An evidence for regulatory cross-talk between aryl hydrocarbon receptor and glucocorticoid receptor in HepG2 cells.

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Short title: Cross talk between AhR and GR.

Summary

Aryl hydrocarbon receptor (AhR) and glucocorticoid receptor (GR) play crucial role in the regulation of drug metabolizing enzymes and in many essential physiological processes. Cellular signaling by these receptors shares several functional and regulatory features. Here we investigated regulatory cross-talk between these two receptors. Human hepatoma cells (HepG2) were the model of choice. We analyzed the effects of dexamethasone (DEX) and dioxin (TCDD) on (i) Expression of AhR and GRα mRNAs; (ii) Levels of AhR and GR proteins; (iii) Transcriptional activities of AhR and GR in reporter assays; (iv) 7-Ethoxyresorufin-*O*-deethylase activity (EROD). We show that both DEX and TCDD affected AhR and GR mRNAs expression, proteins levels and transcriptional activities in HepG2 cells. These effects on cellular signaling by AhR and GR comprised up-/down-regulation of gene expression and ligand-dependent protein degradation. We conclude that interactive regulatory cross-talk between GR and AhR receptors in HepG2 cells defines possible implications in physiology and drug metabolism. Future research should be focused on the investigation of AhR-GR cross-talk in various normal human cells and tissues both *in vitro* and *in vivo*.

Keywords: Cellular signaling; Nuclear receptors; Xenobiotics; Dioxin; Glucocorticoids; Transcriptional control

Introduction

Nuclear receptors are distantly related regulatory proteins that share certain similarities in terms of sub-cellular localization, translocation requirements, DNA binding site sequences and co-activators. A subordination exists between certain members with possible as yet unidentified participants with a say in the matter constituting a tangle of receptor network within a particular cell type (Pascussi *et al.*, 2003; Dvorak *et al.*, 2005b). Only a limited number of receptors are involved in xenobiotic metabolism. Huge knowledge is available on the role of pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR) in drug metabolism. The role of other nuclear receptors or ligand-activated transcriptional factors in the biotransformation enzymes expression still is under intense scrutiny. Namely retinoic X receptor (RXR), farnesyl X receptor (FXR), liver X receptor (LXR), vitamin D receptor (VDR) and glucocorticoid receptor (GR) were found to influence xenobiotics metabolizing enzymes expression. The cross-talk between typical xenoreceptors and several endogenous receptors was described (Pascussi *et al.*, 2004). Within this ingenious signaling network, the putative interaction between AhR and GR receptors seems to be of certain superiority, because the expression of several receptors such as RARs, RXRs, PXR, CAR etc. is subject of regulation by AhR or GR (Pascussi *et al.*, 2003; Pascussi, 2004; Dvorak *et al.*, 2005a).

Interestingly, the information on possible interactions between AhR and GR receptors in terms of cellular signaling and regulation of drug metabolizing enzymes are ambiguous. Several lines of evidence indicate that AhR and GR could influence reciprocally each other both *in vivo* and *in vitro*. Very first investigations were focused on the comparison of molecular, structural and physical-chemical properties of the two receptors with findings that there exist certain similarities (Cuthill *et al.*, 1987; Denis *et al.*, 1988; Denis *et al.*, 1989). Another array of papers deals with the effects of administration of corticoids and exposure to dioxin on the induction of cleft palate in mammalian embryos (Pratt, 1985; Abbott *et al.*, 1994; Abbott, 1995; Abbott *et al.*, 1998; Abbott *et al.*, 1999). Several *in vivo* studies described the influence of TCDD administration on GR content and activity (Csaba *et al.*, 1991; Lin *et al.*, 1991; Mizuyachi *et al.*, 2002; Aluru and Vijayan, 2004; Aluru *et al.*, 2005).

The data on possible cross-talk between AhR and GR *in vitro* are much more controversial than that *in vivo*. First indication that GR could modulate activity of AhR and *vice versa* comes from the observation that glucocorticoid dexamethasone (DEX) enhances TCDD-inducible expression of CYP1A protein and catalytic activity (EROD) in fish Poeciliopsis lucida hepatocellular carcinoma cells (Celander *et al.*, 1996). Similarly, the study

on H4IIE rat hepatoma cells demonstrated that TCDD-dependent CYP1A1 induction is augmented by dexamethasone. This potentiation was found to depend on posttranscriptional processing (Lai *et al.*, 2004). Synergistic effects of DEX on TCDD-dependent activation of AhR were reported by several other authors (Mathis *et al.*, 1986a; Mathis *et al.*, 1986b; Hines *et al.*, 1988). Contrary, TCDD-inducible induction of CYP1B1 mRNA in mouse mammary fibroblasts was suppressed by DEX (Brake *et al.*, 1998).

