Molecular Cloning and Sequencing of the Hepatic AH Receptor from the Most TCDD-Resistant Rat Strain Reveals Altered C-Terminal End

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Abstract

Han/Wistar (*Kuopio*; H/W) rats are extremely resistant to the acute lethality of TCDD with an LD_{50} value of >9600 μg/kg. Recent studies revealed that the AH receptors of these rats are exceptionally small (apparent molecular mass ≈98 kDa vs. 106 kDa in most other rats) and show peculiar physicochemical properties. To gain further insight into the molecular structure of these receptors, we used RT-PCR to clone the coding region of the AH receptor cDNA in several fragments. For comparison, relevant segments were also cloned from TCDD-sensitive Long-Evans (*Turku AB*; L-E) and Sprague-Dawley (SD) rats. Sequencing of the clones gave structures identical to the previously reported "rat" AH receptor for L-E and SD rats. However, the H/W rat receptor exhibited two departures from this sequence: First, there was a point mutation in the second base of a codon at nucleotide 1520 (T→C) resulting in conversion from valine to alanine at the translation step. Second, the reason for the exceptionally small receptor protein in H/W rats appeared to reside at the 3' end of the cDNA where 3 different products were obtained with PCR. One of them had a major deletion of nucleotides 2326-2454, the 3' end exactly corresponding to the 3' end of exon 10 in mice; the genomic structure of rat AH receptor gene is not yet known. Another product displayed otherwise normal structure except for 29 extra nucleotides being inserted again at the 3' end site of exon 10 in mice (between bases 2454-5). This extra stretch of unknown origin contained a premature stop codon which would lead to the formation of a protein molecule of similar molecular mass to the one stemming from mRNA with the deletion. The third product was of equal size to that in L-E and SD rats and may represent the normal sequence. However, western blots showed that these H/W rats produced only the small ≈98 kDa receptor protein. Thus, TCDD-resistant H/W rats have unique AH receptors with altered C-terminal ends.

Introduction

The AH receptor is an indispensable mediator of most, if not all, biological effects of TCDD[1]. H/W rats are extremely resistant to the acute lethality of TCDD, but their sensitivity to a number of other responses, including CYP1A1 induction, does not appreciably differ from that of other strains[2]. Recent studies disclosed that the AH receptors of H/W rats are exceptionally small (≈98 kDa vs. 106 kDa in most other rats)[3] and have peculiar physicochemical properties[4]. This

prompted us to examine the primary structure of the H/W rat AH receptors in detail by molecular cloning and sequencing of the whole coding region of the corresponding cDNA. For comparison, relevant segments were also cloned from TCDD-sensitive L-E and SD rats.

Experimental Methods

Male rats (female for SD) at the age of 13 to 14 weeks were used. For validation of the findings, additional male H/W rats that were 5 weeks old were studied. The rats were killed by decapitation. A piece of liver weighing approximately 1 g was rapidly removed by a sterile technique, flash-frozen in liquid nitrogen and stored at -80°C until analysis. The liver samples were homogenized frozen and total RNA was isolated from the homogenate by the phenol-chloroform-guanidine isothiocyanate method[5]. RT-PCR was performed with AMV reverse transcriptase (Boehringer-Mannheim, Mannheim, Germany) and either Taq (Pharmacia Biotech, Uppsala, Sweden) or AmpliTaq Gold (Perkin-Elmer - Roche, Branchburg, NJ, USA) DNA polymerase. Random primers were used for RT. For PCR, the primers were designed on the basis of a published sequence for SD rat AH receptor[6]. The coding region of the H/W rat AH receptor cDNA was obtained in 5 fragments (from 5' to 3' end: -29 or 0 to 839; 810 to 1660; 1561 or 1884 to 2609). The PCR products were cloned by blunt-end cloning into pCR-Script SK(+) Amp plasmids (Stratagene, La Jolla, CA, USA). The inserts were sequenced with an A:L:F-automated DNA sequencer (Pharmacia Biotech) using either AutoRead sequencing kit (Pharmacia Biotech) or Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham, Cleveland, OH, USA).

Each clone was first sequenced from a H/W rat. If any differences from the published SD rat receptor base order were detected (or if there were compression areas), the reciprocal strand of the DNA double helix was analyzed on the same site or, if the clone was too large for this, another fragment over the same region was amplified with slightly different primers. If either of these confirmed the alteration, this region was examined in another H/W rat. If an identical change was found, its strain specificity was assessed by cloning and sequencing this region from L-E and SD rats.

Results and Discussion

The 5' end of the H/W rat AH receptor, encompassing the basic helix-loop-helix and PAS domains, proved to be identical to that in SD rats. This was not surprising in light of the previous data demonstrating that, compared with TCDD-sensitive rats, the AH receptors of H/W rats show similar dioxin responsive element binding and the same is also true for ligand binding affinity[4]. Outside these two domains, a strain-specific point mutation was detected at nucleotide 1520 where T was replaced by C, resulting in an amino acid change from valine to alanine. Although the biological consequences of this alteration are unknown at present, it is improbable that a shift from one nonpolar hydrophobic amino acid to another of the same kind in a hypervariable region would be of major significance.

Another change much more likely to be of biological importance was seen in the clone spanning the last 726 nucleotides at the 3' end of the coding region of H/W rat AH receptor cDNA. In contrast to the single strong band obtained by PCR in L-E and SD rats from this region, 3 bands were discernible in all 4 H/W samples analyzed with either the top or bottom band displaying highest intensity (Fig. 1). Two of these products were successfully cloned. One of them (probably

representing the bottom band) had a major deletion of 129 bases (nucleotides 2326-2454). The ends of the deletion matched codon borders so that there would be no frame-shift in the translated protein. Interestingly, the 3' end of the deletion was found to to be the exact 3' end site of exon 10 in mice, the only rodent in which the genomic organization of the AH receptor gene is known[7]. This could result from alternative mRNA splicing. On the other hand, exon 10 is a large exon in mice comprising a total of 1265 bases[7]. Therefore, the present data suggest that exon 10 may be split into smaller subunits in rats and prompt clarification of the genomic structure of the AH receptor gene in this species.

