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Review

Phenobarbital-induced expression of cytochrome P450 genes★

Piotr Czekaj✉

II Department of Histology and Embryology, Silesian Medical Academy, Katowice, Poland

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In contrast to the well-known Ah receptor-mediated regulation of the *CYP1A1* gene by polycyclic aromatic hydrocarbons, the molecular mechanism by which phenobarbital (PB) and PB-like inducers affect transcription of *CYP* genes remains unknown; no receptor for these chemicals has been found to date. However, in the last 5 years PB-responsive sequences have been identified in the 5′ flanking regions of several *P450* genes. The phenobarbital-responsive enhancer unit (PBRU) of *CYP2B* gene family members contain two potential nuclear receptor binding sites (NR1 and NR2) that flank a nuclear factor 1 (NF-1) binding motif. The nuclear factors that regulate PBRU activity have not yet been characterized. It seems that PB may activate multiple nuclear orphan receptors to induce various *CYP* genes. *CYP2B* and *CYP3A* genes appear to be targets for the orphan receptors CAR and PXR, respectively. It is also possible that the pleiotropic effects of PB can, in part, be explained by the ability of the CAR–RXR heterodimer to bind to a variety of nuclear receptor binding motifs. The induction of cytochromes P450 may result in interactions between xenobiotics and in the interference of xenobiotic metabolism and endogenous signalling pathways.

The superfamily of cytochrome P450 comprises about 500 microsomal, mitochondrial and bacterial proteins that are involved in the metabolism of endo- and exogenous com-

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✉II Department of Histology and Embryology, Silesian Medical Academy, Medyków 18, 40-752 Katowice, Poland; e-mail: pcz@slam.katowice.pl

Abbreviations: Ah, aryl hydrocarbon; CAR, constitutively activated receptor; CYP, cytochrome P450; NE, negative element; NF-1, nuclear factor-1; NR, nuclear receptor binding site; PB, phenobarbital; PBRU, phenobarbital-responsive enhancer unit; PCN, pregnenolone 16α-carbonitrile; PE, positive element; PXR, pregnane X receptor; ROS, reactive oxygen species; RXR, retinoid X receptor; SRC-1, steroid receptor coactivator protein-1.

pounds [1]. Families 1, 2, and 3 play an essential role in the biotransformation of xenobiotics in humans, being responsible for the first step of detoxication. During biotransformation, a hydrophobic substrate undergoes monooxygenation to form a hydrophilic metabolite that is easily excreted in urine or bile. Paradoxically, biotransformation often results in the metabolic activation of a substrate, as in the case of procarcinogens, promutagens and prodrugs [2, 3]. The resultant active metabolites may produce a mutagenic or carcinogenic effect by forming DNA-adducts [4, 5]. Reactive oxygen species (ROS) are also potentially dangerous by-products of biotransformation. ROS modify the purine and pyrimidine bases of DNA, thus inducing point mutations and breaks within DNA strands [6–8].

Taking into account the metabolic and genetic results of monooxygenase activation, the fact that the monooxygenase system can either be inhibited or induced by xenobiotics is of great clinical significance [9–11]. The term induction denotes a dose-dependent increase in monooxygenase activity associated with an increase in the level of CYP protein. The molecular mechanisms of P450 induction have not yet been clarified. However, it is known that an increase in the level of protein is not always paralleled by an increased rate of gene transcription because the level of protein can be regulated at the translational and posttranslational levels, as in the case of CYP2E1 [12]. A substrate may be necessary to induce monooxygenation, but not necessarily. An inducer may affect one or more P450 isoforms and the effects of inducers may sometimes overlap.

Induction of P450-dependent activities enhances the metabolic activation of substrates, thus indirectly determining their toxic, mutagenic and pharmacologic effects. The level of induction and the clinical outcomes vary from person to person, depending on genetic, dietary, physiological and environmental factors. The genetic polymorphism of P450 isoforms results in their different responses to inducers. Some P450 isoforms do not have any polymorphic variants (e.g., CYP3A4), while for other isoforms it is their polymorphism but not induction that is responsible for the expression of an individual gene (e.g., CYP2D6). Generally, most P450 isoforms are both polymorphic and inducible [3, 13].

