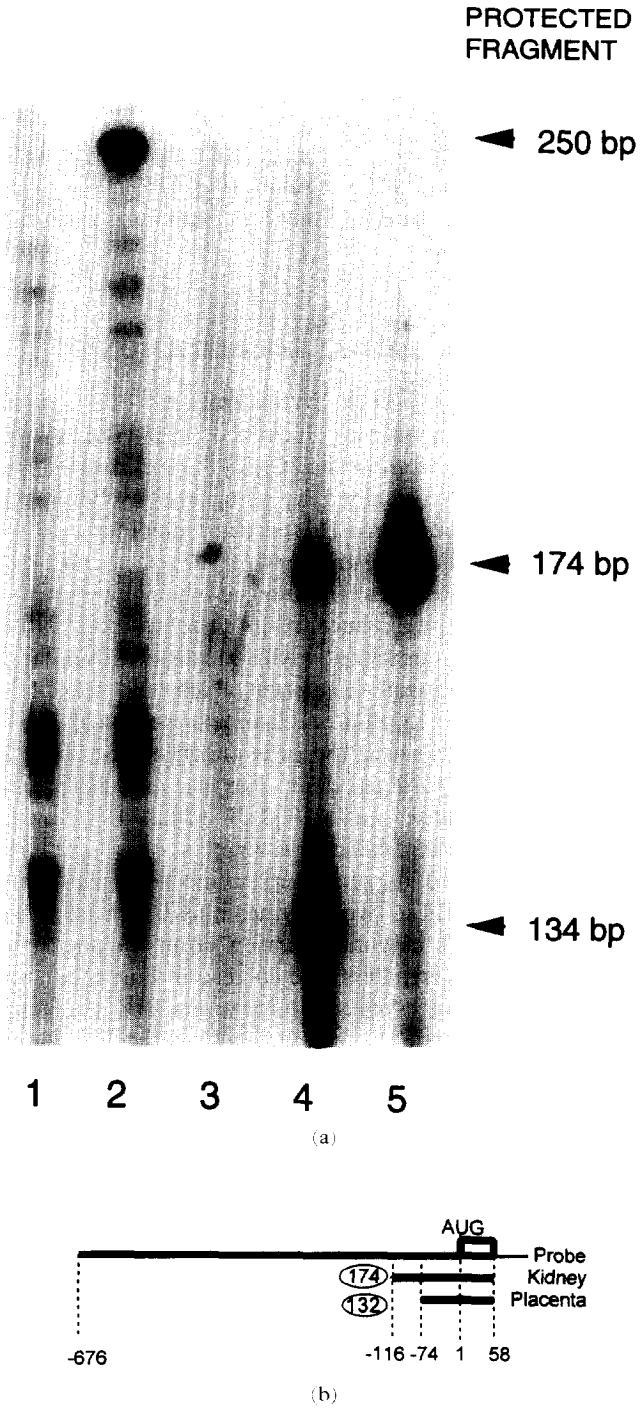

Fig. 5. Ribonuclease protection analysis. (a) Experimental results. Lanes 1 and 2 represent hybridization of radiolabeled mouse β-globin antisense RNA with 10 μg of yeast tRNA or 2.5 μg of mouse liver RNA, respectively. A protected fragment of 250 bp is observed in lane 2. Lanes 3, 4 and 5 represent hybridization of an antisense 11β-HSD K genomic fragment with 10 μg of yeast tRNA, human placenta RNA or kidney RNA respectively. Mobilities of protected fragments are deduced by comparison with the β-globin control and with a sequencing reaction run in parallel (not shown). (b) Schematic representation. The probe consists of −676 nucleotides upstream and 58 nucleotides downstream from the initiating AUG with some vector sequences shown by thinner line. The length of the protected fragment from kidney and placenta are shown in circles.

16q22.1 [32] possibly near the 11β-HSD K gene. The significance of this is not established.

CONCLUSION

The expression of the 11β-HSD K isozyme in kidney and colon, which are both mineralocorticoid target tissues, is consistent with a role in protecting the mineralocorticoid receptor from the binding of circulating glucocorticoids mainly the cortisol. Since this presentation, we found mutations in the 11β-HSD K gene in patients from 8 out of 9 kindreds with the syndrome of AME; these mutations affected enzymatic activity or pre-mRNA splicing [33].

Polymorphisms in 11β-HSD activity have been hypothesized to be a risk factor for the development of essential hypertension. It may be of interest to isolate polymorphic markers linked to 11β-HSD K in order to carry out affected family member analysis of hypertensive kindreds.

The role of this isozyme in the adrenal gland is less apparent, particularly because concentrations of glucocorticoids within the adrenal cortex are in the micromolar range and should easily saturate the enzyme. Perhaps this isozyme prevents glucocorticoids secreted by the zona fasciculata from entering the zona glomerulosa of the adrenal cortex (the site of mineralocorticoid synthesis). Testing of this hypothesis will require localization of 11β-HSD K expression within the adrenal gland.

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