Toxicology 254 (2008) 97–105

Contents lists available at ScienceDirect

Toxicology

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

Dexamethasone suppresses the expression of multiple rat carboxylesterases through transcriptional repression: Evidence for an involvement of the glucocorticoid receptor☆

Deshi Shi[a,b], Jian Yang[a], Dongfang Yang[a], Bingfang Yan[a,*]

[a] Department of Biomedical Sciences, Center for Pharmacogenomics and Molecular Therapy, University of Rhode Island, Kingston, RI 02881, United States
[b] College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China

ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form
15 September 2008
Accepted 19 September 2008

Keywords:
Dexamethasone
Carboxylesterases
Hydrolysis
Transrepression
Glucocorticoid receptor

ABSTRACT

Carboxylesterases play important roles in the metabolism of xenobiotics and detoxication of insecticides. Without exception, all mammalian species studied express multiple forms of carboxylesterases. Several rat carboxylesterases are well-characterized including hydrolase A, B and S, and the expression of these enzymes is significantly suppressed by glucocorticoid dexamethasone. In this study, we used multiple experimental systems and presented a molecular mechanism for the suppression. Rats receiving one or more daily injections of dexamethasone consistently expressed lower HA, HB and HS. The suppression occurred at the levels of mRNA, protein and hydrolytic activity. In hepatoma cell line H4-II-E-C3, nanomolar dexamethasone caused significant decreases in HA, HB and HS mRNA, and the decreases were abolished by antiglucocorticoid RU486. Additionally, dexamethasone at nanomolar concentrations repressed the promoters of carboxylesterases, and the repression was reduced by glucocorticoid receptor-β, a dominant negative regulator of the glucocorticoid receptor (GR). In contrast, co-transfection of the pregnane X receptor (PXR) increased the reporter activities, but the increase occurred only at micromolar concentrations of dexamethasone. These findings establish that both GR and PXR are involved in the regulated expression of rat carboxylesterases by dexamethasone but their involvement depends on the concentrations.

1. Introduction

Carboxylesterases play an important role in the metabolism of endogenous lipids and foreign compounds containing such functional groups as carboxylic acid ester, amide and thioester (Satoh and Hosokawa, 2006; Shi et al., 2006). In addition to hydrolysis, some carboxylesterases catalyze transesterification reaction, which accounts for the conversion of anti-platelet agent clopidogrel (a methyl ester) to ethyl clopidogrel (the corresponding ethyl ester) (Tang et al., 2006). While carboxylesterase activity is widely distributed in mammalian tissues, the highest level is present in liver microsomes (Satoh and Hosokawa, 2006). The abundant presence of carboxylesterases in the liver is linked to certain cellular structural roles, particularly in directing protein trafficking (Ovnic et al., 1991). For example, egasyn, a liver microsomal carboxylesterase, binds to β-glucuronidase via its active site, and such binding results in sequestration of this enzyme within the endoplasmic reticulum (Ovnic et al., 1991). Organophosphorus insecticides target the active site and release egasy-complexed β-glucuronidase into the blood (Satoh et al., 1999). Organophosphorus compounds such as fenitrothion at nanomolar concentrations cause significant increases of β-glucuronidase in the blood, thus serving as a sensitive biomarker for the exposure to these insecticides (Satoh et al., 1999).

Mammalian species express multiple forms of carboxylesterases (Satoh and Hosokawa, 2006). The well-characterized examples include rat hydrolase A, B and S (HA, HB, HS) (Yan et al., 1995a,b, 1994; Morgan et al., 1994), and *h*uman *c*arboxyl*e*sterase HCE1 and HCE2 (Schwer et al., 1997; Kroetz et al., 1993). These carboxylesterases generally have a sequence identity of ~70% with an exception of HCE2, which shows ~50% identity with other

Abbreviations: AGP, α-1 acid glycoprotein; CYP, cytochrome P450; HA, hydrolase A; HB, hydrolase B; HS, hydrolase S; MEME, minimum essential medium eagle; GAPDH, glyceradehyde-3-phosphate dehydrogenase; HCE, human carboxylesterases; PXR, pregnane X receptor; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; Tat, tyrosine aminotransferase.

☆ This work was supported by NIH grants R01ES07965, R01GM61988 and F05AT003019.

* Corresponding author at: Department of Biomedical and Pharmaceutical Sciences, Center for Pharmacogenomics and Molecular Therapy, University of Rhode Island, Kingston, RI 02881, United States. Tel.: +1 401 874 5032;
fax: +1 401 874 5048.
E-mail address: byan@uri.edu (B. Yan).

