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The CYP2B2 5′ flank contains a complex glucocorticoid response unit

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ABSTRACT

Rat CYP2B1 and CYP2B2 and mouse CYP2B10 are dramatically induced by phenobarbital (PB) in liver. PB responsiveness requires the constitutive androstane receptor (CAR). However, dexamethasone treatment can also induce *CYP2B* genes in both rat and mouse liver. Three regions have been shown to be involved in conferring dexamethasone responsiveness on *CYP2B2* reporter constructs. They are the PB response unit, a functional glucocorticoid response element at −1.3 kb in the 5′ flank and a weak element in the basal promoter. We report here the identification, by deletion analysis of the *CYP2B2* 5′ flank, of new glucocorticoid response elements or accessory factor sites. Moreover, we show that CAR acts as an accessory factor in the dexamethasone response in vivo of CYP2B10 protein in mice, by increasing both the basal and induced levels. We propose a model to explain the dexamethasone responsiveness of the *CYP2B2* gene in which induction is mediated by a complex glucocorticoid response unit.

1. Introduction

Hepatic cytochrome P450 (CYP) enzymes are well known for their inducibility in response to xenobiotics [1,2]. In mouse liver the *Cyp2b10* gene and in rat liver the *CYP2B1* and *CYP2B2* genes are highly responsive to phenobarbital (PB) and PB-like inducers [1,2]. PB responsiveness is conferred by the PB response unit (PBRU), a 163-bp fragment located at −2317/−2155 in the rat *CYP2B2* 5′-flank [3,4]. Three DR-4 sites are located in the PBRU, NR1, NR2 and NR3, which are recognized by heterodimers of the constitutive androstane receptor (CAR) and the retinoid X receptor (RXR) [5–8]. CAR is normally retained in the cytoplasm of untreated hepatocytes and becomes concentrated in the nucleus in response to PB treatment [9]. There, it is thought to activate transcription of its target genes by binding to the NR sites [6,8,10,11].

Induction of *CYP2B* genes by dexamethasone (DEX) was described some time ago [12–14]. Although the specific mechanisms involved in DEX responsiveness are still unclear, the basal level of hepatic CYP2B10 protein is reduced in mice with a targeted glucocorticoid receptor (GR) gene disruption, and the CYP2B10 protein is not inducible by DEX in such

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Abbreviations: CAR, constitutive androstane receptor; DEX, dexamethasone; DMSO, dimethyl sulfoxide; EMSA, electrophoretic mobility shift assays; FBS, fetal bovine serum; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GRU, glucocorticoid response unit; HNF-3, hepatocyte nuclear factor 3; oligo, oligonucleotide; PB, phenobarbital; PBRU, phenobarbital response unit; RXR, retinoid X receptor.

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animals [15]. We have shown elsewhere that DEX induction of *CYP2B2* and *Cyp2b10* reporter genes is mediated by GR and that CAR acts as an accessory factor, by stimulating this response (Audet-Walsh and Anderson, submitted for publication). For *CYP2B2* reporters, the roles of the PBRU and of a previously reported glucocorticoid response element (GRE) situated at −1.3 kb [16], referred to here as GRE1.3, were studied. Both were found to be necessary for maximal DEX responsiveness. However, the results suggested that other sites, GREs or accessory sites, are also required for maximal responsiveness. We report here the identification of new elements in the *CYP2B2* 5′ flank that are necessary to obtain maximal DEX responsiveness, indicating that *CYP2B2* induction by DEX is mediated through a complex glucocorticoid response unit (GRU).

2. Materials and methods

2.1. Materials and animals

DEX was from Sandoz (Boucherville, Canada) and was diluted in dimethyl sulfoxide (DMSO) (1:100, v/v) for cell culture treatment. All enzymes were from Fermentas (Burlington, Canada). Waymouth's medium, minimum essential medium (α-MEM), fetal bovine serum (FBS), penicillin-streptomycin and gentamicin were from Invitrogen (Burlington, Canada). Oligonucleotide (oligo) primers, DMSO and other chemicals were from Sigma–Aldrich (Oakville, Canada). C57-Black/6 mice (20–25 g) were from Charles River (St-Constant, Canada). Mice in which the *Car* gene has been subjected to targeted inactivation [17] were provided by Dr. David Moore. Animals were treated in accordance with the requirements of the Comité de protection des animaux du CHUQ/CPAC.