Unfavorable effects of metabolic activation of xenobiotics are usually neutralized by conjugation during phase II detoxication. Excessive induction of phase I enzymes, polymorphism of genes encoding phase II enzymes, and different responses of phase II enzymes to the same inducers may strike the balance between metabolic activation and detoxication [14]. To sum up, the net effect of a xenobiotic depends on the following determinants: exposure, absorption, activation, detoxication and DNA repair (Fig. 1).

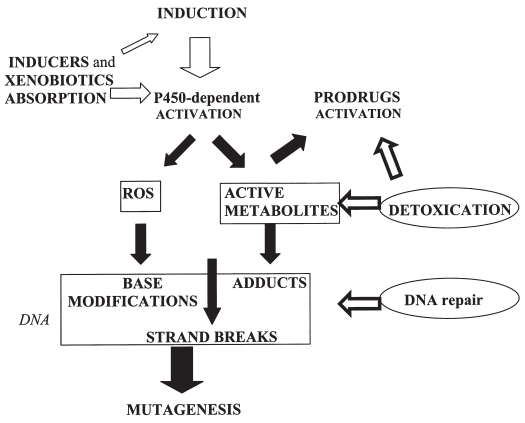

Figure 1. Metabolic and genetic effects of induction of cytochrome P450-dependent monooxygenases.

The net effect of this induction is dependent on inducer/substrate absorption, activation, detoxication and DNA repair. ROS, reactive oxygen species.

PB AND PB-LIKE INDUCERS

Although it has been known for many years that phenobarbital (PB) modifies xenobiotic metabolism, the mechanism by which it induces P450-dependent reactions is still unclear. PB exerts its action not only on phase I

enzymes (CYP 1, 2 and 3) but it also has a pleiotrophic effect on the liver, including an increase in liver mass, proliferation of the smooth endoplasmic reticulum, promotion of liver tumors, increased activities of phase II enzymes (uridine-diphospho(UDP)-glucuronosyl transferase, glutathione *S*-transferase, epoxide hydrolase, and aldehyde dehydrogenase) and enzymes involved in heme synthesis, lipid metabolism, and so on. About 50 genes in total are activated by PB in the liver [15, 16].

Phenobarbital is the prototype of a large group of xenobiotics-inducers, called PB-like inducers, that regulate gene transcription. PB-like inducers include drugs (barbiturates, phenytoin, carbamazepine, clotrimazole, lovastin, primidone, cyclophosphamide, and iphosphamide), pesticides (dieldrin, chlordane, methoxychlor), solvents (acetone, pyridine), plant products (camphor, diallylsulfide, isosafrole) and other compounds, such as 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP: first isolated as a pesticide contaminant; known as an extremely powerful PB-like inducer in mice but not in rats and guinea pigs), polychlorinated biphenyls, acetaminofluorene and *trans*-stilbene oxide [17–20].

It is difficult to explain the mechanism by which PB induces P450 because PB influences cytochromes belonging to several P450 families, such as CYP2A, 2B, 2C and CYP3A, though the last seems to be affected in a different way because it is induced by a completely different group of inducers [2, 13, 21]. CYP2 inducers (and CYP3A inducers) are structurally unrelated compounds of different sizes and inducing potencies. The features of PB-like inducers differ from species to species. Such a great variety of interactions between these inducers and their target genes was not conducive to determining whether the mechanism of PB-dependent induction is unspecific, receptor-mediated, or whether other mechanisms also play a role in this type of induction. Is was suggested that PB indirectly affected gene expression by stimulating the synthesis of steroids and steroid-induced activation of transcription *via* a steroid receptor [22]. Pinkus *et al.* [23] suggested that the regulation of PB-dependent *CYP* genes is mediated by transcription factor AP-1. In this mechanism PB induces the Fos/Jun heterodimeric (AP-1) complex bound to the enhancers of genes of glutathione *S*-transferase Ya and quinone reductase, the phase II enzymes of xenobiotic metabolism.

