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Induction of nuclear transcription factors, cytochrome P450 monooxygenases, and glutathione *S*-transferase alpha gene expression in Aroclor 1254-treated rat hepatocyte cultures

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Abstract

Aroclor 1254 is a complex mixture of polychlorinated biphenyls and is well known for its potency to induce drug-metabolising enzymes, but little is known about its ability to modulate gene expression of transcription factors, which code for proteins that bind to the regulatory elements of DNA and facilitate transcriptional activation. We therefore investigated the gene expression of the liver-specific transcription factors CCAAT/enhancer-binding protein α (c/EBPα), hepatic nuclear factor (HNF) 1 and 4, and major cytochrome P450 (CYP) isozymes in addition to glutathione *S*-transferase alpha 2 (GSTA-2) in cultures of primary rat hepatocytes. We found highly significant and dose-dependent increases of c/EBPα (up to 62-fold), HNF-1 (up to 7-fold), HNF-4 (up to 8-fold), and 50- and 4-fold inductions of GSTA-2 and CYP monooxygenases, respectively. Based on the ethoxyresorufin-*O*-deethylase assay, the gene expression and enzyme activity for CYP1A1 were in good agreement, but for other CYP isozymes similar correlations could not be obtained. In conclusion, the simultaneous induction of liver-specific TFs and of several detoxifying enzymes may point to a coordinate genomic response in cultures of rat hepatocytes upon treatment with Aroclor 1254.

Keywords: Transcription factor; CYP monooxygenase; Glutathione *S*-transferase; Gene expression; Protein activity; Hepatocytes

1. Introduction

For transcriptional activation, binding of transcription factors to distinct regions of DNA (promoter and enhancer regions) is of paramount importance [1]. Some of these TFs are tissue-specific, but others are required for gene expression in a variety of tissues. The protein/DNA interaction of TFs can be studied by the *in vivo* footprinting procedure [2], and suitable oligonucleotide probes can be designed to permit PCR-based amplification of mRNA transcripts [3]. Gene expression of downstream target genes regulated by these factors can be studied as well.

There are several TFs important for the onset of liver-specific gene expression in committed stem cells leading to hepatocellular differentiation [4]; in the case of the rat, and shortly after implantation, HNF-3α as well as HNF-3β and HNF-γ are expressed to commit cells towards hepatocyte development [5]. In late stage embryos, C/EBPα, β, and γ as well as HNF-1α and HNF-1β are additionally expressed. This leads to the formation of hepatoblasts and the differentiation of progenitors shortly after birth [6]. Further TFs are usually recruited in mature hepatocytes and this is followed by liver-specific gene expression, such as the albumin gene [7], cytochrome P450-dependent monooxygenases [8], UDP glucuronyl transferases [9], and glutathione *S*-transferases [10]. The latter genes/enzymes play an important role in the endogenous metabolism of fatty acids, bile acids, vitamin D, and steroids [11–13] and are of critical importance in the metabolism of drugs and the detoxification of xenobiotics.

The molecular mechanisms leading to enhanced gene expression of CYP monooxygenase genes remain largely uncertain, although information on the transcriptional activation of the aryl hydrocarbon (*Ah*) gene battery is sufficient to understand the role of the Ah TF in facilitating gene

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Abbreviations: Ah, aryl hydrocarbon; c/EBP, CCAAT/enhancer-binding protein; cDNA, complementary DNA CYP, cytochrome P450; EROD, ethoxyresorufin-*O*-deethylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSTA, glutathione *S*-transferase alpha; HNF, hepatic nuclear factor; RT–PCR, reverse transcription–polymerase chain reaction; and TF, transcription factor.

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expression of CYP1A1 on the basis of a basic helix–loop–helix interaction with the CYP1A1 DNA [14].

We previously reported the concomitant P450 isozyme and proto-oncogene protein and mRNA expression in rat liver upon treatment with Aroclor 1254 [15,16]. In the present study, we wanted to investigate the ability of this polychlorinated biphenyl (PCB) mixture to induce gene expression of transcription factors HNF-1, HNF-4, and c/EBPα as well as of major CYP isozymes in cultures of primary rat hepatocytes [8, 17–24]. Overall, this should lead to a better understanding of the simultaneous gene expression of liver-specific TFs and xenobiotic-metabolising enzymes regulated by these factors.

2. Materials and methods

2.1. Animals

All animal procedures described in this report were approved by the local authorities. Male Sprague–Dawley rats weighing 211 $\pm$ 11 g were obtained from Charles River. Food and water was given *ad lib*. Rats were anaesthetised with Ketamin (anaesthetic) and xylazin hydrochloride (muscle relaxant) with 0.1 mL of Ketamin per 100 g body weight and 0.05 mL of xylazin hydrochloride per 100 g body weight. In addition, 2000 IU heparin were given intraperitoneally prior to surgery.

