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Primary structure and inducibility by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) of aryl hydrocarbon receptor repressor in a TCDD-sensitive and a TCDD-resistant rat strain

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Abstract

The aryl hydrocarbon receptor repressor (AHRR) is a negative regulator of AH receptor (AHR), which mediates most of the toxic and biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). AHR has been shown to be the major reason for the exceptionally wide (ca. 1000-fold) sensitivity difference in acute toxicity of TCDD between two rat strains, sensitive Long–Evans (*Turku/AB*) (L–E) and resistant Han/Wistar (*Kuopio*) (H/W), but there is another, currently unknown contributing factor involved. In the present study, we examined AHRR structure and expression in these rat strains to find out whether AHRR could be this auxiliary factor. Molecular cloning of AHRR coding region showed that consistent with AHRR proteins in other species, the N-terminal end of rat AHRR is highly conserved, but PAS B and Q-rich domains are severely truncated or lacking. Identical structures were recorded in both strains. Next, the time-, dose-, and tissue-dependent expression of AHRR was determined using quantitative real-time RT-PCR. In liver, AHRR expression was very low in untreated rats, but it increased rapidly after TCDD exposure (100 μg/kg). Testis exhibited the highest constitutive expression of AHRR, whereas kidney, spleen, and heart showed the highest induction of AHRR in response to TCDD treatment. Again, no marked differences were found between H/W and L–E rats, implying that AHRR is not the auxiliary contributing factor to the strain difference in TCDD sensitivity. However, simultaneous measurement of CYP1A1 mRNA reinforced the view that AHRR is an important determinant of tissue-specific responsiveness to TCDD.

Keywords: Aryl hydrocarbon receptor repressor; Aryl hydrocarbon receptor; Aryl hydrocarbon receptor nuclear translocator; CYP1A1; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Cloning; Rat; Species differences

Dioxins, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are ubiquitous environmental contaminants, which cause various biological and toxic responses in experimental animals [1]. Most of these effects are mediated by an intracellular protein, aryl hydrocarbon receptor (AHR), which is a highly conserved transcription factor belonging to a rapidly growing family of signal transduction molecules consisting of basic-helix–loop–helix (bHLH) and PAS domains. In the absence of ligand, the AHR is associated with hsp90 and some other proteins like XAP2 and p23 [2,3]. Upon exposure to TCDD, the AHR translocates into the nucleus and dissociates from the cytosolic chaperones. There it heterodimerizes with another bHLH/PAS protein, AH receptor nuclear translocator (ARNT). This complex then binds to its specific DNA recognition sites, known as xenobiotic response elements (XREs), located upstream of AHR-responsive genes to activate the transcription of these genes [4,5].

Aryl hydrocarbon receptor repressor (AHRR), also a member of the bHLH/PAS family, is a recently discovered negative regulator, which has been identified in mouse, human, and Atlantic killifish (*Fundulus*

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heteroclitus) [6–10]. In mice, AHRR binds to ARNT in competition with AHR. The AHRR–ARNT complex is capable of binding to XREs but not of transactivating the regulated genes. Furthermore, AHRR shows an inherent ability to repress the transactivation potency of ARNT [6]. AHRR is itself induced by liganded AHR–ARNT complex, and then the expressed AHRR, in turn, inhibits function of the AHR [6]. This negative feedback regulation of AHR function by AHRR seems to be conserved across mammals and fish [10]. In the tissues of untreated mice, essentially no basal expression of AHRR mRNA was detected, but after 3-methylcholanthrene treatment AHRR mRNA levels were induced in lung, thymus, heart, liver, kidney, and intestine [6]. In humans, AHRR is constitutively expressed in various normal tissues, especially in testis [11,12].

There are large inter- and intraspecies differences in susceptibility to the toxic effects of TCDD. In our laboratory, a 1000-fold sensitivity difference to acute lethality of TCDD has been established between sensitive Long–Evans (*Turku/AB*) (L–E) and resistant Han/Wistar (*Kuopio*) (H/W) rat strains. The principal reason for TCDD resistance in H/W rats is a mutated AHR [13,14]. However, there appears to be another gene product that contributes to dioxin resistance [14,15]. This unknown "gene B" is not ARNT, because there were no differences between these strains in the structure or expression of ARNT or ARNT2 (a protein closely related to ARNT but expressed only in the CNS and kidney) [16]. Therefore, we cloned and sequenced AHRR cDNAs from H/W and L–E rats and determined the time-, dose-, and tissue-dependent expression of AHRR mRNA by quantitative RT-PCR analysis to find out if AHRR could be this auxiliary factor involved in the large interstrain sensitivity difference in TCDD toxicity. In the expression analysis, CYP1A1 was also analysed to get a measure of the degree to which AHRR is capable of modulating the effects of TCDD in vivo.

Materials and methods

Animal husbandry. Young adult male L–E and H/W rats were housed in single-rat stainless steel wiremesh cages in an artificially illuminated animal room with a constant temperature of 21.5 ± 1 °C, humidity $55 \pm 10\%$, and a 12/12-h light/dark rhythm (lights on at 7 a.m.). In the dose–response study, rats of both strains were exposed i.g. to 1 ng/kg–100 μg/kg TCDD (dissolved in corn oil) 19 h before decapitation. The time-course of AHRR induction was examined by exposing L–E and H/W rats to 100 μg/kg TCDD 3, 6, 19, 24 h or 4 days before killing. TCDD was purchased from Ufa-Institute (Ufa, Russia) and was over 98% pure as assessed by gas chromatography–mass spectrometry. The rats were killed by decapitation and various tissues were rapidly removed, flash-frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