Attempts at identifying a barbiturate receptor have failed, so far. More promising are studies of regulatory DNA sequences in the upstream regions of PB-inducible genes and studies of proteins that bind to these sequences.

PHENOBARBITAL-RESPONSIVE SEQUENCES IN DNA

In the early nineties, Fulco [24] investigated the promotor regions of $P450_{BM\text{-}1}$ (*CYP106*) and $P450_{BM\text{-}3}$ (*CYP102*) genes in *Bacillus megaterium*; the two genes are responsible for monooxygenation of fatty acids, amides and alcohols. The protein product of *CYP102* expression resembles the liver microsomal system as it contains P450 and NADPH–P450 reductase domains. Both the genes were responsive to barbiturate inducers that are not substrates for these cytochromes. PB-inducible expression requires a 1-kbp fragment located at the 5′ end of DNA. This fragment contains operator sequences and sequences that are necessary for the synthesis of negative and positive protein regulators. Deletion analysis revealed that the initial 300-bp fragment contains a 17-bp consensus nucleotide segment, the so-called Barbie box.

The Barbie box sequence and palindromic sites in the operators of *CYP106* and *CYP102* genes are constitutively blocked by the repressor protein Bm3R1 with a helix-turn-helix motif [25, 26]. The sequence encoding Bm3R1 is located immediately upstream of the *CYP102*

gene and they together form a bicistronic operon. Both *BM3R1* and *CYP102* genes are either repressed by Bm3R1 protein or cotranscriptionally induced by barbiturates. Barbiturates induce *CYP106* and *CYP102* expressions by derepression of these genes triggered *via*: (1) direct binding of PB to Bm3R1 protein; (2) PB-stimulated competition between negative and positive DNA transcription regulators; and (3) direct interaction between positive factors (e.g., Bm1P1 and Bm1P2) and Bm3R1, which disables Bm3R1 to bind to the DNA [24–27] (Fig. 2).

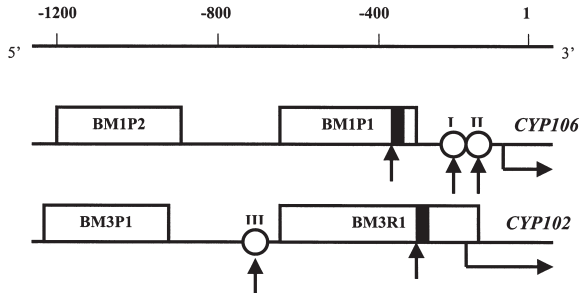

Figure 2. Structure of regions participating in the regulation of expression of *CYP106* and *CYP102* in *Bacillus megaterium*.

Vertical arrows indicate sites that are constitutively blocked by protein product of *BM3R1* sequence (negative factor). I, II, III indicate *CYP106* and *CYP102* gene operator sequences. Barbie-box elements are shown as black boxes within the coding region for Bm1P1 and Bm3R1. Derepression of these sites following PB induction results from the direct interaction of PB and positive factors (Bm1P1, Bm1P2, Bm3P1) with Bm3R1 protein or by competition of positive factors for the binding sites (BMs).

The Barbie box sequence is located in the 5′-flanking regions of almost all PB-inducible genes in various species [13]. Its conservative nature and wide distribution in nature suggested that it plays an important role in the mechanism of PB-dependent induction shared by *Procaryota* and *Eucaryota*. Moreover, the Barbie box sequence has similar locations in the *CYP106* and *CYP102* genes of *B. megaterium* and in the rat *CYP2B1/2* gene (Fig. 3). Synthetic oligonucleotides containing such a sequence bind to proteins from either rat liver or bacterial cell extracts [28]. PB alters the DNA binding activity of these proteins and, as a result, the binding of the bacterial proteins is reduced and the binding of the rat liver nuclear proteins is increased. Thus the action of the rat proteins is similar to that of positively acting bacterial factors under the influence of PB. However, it is still unclear whether these proteins show constitutive expression in mammals or, as one can conclude from *in vitro* studies, they are synthesized *de novo* under the influence of PB [29].