2.2. Isolation and cultivation of hepatocytes

For isolation of hepatocytes, a modified method described by Hansen *et al*. [9] was used. In brief, after midline incision the portal vein was cannulated and the liver was first perfused *in situ* with 100 mL calcium-free Krebs-Ringer buffer (KRB) for 10 min, then with 100 mL KRB and EDTA (1 mmol/L). Thereafter, the liver was perfused for 8–10 min with KRB supplemented with collagenase type IV (Worthington) and 0.5 mM calcium chloride (Sigma). Following perfusion, the liver capsule was gently removed, and the dissolved liver tissue was filtrated through a nylon mesh (pore size, 100 μm) and washed twice with the washing buffer (1000 mL Hanks balanced salt solution (PAA Laboratories) supplemented with 2.4 g HEPES (Sigma) and 2 g bovine serum albumin (Sigma). The cell pellet was resuspended in Williams' E medium (Biochrom) supplemented with 5% foetal bovine serum (Biochrom), 9.6 μg/mL of prednisolone, 0.014 μg/mL of glucagon (Novo), 0.16 U/mL of insulin (Hoechst), 200 U/mL of penicillin, and 200 U/mL of streptomycin (GIBCO). Hepatocytes were counted in a hemocytometer in the presence of 0.04% trypan blue solution. Two million hepatocytes per dish were cultured between two layers of collagen in a modification of the method of Dunn *et al*. [25] and were used for further experiments 48 hr post isolation.

2.3. Induction of gene expression

Induction of gene expression was done with Aroclor 1254 (dissolved in dimethylsulphoxide), a complex chemical mixture (>80) of individual polychlorinated biphenyl (PCB) isoforms and congeners at a concentration of 10 μM for 48 hr. Appropriate controls received the vehicle only, e.g. dimethylsulphoxide.

2.4. RNA and cDNA

RNA was isolated from hepatocytes using the Spin or Vacuum Total RNA Isolation System (Promega) according to the manufacturer's recommendation. The quality of isolated RNA was checked using a 1.0% agarose gel. Four micrograms of total RNA from each sample was used for reverse transcription. RNA and random primer (Roche) were preheated for 10 min at 70°. Then, 1× reverse transcriptase–avian myeloblastosis virus (RT–AMV) buffer, dNTPs (Roche, 1.0 nM), 40 U RNAse inhibitor (Stratagene), and 20 U RT–AMV (Promega) were added and diethyl pyrocarbonate-treated water was added to a final volume of 20 μL. Then, reverse transcription was carried out for 60 min at 42° and was stopped by heating to 95° for 5 min. The resulting cDNA was frozen at −20° until further experimentation.

2.5. Thermocycler RT–PCR

For the PCR amplification of cDNA, a 25-μL reaction mixture was prepared containing 10× polymerase reaction buffer, 3 mM $MgCl_2$, 0.4 nM dNTPs (Roche), 400 nM concentration of the 3′ and 5′ specific primer (synthesised by GIBCO), 1 U Taq polymerase (Roche), and 1 μL of cDNA. PCR reactions were carried out in a thermocycler (T3, Biometra) using the following melting, annealing, and extension cycling conditions: denaturation for 30 sec at 94°, annealing for 60 sec at 57°, and extension for 60 sec at 72° (29 cycles) for CYP isoforms and GAPDH. PCR conditions for c/EBPα were: denaturation for 45 sec at 94°, annealing for 45 sec at 57°, and extension for 60 sec at 72° for 30 cycles. PCR conditions for HNF-1 were: denaturation for 45 sec at 94°, annealing for 60 sec at 56°, and extension for 120 sec at 72° for 29 cycles. PCR conditions for HNF-4 and GSTA-2 were: denaturation for 45 sec at 94°, annealing for 45 sec at 57°, and extension for 60 sec at 72° for 27 cycles. Each PCR started with a 2-min denaturation step and ended with a 7-min elongation step. Primer sequences are shown in Table 1. PCR products were separated using a 1.8% agarose gel and were stained with ethidium bromide and photographed on a transilluminator.

2.6. Real-time semiquantitative RT–PCR

cDNA was diluted 1:10 with nuclease-free water and 1 μL of the diluted cDNA was added to the Lightcycler-

Table 1
Oligonucleotide primers used in the RT–PCR

Gene (Accession number)	Forward primer (5′ · · · 3′)	Reverse primer (5′ · · · 3′)	Product length (bp)	Elongation time (sec)	Melting point (°)
CYP1A1 (X00469)	CTGGTTCTGGAT ACCCAGCTG	CCTAGGGTTGGT TACCAGG	331	14	86.2
CYP1A2 (X01031)	GTCACCTCAGGG AATGCTGTG	GTTGACAATCTT CTCCTGAGG	236	10	84.6
CYP2B1/2 (L00316)	GAGTTCTTCTCT GGGTTCCTG	ACTGTGGGTCAT GGAGAGCTG	549	23	86.1
CYP2C11 (U33173)	CTGCTGCTGCTG AAACACGTG	GGATGACAGCGA TACTATCAC	248	11	84.7
CYP2E1 (M20131)	CTCCTCGTCATA TCCATCTG	GCAGCCAATCAG AAATGTGG	473	20	87.5
CYP3A1 (X64401)	ATCCGATATGGA GATCAC	GAAGAAGTCCTT GCTTGC	579	24	85.5
CYP4A1 (M57718)	GGTGACAAAGA ACTACAGC	AGAGGAGTCTTG ACCTGCCAG	344	15	85.4
GSTA-2 (K00136)	CAGGAGTGGAGT TTGAAGAGA	AGAGGGAAAGA GGTCAGAAG	443	19	89.1
GAPDH (X02231)	GGCCAAGGTCAT CCATGA	TCAGTGTAGCCC AGGATG	353	16	90.0
c/EBPα (X12752)	CCCGTGCCCAGC CCTCAT	CACCTTCTGCTG CGTCTCCAC	263	11	88.3
HNF-1 (X54423)	AGGGCGGACTG ATTGAAGAG	CACCTCAGGCTT GTGGCTGTACAG	970	39	92.3
HNF-4 (X57133)	GCCTGCCTCAAA GCCATCAT	GACCCTCCAAGC AGCATCTC	274	12	89.0