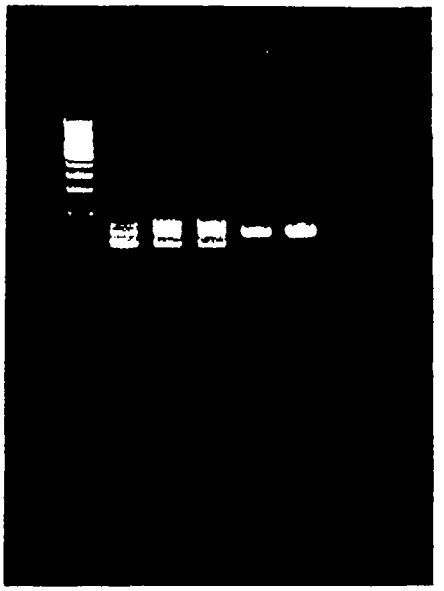

Fig. 1. PCR amplification products from the 3' end of the rat AH receptor coding region. The forward and reverse primers recognized bases 1884-1901 and 2609-2592, respectively, and thus the expected size of the PCR product was 726 nucleotides. Lanes: 1 1 kb DNA MW marker (MBI Fermentas, Vilnius, Lithuania); 2 - 4 H/W rats; 5 L-E rat; 6 SD rat. The rat samples used in PCR were RT products obtained with random primers from total RNA (3.5 μl/50 μl PCR reaction volume).

The other major PCR product at the 3' end in H/W rats was slightly larger than the regular counterpart in L-E and SD rats (Fig. 1). We managed to clone a DNA fragment which had otherwise the normal primary structure of this region, but there was an insertion of 29 additional bases between nucleotides 2454-5, again at the 3' end site of exon 10 in mice (Fig. 2). The extra material contained a premature stop codon and thus this mRNA would result in a protein of fairly similar size to that emanating from the deletion (38 vs. 43 missing amino acids or ca. 5.5 and 4.5 kDa reductions in protein size, respectively). As mentioned above, at the protein level the AH receptor size difference between H/W and L-E (or SD) rats is about 8 kDa when assessed from Western blots after SDS-polyacrylamide gel electrophoresis. However, the match with the calculated molecular mass is seldom perfect and the deletion/insertion change is the most likely reason for the exceptionally small AH receptor typical of H/W rats.

Although in H/W rats there was also a third amplification product of equal size to the single specific product found in L-E and SD rats (Fig. 1), only the ≈98 kDa AH receptor protein was discernible in a Western blot (data not shown). The deviant mRNAs may therefore be preferentially translated in H/W rats.

The C-terminal end of the AH receptor appears to interact with other transcription factors in the nucleus and is responsible for transcriptional gene activation[8]. It is conceivable that this provides one mechanism for selectivity in terms of which genes are activated in which cells. Since H/W rats respond in a virtually normal manner to some effects of TCDD, e.g., CYP1A1 induction, but are more or less indifferent to others, especially acute lethality, their deviant AH receptors may be functionally incomplete. Further studies are needed to elucidate the exact role of these C-terminal AH receptor alterations in the unique response pattern of H/W rats to TCDD.

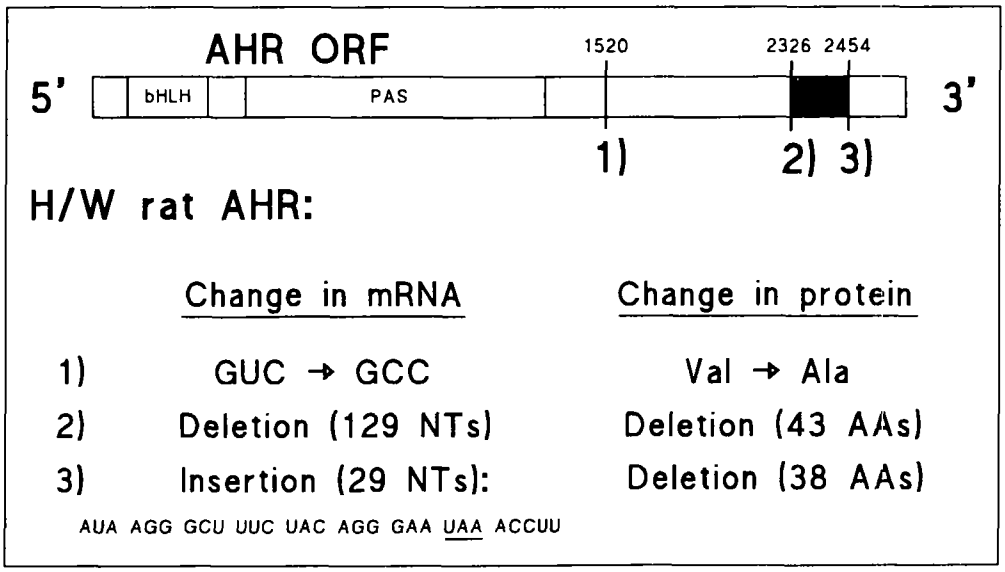

Fig. 2. Schematic illustration of the alterations detected in the primary structure of H/W rat AH receptors. The bar represents the open reading frame of the AH receptor mRNA with the major domains and the sites of changes in H/W rats depicted. The underlined codon in the insertion sequence is a translation termination codon.

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