0300-483X/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2008.09.019

carboxylesterases (Satoh and Hosokawa, 2006). Like many other xenobiotic-metabolizing enzymes (Yang et al., 2008; Choudhary et al., 2004), the expression of carboxylesterases is regulated developmentally as well as by many xenobiotics. Based on Western analysis, neither HA nor HB is expressed in 3-week old or younger rats (Morgan et al., 1994). Both carboxylesterases are induced by phenobarbital and clofibrate, however, the induction is only minimal (15 and 30%) (Yan et al., 1995b; Morgan et al., 1994). Based on immunoprecipitation study, hydrolase A and B together contribute 90% of the hydrolytic activity toward *para*-nitrophenylacetate (Morgan et al., 1994).

In contrast to the induction, suppression of rat carboxylesterases is profound by several chemicals (Poole et al., 2001; Yan et al., 1995b; Morgan et al., 1994). For example, treatment of mature rats with dexamethasone causes as much as 80% decrease in hydrolytic activity toward *para*-nitrophenylacetate (a standard carboxylesterase substrate), the corresponding proteins and mRNAs of HA, HB and HS (Zhu et al., 2000; Yan et al., 1995b; Morgan et al., 1994). The suppression, however, does not occur with pregnenolone-16α-carbonitrile, an antiglucocorticoid (Yan et al., 1995b). In cultured rat hepatocytes, dexamethasone caused significant suppression even at nanomolar concentrations (Zhu et al., 2000). Two major receptors are known to mediate the action of dexamethasone: the glucocorticoid receptor (GR) (Pei, 1996) and the pregnane X receptor (PXR) (Kliewer et al., 1998). The activation of GR requires nanomolar, whereas the activation of PXR requires micromolar concentrations of dexamethasone. In addition, GR is activated by glucocorticoids only, but PXR can be activated by both glucocorticoids and antiglucocorticoids.

The aim of this study was to test the hypothesis that dexamethsaone suppresses the expression of rat carboxylesterases through GR. Rats and hepatoma cell line were treated with dexamethasone at various doses or concentrations. As expected, dexamethasone caused significant and persistent suppression of HA, HB and HS in rats and hepatoma cell line. The suppression, however, was abolished by antiglucocorticoid RU486, but not by protein synthesis inhibitor cycloheximide, suggesting that the suppression is achieved through GR-mediated trans-repression and does not require on-going protein synthesis. In addition, dexamethasone repressed the promoters of rat carboxylesterase, and the repression was reduced by glucocorticoid receptor-β, a dominant negative regulator of glucocorticoid receptor-α. Interestingly, the promoter sequences supporting the repression lack canonical glucocorticoid response elements. These findings establish that glucocorticoid receptor-α is involved in the suppression of rat carboxylesterases but through a nonclassic mechanism.

2. Materials and methods

2.1. Chemicals and supplies

Cortisol, cycloheximide, dexamethasone, equine serum, *para*-nitrophenylacetate, Hanks balanced salt solution and RU486 were purchased from Sigma (St. Louis, MO). Minimum essential medium eagle (MEME) and high fidelity Platinum Taq DNA polymerase were purchased from Invitrogen (Carlsbad, CA). Reporter Assay System was from Promega (Madison, WI). Fetal bovine serum was from HyClone laboratories (Logan, UT). Ketamine HCl was purchased from Fort Dodge Animal Health (Fort Dodge, IA). The antibody against glyceradehyde-3-phosphate dehydrogenase (GAPDH) was from Abcam (Cambridge, UK). The goat anti-rabbit IgG conjugated with horseradish peroxidase was from Pierce (Rockford, IL). Nitrocellulose membranes were from Bio-Rad (Hercules, CA). Unless otherwise specified, all other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Animal treatment

Sprague–Dawley male rats from Charles River (Wilmington, MA) were injected i.p. once daily with dexamethasone (50 mg/kg) in corn oil or the same volume of vehicle (Zhang et al., 1999). At a given time-point for sacrifice, rats were i.p. injected with ketamine (1 ml/kg at 100 mg/ml). After rats were completely anesthetized (~10 min), surgery was performed to expose the liver. The liver was perfused with phosphate buffered saline (37 °C) through the portal vein to remove blood. The perfused liver was then divided into two parts. One part was immediately used for preparing total RNA and the remaining part was frozen at −80 °C for preparing S9 fractions. All rats were allowed free access to Purina Rodent Chow 5001 and water, and the use of animals was approved by the Institutional Animal Care and Use Committee.