Mastermix (0.5 μM of specific primer, 3 mM $MgCl_2$, and 2 μL Master-SYBR-Green, Roche). This reaction mixture was filled up with water to a final volume of 20 μL. PCR reactions were carried out in a real-time PCR cycler (Lightcycler, Roche). After an initial denaturation step at 95° for 30 sec, the PCR reaction was initiated with an annealing temperature of 56–57° for 6 sec followed by an extension phase at 72° for 10–39 sec (see Table 1) and a denaturation cycle at 95° for 1 sec. The PCR reaction was completed after a total of 35–45 cycles and at the end of each extension phase, fluorescence was observed and used for quantitative purposes.

A melting point analysis was carried out by heating the amplicon from 65° to 95°, and a characteristic melting point curve was obtained for each product. In addition, a serial dilution experiment was carried out (N = 3) with cDNA produced from hepatocyte total RNA using a 1:10 dilution regiment to estimate the mRNA expression level of the specific genes. Control samples contained water instead of cDNA, and it should be noted that oligonucleotide dimers were produced in some of the PCR cocktails, but could easily be distinguished by the melting point analysis.

2.7. EROD assay

This assay was carried out essentially as described by Grant *et al*. [26]. Control cells and cells treated with Aroclor 1254 were incubated with 10 μM EROD and 10 μM dicumerol (Sigma), and the supernatants were removed from the cultures at time points 0, 1, 2, 3, and 4 hr. Appropriate aliquots were taken from the samples and treated with ammonium acetate (200 mM, pH 4.5) and with 100 U/mL of β-glucuronidase (Sigma) and incubated at 37° overnight. Five hundred microlitres of glycine buffer (pH 10.3) was added to the sample containing β-glucuronidase, and subsequent fluorometric analysis was carried out on a spectrofluorophotometer (RF-1501, Shimadzu). The other aliquot was treated with ammonium acetate buffer (250 μL) and glycine buffer (500 μL), but without β-glucuronidase. The fluorometric analysis of the resultant product resorufin was done at an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Calibration of the system was performed with appropriate standards at a concentration range of up to 100 nM.

2.8. Testosterone assay

Testosterone has proven most useful as a substrate for distinguishing multiple CYP isoenzymes due to the characteristic metabolite profiles obtained. The different regio- and stereoselective hydroxylation reactions have been associated with single CYP isozymes. To estimate the activity of individual CYP isozymes, hepatocyte cultures are exposed to culture medium containing 100 μM testosterone. Finasteride (25 μM) is added as a 5α-reductase inhibitor because of its contribution to the formation of certain testosterone metabolites.

Testosterone and its metabolites were analysed here by HPLC according to Arlotto *et al*. [27], with slight modifications as described below. 11-α-Hydroxyprogesterone was used as an internal standard for the quantitative determination of testosterone and its metabolites using the following procedures. One microgram of 11-α-hydroxyprogesterone was added to 1 mL of cell culture supernatant. Following addition of 100 μL isopropanol, the samples were extracted with 5 mL ethyl acetate by gentle shaking for 20 min. Extracts were evaporated to dryness and the residues reconstituted in 100 μL of the mobile phase (water/methanol/acetonitrile/, 60/25/15, v/v/v), after which 80 μL of the sample was injected onto the HPLC system (Hewlett-Packard HP1100). The mobile phase was delivered at a flow rate of 1 mL/min using the HP 1100 Quaternary Pump. Chromatographic separation of cytochrome P450-produced testosterone metabolites was carried out at 30° on a C18 Nucleosil column with the dimensions 250 $\times$ 4 mm and a particle size of 5 μm (Macherey–Nagel). Testosterone and metabolites were detected by UV absorption at 238 nm using synthetic reference standards. The mobile phase consisted of water (solvent A), methanol (solvent B), and acetonitrile (solvent C), and analysis was initiated with an isocratic elution of 60% A, 25% B, and 15% C for 12 min, followed by 45% A, 40% B, and 15% C for 3 min, and finally 45% A, 45% B, and 10% C thereafter. The total run time was 30 min per sample. Control cells and cells treated with Aroclor 1254 (10.0 μM, 48 hr) were incubated with 100 μM testosterone and 25 μM finasteride and were analysed at time points 0, 1, 2, 3, and 4 hr.

2.9. Statistical analysis

The Wilcoxon signed rank test was used and differences were considered significant at $P < 0.05$.

3. Results

3.1. Hepatocyte culture

Immediately after seeding, rat hepatocytes were spherical in shape. Following 24 hr in culture, the majority of the cells appeared polygonal and formed confluent monolayers. Non-adherent cells were removed and the second collagen layer was applied as described in the Materials and Methods section. As cells have usually recovered and returned to normal by day 3 in culture, hepatocytes were exposed to 0.1, 1.0, and 10.0 μM Aroclor 1254 72 hr postisolation and were harvested at day five in culture, i.e. after 48 hr of treatment.

