Biochemical Pharmacology, Vol. 55, pp. 235–238, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00476-0

SHORT COMMUNICATION

Effect of Phenobarbital on Hepatic CYP1A1 and CYP1A2 in the *Ahr*-Null Mouse

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ABSTRACT. Studies have suggested that phenobarbital (PB) induces members of the CYP1A subfamily by both transcriptional and post-transcriptional mechanisms. Using the *Ahr*−/− mice, we examined the induction of CYP1A1 and CYP1A2 by PB. CYP1A2 mRNA and protein were induced by PB in the null mice, suggesting that CYP1A2 is regulated by PB by a mechanism independent of the aryl hydrocarbon receptor (AHR). In contrast, the regulation of CYP1A1 is highly dependent on the AHR. BIOCHEM PHARMACOL **55**;2:235–238, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. *Ahr*-null mouse; CYP1A1; CYP1A2; phenobarbital; regulation

Cytochromes P450 play an important role in the metabolism of a wide variety of endogenous and exogenous compounds. Some P450 genes are constitutively expressed, whereas others are induced upon exposure to certain foreign compounds [1–3]. CYP1A2 is constitutively expressed in mammalian liver, and its levels of expression may determine individual biological response to clinically important drugs [4] and potential susceptibility to a wide variety of toxic and carcinogenic compounds including aflatoxin B_1 and food-derived heterocyclic amines [5].

There is compelling evidence that induction of CYP1A1 is solely mediated by the AHR‖. In response to an agonist, the ligand–receptor complex translocates to the nucleus and forms a heterodimer with a nuclear transcription factor called the Arnt. The AHR–Arnt complex then binds to the AHR-response elements located in the 5′-flanking region of target genes [6]. Induction of CYP1A2 can be mediated by an AHR-dependent mechanism as well as an AHR-independent mechanism, depending on the nature of the inducer [7].

PB induces several hepatic cytochrome P450 forms, including those belonging to subfamilies 2B and 3A [8]. However, the cellular mechanism(s) through which PB exerts its inductive effects is unknown. Evidence exists that PB also induces members of the CYP1A subfamily, and these studies suggest both post-transcriptional (e.g. microsomal protein stabilization) as well as transcriptional (e.g. involving the formation of AHR–inducer complex) mechanisms, depending upon the system examined [9–15]. The purpose of the following study was to determine whether the regulation of the *Cyp1a* genes by PB is dependent on the AHR. Levels of CYP1A1 and CYP1A2 mRNAs and proteins were measured after administration of PB in the *Ahr* −/− knockout mouse model [16–18].

MATERIALS AND METHODS

Chemicals

Sodium phenobarbital was obtained from the Amend Drug & Chemical Co. BSA and EDTA were purchased from the Sigma Chemical Co. SDS and acrylamide (ultra pure grade) were obtained from Boehringer Mannheim, and ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) from Bio-Rad Laboratories.

Mice Treatment

Male C57BL/6N × 129/SV mice (4 months old) received either a daily intraperitoneal injection of sodium phenobarbital (80 mg/kg of body weight in sterile PBS) or PBS alone for 4 days and killed 24 hr later. The livers were quickly removed, snap frozen in liquid nitrogen, and stored at −80° until used. For each genotype, eight animals were used (N = 4 mice/treatment group).

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‖ *Abbreviations:* AHR, aryl hydrocarbon receptor; *Ahr*−/− mouse, homozygous for a disrupted *AHR* gene; *Ahr*+/− mouse, heterozygous for the *AHR* gene; *Ahr*+/+ mouse, wild-type mouse; Arnt, AHR nuclear translocator; PB, phenobarbital; and TCDD, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin.

Received 25 March 1997; accepted 7 August 1997.

