SHORT COMMUNICATION

Mineralocorticoid receptor splice variants in different human tissues

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Abstract

The mineralocorticoid receptor (MR), a member of the steroid receptor family, acts as a transcription factor and mediates both aldosterone and cortisol effects. Aldosterone specificity in some tissues results from the inactivation of competing cortisol into cortisone by 11 β -hydroxysteroid dehydrogenase. In other tissues MR and the glucocorticoid receptor show overlapping physiological effects or may act together by forming a heterodimer. An additional MR splice variant (MR+4) has been found in different mRNA samples from rat tissues and human white blood cells, thereby implying additional modes of MR-regulated effects. We therefore looked for the presence of these two MR-mRNA isoforms in human classical aldosterone target tissues and various other tissues. MR-mRNA was found in all samples investigated, thereby showing the expression of MR to be more abundant than has been observed thus far. In addition, the MR+4-mRNA variant was also found in all the tissues examined.

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Introduction

The human mineralocorticoid receptor (MR) mediates aldosterone action in different target tissues, e.g. kidney, colon, and salivary and sweat glands. The hormone–receptor complex acts as a ligand-dependent transcription factor by controlling the expression of several genes, thereby regulating ion balance. The 60–90 kb long MR gene is composed of ten exons including two untranslated exons (1a, 1b) which are controlled by separate promoters. They are alternatively spliced to two 6 kb long mRNA isoforms that differ in their 5′-untranslated region and are expressed in a tissue specific manner (1). Both mRNA subtypes result in the same 107 kDa receptor protein (2–4). In addition, different splice site usage (donor splice site: end of exon 3 or intron c) of the MR-mRNA has been observed in various rat tissues and human white blood cells. This leads to an insertion of 12 bp and to a transcript of 988 (MR+4) amino acids (5).

As a characteristic feature MR and other members of the steroid receptor subfamily (receptors for glucocorticoids, progesterone, androgen and estrogen (6)) contain two zinc fingers which, in case of the wild-type MR, are separated by 45 bp. In the MR+4 variant the additional four amino acid residues enlarge this zinc finger spacing region, suggesting different modes of receptor-mediated gene regulation by these two MR proteins. For this reason we focused our attention on the presence of the

two MR splice variants in ten different human tissues, and established their ratio by mRNA quantification.

Materials and methods

Normal human tissues were obtained at surgery from the University Hospital, Bonn and the Johannes Hospital, Bonn after consent had been obtained in accordance with local institutional guidelines. Samples were either immediately frozen (within 5 min) or microdissected (glandular breast tissue) and stored at –80°C. RNA was extracted by the use of the Trizol reagent (Gibco-BRL, Paisley, Strathclyde, UK). Reverse transcription (RT) was performed with Expand Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the instructions of the manufacturer. The cDNA was amplified with the following primers: P1F (5′CGGCGACCTGTCGTCTAGAAG3′), P2Fn (5′CCAGAAAATGTATCAAGCTCT-AC3′), P3R (5′CCAACTTCTTTGACTTT-CGTG3′), P4Rn (5′CTTCTTTGACTTTTCG-TGCTCC3′). For mRNA quantification a nested, fluorescence-labeled forward primer (P2Fn) was used in the second PCR and the resulting PCR products were separated on a 6% denaturing polyacrylamide gel. Peak areas corresponding to the two MR splice variants were detected on a 373A automatic sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed with the Genescan program (Applied

Figure 1 Ratio of MR-mRNA splice variants in different human tissues. MG=mammary gland.

Biosystems, Version 1.2.1). All samples were examined twice. As a sequence control, an aliquot of the purified PCR product served as a template in a PRISM Ready Reaction Dye Terminator Cycle Sequencing procedure (Applied Biosystems) with AmpliTaq FS (Applied Biosystems) using the 373A sequencer.

Results

In contrast to Northern blotting, the use of nested fluorescence-labeled primers and a semiautomated sequencer allows the detection of mRNA species even at low levels (7). Since the sequence between the two MR-mRNA splice variants differs by only 12 bp (fragment size 290/302 bp), identical amplification kinetics during PCR can be expected, in the absence of any preference for the smaller template. We coamplified both splice variants, and PCR product analysis revealed the presence of the two MR isoforms in all human tissues tested. Ratios obtained from the individual tissue samples are shown in Fig. 1.

As can be seen, not only classical aldosterone target tissues (kidney, skin), but also adrenal and mammary gland, as well as placenta, showed expression of the MR gene and an approximate splice variant ratio of 0.85:0.15 (MR-mRNA:MR+4-mRNA). When compared with aldosterone target tissues samples from brain, those from liver and leukocytes revealed a tendency to a very slight increase in the MR+4-mRNA content. However, different ratios were obtained in various samples of each tissue investigated (e.g. the content of the wild-type MR-mRNA in nine leukocyte samples ranged between 0.71 and 0.87). Interestingly, samples obtained from liver tissue gave nonhomogeneous results. One sample showed a nearly equal ratio (0.48:0.52) of the two MR-mRNA isoforms, whereas

two samples showed ratios of 0.7:0.3 and 0.9:0.1 respectively. In the cases of kidney and skin, the amount of the wild-type MR-mRNA was more than 97%, so MR+4-mRNA content was close to the detection limit. It remains to be elucidated whether these tissues actually express the low levels of MR+4 transcript. In addition, we have found both MR-mRNA splice variants in a pool of three samples of prostate (ratio 0.79:0.21) and two samples of ovarian tissue (ratio 0.87:0.13) (data not shown).

Discussion

RT-PCR technology combined with nested PCR enabled us to detect even low levels of MR-mRNA transcripts in tissues so far not known to express the receptor (e.g. placenta). These are the first data on the expression of MR-mRNA, in parallel with the presence of placental 11 β -hydroxysteroid dehydrogenase type 2 observed by Petrelli *et al.* (8). In addition we have demonstrated in a variety of human tissues the splice variant previously shown to be present in rat tissues and in human white blood cells (5).

All tissues tested showed the MR+4-mRNA at lower abundance than wild-type MR-mRNA, and in one sample from kidney and skin the MR+4 RT-PCR product was close to the detection limit. The finding of almost equal amounts of both splice variants in one sample of human liver is supported by comparable findings obtained from rat liver (5). Investigations of the relative stability of rat brain MR and glucocorticoid receptor (GR) mRNA showed positive signals corresponding to both receptors 24 h postmortem (9). The liver data may therefore represent a particular physiological state for the tissue sample rather than an artefact of mRNA degradation over the time of sample collection and

freezing. It is also possible that the data, despite being obtained from healthy tissue samples, might reflect altered cell metabolism in response to a particular disease state. We detected both splice variants in placenta, prostate and ovarian tissue. So far it is unknown if the variant mRNA is translated into receptor protein and, if so, what function MR might have in these tissues.

Alternative splicing is not confined to the MR, but has been found to be present in other corticosteroid receptors. For example, compared with the classical GR found in Old World primates and man (10), the GR of the New World primate marmoset also contains an additional amino acid (arginine; GR+1) in the inter-finger region. In the rainbow trout (*Onchorhynchus mykiss*) another GR splice variant with nine amino acids added (GR+9) has been found exclusively in gill, intestine, skeletal muscle, kidney and liver (11); mRNA obtained from fish testis contains two GR splice variants, GR+9 and GR, lacking this insert (12). The observed MR and GR mRNA isoforms in all cases arise from the alternative use of a donor splice site between exon 3 and 4, and the resultant difference at the protein level may represent another pathway of steroid receptor action.

Further experiments to address these questions might also explore differences in sex- and/or age-dependent ratios of the two MR isoforms.

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