RT-PCR for cloning. The liver samples were homogenized and total RNA was isolated using GenElute Mammalian Total RNA Miniprep kit (Sigma–Aldrich, St. Louis, MO) or Trizol reagent (Life Technologies, Eggenstein, Germany). cDNA was synthesized with Omniscript reverse transcriptase (Qiagen, Hilden, Germany). PCR was performed with DyNAzyme EXT DNA polymerase blend (Finnzymes, Espoo, Finland) or with FastStart DNA polymerase (Roche, Mannheim, Germany) (see below) on either Uno II or TGradient thermocycler (Biometra, Göttingen, Germany). The "touchdown" method was applied throughout all reactions: the annealing temperature was set at about 5 °C above the calculated melting temperature of primers for the first cycle and then decreased by 1 °C/cycle down to the desired final temperature (determined with the aid of the gradient feature of the TGradient thermocycler). A total of 35 cycles were usually run.

PCR products were cloned by blunt-end cloning into pCR-Script SK(+) Amp plasmid (Stratagene, La Jolla, CA). XLB-1 supercompetent cells were used in transformations (Stratagene). The plasmids were purified by Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI). The inserts were sequenced with an A.L.F. or A.L.Fexpress DNA sequencer (Amersham–Pharmacia Biotech, Uppsala, Sweden) using either Thermo Sequenase fluorescent labelled primer cycle sequencing kit or Thermo Sequenase CY5 Dye Terminator Kit (Amersham–Pharmacia Biotech). All ambiguities were resolved by auxiliary clonings. The entire coding region was cloned and sequenced from H/W and L–E rats.

Cloning and sequencing of AHRR. The first clone of AHRR was produced from liver cDNA of L–E rat using primers AHRRcons-F (ACC-GGG-ACC-GCC-TCA-A) and AHRRcons-R (CTT-CTT-CTG-TCC-AAA-CAG-GAA). Next two clones (1100 and 1300 bp) of the middle part of AHRR cDNA were amplified using primers AHRR1100-F (CCT-GAC-TAT-GCA-GTT-CCA-AGG), AHRR1100-R (GGC-TCT-GTT-TTC-AGG-TGC-ATC), and AHRR1300-R (TCC-AGA-GGC-TCA-CGC-TTA-AC).

To obtain the 5′ end structure of the AHRR, mRNA was enriched from liver total RNA employing the MicroPoly(A)Purist Kit (Ambion, Austin, TX). cDNA was generated with the AHRR5′-RT primer (CTT-CAG-GTG-ACA-GAG-CTG-GA) using Omniscript reverse transcriptase (Qiagen). Single-stranded DNA was purified from RNA by RNase (MBI Fermentas, Vilnius, Lithuania) treatment followed by alkaline hydrolysis and PelletPaint (Novagen, Madison, WI) precipitation. The ENR-LIG oligo (CTT-ACT-CCC-GGG-TTG-TGC-GGT-GCA-GTT-TC) having its 5′ end phosphorylated and its 3′ end blocked was then joined to the 3′ end of the newly synthesized cDNA with T4 RNA ligase (MBI Fermentas) incubating the reaction at room temperature for 2 days. The ligation products were purified with Qiaquick (Qiagen) and subjected to PCR amplification with ENR1-F (GGA-AAC-TGC-ACC-GCA-CAA-C) and AHRR5′REV-1 (ATG-ATG-TCG-GGT-GAA-AAT-GG) as primers. The products were again purified with Qiaquick followed by another PCR amplification step with ENR2-F (CGC-ACA-ACC-CGG-GAG-TAA-G) and AHRR5′REV-2 (CCA-GGT-GGT-CCA-GCT-CTG-TAT) as primers.

To obtain 3′ end of the AHRR open reading frame together with a part of the untranslated region, cDNA was produced from poly(A)$^+$-enriched (MicroPoly(A)Purist, Ambion) liver RNA with the Anchor-RT primer and Omniscript reverse transcriptase (Qiagen). A total of three seminested PCRs were then conducted with HotStarTaq DNA polymerase (Qiagen) using Anchor-R (CCA-CCT-ACC-CAC-TAC-CCC-TCT-CA) as the reverse primer in each case. The forward primers employed were (in the order of use): AHRR3′1-F (AAG-TCA-GCA-TCC-CTC-CTT-GGA-C), AHRR3′2-F (TTG-GAC-CAA-GAC-TGC-AGA-GC), and AHRR3′3-F (CCA-AGA-CTG-CAG-AGC-TCC-TA).

Finally, the cDNA cloning of L–E AHRR was verified by producing three fragments covering the entire coding region using primers AHRR67-F (CCC-AGG-AGC-AAA-GAT-GAT-GA), AHRR867-R (AAA-CAA-GGA-GAG-CCG-AGG-AG), AHRR713-F (CTT-CTT-GAC-ACG-CTG-CTT-CA), AHRR1558-R (ACT-TCC-CTG-GGG-GAT-ATG-AC), AHRR1493-F (AGA-CCC-TTG-CCA-GAT-ATC-CA), and AHRR2287-R (CAC-AGG-AAA-TCT-CAG-CCT-CA). These same primers were used to clone the cDNA of H/W rat AHRR. In both strains, the coding region was resolved from two rats with identical outcomes.