3.2. Gene expression of nuclear transcription factors

Semiquantitative RT–PCR was carried out with the Lightcycler (Roche) as described in the Materials and Methods section. Results for control rat hepatocyte cultures were set to 100% and, as shown in Fig. 1, a dose-dependent but not linear increase in the expression of the *c/EBPα* gene was observed, the level being approximately 10-, 52-, and 61-fold at 0.1, 1.0, and 10.0 μM Aroclor 1254, respectively. When compared to controls, a dose-dependent and linear increase in the expression of HNF-1 was observed, with 2-, 4-, and 6-fold increases at the 0.1, 1.0, and 10.0 μM Aroclor 1254 dose levels. Similar to this increase in HNF-1, a dose-dependent rise in the gene expression of HNF-4 was observed, the level being approximately 2-, 3-, and 8-fold, respectively, when compared to controls.

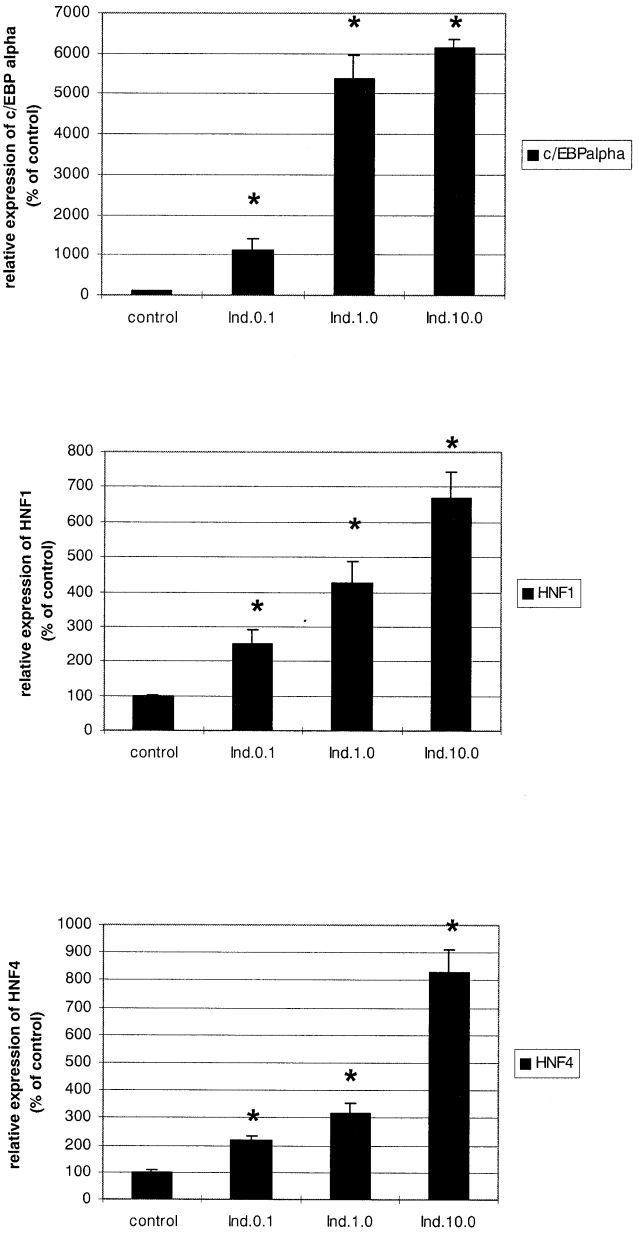

Fig. 1. Semiquantitative RT–PCR gene expression of c/EBPα, HNF-1, and HNF-4 in rat hepatocyte cultures upon treatment with ascending doses of Aroclor 1254. Data represent means $\pm$ SEM of N = 3 different cultures with approx. 2 million cells per culture dish. *, $P = < 0.05$. Ind, treated with 0.1 μM Aroclor 1254.