The aim of this work was to confirm/refute the existence of cross-talk between AhR and GR *in vitro*. Human hepatoma cells (HepG2) were the model of choice for this pilot study, because: (i) These cell line is of human origin; (ii) HepG2 cell line is equipped at least with functional endogenous AhR and perhaps GR. The effects of DEX and TCDD on AhR and GR were assessed as: i) Expression of AhR and GRα mRNAs; ii) The levels of AhR and GR proteins; iii) Transcriptional activity of AhR and GR in reporter assay in transiently transfected cells; iv) 7-Ethoxyresorufin-*O*-deethylase activity (EROD). We used single concentrations of DEX (100 nM) and TCDD (5 nM) in the experiments, ensuring saturation of GR and AhR receptors, respectively.

Materials and methods

Chemicals

Dulbecco`s modified Eagle`s medium, foetal calf serum, penicillin, streptomycin, L-glutamine, non-essential amino acids, sodium pyruvate, dicumarol, 7-ethoxyresorufin, dexamethasone, Triton X-100, and Kodak X-Omat AR photographic film were purchased from Sigma Chemicals (St. Louis, MO, USA). 2,3,7,8- tetrachlorodibenzo-p-dioxin was purchased from Ultra Scientific (RI, USA). Trizol® Reagents, was purchased from GibcoBRL Life Technologies (Cergy Pontoise, France). Complete™ protease inhibitor cocktail tablets and FuGENE 6 transfection reagent were purchased from Roche Diagnostics GmbH (Mannheim, Germany). Secondary horseradish peroxidase conjugated antibody, and Western Blotting Luminol Reagent were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were of the highest grade commercially available.

Cell cultures

Human hepatoma cells HepG2 (ECACC No. 85011430) were cultured in Dulbecco`s modified Eagle`s medium (DMEM) supplemented with 10% FCS, 100 U/mL streptomycin, 100 µg/mL penicillin, 4 mM L-glutamine, non-essential amino acids, and 1 mM sodium pyruvate. Cultures were maintained at 37°C in 5% CO_2 (air:CO_2, 95:5) humidified incubator.

Protein analyses

HepG2 cells were seeded on 6-well dishes in a density of 1 x 10^6 cells/well using culture media enriched with foetal calf serum (10% v/v). Following 16 h of stabilization, the medium was exchanged for a serum-free one and the cells were treated 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. Total protein extracts were prepared as follows: Cells were washed twice with 1 mL of ice-cold PBS and scraped into 1 mL of PBS. The suspension was centrifuged (1500 g/ 5 min/ 4°C) and the pellet was resuspended in 120 µL of ice-cold lysis buffer (10 mM Hepes pH 7.9; 10 mM KCl; 1.5 mM $MgCl_2$; 0.5 mM DTT; 0.1% v/v NP-40; anti-protease cocktail, 0.2% w/v sodium dodecylsulfate). The mixture was incubated for 20 min on ice and then centrifuged (12,000 g/ 10 min/ 4°C). Supernatant was collected and the protein content in extracts was determined by the biscinchoninic acid method.

The extracts were analyzed on SDS-PAGE gels (7.5%) according to the general procedure. Protein transfer onto nitrocellulose membrane was carried out, the membrane was stained with Ponceau S red for control of transfer and then saturated with 8% non-fat dried milk overnight. Blots were probed with primary antibodies against: human glucocorticoid receptor (sc-1003; GR(E-20)X rabbit polyclonal; dilution 1/1000); aryl hydrocarbon receptor (sc-5579; Ah Receptor (H-211) rabbit polyclonal; dilution 1/500), and α-actin (sc-1616; Actin (I-19) goat polyclonal; dilution 1/1000); all purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA). Chemiluminescence detection using horseradish peroxidase conjugated secondary antibodies and an Amersham ECL kit was performed. The intensity of bands after WB analyses was quantified by densitometry.

mRNA analyses

HepG2 cells were plated on Petri dishes (100 mm I.D.) in a density of 4 x 10^6 cells/well using culture media enriched with foetal calf serum (10% v/v). Following 16 h of stabilization, the medium was exchanged for a serum-free one and the cells were treated 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. Concentration of RNA was quantified by spectrometry at 260 nm and purity was assessed from the ratio of absorbances A_{260nm}/A_{280nm}. Reverse transcription (RT) was performed on 1