Expression of AHRR and CYP1A1. Tissues were homogenized and total RNA was isolated using GenElute Mammalian Total RNA Miniprep kit (Sigma–Aldrich) or Trizol reagent (Life Technologies). cDNA was synthesized with Omniscript reverse transcriptase (Qiagen) from 1.2 μg of total RNA using random hexamers (Roche). Quantitative RT-PCR analyses were performed on cDNA originating from 15 ng of total RNA employing QuantiTect SYBR Green PCR kit (Qiagen) and Rotor-Gene 2000 Real-Time Amplification System (Corbett Research, Mortlake, NSW, Australia). The expression levels of AHRR and CYP1A1 were detected from the same samples using primers AHRR3′1-F (AAA-GTC-AGC-ATC-CCT-CCT-TG), AHRRexp3′-R (CCC-ATC-AGA-TCC-TTT-GGA-TG), and CYP1A1-F (CCA-TAT-GCT-TTG-GCA-GAC-GTT-A), CYP1A1-R (TCA-AAC-CCA-GCT-CCA-AAG-AG). The expression levels were related to mRNA concentrations of the house-keeping gene β-actin (whose levels were first verified not to be affected by the TCDD treatments) using primers β-ACTIN-F (CAC-GGC-ATT-GTA-ACC-AAC-TG) and β-ACTIN-R (GAG-CGC-GTA-ACC-CTC-ATA-GA) to normalize the amount of cDNA in PCRs.

The PCR was initiated with an incubation step of 15 min at 95 °C to activate HotStartTaq DNA polymerase. This was followed by 45 cycles of denaturation at 94 °C for 20 s, annealing at 57 °C for 20 s, and extension at 72 °C for 20 s. Melting curve was run at 55–99 °C to verify the specificity of PCR products. Standard curves were generated to determine the amount of template in each reaction. Control plasmids for standard curves were created by amplifying 150–200 bp fragments of AHRR, CYP1A1, and β-actin by conventional PCR and by cloning the PCR products into pCR-Script SK(+) Amp plasmid. Plasmid DNAs were linearized and quantified by spectrophotometry for amplification. PCRs were carried out in duplicate or triplicate and the dilutions used for AHRR were 10^{-12}–10^{-8}, for CYP1A1 10^{-9}–10^{-4}, and for β-actin 10^{-8}–10^{-4}. Negative controls containing all the components of the reaction mixture but water replacing the template were included in each run.

Statistics. In the time-course (time points 3, 6, and 19 h) and dose–response analysis with three or more groups, statistical comparisons were performed by one-way analysis of variance (ANOVA) if the variances were homogeneous. Duncan's multiple range test was employed for multiple comparisons post hoc if ANOVA showed a statistically significant difference ($p < 0.05$) among the groups. In the case of nonhomogeneous variances, the Kruskal–Wallis nonparametric ANOVA followed by the Mann–Whitney U test was used. The time-course data from days 1 to 4 were broken down strainwise and assessed statistically by Student's t test.

Results

The first cDNA clones of AHRR were produced by RT-PCR using primers selected from highly conserved regions. The sequence information obtained from these was then utilized in subsequent amplifications. Modified RACE techniques were used to unravel 5′ and 3′ ends. In all, 10 cDNA fragments covering the entire coding region of AHRR were produced from L–E rat. After that, AHRR of the H/W rat was cloned and sequenced in three duplicate or triplicate pieces to find out if there are differences between these rat strains in the structure of AHRR cDNA, but the sequences proved to be identical.

The coding region of rat AHRR cDNA contained 2106 nucleotides encoding a deduced protein of 701 amino acids (Fig. 1). Sequence comparison of rat, mouse, human, and *Fundulus* AHRR proteins revealed significant sequence identity in the N-terminal region containing bHLH and PAS A domains (Fig. 2). Considered as a whole, the rat AHRR protein was 95% identical to the mouse AHRR, but only 56 and 32% identical to human and *Fundulus* AHRRs, respectively. At the same time, it shared only 24% identity to rat AHR (Fig. 3). Again, the N-terminal end, containing bHLH and PAS A domains, was highly conserved. The most conspicuous departure from the AHR structure occurred in PAS B and Q-rich regions, which were notably shorter with functionally crucial parts missing in AHRR. A phylogenetic analysis of AHR, AHRR, and ARNT amino acid sequences (Fig. 4) implied that AHRR has diverged from an ancestral AHR gene later than ARNT.

The expression of AHRR was analyzed using quantitative real-time RT-PCR. The expression levels were related to β-actin mRNA concentrations to normalize for variations in pipetting and RNA quantitation. In untreated rat livers, AHRR mRNA levels were close to the detection limit of the method, about 1000-fold and 100,000-fold lower than the expression levels of CYP1A1 and β-actin mRNAs, respectively (Figs. 5–7). The constitutive expression levels of AHRR mRNA were significantly higher in H/W rats than in L–E rats in pooled control samples from three independent experiments ($p = 0.006$, $n = 14$).

To determine whether AHRR expression is modulated by TCDD in these strains, both the time-course and dose–responsiveness of AHRR expression in liver were analyzed after TCDD treatment. In the time-course study, H/W and L–E rats were treated with 100 μg/kg TCDD. In both rat strains, this dose of TCDD caused an upregulation of hepatic AHRR expression, which was significant at all time points (Fig. 5). The maximal induction levels were seen 1 day after TCDD exposure (5-fold in H/W rat and 23-fold in L–E rat with no statistically significant difference in the absolute induced values).

