Toxicology and Applied Pharmacology 236 (2009) 166–182

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Transgenic mouse lines expressing rat AH receptor variants — A new animal model for research on AH receptor function and dioxin toxicity mechanisms☆

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ARTICLE INFO

Article history:
Received 19 September 2008
Revised 9 January 2009
Accepted 12 January 2009
Available online 23 January 2009

Keywords:
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD
AH receptor
Transgenic animals
Strain differences
Enzyme induction
Acute lethality
Liver toxicity

ABSTRACT

Han/Wistar (*Kuopio*; H/W) rats are exceptionally resistant to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity mainly because of their mutated aryl hydrocarbon receptor (*AHR*) gene. In H/W rats, altered splicing of the AHR mRNA generates two AHR proteins: deletion (DEL) and insertion (INS) variants, with the INS isoform being predominantly expressed. To gain further insight into their functional properties, cDNAs of these and rat wild-type (rWT) isoform were transferred into C57BL/6J-derived mice by microinjection. The endogenous mouse AHR was eliminated by selective crossing with *Ahr*-null mice. A single mouse line was obtained for each of the three constructs. The AHR mRNA levels in tissues were generally close to those of C57BL/6 mice in INS and DEL mice and somewhat higher in rWT mice; in testis, however, all 3 constructs exhibited marked overexpression. The transgenic mouse lines were phenotypically normal except for increased testis weight. Induction of drug-metabolizing enzymes by TCDD occurred similarly to that in C57BL/6 mice, but there tended to be a correlation with AHR concentrations, especially in testis. In contrast to C57BL/6 mice, the transgenics did not display any major gender difference in susceptibility to the acute lethality and hepatotoxicity of TCDD; rWT mice were highly sensitive, DEL mice moderately resistant and INS mice highly resistant. Co-expression of mouse AHR and rWT resulted in augmented sensitivity to TCDD and abolished the natural resistance of female C57BL/6 mice, whereas mice co-expressing mouse AHR and INS were resistant. Thus, these transgenic mouse lines provide a novel promising tool for molecular studies on dioxin toxicity and AHR function.

Introduction

Dioxins are lipophilic, persistent and wide-spread environmental contaminants arising from various industrial activities and thermal reactions including metal and electronic waste recycling, ore sintering, as well as backyard and landfill fires (Cieplik et al., 2003; Hedman et al., 2005; Fiedler, 2007; Li et al., 2007; Nguyen et al., 2003). The most potent congener of dioxins is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In laboratory animals, it brings about an exceptionally wide range of behavioral, biochemical and morphological effects encompassing e.g. a wasting syndrome and alterations in feeding behavior, endocrine disruption, impairment of the immunological system, liver toxicity, induction of specific Phase I and Phase II biotransformation enzymes, as well as carcino- and teratogenicity (Okey 2007; Pohjanvirta and Tuomisto, 1994; Birnbaum and Tuomisto, 2000; Knerr and Schrenk, 2006). In humans, exposure to TCDD and other dioxins at levels somewhat higher than those currently prevailing in the general population of Western countries has epidemiologically been associated with tooth abnormalities and altered gender ratio in offspring (Alaluusua and Lukinmaa, 2006; Mocarelli et al., 2000). After occupational or accidental exposure to still higher concentrations, evidence for other ailments including cancer, porphyria, changes in serum biochemical variables, chloracne, cardiovascular dysfunction and impaired glucose tolerance (or even diabetes) has been obtained (Pelclová et al., 2006; Schecter et al., 2006; Sweeney and Mocarelli, 2000; Consonni et al., 2008).

Virtually all biological effects of dioxins are mediated by a cytosolic protein with functional reminiscence of the nuclear receptor superfamily: aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated transcription factor which structurally belongs to the bHLH/PAS proteins. In an inactive state, it is physically associated with a chaperone complex consisting of two molecules of heat shock protein-90, the immunophilin-like X-associated protein 2 (XAP2) and p23 (Petrulis and Perdew, 2002; Harper et al., 2006; Furness et al., 2007). Binding of ligand transforms the receptor disclosing its nuclear localization signal and the receptor moves into the nucleus detaching from the chaperones and heterodimerizing with a structurally related protein, AHR nuclear translocator (ARNT). The AHR/ARNT-dimer then

☆ Preliminary results were reported in 46th Annual Meeting of the Society of Toxicology, Charlotte, NC, USA, March 25–29, 2007 (The Toxicologist 2007, 96, 278 [abstr. 1344]).

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doi:10.1016/j.taap.2009.01.005