μg of total RNA using MMLV reverse transcriptase (Finnnzyme, Espoo, Finland) in a reaction volume of 12 μl containing 1× reaction buffer, 5 mM $MgCl_2$, 0.5 mM dNTP mixture, 7.5 μM of oligo$(dT)_{18}$VN (Generi-Biotech, Hradec Kralove, Czech Republic), 1U/μl of RNase inhibitor TaKaRa (Otsu, Japan), 10U/μl of MMLV reverse transcriptase, and 1 μg of RNA. RT was performed in a thermal Palm-cycler (Corbett Research, Mortlake, Australia) with a profile of 25°C for 10 min and 42°C for 70 min. Samples were aliquoted and stored at -80°C until analysis. Real-time PCR was performed using an iCycler (Bio-Rad, Hercules, CA) using SYBR®Green chemistry. Mastermix of a volume 25 μl contained 0.035 U/μl of HotStart *Taq*Polymerase (QIAGEN, Valencia, CA), 3 mM $MgCl_2$, 200 nM fluorescein, 1x buffer, 200 nM dNTP, SybreGreen 1:2500 (Bio-Rad) and 300 nM of each primer. After initial activation of polymerase for 14 minutes at 94°C, samples underwent an additional 40 cycles at 94°C for 10 seconds, 60°C for 20 seconds, and 72°C for 25 seconds, followed by a melting curve analysis. Amplicons were visualized using 2% agarose gel electrophoresis. The primer sequences for hAhR (forward primer: 5'-3': TGGACAAGGAATTGAAGAAGC; reverse primer: 5'-3': AAAGGAGAGTTTTCTGGAGGAA), for hGRα (forward primer: 5'-3': AAACCTTACTGCTTCTCTCTTCA; reverse primer: 5'-3': GTTAAGGAGATTTTCAACCACTTC) and for housekeeping gene hHPRT (forward primer: 5'-3': CTGGAAAGAATGTCTTGATTGTGG; reverse primer: 5'-3': TTTGGATTATACTGCCTGACCAAG). All samples were run in quadruplicates and CT was automatically calculated. These transcripts were extensively optimized, run simultaneously with RNA- and RT-negative controls, and agarose gel electrophoresis was used to confirm the specificity of the priming. Primers were designed using Vector NTI software (Invitrogen). The data were normalized per GAPDH content using delta-delta method. Pfapfl coefficient was considered 2. Statistical calculations were performed in MS Excell using non-pair Student´s t-test.

Transfection assays

The pTAT-(GRE)2-TK-luc plasmid containing two copies of the consensus GRE (glucocorticoid responsive element) upstream of a minimal herpes simplex virus thymidine kinase promoter and a luciferase reporter gene and pTXINV-luc plasmid containing two inverted repeats of the XRE (xenobiotic responsive element) upstream of the thymidine kinase promoter and luciferase reporter gene (Daujat *et al.*, 1996; Backlund *et al.*, 1997) were kindly provided by Dr. L. Poellinger (Karolinska Institute, Stockholm, Sweden). For reporter

assays, HepG2 cells were transiently transfected by lipofection (FuGENE 6) with 300 ng of luciferase reporter construct pTAT-(GRE)2-TK-luc or pTXINV-luc. Following 16 h of stabilization, the medium was exchanged for a serum-free one and the cells were treated in serum free medium for 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. After the treatments, cells were lysed and luciferase activity was measured and standardized per milligram of protein.

EROD assay

HepG2 cells were plated on 96-well dishes at a density of $2.4x10^4$cells/cm^2 in culture medium supplemented with 10% FCS and stabilized for 24 h. The medium was exchanged for a serum-free one and the cells were treated for 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. The catalytic activity of 7-ethoxyresorufin-O-deethylase (EROD) in cell cultures was measured as described elsewhere (Donato *et al.*, 1993). Briefly, monolayers were washed with PBS and the serum free medium containing 8 µM 7-ethoxyresorufin and 10 µM dicumarol (to inhibit cytosolic diaphorase) was applied to cells. Following 30 min of incubation at 37°C, an aliquot of 75 µL of the medium was mixed with 125 µL of methanol and fluorescence was measured in 96-well plate with 530 nm excitation and 590 nm emission filters. The resorufine formation was linear up to 60 min. The data were expressed as the ratio of treated over control values (DMSO treated cells).

Statistics

The results were expressed as means ± standard deviations. A Student´s t-test was applied to all analyses. All calculations were performed using MS Excel 2000.

Results

Effects of DEX and TCDD on AhR and GR mRNAs expression

First we analyzed influence of DEX and TCDD on AhR and GR genes expression. HepG2 cells were treated 24 h with 100 nM DEX, 5 nM TCDD and/or with mixture of TCDD and DEX. The levels of AhR and GR mRNAs were analyzed by real-time PCR. Both TCDD and DEX down-regulated AhR mRNA (30-40% decrease). Interestingly, combination of DEX + TCDD had only moderate inhibitory effect on AhR mRNA (5-10% decrease) (**Fig. 1A**).