Northern Hybridization

Total RNAs from control and PB-treated mice were isolated from liver tissue using the guanidinium thiocyanate extraction method followed by cesium trifluoroacetic acid centrifugation as described [19]. Twenty micrograms of purified RNA was subjected to electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde. The gels were washed with deionized water, stained with ethidium bromide, destained with deionized water for several hours, soaked in 20× SSC (3 M NaCl and 0.15 M sodium citrate, pH 7.0) for 30 min, and then transferred to GeneScreen Plus (Dupont) nylon membranes using 20× SSC. The filters were baked at 80° for 2 hr under vacuum. The membranes were prehybridized in a solution of 1% BSA, 7% SDS, 0.5 M sodium phosphate buffer, pH 7.0, and 1 mM EDTA for 3 hr at 65°. Fragments of mouse CYP1A2 (1.5 kb) and CYP1A1 cDNAs (3 kb) that represent unique regions of the 3′ end of the mRNAs [20] were labeled by random primers using [^{32}P]dCTP (Pharmacia Biotech). These probes were demonstrated previously to be specific for CYP1A1 and CYP1A2 mRNAs [21]. RNA hybridization was carried out at 65° for 20 hr, and membranes were washed first with 2× SSC/0.5% SDS (30 min, 65°) and then with 0.1× SSC/0.5% SDS (65°, 3 hr). Filters were air-dried and subjected to autoradiography (Kodak X-OMAT AR-5 film) with an intensifying screen. After exposure to film, the blot was boiled for 5 min in 0.1× SSC/0.5% SDS to remove the cDNA probes and rehybridized with a mouse β-actin cDNA to confirm that all lanes had equivalent amounts of undegraded RNA.

Western Blot

Hepatic microsomal proteins were prepared as described [22]. Microsomes were resuspended in glycerol phosphate buffer (25% 200 mM KPO_4, 40% glycerol, 35% 1.15 KCl), frozen, and later used for western blots. Aliquots from stored microsomes were thawed on ice, and total microsomal protein concentrations were determined by the bicinchoninic acid (BCA) method (Pierce Chemical Co.). Liver microsomes (10 μg) were subjected to electrophoresis on SDS–PAGE, transferred onto nitrocellulose membranes, and probed with monoclonal antibodies (Mab 22 specific for CYP1A2 [23] or Mab 1-36-1 specific for CYP1A1 [24]). Monoclonal antibody binding was detected using alkaline phosphatase-conjugated goat anti-mouse antibodies or horseradish peroxidase conjugated goat anti-mouse antibodies with an enhanced chemiluminescence substrate (Kirkegaard & Perry Laboratories Inc.).

RESULTS AND DISCUSSION

The expression of CYP1A2 mRNA before and after exposure to PB is shown in Fig. 1. CYP1A2 mRNA was extremely low in control *Ahr*−/− mice, in agreement with previous studies [7, 16]. Administration of PB resulted in an increase in CYP1A2 mRNA in the *Ahr*−/− mice (Fig. 1). CYP1A2 mRNA from PB-treated *Ahr*+/− mice was also increased (Fig. 1). These data suggest that the cellular mechanism(s) responsible for the increase in CYP1A2 mRNA expression is independent of the AHR.

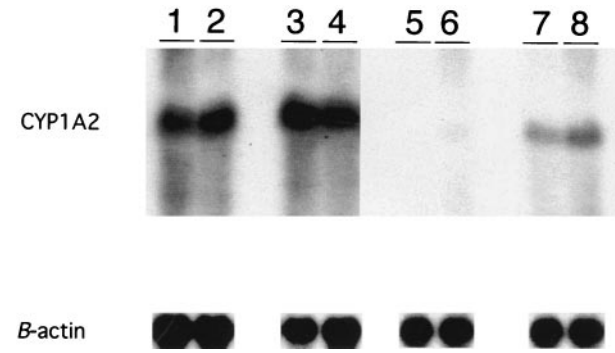

FIG. 1. PB induction of CYP1A2 mRNA in *Ahr*+/− and *Ahr*−/− mice. Twenty micrograms of total liver RNA was loaded in each lane. Lanes 1 and 2, *Ahr*+/− liver RNA from control mice (PBS); lanes 3 and 4, *Ahr*+/− liver RNA from PB-treated mice; lanes 5 and 6, *Ahr*−/− RNA from control mice (PBS); and lanes 7 and 8, *Ahr*−/− liver RNA from PB-treated mice. Four animals were used per group; for comparative purposes, two mice from each group are shown. β-Actin is shown as a control for RNA loading.