In the dose–response study (conducted 19 h after a single i.g. dose of TCDD), large variations were seen among individual rats in the expression of liver AHRR mRNA, especially at TCDD dose 0.01 μg/kg in H/W rats (Fig. 6). When the expression of CYP1A1 was measured using the same cDNA samples, the interindividual variation was strikingly less. The lowest dose, 0.001 μg/kg TCDD, tended to increase AHRR mRNA levels slightly but not significantly. Statistically significant inductions of AHRR mRNA were seen in H/W rats at doses 0.01–100 g/kg and in L–E rats at doses 1–100 μg/kg. CYP1A1 mRNA levels remained unaffected at the lowest dose of 0.001 μg/kg TCDD and the first significant increase of CYP1A1 mRNA occurred at TCDD dose 0.01 μg/kg in both strains. At higher doses, CYP1A1 expression followed a steep dose–response curve with maximal induction appearing at the highest dose tested, 100 μg/kg. In distinct contrast, AHRR

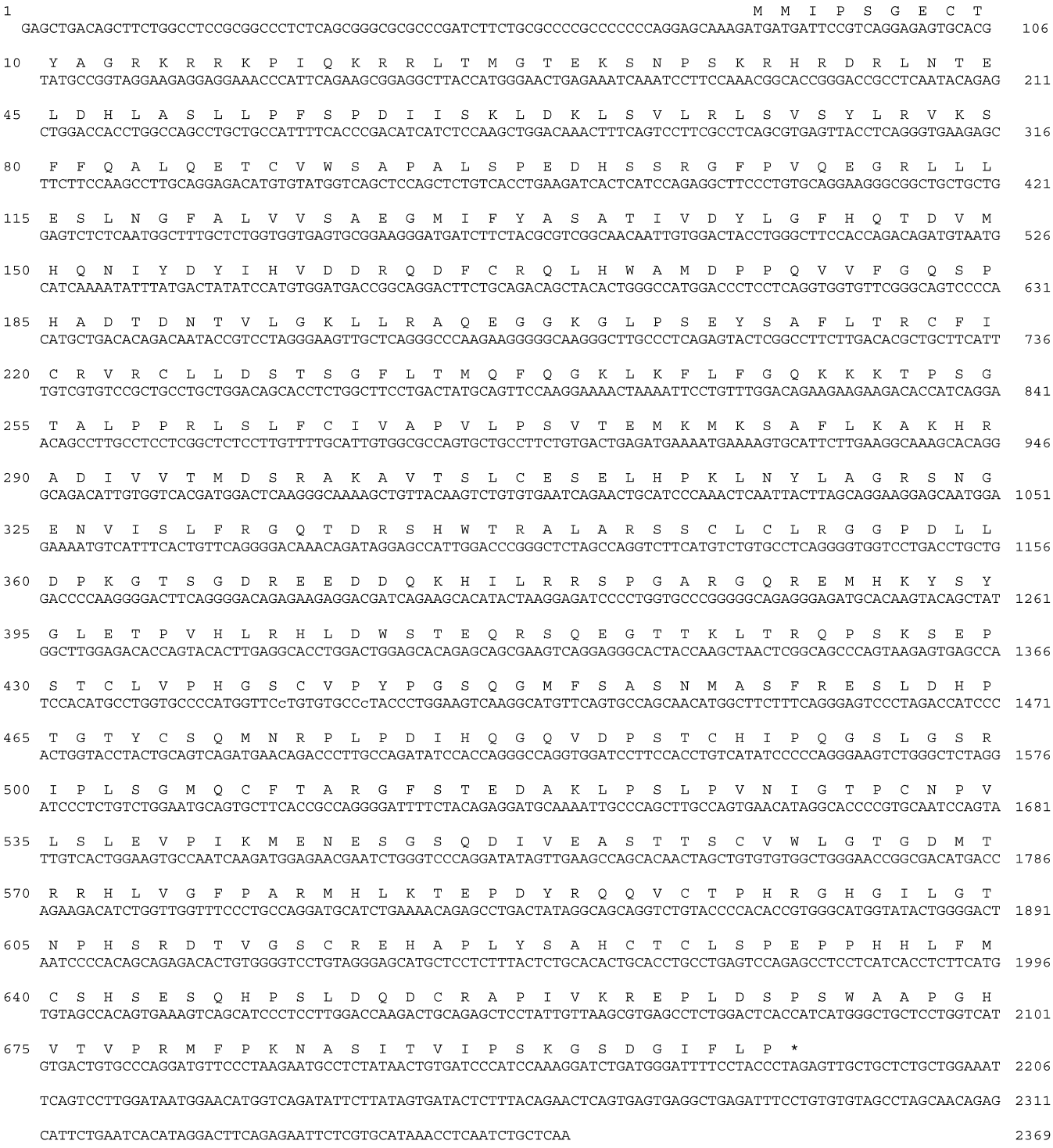

Fig. 1. Nucleotide and deduced amino acid sequences of rat AHRR cDNA (GenBank Accession No. AY367561). Nucleotides are numbered on the left and amino acids on the right. The termination codon is indicated with an asterisk (*).

expression exhibited a vague dose–responsiveness. In L–E rats, AHRR mRNA levels were lower at 0.01 and 0.1 μg/kg than at 0.001 μg/kg, but reached the maximum at 1 μg/kg which sustained at the two higher doses tested. In H/W rats, the maximum levels appeared to be attained already at 0.01 μg/kg. There were no marked differences between L–E and H/W rats in the maximal concentrations of AHRR mRNA; also CYP1A1 exhibited a highly similar overall induction pattern.