TCDD did not alter the levels of GRα mRNA but DEX diminished the expression of GRα mRNA (40% decrease). This diminution was reverted when HepG2 cells were incubated with combination of DEX + TCDD (**Fig. 1B**).

Effects of DEX and TCDD on AhR and GR proteins levels

While the analyses of AhR and GR mRNAs reveal about the effects of tested substances on gene expression of the two receptors, the analyses of AhR and GR protein content comprise additionally information on the stability/degradation of the protein products – receptors. HepG2 cells were treated 24 h with 100 nM DEX, 5 nM TCDD and/or with combination of TCDD and DEX. Total protein extracts were isolated and subjected to western blot analyses. TCDD caused decrease of AhR protein (cca 50% decrease; **Fig. 2A**) probably due to ligand dependent protein degradation. Interestingly, co-treatment with DEX+TCDD partly reversed decrease of AhR protein, whereas basal level of AhR was not affected by DEX (**Fig. 2A**). GR protein was detected in variety of cell lines, however, there are no reports on GR functions and regulation in HepG2 cells. Here, we observed intensive glucocorticoid-dependent (by DEX) degradation of GR protein in HepG2 cells (more than 90% decrease; **Fig. 2B**). Co-treatment with DEX+TCDD partly reversed decrease of GR protein by DEX (**Fig. 2B**). In addition, basal level of GR protein was slightly increased by TCDD.

Effects of DEX and TCDD on AhR and GR transcriptional activities

HepG2 cells were transiently transfected with pTXINV (XRE-luc; TCDD-responsive) and/or with pTAT-(GRE)2-tkLUC (GRE-luc; glucocorticoid-responsive) reporter plasmids. Following stabilization period, cells were challenged with 100 nM DEX, 5 nM TCDD and/or with mixture of TCDD and DEX for 24 h. Transcriptional activities of GR and AhR receptors were monitored as luciferase activity. Incubation of HepG2 cells transfected with XRE-luc reporter with TCDD resulted in approx. 35 fold induction of AhR-dependent luciferase activity. Dexamethasone did not activated AhR-dependent luciferase whereas co-treatment with DEX+TCDD significantly inhibited (cca 30% decrease) TCDD-mediated induction of luciferase (**Fig. 3A**). Surprisingly, GR-dependent activity of luciferase in HepG2 cells transfected with GRE-luc reporter was induced (cca 2 fold induction) not only by glucocorticoid DEX but with similar potency also by TCDD. This effect was significantly augmented when the cells were co-treated with DEX+TCDD (**Fig. 3B**).

Effects of DEX and TCDD on EROD activity

Finally, we examined the capability of TCDD and DEX to induce enzymatic activity of AhR-regulated CYP1A1 (EROD activity) in HepG2 cell line. Cells were treated 24 h with 100 nM DEX, 5 nM TCDD and/or with mixture of TCDD and DEX. TCDD caused an explicit induction of EROD activity (the average increase 6 – 7 fold), whereas DEX had no effect (**Fig. 4**). Both basal and TCDD-induced EROD activity was diminished by treatment with DEX (cca 20% decrease), which is in accordance with the inhibition of TCDD-dependent AhR transcriptional activation in HepG2 cells by DEX (**Fig. 3A**).

Discussion

In present study we bring the evidence that there exists a regulatory cross-talk between cellular signaling by AhR and GR receptors. It is supported in particular by findings that: (i) Dexamethasone (DEX) and dioxin (TCDD) cross-induced the expression of luciferase gene fused to xenobiotic-responsive element (XRE) and glucocorticoid responsive element (GRE), respectively. (ii) DEX modulated TCDD-induced transcriptional activity of AhR. (iii) DEX modulated TCDD-elicited degradation of AhR protein. (iii) Both DEX and TCDD modulates expression of AhR and GRα mRNAs. All the data are summarized in a table for better orientation (Table 1). In addition, we show that HepG2 cells are equipped with GR protein that is degraded in the presence of the ligand and that GRE-luc reporter was trans-activated by DEX in HepG2 cells.

Available literary data together with our data presented here indicate that the interactions between AhR and GR *in vitro* are determined in particular by: **(i)** differences between cellular signaling in normal and transformed cells; it is well known that transformed cells have often altered signaling pathways and regulatory mechanisms as compared to the normal ones. **(ii)** differences between proliferating and non-proliferating (quiescent) cells; numerous studies demonstrated cell cycle dependency of AhR (Scholler *et al.*, 1994; Santini *et al.*, 2001; Bonzo *et al.*, 2005) and GR (Cidlowski and Cidlowski, 1982; Hsu *et al.*, 1992; Bodwell *et al.*, 1998; Abel *et al.*, 2002) function, when stability, transcriptional activity, phosphorylation status and ligand binding capacity of these receptors oscillates throughout cell cycle. **(iii)** cell type specificity.