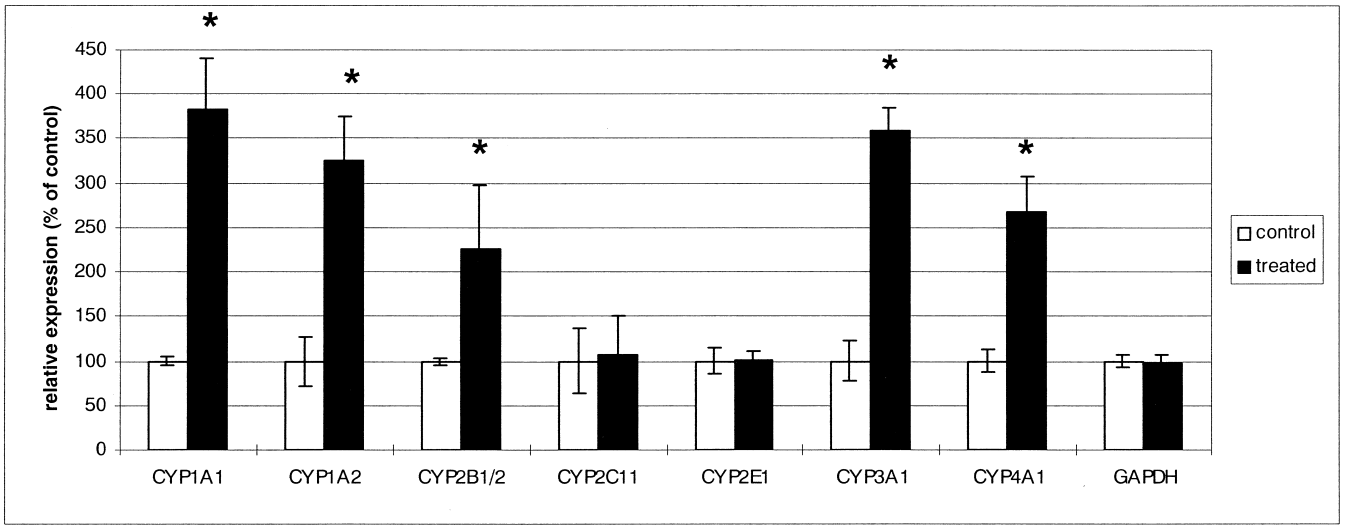

Fig. 2. Semiquantitative RT–PCR gene expression of individual CYP monooxygenase genes in rat hepatocyte cultures upon treatment with Aroclor 1254 (10 μM). Data represent means ± SEM of N = 3 different cultures with approx. 2 million cells per culture dish. *, $P = < 0.05$.

3.3. *Expression of* CYP *genes*

When compared to controls, approximately 4- and 2-fold increases were observed in the mRNA expression of *CYP1A1*, *CYP1A2*, and *CYP3A1* (4-fold) and *CYP2B1/2* and *CYP4A1* (2-fold), whereas for all other genes, e.g. *CYP2C11*, *CYP2E1*, and the housekeeping gene *GAPDH*, no significant changes in mRNA expression were evident (Fig. 2).

3.4. *Gene expression of* GSTA-2

The gene expression of *GSTA-2* is shown in Fig. 3. A non-linear increase in the mRNA expression of this particular gene was noted with an ascending dose level, the increase being approximately 20-, 22-, and 50-fold, respectively. A summary of all PCR-amplified genes visualised by ethidium bromide-stained agarose gels is shown in Fig. 4.

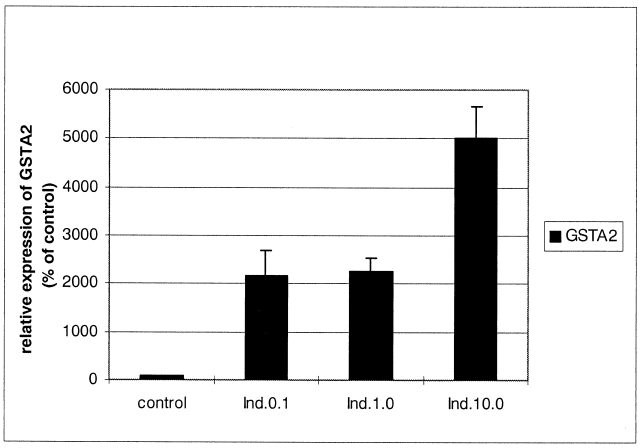

Fig. 3. Semiquantitative RT–PCR gene expression of GSTA-2 in rat hepatocyte cultures upon treatment with ascending doses of Aroclor 1254. Data represent means ± SEM of N = 3 different cultures with approx. 2 million cells per culture dish. *, $P = < 0.05$. Ind, treated with 0.1 μM Aroclor 1254.

3.5. *Protein activity* CYP1A1

Rat hepatocytes were incubated with the *CYP1A1* substrate ethoxyresorufin as described in the Materials and Methods section. As shown in Fig. 5A, the amount of resorufin produced in these cultures declined steadily with time and was close to control values after 7 hr of incubation. When, however, these samples were treated with the enzyme β-glucuronidase, a linear and dose-dependent increase in EROD activity could be observed, with an approximate 12-fold increase in activity at the highest dose level (Fig. 5B).

3.6. *Protein activity of multiple CYP isozymes (testosterone assay)*

Incubation of rat hepatocytes with testosterone produced the following results. With the exception of 7-alpha-testosterone, there was a time but not dose-dependent increase in the formation of testosterone metabolites, as shown in Fig. 6. Noticeably, treatment with Aroclor 1254 at all dose levels did not alter the testosterone metabolite pattern and/or enhanced production of a particular hydroxylation product. The rate of metabolite formation was in the order of androstendione > 16-alpha-testosterone > 2-alpha-testosterone > 6-beta-testosterone > 16-beta-testosterone > 7-alpha-testosterone (see Fig. 6).