To gain some insight into the distribution of constitutive AHRR expression in the body as well as into its inducibility, heart, liver, kidney, spleen, and testis were

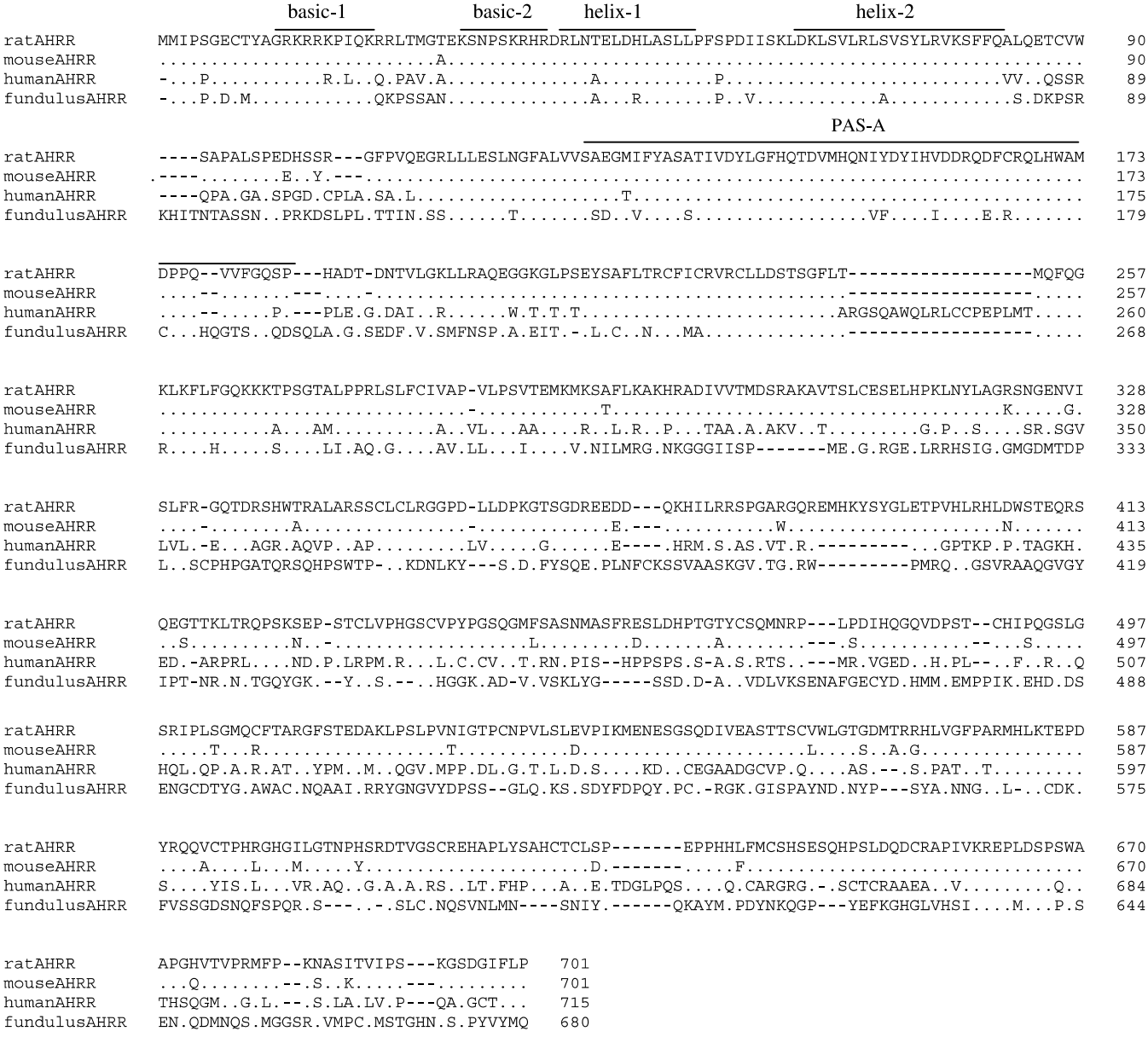

Fig. 2. Comparison of rat, mouse, human, and *Fundulus* amino acid sequences. Dots indicate identities while dashes denote gaps inserted to improve alignment. The basic-helix–loop–helix and PAS A regions are marked by solid bars above the domains. The sequence alignment was performed using Clustal W (1.82). The GenBank accession numbers of the AHRR sequences used here are: AY367561 (rat), NM-009644 (mouse), NM-020731 (human), and AF443441 (*Fundulus heteroclitus*).

analyzed from L–E and H/W rats 19 h after exposure to corn oil or 50 μg/kg TCDD (Fig. 7). The highest constitutive levels of AHRR mRNA were recorded in testis, but there was only a slight induction after TCDD in that organ. Concomitantly, the expression of CYP1A1 was very low before and after TCDD treatment. After TCDD exposure, the highest expression of AHRR was detected in kidney, spleen, and heart, in which CYP1A1 induction was relatively low. Conversely, liver displayed the lowest AHRR levels in response to TCDD but the highest induction of CYP1A1. Again, H/W and L–E rats showed indistinguishable expression patterns.

Discussion

Sensitivity to the toxic effects of TCDD varies widely among animal species and even within species, which complicates dioxin risk assessment. For example, L–E rats are highly sensitive to TCDD in contrast to H/W rats which can tolerate over 1000-fold higher TCDD doses. This difference is mainly based on reconstructed AHR in the H/W strain, but there is also another, currently unknown gene involved [13,14]. In the present study, AHRR cDNA was cloned from both of these rat strains to probe the possible role of AHRR structure in