Apparent discrepancies between the effects of ligands and their combinations of the studied receptors results in certain difficulty in data interpretation. For instance, TCDD down-regulates AhR mRNA in HepG2 cells (Fig1A), simultaneously decreases AhR protein in HepG2 cell (probably also via degradation – Fig 2A), but transcriptional activity is

progressively increasing (Fig 3A). Like this, the interpretation of combined effects of DEX and TCDD should be done with prudence. In addition, the effects of DEX, TCDD and their combinations on AhR and GR mRNAs is rather modulation than robust up-/down-regulation.

Essential question is: Why such an interactive regulation between AhR and GR exists? AhR and GR share several structural and functional features in terms of association of these receptors with chaperones in cytosol, ligand-dependent cytosol to nucleus translocation, proteaome-mediated degradation as negative feed back etc. It has been a dogma for long time, that GR is typical steroid receptor, essential for variety of endogenous functions. However, GR plays important role in regulation of drug-metabolizing enzymes, as it controls expression of PXR, CAR and RXR receptors (Pascussi *et al.*, 1999; Pascussi *et al.*, 2000a; Pascussi *et al.*, 2000b). Similarly the role of AhR receptor in living organisms is ambiguous. It has long been considered as xenoreceptor regulating phase I and phase II biotransformation enzymes. In addition it has been considered as malicious fellow, when its activation, e.g. by exposure to dioxin, leads to a number of toxic effects, in particular to tumor promotion and immune-suppression (Barouki and Morel, 2001). On the other hand, it is essential factor, when its absence results in severe phenotypic abnormalities (Mimura and Fujii-Kuriyama, 2003). In other words, sustained activation of AhR by endogenous ligands is essential for the correct development and function of living organisms. The exogenous activation of AhR is responsible for chemically induced carcinogenesis and other pathological responses. Taken together, both AhR and GR play important roles in physiological processes as well as in drug metabolism or carcinogenesis.

Apart from mechanistic point of view, our data have possible implications in physiology and drug metabolism. Since there exist striking differences between normal and transformed cells, between proliferating and quiescent and cell type specificity in the interactions between AhR and GR, future research should be focused on the investigation of AhR-GR cross-talk in normal human cells and tissues both *in vitro* and *in vivo*. In this context, the most attractive models for these studies would be human hepatocytes, placental, intestinal, kidney and lung cells and tissues. For instance, based on the presumption that AhR – GR cross-talk exists, there should be definitely differences in the biological effects of clinically used glucocorticoids in non-smokers and smokers (TCDD-like effects of polycyclic aromatic hydrocarbons). If it is truth, it would be alerting issue regarding the massive use of glucocorticoids in asthma, pregnancy, oncology, immunology etc.

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Figure legends

Figure 1. Effects of DEX and TCDD on AhR and GR mRNAs expression. Cells were treated 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. The levels of AhR, GRα and hHPRT mRNAs were determined by RT-PCR as described in the Methods section. The data were normalized on the hHPRT mRNA level. Bar graphs represent means ± SD of three independent experiments. * - the value significantly different from the control value (0 μM) at $p < 0.05$. **Panel A**: Analyses of AhR mRNA; **Panel B**: Analyses of GRα mRNA.

Figure 2. Effects of DEX and TCDD on AhR and GR proteins levels. Shown are representative Western blots of AhR and GR. Cells were treated 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. Total protein extracts were isolated and after western blot analysis the membrane was probed with anti AhR, anti GR and anti actin antibodies. A similar profile was obtained from three independent experiments. **Panel A**: Analyses of AhR protein; **Panel B**: Analyses of GR protein.

Figure 3. Effects of DEX and TCDD on AhR and GR transcriptional activities. Bar graphs summarizing the GR and AhR transcriptional activities in transiently transfected HepG2 cells. Cells transfected with pTAT-(GRE)2-TK-luc and/or pTXINV plasmid were incubated for 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. The chemiluminescent activities of luciferase reporter genes in cell lysates were measured and normalized to protein concentration. Bar graphs represent the means ± SD of five independent transfection experiments. In each experiment, six parallel samples were analyzed. * - value is significantly different from the activity of DMSO-treated cells ($p<0.05$). **Panel A**: HepG2 cells transfected with pTXINV; # - value is significantly different from the activity of TCDD-treated cells ($p<0.05$); **Panel B**: HepG2 cells transfected with pTAT-(GRE)2-TK-luc; # - value is significantly different from the activity of TCDD- and/or DEX-treated cells ($p<0.05$).