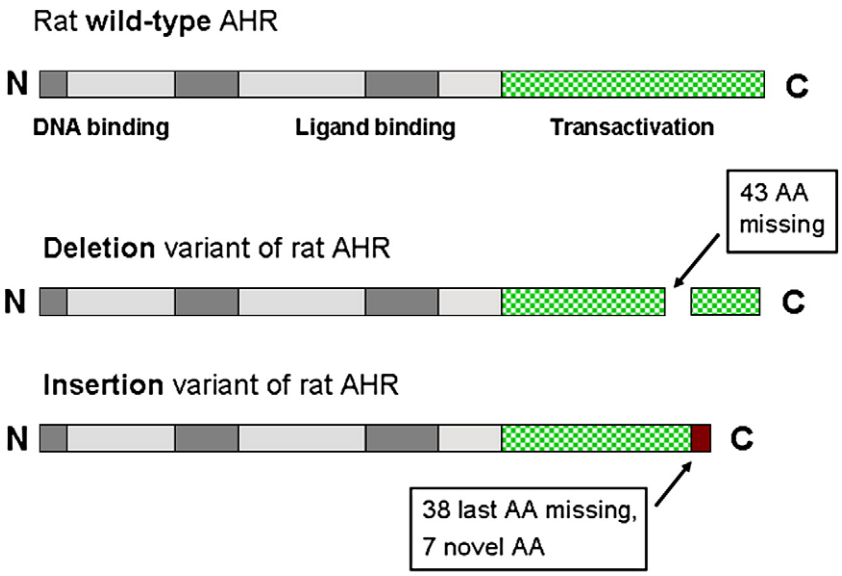

Fig. 1. Main features of rat AHR isoforms. TCDD-sensitive rat strains such as Sprague-Dawley and Long-Evans (*Turku/AB*) express almost exclusively the wild-type form of the receptor, whereas the highly TCDD-resistant Han/Wistar (*Kuopio*) strain predominantly expresses the insertion variant (ca. 85%) but also, to a smaller degree (ca. 15%), the deletion variant of the AHR (Moffat et al., 2007).

binds to the DNA at specific response elements (AHRE-I and AHRE-II) residing in promoter and/or enhancer regions of genes regulated by the AHR (Whitlock 1999; Boutros et al., 2004). Physically interacting with transcriptional co-activators, the DNA-bound AHR is capable of upregulating gene expression; the mechanisms of gene repression are still obscure (Furness et al., 2007). AHR is finally degraded by the ubiquitin-26S proteasome system (Ma, 2001).

The AHR protein has a modular structure. The domains responsible for DNA binding and heterodimerization are located towards the N-terminus, while the ligand-binding domain is in the mid-region. The C-terminal end harbors a large transactivation domain (TAD) which comprises several interacting subunits (Ma, 2001).

The acute toxicity of TCDD is characterized by exceptionally large variation in sensitivity among laboratory animals. About 1000-fold differences have been reported not only between species (with guinea pigs and hamsters representing the extremes in mammals) but also within species: the LD50 value for Long-Evans (*Turku/AB*; L-E) rats is 9.8 (females) or 17.7 μg/kg (males) whereas it is over 9600 μg/kg for both genders of Han/Wistar (*Kuopio*; H/W) rats (Pohjanvirta et al., 1993; Unkila et al., 1994; Pohjanvirta and Tuomisto, 1994). Diverse studies some 20 years ago already demonstrated that this rat strain difference does not arise from kinetic reasons (Pohjanvirta et al., 1990), appears to be specific to agents acting through the AHR (Unkila et al., 1992), is inherited as an autosomal dominant trait (Pohjanvirta 1990), and diminishes with reducing binding affinity to the AHR among dioxins (Pohjanvirta et al., 1993). Moreover, it is not universal: H/W rats show normal susceptibility to a number of biochemical and even toxic impacts of TCDD (dubbed type I effects) including induction of drug-metabolizing enzymes, changes in vitamin A status and thymic atrophy (Pohjanvirta and Tuomisto 1994 and references therein; Simanainen et al., 2002).

A logical explanation for this inter-strain divergence was provided by the exciting finding that the AHR of the TCDD-resistant H/W rats is remodeled: a point mutation at the first nucleotide of intron 10 results in altered splicing of AHR mRNA generating 3 variant mRNA species, a deletion (DEL) and two insertion variants (Pohjanvirta et al., 1998). As the insertions will translate identically, there are two

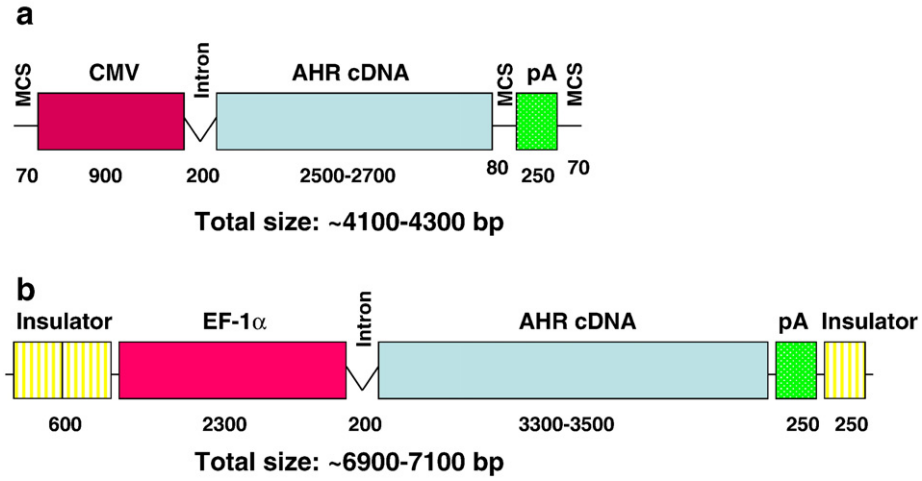

Fig. 2. Principal elements of the two sets of rat AHR expression constructs tested *in vivo*. (a) Initial constructs. (b) Final constructs. The initial constructs worked well *in vitro* but failed to be expressed *in vivo*. Thus, all the data presented in this paper are derived from the second-generation (final) constructs. The numbers below the schematic structures indicate the approximate sizes (in bp) of the subunits. Abbreviations: MCS, multiple cloning site; CMV, cytomegalovirus immediate-early enhancer/promoter region; pA, SV40 late polyadenylation signal; EF-1α, human eukaryotic translation elongation factor-1α1 promoter region.