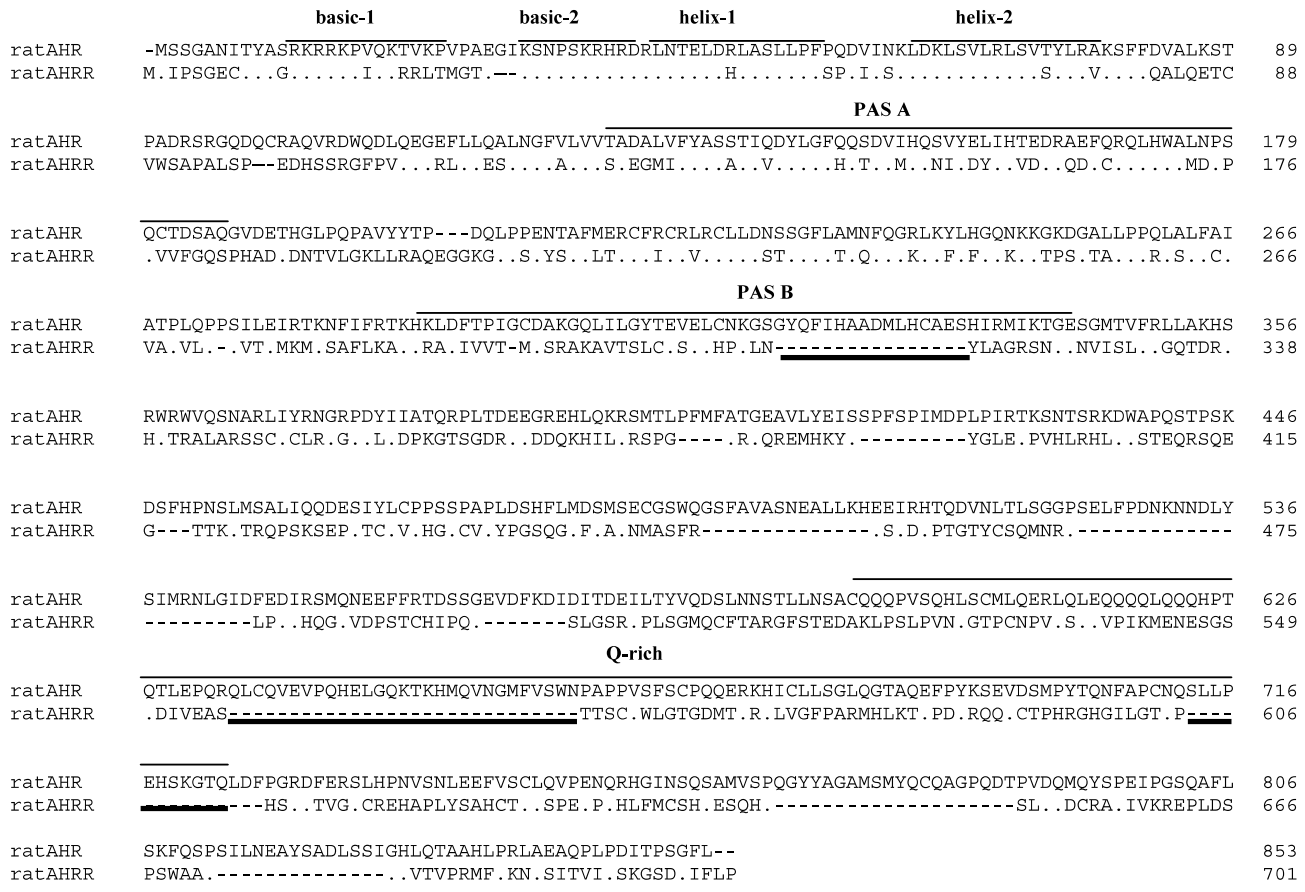

Fig. 3. Alignment of rat AHR and AHRR amino acid sequences. Identical amino acids are marked with dots. The basic-helix–loop–helix region, PAS domains, and glutamine (Q)-rich domain are indicated by lines above the domains. The large deletions in AHRR at PAS B and Q-rich regions are underlined. The sequence alignment was performed using Clustal W (1.82).

the strain-specific sensitivity to TCDD. However, the sequences proved to be identical indicating that altered structure of AHRR does not contribute to dioxin sensitivity in this rat model.

The deduced amino acid sequence of rat AHRR shows a high degree of sequence similarity to the rat AHR protein with regard to bHLH and PAS A domains. However, PAS B domain, which constitutes part of the ligand binding domain in AHR, is missing in rat AHRR. Also mouse, human, and killifish possess the same deletion in their AHRRs and probably this structural feature is the reason why AHRR is not capable of binding ligands [6,10]. Another domain of rat AHR severely truncated in AHRR is Q-rich region which has been shown to be important in the transactivation function of AHR [17]. This is a likely reason for the fact that AHRR is not itself transcriptionally active but functions as a transcriptional repressor [5,6]. Despite deviations in PAS B and Q-rich domains, AHRR can form a stable and active heterodimer with ARNT [18] and thus compete with AHR for heterodimerizing with ARNT.

The phylogenetic analysis of some AHR, AHRR, and ARNT amino acid sequences reveals that AHRR proteins form their own clade distinct from existing AHR, ARNT, and intervertebrate AHR clades. More specifically, both AHR and ARNT clades divide into two groups of which the AHR2 group only contains fish sequences [19], and ARNT1 and ARNT2 groups differentially expressed proteins [20]. It has been suggested that AHRR diverged from AHR as a result of a gene duplication and soon thereafter lost its transactivation function but acquired repressor activity [19]. The tree implies that this has probably occurred later than the divergence of ARNT from AHR.

Expression of AHRR was increased by TCDD in both L–E and H/W rats. However, compared with that of CYP1A1, AHRR induction was of lesser magnitude and exhibited larger interindividual variation, especially in H/W rats. In this strain, the near-maximum expression in liver was already attained at 10 ng/kg, whereas doses of 1 μg/kg and above were needed for maximal induction in L–E rats. These maximal expression levels were, however, similar in H/W and L–E rats. In both strains, the induction evolved rapidly becoming manifest already 3 h after exposure. This is in keeping with findings in human HepG2 cells, where 3-methylcholanthrene proved to induce AHRR mRNA expression more rapidly than that of CYP1 family [11].