Studies indicate that two 5′-flanking regions, namely the proximal promoter (to –0.8 kbp) to the Barbie box and distal region (between –0.8 and –2.4 kbp), may be important for the PB-induction of *CYP* genes in mammals and birds. However, experimental data do not support the role of the Barbie box sequence in the PB-dependent induction of vertebrate genes, thus questioning the existence of close analogy between the models of *P450* gene induction in vertebrates and bacteria [30–32]. For example, the positively acting bacterial factors do not appear to require the Barbie box sequence because they can bind to mutated Barbie elements. In several transcription assays, constructs containing the proximal promoters did not respond to PB, and the proximal promoter regions of *CYP2B1/2*, including the Barbie box sequence, were not detected as a protein binding region after PB-treatment. In transgenic mice that contained the rat *CYP2B2* gene the proximal promoter elements were expressed constitutively and were not induced by PB, while the expression of transgenes containing the distal elements was PB-dependent. Moreover, mammals and bacteria differ in the transcriptional activity and in the structure and dose of the PB-like inducers. There are also substantial differences in the structure and composition of bacterial DNA and vertebrate chromatin and transcriptional activation, the last involving structural changes in vertebrates. The Barbie box is not present in

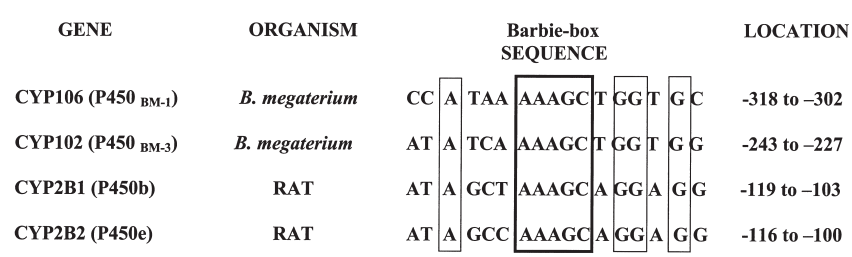

Figure 3. Comparison of Barbie box nucleotide sequences and their locations in the 5′-flanking regions of PB-dependent genes in *Bacillus megaterium* and rat.

Homologous sequences in all four genes are boxed. The central core sequence is AAAGC (modified [24]).

the upstream enhancer regions of mouse *Cyp2b10*, rat *CYP2B1/2* and chicken *CYP2H1* genes, which are all PB-responsive. In the chicken *CYP2H1* gene, a proximal promoter region of 160 bp binds multiple liver-enriched transcription factors and responds to constitutive, but not PB-induced, activation [27, 33–35].

PROXIMAL PROMOTER

Studies of a promoter fragment from +1 to –179 have shown that an NF-1-like sequence, C/EBP sequence and TATA box form a core promoter which is involved in the constitutive expression of *CYP2B1/2* [27, 34]. The proteins that bind to this NF-1-like sequence and TATA have not been characterized, whereas different complexes of C/EBP family members and DNA that activated transcription were observed. C/EBP probably participates in PB-induced expression but it indirectly mediates the inducer response. Region –69 to –89 functions as a positive element (PE) and contains the Barbie box and a G-rich sequence. Reports on protein binding to either the Barbie box or the PB-dependent positive element are contradictory, and in some studies mutations of the Barbie box did not reduce *CYP* expression whereas mutations in the G-rich sequence (BTE) and C/EBP reduced *CYP* expression by 65% and 80%, respectively, in untreated rats and HepG2 cells [31, 36]. Based on these studies, BTE and C/EBP seem to be the major positive elements. In contrast to the other elements of the proximal promoter, the G-rich and C/EBP elements of *CYP2B1/2*, *Cyp2b9* and *Cyp2b10* are very similar [27].