4. Discussion

We investigated the gene expression of liver-enriched transcription factors and major CYP isozymes in cultures of

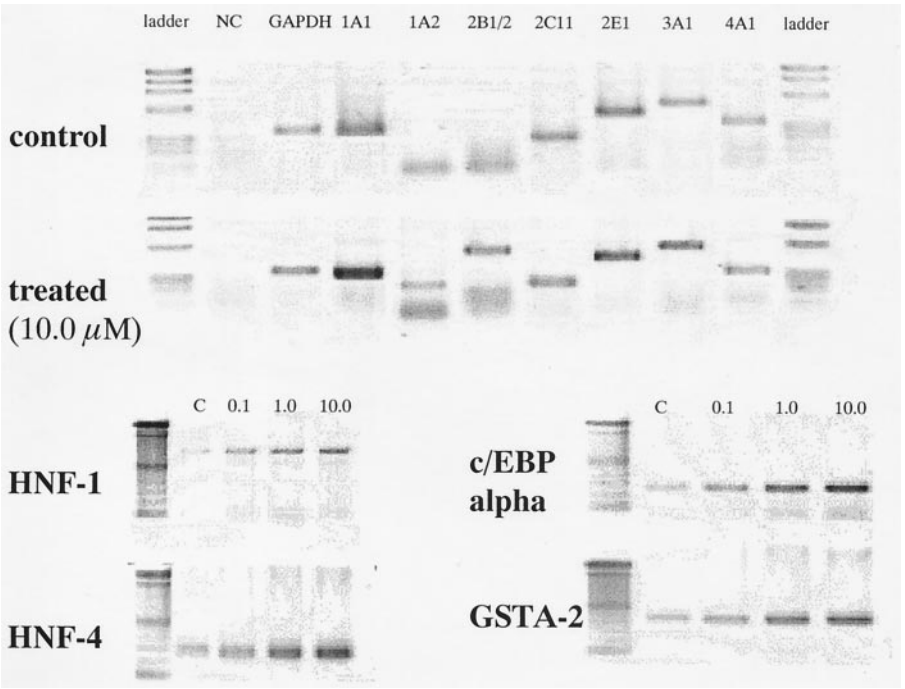

Fig. 4. mRNA expression of transcription factors and target genes of hepatocyte cultures in ethidium bromide-stained agarose gels. (NC, negative control; C, control).

primary rat hepatocytes. In the case of CYP1A1, transcript levels and enzyme activities correlated well, but this was not seen with any of the other CYP isozymes investigated in this study (see testosterone hydroxylation data in Fig. 6). The CYP1A1 substrate 7-ethoxyresorufin is also subject to extensive glucuronidation [26], and thus treatment of rat hepatocyte culture media with β-glucuronidase became necessary. This allowed an estimate of overall product release (see Fig. 5B), with a dose- but not time-dependent increase in product formation being observed in cultures of rat hepatocytes (see Fig. 5B). Aroclor 1254 resulted in very significant dose-dependent inductions of c/EBPα (up to 62-fold), HNF-1 (up to 7-fold), HNF-4 (up to 8-fold), and GSTA-2 (50-fold), but most CYP isozymes were only marginally increased at the mRNA and protein level.

RT–PCR analysis sometimes yielded more than one amplicon, but non-specific product formation could be distinguished on the basis of molecular size (see Fig. 4, where the lower bands for CYP1A2 and CYP2B1/2 are non-specific); in the case of the Lightcycler, primer–dimer formation and/or other non-specific product formation could be detected by the characteristic melting curves. Quantification of rare mRNA transcripts (e.g. CYP1A2 and CYP2B1/2) is difficult, particularly if standard PCR procedures are used. We therefore employed real-time PCR and followed the cDNA synthesis during the amplification procedure. This permitted a good estimation of mRNA transcripts for genes that are normally down-regulated. In the case of *CYP1A2* and *CYP2B1/2* and after 29 cycles of cDNA amplification, no mRNA copies could be determined when visualised on ethidium bromide-stained agarose gels. In contrast, the Lightcycler offers the unique opportunity to determine rare gene copies in an accurate fashion, and thus the induction factors determined for the CYP monooxygenases are less than expected when Fig. 2 (real-time PCR) and Fig. 4 (ethidium bromide-stained agarose gel) are compared.

Rather than studying a time–course at one particular Aroclor 1254 dose, we wanted to investigate the dose–response relationship. Indeed, we show a dose-dependent mRNA induction for all TFs, albeit at different levels. This points to a selective responsiveness when individual liver-enriched TFs are compared. In this study, all strongly induced genes are members of the aryl hydrocarbon-responsible gene family (*CYP1A1*, *CYP1A2*, and *GSTA-2*) and are known to be induced by Aroclor 1254 [9,15,16]. The Ah receptor is a transcription factor which forms with its nuclear counterpart ARNT (Ah receptor nuclear translocator protein) a basic helix–loop–helix motif to facilitate gene expression of the Ah receptor-responsible gene family [28]. Thus, the strong induction of *GSTA-2* and *CYP1A1* can be explained, at least in part, in terms of Ah receptor-mediated transcriptional activation, whereas for the other CYP isozymes, the molecular mechanisms leading to enhanced gene expression remain largely unknown. Therefore, no firm conclusions can be drawn on the basis of the data on *CYP* gene expression and testosterone metabolism.