The expression of CYP1A1 mRNA was also examined and found to be undetectable after PB treatment in *Ahr*+/+, *Ahr*+/−, and *Ahr*−/− mice (Fig. 2). This is in contrast to earlier studies showing less than 3-fold induction of CYP1A1 by PB [10–16]. The reasons for the differences in the results of our study and those of others are not known at the present time but may involve route and time of inducer administration and specificities of antibodies and cDNA probes employed for measurements of CYP1A1. Our observation is in support of two

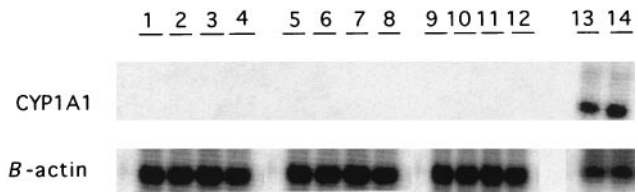

FIG. 2. PB induction of CYP1A1 mRNA in *Ahr*+/+, *Ahr*+/−, and *Ahr*−/− mice. Twenty micrograms of total liver RNA was loaded in each lane. Lanes 1 and 2, *Ahr*+/+ liver RNA from control mice (PBS); lanes 3 and 4, *Ahr*+/+ liver RNA from PB-treated mice; lanes 5 and 6, *Ahr*+/− liver RNA from control mice (PBS); lanes 7 and 8, *Ahr*+/− liver RNA from PB-treated mice; lanes 9 and 10, *Ahr*−/− RNA from control mice (PBS); lanes 11 and 12, *Ahr*−/− liver RNA from PB-treated mice; and lanes 13 and 14, liver RNA from TCDD and benzopyrene-treated *Ahr*+/+ mice used as a positive control. Four animals were used per group. For comparative purposes, two mice from each group are shown. β-Actin is shown as a control for RNA loading.

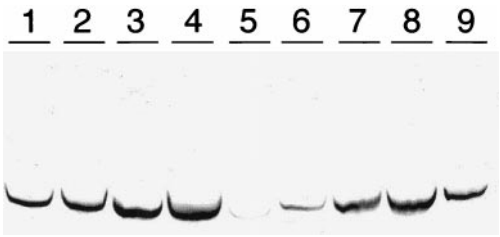

FIG. 3. Western immunoblotting of CYP1A2 protein in *Ahr*+/− and *Ahr*−/− mice. Ten micrograms of microsomal protein was loaded in each lane. Lanes 1 and 2, *Ahr*+/− liver microsomes from control mice (PBS); lanes 3 and 4, *Ahr*+/− liver microsomes from PB-treated mice; lanes 5 and 6, *Ahr*−/− liver microsomes from control mice; and lanes 7 and 8, *Ahr*−/− liver microsomes from PB-treated mice. Lane 9, positive control mouse CYP1A2 protein expressed in vaccinia virus. Four animals were used per group; for comparative purposes, two mice from each group are shown.

recent studies in which PB did not affect CYP1A1 mRNA [9, 25].

The induction of CYP1A2 was investigated further by immunoblotting with monoclonal antibody highly specific for CYP1A2 protein (Mab 22). The constitutive levels of CYP1A2 were decreased in *Ahr*−/− mice as compared with control mice (Fig. 3), in agreement with previous data [16, 18]. However, after PB treatment, microsomal CYP1A2 apoprotein in the *Ahr*−/− animals was induced markedly when compared with control mice (Fig. 3). This increase in the amount of protein correlates with the increase in mRNA for CYP1A2 (Fig. 1). Immunoblot analysis was also done with Mab 1-36-1 to determine the amount of CYP1A1 apoprotein in response to PB. CYP1A1 apoprotein was undetectable after PB administration (data not shown). Therefore, our data suggest that CYP1A2 is regulated by PB independently of the AHR, whereas the regulation of CYP1A1 is unaffected by this drug.

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