Figure 4. Effects of DEX and TCDD on EROD activity. HepG2 cells were treated 24 h with dexamethasone (DEX; 100 nM final concentration), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 5 nM final concentration), TCDD+DEX and with DMSO as vehicle for control. CYP1A1 activity (7-ethoxyresorufin-O-deethylase; EROD) was measured by fluorescence spectrophotometry as described in the Methods section. Bar graph represent the means ± SD of three independent experiments. * - value is significantly different from the activity of DMSO-treated cells ($p<0.05$); # - value is significantly different from the activity of TCDD-treated cells ($p<0.05$).

Table 1. Summary of the effects of TCDD and DEX on GR and AhR protein levels, mRNA levels and transcriptional activities. ↑↑↑ = strong increase; ↑↑ = medium increase; ↑ = weak increase; ↓↓↓ = strong decrease; ↓↓ = medium decrease; ↓ = weak decrease; 0 = no effect; NA = not applicable.

Abbreviations: AhR, Aryl hydrocarbon receptor; BNF, beta-naphtoflavone; CAR, constitutive androstane receptor; CYP, Cytochrome P450; DEX, dexamethasone; DTT, dithiothreitol; EROD, 7-Ethoxyresorufine-*O*-deethylase; FXR, farnesyl X receptor; GR, Glucocorticoid receptor; GRE, glucocorticoid responsive element; HepG2, Human hepatoma cells; LXR, liver X receptor; PXR, pregnane X receptor; RU486, mifepristone; RXR, retinoic X receptor; TCDD, 2,3,7,8- tetrachlorodibenzo-p-dioxin; VDR, vitamin D receptor; XRE, xenobiotic-responsive element

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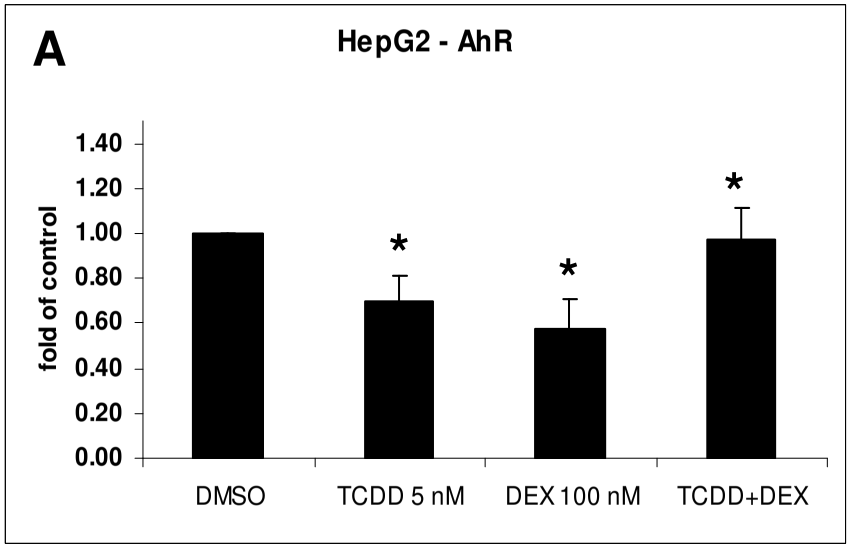
A
HepG2 - AhR
fold of control
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
*
*
DMSO
TCDD 5 nM
DEX 100 nM
TCDD+DEX

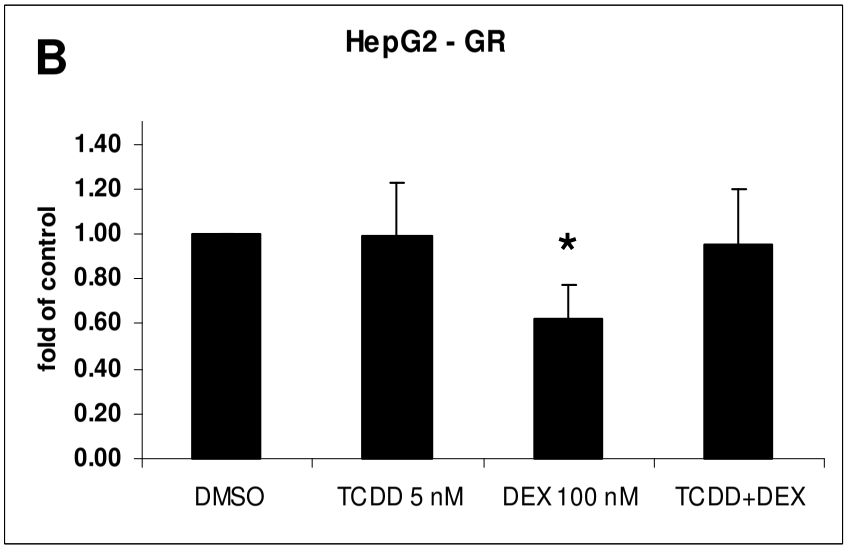
B
HepG2 - GR
fold of control
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
DMSO
TCDD 5 nM
DEX 100 nM
TCDD+DEX