Region –160 to –126 of the proximal promoter contained a negative element (NE) that bound 68- and 44-kDa proteins in nuclei from PB-treated rats. The PE and NE proteins purified by affinity chromatography appear to be identical to those obtained by SDS/PAGE analysis, where they migrate as a 26–28 kDa protein [34]. A 94–100 kDa protein also interacts with the positive element in response to PB treatment. The binding of proteins to PE or NE is accompanied by phosphorylation or dephosphorylation, respectively, though the PE can bind both phosphorylated and dephosphorylated proteins. It is likely that the constitutive transcription of *CYP2B1/2* occurs when a dephosphorylated protein is bound to NE. PB is likely to mediate the phosphorylation of a factor that is detached from the NE and then is bound to the PE. Afterwards, transcriptional activity is mediated by the binding a 65-kDa protein to the PE and by the interaction of a 94–100 kDa protein with an upstream enhancer.

To sum up, it was proposed that the proximal promoter of *CYP* responds to PB by binding proteins to its either positive or negative elements [27, 34] (Fig. 4).

PHENOBARBITAL-RESPONSIVE ENHANCER UNIT (PBRU)

Studies of proximal and distal regulatory elements showed that proximal promoter se-

quences are not sufficient for PB-dependent expression of the *CYP2B1/2* gene. As was shown in transgenic mice, the distal regulatory elements required for a response to PB are located upstream of –800 [30]. In the absence of the distal region, promoter fragments of *CYP2B1* and *CYP2C1* did not exhibit any positive response to PB [31]. This region is also responsible for liver-specific gene expression. More precise studies of the DNase I hypersensitivity of the *CYP2B1/2* genes, by transfection into primary hepatocytes and *in situ* transient transfection of liver, defined the presence of PB-responsive elements between –2.2 and –2.4 kbp, which are functionally independent of proximal promoter elements [27]. These sequences constitute the phenobarbital-responsive enhancer unit (PBRU; or PBRE Module – PBREM) (Fig. 5).

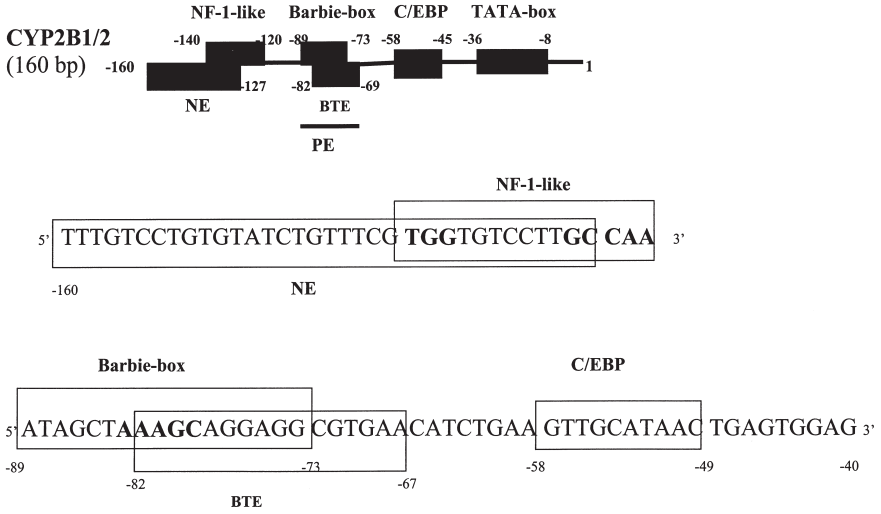

Figure 4. Structure of proximal promoter region in rat *CYP2B1/2* gene.

Proximal promoter elements are insufficient for the PB-dependent activation of *CYP* genes. In the mouse *Cyp2b9* and *Cyp2b10* genes the Barbie box is split by a 42-bp insertion (not presented). It indicates that the Barbie box is not a conserved region in mammals. NE, negative element; PE, positive element.