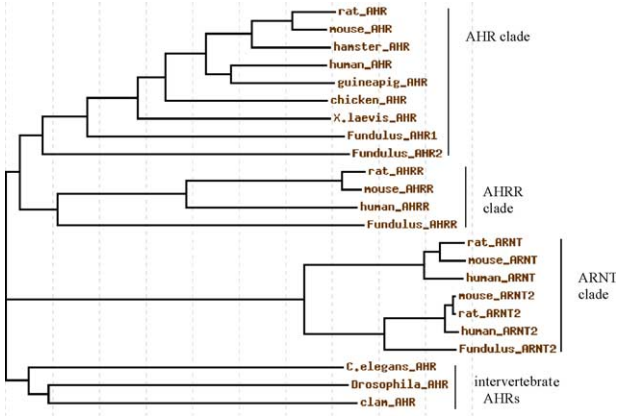

Fig. 4. Phylogenetic analysis of AHR, AHRR, and ARNT proteins. The tree was constructed using Advanced GeneBee Clustal W 1.75 software. The GenBank accession numbers of the sequences used are: rat AHR (NM-013149), mouse AHR (NM-013464), hamster AHR (AF275721), human AHR (NM-001621), guinea pig AHR (AY028947), chicken AHR (AF260832), *Xenopus laevis* AHR (AB109555), *Fundulus heteroclitus* AHR1 (AF024591), rainbow trout AHRα (AF065137), rainbow trout AHRβ (AF065138), *Fundulus heteroclitus* AHR2 (U29679), rat AHRR (AY367561), mouse AHRR (NM-009644), human AHRR (NM-020731), *Fundulus heteroclitus* AHRR (AF443441), rat ARNT (NM-012780), mouse ARNT (NM-009709), human ARNT (M69238), mouse ARNT2 (NM-007488), *Fundulus heteroclitus* ARNT2 (AF402781), rainbow trout ARNTa (U73840), rainbow trout ARNTb (U73841), *Caenorhabditis elegans* AHR (AF039570), *Drosophila melanogaster* AHR (AF050630), and clam AHR (AF261769).

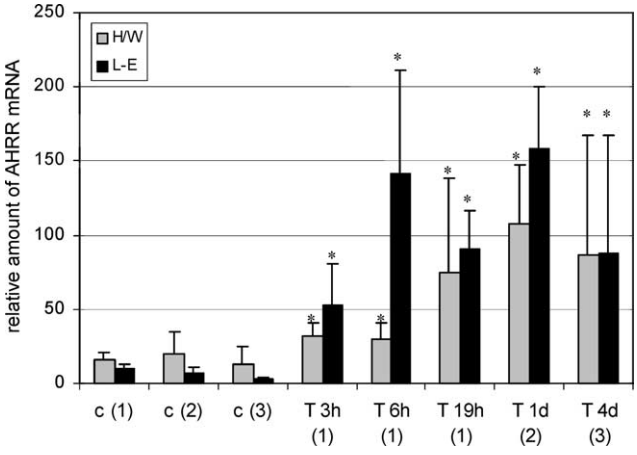

Fig. 5. Expression of AHRR mRNA in rat liver as a function of time. H/W and L–E rats were treated with 100 μg/kg TCDD in three independent experiments (1–3) and killed at 3, 6 or 19 h (1); 1 day (2); or 4 days (3) after exposure. Controls of each experiment are included. Expression levels of AHRR are given relative to β-actin mRNA concentrations. Columns represent means ± SD of four to six individual rats. Statistically significant differences ($p > 0.05$) vs control are depicted with asterisks (*).

The 5′-flanking sequence of the mouse AHRR gene has been reported to contain three copies of functional XREs, whose binding affinities to the AHR/ARNT heterodimer, however, are lower than those of XREs in

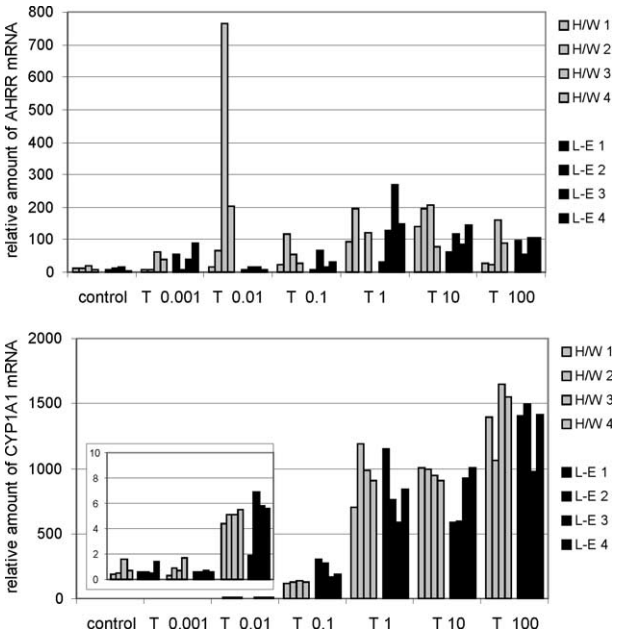

Fig. 6. Expression of AHRR and CYP1A1 mRNA in rat liver as a function of TCDD dose. H/W and L–E rats were treated with 0.001–100 μg/kg TCDD 19 h before tissue removal. Expression levels are given relative to β-actin mRNA concentrations. The columns represent data from individual rats (in the same order in both panels) to reveal the large variation in AHRR responses. Note the different *Y*-axis scale in the CYP1A1 inset for doses 0–0.01 μg/kg.