Figure 1

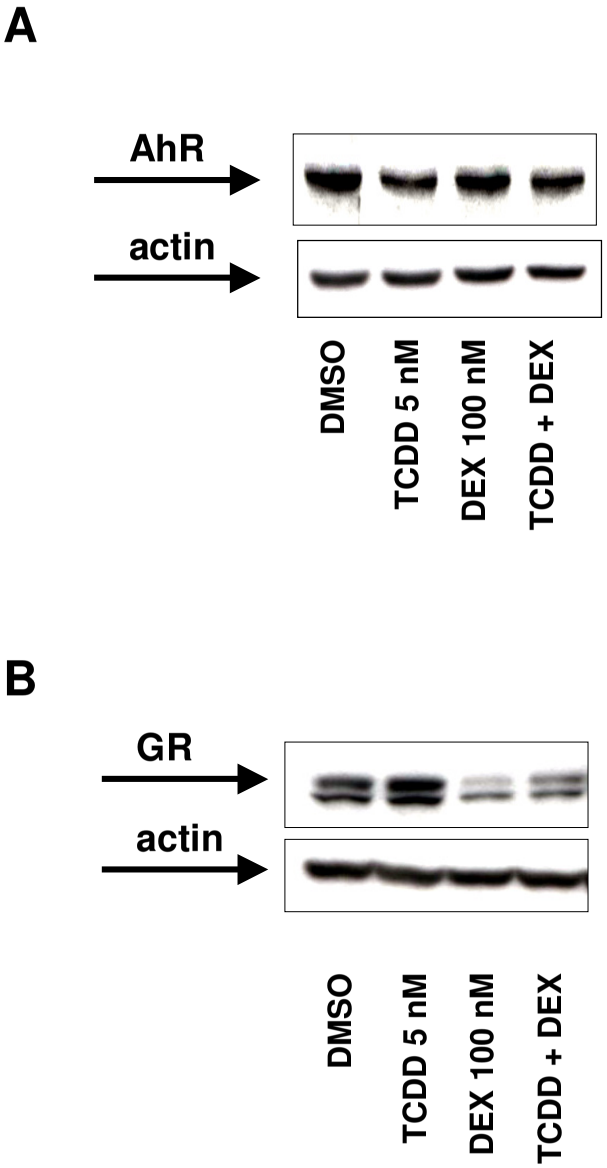
A
AhR
actin
DMSO
TCDD 5 nM
DEX 100 nM
TCDD + DEX
B
GR
actin
DMSO
TCDD 5 nM
DEX 100 nM
TCDD + DEX