A PBRU that confers PB inducibility on a *cat* reporter gene has been identified in the *CYP2B2* 5′-flanking region as a 163-bp *Sau*3AI fragment with transcriptional enhancer properties situated between –2318 and –2155 bp upstream from the gene transcription start point [37]. A study using *in situ* DNA injection into rat liver has confirmed that *Sau*3AI confers PB-responsiveness on a heterologous promoter [36]. Further studies using transfection analysis, deletion analysis and DNase I footprinting have shown that *Sau*3AI (PBRU) is a multicomponent enhancer that contains cognate recognition sequences and interacts with multiple regulatory proteins [38].

The functional nuclear factor 1 (NF-1) motif, composed of the **TGG**CACAGT**GCCA** sequence, plays a key role in the PB-dependent expression of *CYP2B2* [18, 27, 39–41]. The flanking sequences of NF-1 are responsible for the specific binding of NF-1 factor, and their mutations reduced the expression of *CYP2B2* after PB induction. NF-1 is one of the positive elements in the PBRU, for which appropriate factors have been identified, and is able to bind 12 constitutively expressed proteins – products of four NF-1 genes. The choice of a factor may depend on sequences flanking the NF-1 site and/or modifications of a PB-dependent NF-1 isoform. It is likely that other unknown positive and negative factors as well as cofactors that do not bind directly to the DNA are involved in induction.

A region homologous to the rat PBRU has been described in the mouse *Cyp2b10* gene (–2397 to –2265) with 91% sequence identity [42]. Afterwards, the mouse gene was found to have a 51-bp multicomponent enhancer element (–2339 to –2289) that contained a NF-1 motif flanked by nuclear receptor binding sites (NRs). This element showed almost 100% identity to the rat PBRU of *CYP2B1/2* genes [18]. These sequences are not conserved in all representants of the *CYP2B* family. The po-

tentially critical corresponding NR and NF sequences of the PB-nonresponsive mouse *Cyp2b9* gene (downstream to –838; 83% identity to the *CYP2B2* PBRU) showed multiple mutations, that abolished the PB-dependent PBRU activity [42].

The NR binding sites of both rat *CYP2B1/2* and mouse *Cyp2b10* genes are composed of imperfect direct repeats of the consensus for nuclear receptors, AGGTCA-like half sites spaced by 4 bp (DR4). Similarly constructed NR motifs of 16-bp, but with opposite half-sites 5′ to 3′ orientation and different location from –5.8 to –1.1 kbp [44]. It was reported to mediate PB induction of a heterologous promoter in primary cultures of chicken hepatocytes. It contains two separate enhancer regions (–5900 bp to –4550 bp and –1956 bp to –1400 bp). The latter region (556-bp) did not contain the Barbie box [33]. Instead, it had a 240-bp fragment (–1640 to –1400 bp) containing several transcription factor binding sites, e.g. an E-box-like element binding USF-like protein, a CCAAT-box motif binding C/EBP-related protein and a NF-1 motif. The proteins binding to the 240-bp fragment are different from those recently reported to be required for the activity of the PB-responsive enhancer domains of rodent *CYP2* genes [45]. The fourth identified sequence did not match any known transcription factor-binding site and binds to unknown protein complexes. All the above-mentioned binding sites of *CYP2H1* are necessary for maximal induction and no site alone is critical.