2.2. Plasmids and plasmid constructs

pSG5-hGR, an expression vector for human GR, was from J.M. Pascussi. The pGL3-2B2X vector (herein referred as pGL3-2B2-Luc), based on the pGL3-Basic vector (Promega, Montréal, Canada) and containing 2.5 kb of the *CYP2B2* 5′-flank, including the natural promoter and the PBRU, subcloned upstream of the *luc* gene, has been described [18]. The different deletion constructs were prepared by amplification of pGL3-2B2-Luc with primers (see Table 1) flanking the region to be deleted, both having the same restriction site EcoRI [19]. The PCR products were digested with both EcoRI and DpnI and finally ligated with T4 DNA ligase to obtain the new constructs (Fig. 1). For the construction of the −1180/−1137 construct, the pGL3-120 plasmid [18] was amplified and the −1180/−1137 fragment was fused to the reverse primer; both primers (see Table 1) had the same restriction site (XhoI) and PCR products were treated as for the different deletion constructs. All plasmids were purified with purification kits (Qiagen, Mississauga, Canada), and the relevant regions were subjected to DNA sequencing by the DNA sequencing service of the Centre de recherche du CHUL (Québec, Canada).

2.3. Cells and transfection assays

HepG2 cells and H4IIEC3 cells were cultured and maintained (5% CO_2/37 °C) in medium B [20] supplemented with 10% (v/v) FBS plus antibiotics (gentamicin at 50 mg/ml or penicillin-streptomycin at 100 U/ml and 100 μg/ml, respectively). All transfections were performed in duplicate with GeneJuice® reagent (VWR International, Montréal, Canada) as described elsewhere (Audet-Walsh and Anderson, submitted for publication). Unless noted otherwise, each transfection was performed at least three times. DEX treatments were for 24 h for HepG2 cells and 48 h for H4IIEC3 cells; after 24 h the H4IIEC3 cells were washed once with HEPES buffer (6.4 mM KCl, 10 mM HEPES, 0.15 M NaCl, pH 7.6) and fresh medium was added. After the DEX treatments, the medium was removed, cells were washed once with HEPES buffer, 120 μl of passive lysis buffer (Promega) was added, and the cells were harvested by scraping and lysed by freeze–thaw treatment. Luciferase activity of each sample was assayed by luminometry with the Dual Luc kit (Promega). The firefly luciferase values were divided by the *Renilla* luciferase values to obtain the relative

Table 1 – Oligos used to make the different *CYP2B2* reporter constructs

Name	Sequence
Universal 2B2 forward primer	ATGAATTCTCGAGCCCGGGCTAGCACGC
−1759	ATGAATTCAGTTGAGGCAAGTTGACCACA
−1370	ATGAATTCCAATAATATCAGTTAGGGTACA
−1180	ATGAATTCAGGGAACCATTTGTCATTAGACA
−1148	ATGAATTCGAGACTATCTTTGTTAGGTTCACTATTTCT
−940	ATGAATTCCCTGATTTCTTACAGAACCCAA
−740	ATGAATTCAAGTTAGACCCGGGGCCCCAAC
−600	ATGAATTCCAGAGAGTGAAATGGGGACTC
−540	ATGAATTCAGGAACCAACAGACGGAGACAA
−500	ATGAATTCCTATTCTTGTCAACTCAAACAT
−440	ATGAATTCGCCCCAATAATTTAAGATTATA
−381	ATGAATTCCCAGTGCATCTAGACTCAGACAA
−240	ATGAATTCTAAGTAAACAGAGCTGACAAAA
−192	ATGAATTCACATAAAACAAGAGGCCTAAGT
−145	ATGAATTCCTGTTTCGTGGTTTCCTTGCC
Forward primer −1180/−1137	AACTCGAGCCCGGGCTAGCACGCGTAA
Reverse primer −1180/−1137	AACTCGAGAGGGAACCATTTGTCATTAGACACAGTGTTCAGAGAC-TATCTTTCTATGGTGTGGGTAAGGGAATGAG