Figure 2

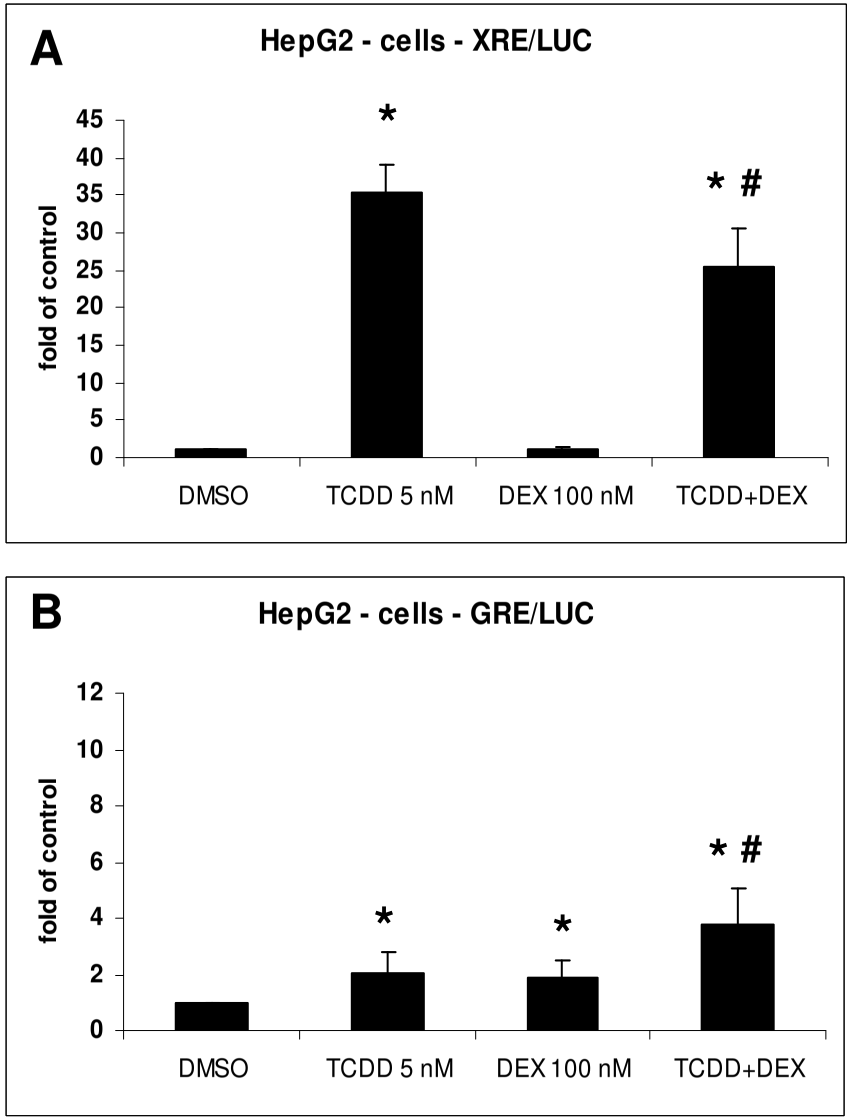
A
HepG2 - cells - XRE/LUC
fold of control
45
40
35
30
25
20
15
10
5
0
*
* #
DMSO
TCDD 5 nM
DEX 100 nM
TCDD+DEX
B
HepG2 - cells - GRE/LUC
fold of control
12
10
8
6
4
2
0
*
*
* #
DMSO
TCDD 5 nM
DEX 100 nM
TCDD+DEX

Figure 3

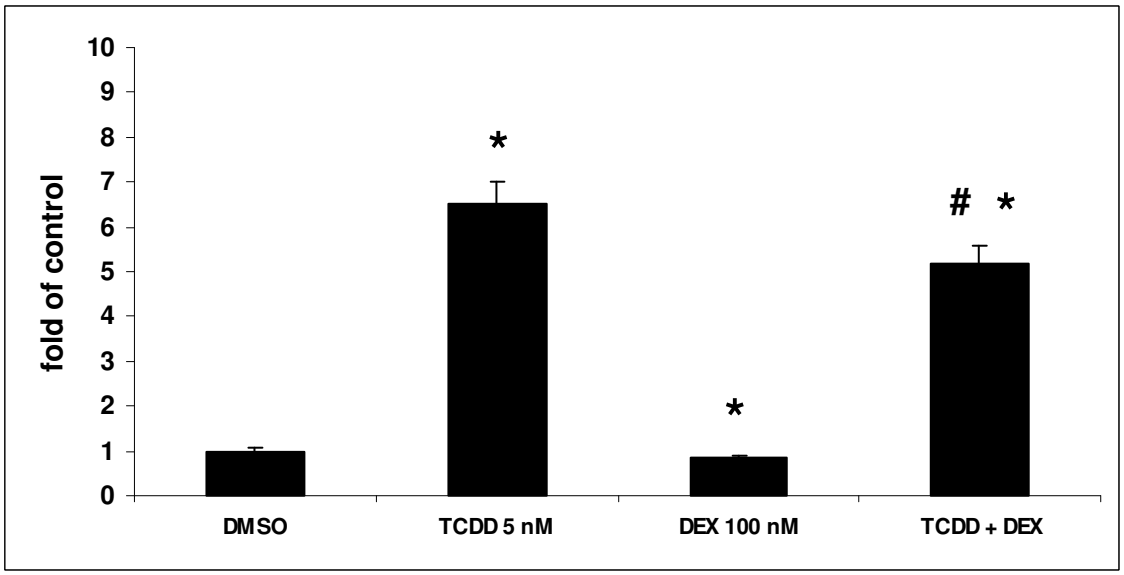
fold of control
10
9
8
7
6
5
4
3
2
1
0
*
*
*
DMSO
TCDD 5 nM
DEX 100 nM
TCDD + DEX

Figure 4

	HepG2					
	GR			AhR		
	DEX	TCDD	T+D	DEX	TCDD	T+D
protein	↓↓↓	↑	↓↓	0	↓↓	↓
mRNA	↓	0	0	↓	↓	0
reporter assays	↑	↑	↑↑	0	↑↑↑	↑↑
Enzyme activity of target gene CYP1A1 (EROD)	NA	NA	NA	↓	↑↑↑	↑↑

Table 1