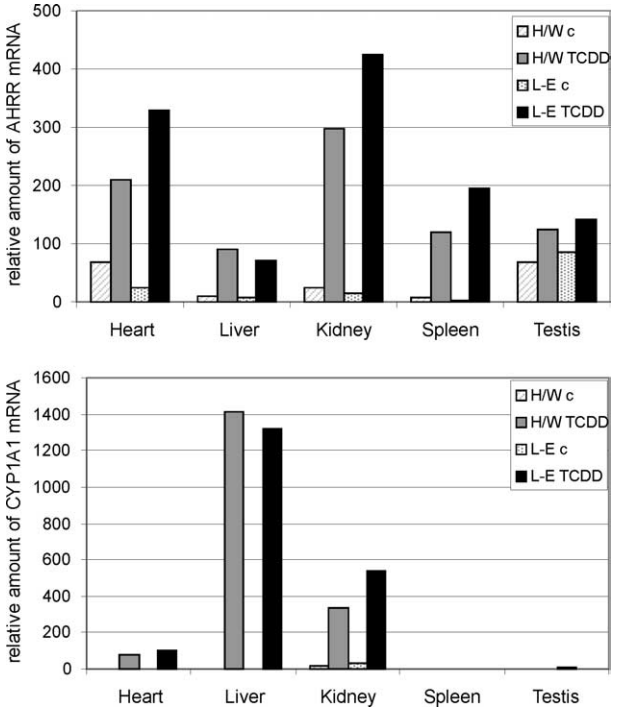

Fig. 7. The tissue-specific expression of AHRR and CYP1A1 mRNA. H/W and L–E rats were treated with 50 μg/kg TCDD 19 h prior to tissue removal. Columns represent means of two individual rats. AHRR expression levels are given relative to β-actin mRNA concentrations.

the CYP1A1 gene [21]. This might partly explain the differences between AHRR and CYP1A1 induction levels seen in this study in rats and previously reported in human cell lines [11]. The induction profile of AHRR did not fully parallel that of CYP1A1, mainly because of the wide interindividual variation in the expression of AHRR mRNA. The variation was not due to poor quality of cDNA samples, because no such variation was found in CYP1A1 and β-actin measured from the same cDNAs.

The constitutive expression levels of AHRR were so low that without the exceptionally sensitive quantitative PCR methodology they would have been almost impossible to detect. Baba et al. [21] have suggested that the low AHRR mRNA levels occurring under normal conditions in cells are due to divergent GC box sequences in the AHRR promoter region. It could be worthwhile to sequence these promoter regions also from H/W and L–E rats to find out if the structural variation in GC box sequences could be the reason for the strain difference found in the constitutive expression of AHRR. Nevertheless, although H/W rats were observed to have higher constitutive levels of hepatic AHRR mRNA, in L–E rats the magnitude of induction by TCDD was somewhat larger compensating for the lower initial levels.

The constitutive and inducible expression of AHRR varies across tissues. For example, AHRR mRNA levels could not be detected by semiquantitative RT-PCR in lung, thymus, heart, liver, kidney or intestine from untreated mice, but became discernible after 3-methylcholanthrene exposure especially in heart and lung [6]. On the other hand, in mouse pituitary, a high basal expression of AHRR mRNA as well as induction of AHRR mRNA by TCDD has been reported [22,23]. AHRR mRNA has also been detected in various fetal and adult tissues in humans [8,11]. In the present study, testis and heart showed the most abundant expression of AHRR mRNA in untreated rats, while TCDD increased AHRR mRNA levels most in kidney, spleen, and heart. The patterns of induction in these tissues were again similar in both rat strains. Overall, our findings imply that AHRR expression is not a contributing factor to the strain difference in TCDD sensitivity. In agreement with this contention, it has been reported that altered transcriptional regulation of AHRR expression is not associated with TCDD resistance in adult fish [10].

AHRR inhibits the function of AHR [6], and it has been suggested that the high expression level of AHRR might underlie the low induction of CYP1 family in human HeLa cells [11]. In human hepatoma cells, the increase of AHRR mRNA by OSM cytokine has been shown to decrease the expression of CYP1A2 [24]. Furthermore, high constitutive expression of AHRR has been reported to repress the induction of CYP1A1 in human fibroblasts [25]. In transgenic mice expressing constitutively active AHR, AHRR is also constitutively expressed in stomach glandular cells, which lack CYP1A1 induction after TCDD treatment [26]. Interestingly, mouse liver and thymus, which are known to be highly susceptible to toxic effects of TCDD, have proved to express AHRR mRNA poorly in response to inducers [6].

Hence, there are accumulating data on an inverse relationship between AHRR expression and sensitivity to induction of xenobiotic-metabolizing enzymes caused by TCDD. The present findings can also be regarded as supporting evidence of this phenomenon. In liver, a low induction of AHRR was recorded together with a strikingly pronounced induction of CYP1A1. Conversely, testis exhibited the highest constitutive expression of AHRR, but no detectable CYP1A1 induction, similar to what has been reported for human testis [11]. Moreover, kidney, spleen, and heart, which are not sensitive tissues to TCDD toxicity in rats, expressed the highest levels of AHRR mRNA after TCDD treatment.

In conclusion, we cloned and sequenced rat AHRR, whose N-terminal end showed high sequence identity to other AHRRs and also to rat AHR. PAS B and Q-rich domains were lacking or rudimentary, which probably explains the inability of AHRR both to bind ligand and to transactivate DRE-regulated genes. The expression of AHRR was very low in untreated rats, but it increased rapidly after TCDD exposure. There were no marked differences in the expression of AHRR between H/W and L–E rats before or after TCDD treatment in any of the tissues examined, suggesting that AHRR may not contribute to dioxin sensitivity. However, the simultaneous determination of CYP1A1 mRNA suggested that AHRR may play a modulatory part in CYP1A1 regulation. It can further have a protective role against TCDD toxicity in certain tissues such as kidney, heart, and spleen.

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