Our study provides new insight into the simultaneous gene expression of nuclear transcription factors and downstream target genes, and evidence is presented to suggest a differential genomic response when individual transcription factors are studied. Presumably, the high expression of c/EBPα is linked to its ubiquitous role in transcriptional

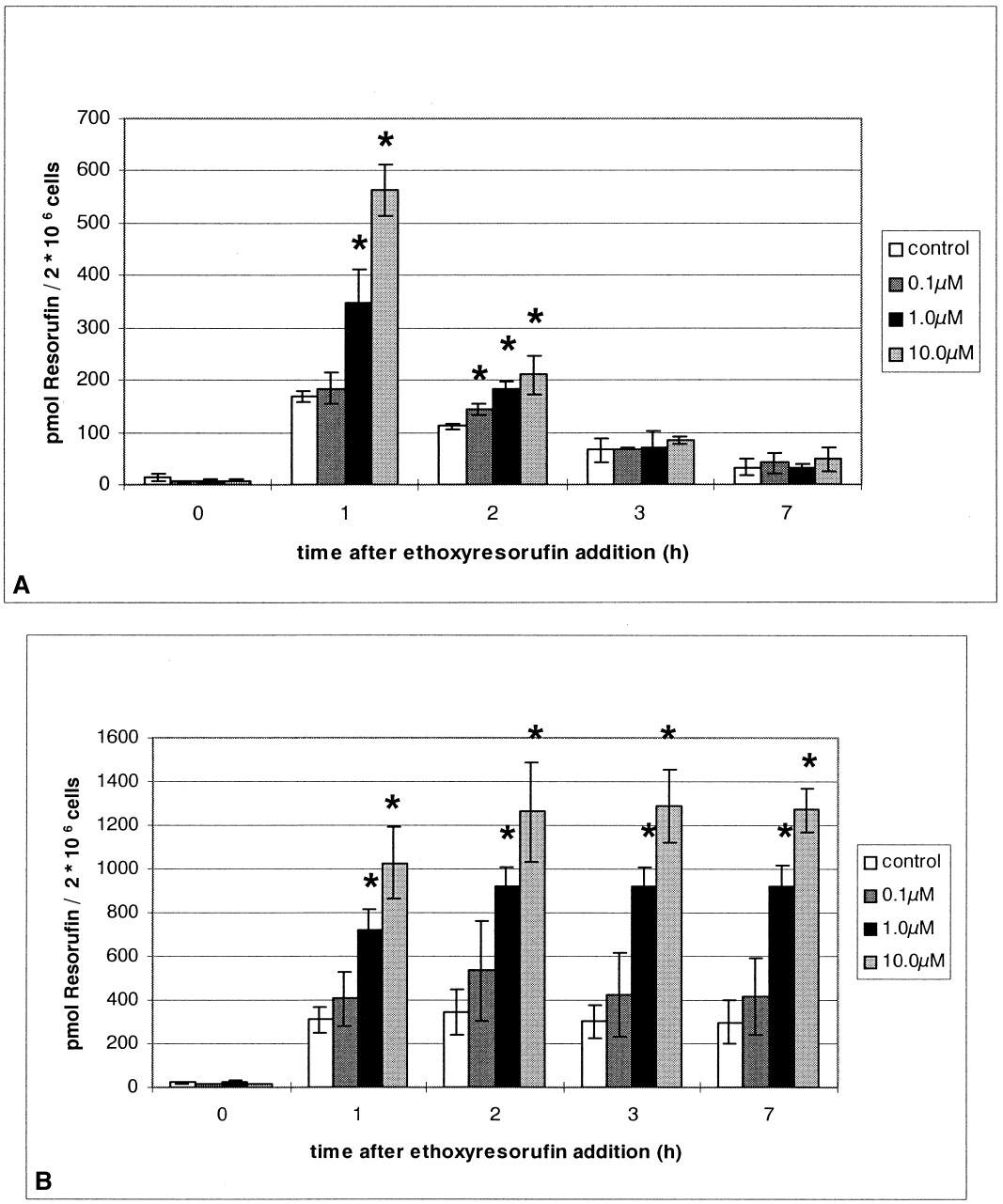

Fig. 5. (A) CYP1A1 protein activity in rat hepatocyte cultures upon treatment with ascending doses of Aroclor 1254. Data represent means ± SEM of N = 3 different cultures with approx. 2 million cells per culture dish. *, $P = < 0.05$. (B) CYP1A1 protein activity in rat hepatocyte cultures upon treatment with ascending doses of Aroclor 1254 after incubation with β-glucuronidase. Data represent means ± SEM of N = 3 different cultures with approx. 2 million cells per culture dish. *, $P = < 0.05$.

activation of many different genes, and this TF is also involved in the gene regulation of most CYP isozymes [17,19,20,22,23,29]. It is intriguing that expression of transcription factor genes can be highly modulated by Aroclor 1254, but further experiments are needed to draw firm conclusions regarding the mechanisms underlying these events.

Although there is limited information on the genome-wide number of target genes regulated by individual transcription factors, it has been shown that *CYP1A2*, *CYP2C13*, and *CYP2E1* have common binding sites for HNF-1 [18,20,28] and similarly, that HNF-4 regulates, at least in part, *CYP2A4*, *CYP2D6*, *CYP2C9*, *CYP2C13*, and *CYP3A1* [21,24,30–32]. With the exception of *CYP4A1*, binding sites for c/EBPα are common to all CYP isozymes investigated in this study [17,19,20,22,23,29] and transcription factors may play a dual role in target gene expression as shown in the case of HNF-4, where expression of *CYP2C13* can be negatively regulated by this particular transcription factor [29]. Furthermore, in co-transfection studies, no co-