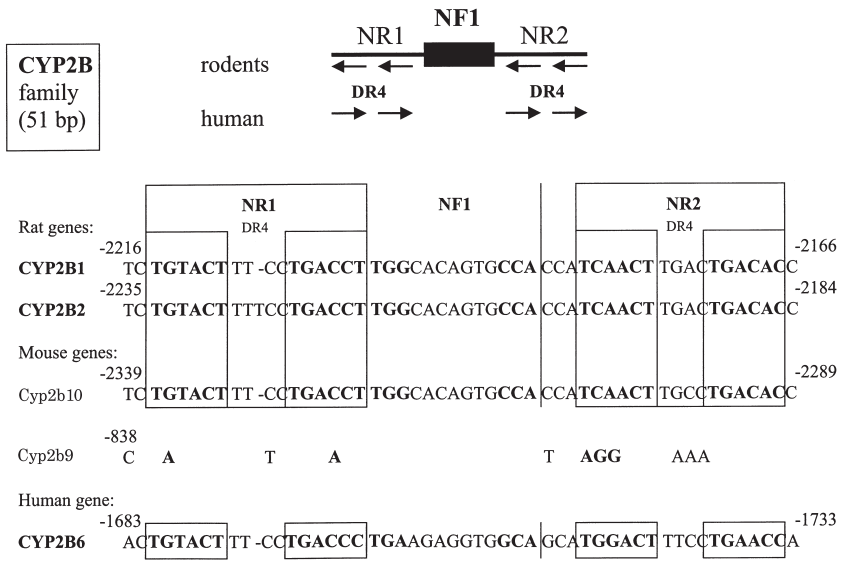

Figure 5. Structure of PBRUs in mammalian *CYP2B* genes.

PBRUs seem to be indispensable for PB-dependent activation of *CYP* genes. In the 51-bp enhancer element from noninducible mouse *Cyp2b9*, differences in the sequence compared to *Cyp2b10* PBRU are indicated. NF1, nuclear factor-1 binding site; NR1 and NR2, nuclear receptor binding sites; DR4, direct repeat spaced by 4 bp.

cation (–1733 to –1683), have been described in the human *CYP2B6* gene [40]. The human NR2 shows 87% identity to NR1. The NR1 sequences of human and mouse PBRUs differ in 1 base, making NR1 the most conserved site among human, mouse, and rat PBRUs. Studies of human *CYP2B6* have also shown that NR1 alone is sufficient to confer PB responsiveness to CAR-mediated transactivation. Both the sequence, orientation and spacing of NR half sites are important in choosing a receptor protein that binds to NR, and these features appear to dictate selective transcriptional effects [43]. The features of NR indicate that heterodimerization conditions effective expression of *CYP2B* genes.

Another 5′ flanking region that responds to PB has been identified in the chicken *CYP2H1*

PUTATIVE PHENOBARBITAL RECEPTORS

The critical question for PBRU regulation is whether there is specific receptor binding after inducer treatment. Compared with the

well-known Ah receptor-mediated regulation of the *CYP1A1* gene, no receptor for PB and PB-like inducers has been found to date [35]. However, searches are in progress for known orphan receptor proteins that are expressed and bound to PBRUs after PB administration.

In vertebrates, orphan receptors are a group of about 30 proteins, whose ligands have not yet been identified [46]. One of them, the nuclear orphan receptor CAR (constitutively activated receptor), is a mammalian and yeast receptor which, as a heterodimer with retinoid X receptor (RXR), can activate a set of retinoic acid response elements (RAREs) consisting of direct repeats related to the hexamer AGGTCA [43, 47]. CAR is transcriptionally active in the absence of exogenous ligand and its constitutive activity was specifically blocked by testosterone 5α-reduced metabolites with a 3α-hydroxy group, namely 5α-androst-16-en-3α-ol (androstenol) and 5α-androstan-3α-ol (androstanol) [48], both of which are unable to activate the androgen receptor [49]. The CAR ligand binding domain probably assumes an active conformation in the absence of the ligand and is shifted toward an inactive conformation on binding the naturally occuring inverse agonists. The activation of CAR may be explained by the ligand-independent recruitment of a co-activator, such as steroid receptor coactivator protein-1 (SRC-1), followed by androstane-induced co-activator-release. On the other hand, CAR repression by testosterone metabolites did not affect the CAR- RXR-DNA binding and CAR-RXR binding in several *in vitro* and *in vivo* investigations. Thus, endogenous androstanes (inhibitors) antagonize putative endogenous CAR ligands (activators) or CAR acts as a ligand-independent transcriptional activator, which undergoes exogenous ligand-mediated deactivation [50]. It is most likely that physiological ligands of CAR have a steroid-like structure.