2.3. Cell culture and treatment

Rat hepatoma line H4-II-E-C3 was purchased from the American Type Culture Collection (Rockville, MD). The hepatoma cells were maintained in MEME containing 10% fetal bovine serum, 5% equine serum, penicillin (100 units per ml)/streptomycin (100 μg/ml), 1× non-essential amino acids and 1 mM sodium pyruvate. Cells were usually seeded at a density of 5×10^5 cells/well (12-well plates) in normal medium. After an overnight incubation, treatment was started with dexamethasone or the same volume of DMSO. In some cases, repeated treatment was performed 24 h after the initial treatment with fresh medium containing dexamethasone at the same concentration. The duration and concentration of treatment are specified in figure legends.

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated with an RNA-Bee (Friendswood, TX) according to the manufacturer's manual, and the integrity of the RNA was confirmed by formaldehyde gel electrophoresis. Total RNA (1 μg) was subjected to the synthesis of the first strand cDNA in a total volume of 25 μl with random primers and M-MLV reverse transcriptase. The reactions were conducted at 25 °C for 10 min, 42 °C for 50 min and 70 °C for 10 min. The cDNAs were then diluted 8 times and quantitative PCR was conducted with *TaqMan* Gene Expression Assay (Applied Biosystems, Foster City, CA). The *TaqMan* assay identification numbers were: HA, Rn00591030_m1; HB, Rn01774462_m1; HS, Rn00592205_m1; tyrosine aminotransferase (Tat), Rn00562011_m1; rat GAPDH, Rn99999916_s1; and rat polymerase II, Rn01752026_m1. The PCR amplification was conducted in a total volume of 20 μl containing universal PCR master mixture (10 μl), gene-specific *TaqMan* assay mixture (1 μl), and cDNA template (6 μl). Cycling profile was 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, as recommended by the manufacturer. The mRNA levels were normalized according to the level of GAPDH and RNA polymerase II (selected samples) (Radonić et al., 2004). Amplification and quantification were done with the Applied Biosystems 7900 Real-Time PCR System.

2.5. Preparation of S9 fractions and hydrolysis of para-nitrophenylacetate

Frozen livers (perfused) were thawed in homogenization buffer (50 mM Tris–HCl, pH 7.4, 150 mM KCl and 2 mM EDTA) and then homogenized with 6 passes of Teflon pestle driven by a Wharton stirrer. The homogenates were centrifuged at $10{,}000 \times g$ for 20 min at 4 °C. The S9 fractions (supernatant) were assayed for the hydrolysis of *para*-nitrophenylacetate as described previously (Yang et al., 2007). Sample cuvette (1 ml) contained 20 μg S9 fractions in 100 mM potassium phosphate buffer (pH 7.4), and 1 mM substrate at room temperature. Reactions were initiated by adding *para*-nitrophenylacetate (10 μl of 100 mM stock in acetonitrile) and hydrolytic rate was recorded from an increase in absorbance at 400 nm. The extinction coefficient (E_{400}) was determined to be 13 mM^{-1} cm^{-1}, and non-enzymatic hydrolysis was subtracted (Yang et al., 2007).

2.6. Reporter constructs

Luciferase reporters harboring rat carboxylesterase promoters at varying length (HB and HS only) were prepared by inserting the corresponding genomic fragment into the pGL4.10 vector. To prepare HB reporters, a genomic fragment from −3590 to −172 (relative to the translation initiation codon) was amplified by PCR with high fidelity Platinum Taq DNA polymerase and rat genomic DNA as the template. The fragment was generated with forward primer HB-3590XhoIs (5′-ccttatctcgagtcgttgaactgacaatgttactat-3′) and reverse primer HB-172BglIIa (5′-aagccagatctcttccaagtttacctggctttatct-3′). This fragment was digested with Xho I and Bgl II and ligated to the pGL4.10 vector pretreated with the same endonucleases. This reporter was designated as HB-3590-Luc. To prepare 5′ truncated constructs of this reporter, the corresponding genomic DNA fragments were amplified by PCR with the HB-3590-Luc reporter as the template. These 5′ deleted genomic fragments were amplified with the same reverse primer but different forward primers (Table 1). Similarly, the PCR-amplified fragments were ligated to the pGL4 vector. To prepare the HB-4822-Luc reporter (containing the longest HB genomic sequence among all HB reporters), a further upstream genomic fragment was generated with forward primer (HB-4822KpnIs) and reverse primer (HB-3537NheIa). This fragment was digested with Kpn I and Nhe I (at −3610 to −3616) and ligated to the HB-3590-Luc pretreated with the same restriction endonucleases to produce the HB-4822-Luc