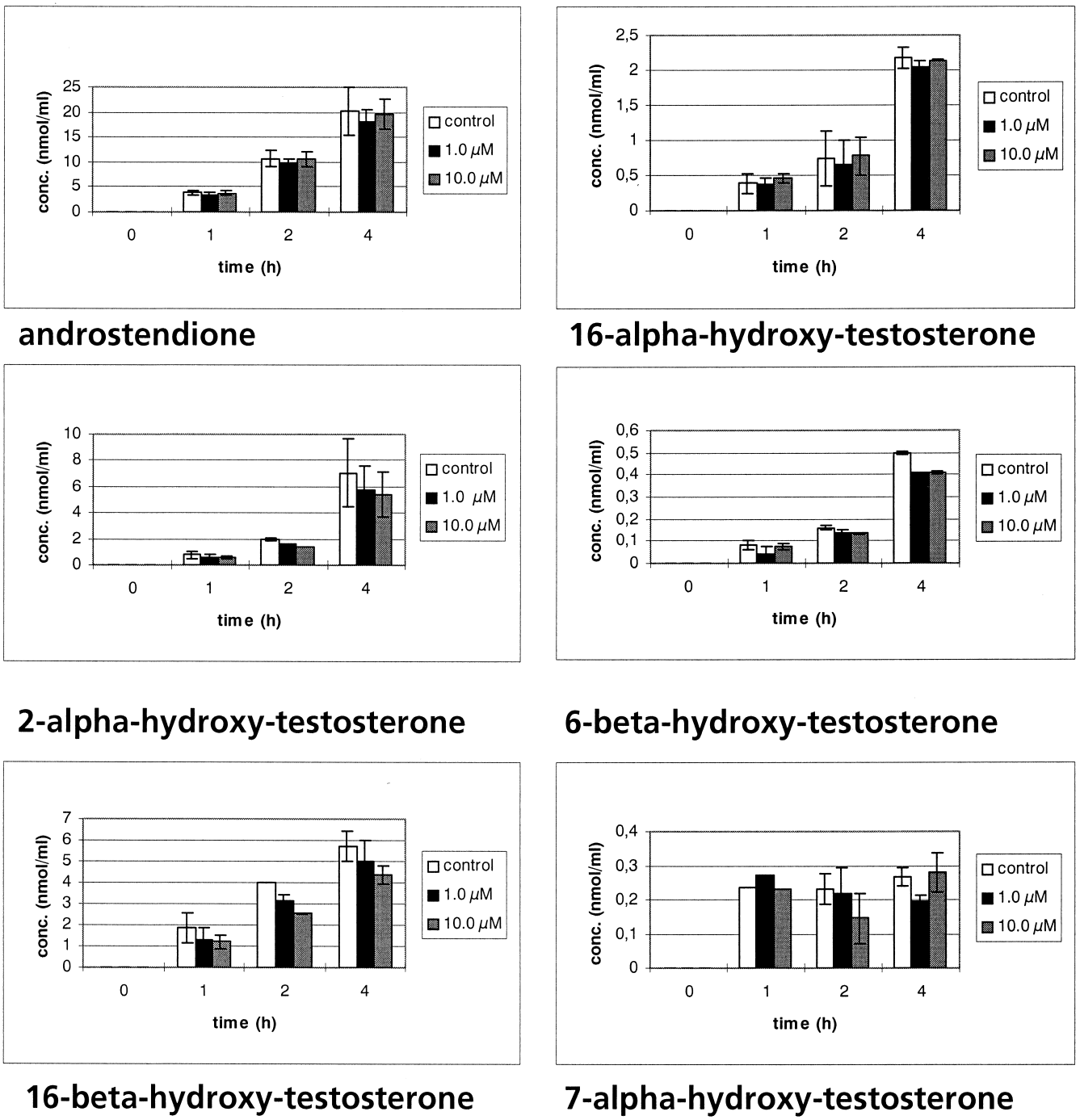

Fig. 6. The formation of individual testosterone metabolites upon treatment of rat hepatocyte cultures with two dose levels of Aroclor 1254. Data represent means ± SEM of N = 3 different cultures with approx. 2 million cells per culture dish.

operativity between two transcription factors could be demonstrated, and elements upstream of the HNF-1 binding site of the *CYP2E1* gene were found to affect the activity of the promoter negatively in a transfection assay [20]. Furthermore, transfection of the rat *CYP2C13* gene promoter involves HNF-1, HNF-3, and members of the orphan receptor subfamily and although HNF-4, apolipoprotein regulatory protein 1 (ARP-1), EAR-2, and ovalbumin upstream promoter (COUP)-TF bind to the CYP2C13 promoter, overexpression of EAR-2 and COUP-TF, but not of HNF-4 or ARP-1, results in the potentiation of the HNF-3- and HNF-1-supported gene activity in non-liver cells [33].

The elucidation of the mechanisms responsible for transcriptional regulation is one of the major challenges in molecular biology; in future studies, protein/DNA and protein–protein interactions will be described, the sum of which will largely determine the degree of transcriptional activation.

In conclusion, our investigation provides new insight into the simultaneous gene expression of nuclear transcription factors and downstream target genes regulated by these factors. We show induction of liver-enriched TFs to be very sensitive to treatment with Aroclor 1254. It is tempting to speculate that the genomic response to Aroclor 1254 follows a coordinate pattern, with TFs and downstream target genes being sequentially activated.

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