It has been shown, that after PB treatment, the CAR-RXR heterodimer functions as a *trans*-acting factor responsible for the NR1-mediated transcription activity of PBRU in the liver, because the binding of CAR and RXR to NR1 increased sharply *in vivo* and occured in accordance with the subsequent accumulation of *Cyp2b10* mRNA [39]. Also, transient transfections of HepG2 cells with CAR-expressing plasmids showed that the human *CYP2B6* gene can be regulated by CAR and a number of known PB-like inducers by binding to the NR1 site [40]. It is also important that the *in vitro* CAR-RXR heterodimer bound to the ER6 responsive element of the human *CYP3A4* gene and mediated PB-dependent expression in a stable HepG2 cell line. The latter observation indicates that CAR can bind to distinct response elements and thus it can mediate pleiotropic effects of PB. However, the direct binding of the inducer to CAR has not been demonstrated, and it remains unclear how PB-like compounds can activate the binding of CAR to PBRU: Does this happen by interferring with the binding of antagonists (androstenol and androstanol), or in a different way?

PXR is another orphan receptor that is induced by PB [51]. PXR (pregnane X receptor) displayed a high selective expression in the liver and intestinal epithelium of the embryo and the adult – the same in which *CYP3A* gene expression is induced. Weaker receptor protein expression was detected in kidney and stomach. PXR is activated by structurally unrelated inducers of human CYP3A4 and rat CYP3A1 (macrolide antibiotics, antimycotics, some natural steroids, synthetic glucocorticoids, antiglucocorticoids, and PB-like inducers) [13]. Human, rat and mouse PXRs are usually activated by the same inducers but human and animal homologues of PXR can differ significantly in ligand binding properties, e.g., rifampicin is a stronger inducer, while pregnenolone 16α-carbonitrile (PCN) is a weaker inducer of the human receptor compared with the mouse receptor. Different levels of PXR activation are a probable cause of species differences in *CYP3A* expression. Many well-known inducers of *CYP3A4* acti-

vated the human PXR in transfection studies. Physiologically, PXR probably regulates steroid homeostasis because its natural activators are endogenous C21 steroids (e.g., the progesterone metabolite, 5β-pregnane-3,20-dione), corticosteroids and estrogens. Moreover, it has been shown that PXR increases *CYP3A* gene transcription after heterodimerization with RXR [50–52].

PXR exists as two isoforms, namely PXR1 (431 amino acids) and PXR2 (390 amino acids) [53]. The difference in length results from the lack of a single exon in PXR2. The lacking DNA segment encodes 41 amino acids in the ligand binding domain and is responsible for the greater responsiveness of PXR1 to natural and synthetic steroids.

The ligand binding and DNA binding domains of PXR display 39% and 64% homologies, respectively, to the vitamin D receptor (VDR). Such a great structural similarity of DNA binding domains of VDR and some orphan receptors indicates that PXR isoforms may bind to similar hormone response elements and form similar heterodimers. It has been shown that PXRs bind specifically as RXR-heterodimers to the half-site sequence AGTTCA separated by a three-nucleotide spacer (DR-3) within the *CYP3A1* and *CYP3A2* genes or by an inverted ER6-type repeat within the *CYP3A4* gene promoter [51]. This motif is not typical of the glucocorticoid response element specific for the glucocorticoid receptor (GR) [54] but can be activated by PXR in response to GR agonists and antagonists, such as dexamethasone, PCN and RU486. As in the case of CAR, SRC-1 has also been shown to be necessary for optimal transcriptional activation, and to interact with PXR in the presence of its ligands in a dose-dependent manner [53].

The fact that the inducer binds to the receptors that are constitutively responsible for the regulation of their target genes by endocrine factors and the fact that these receptors heterodimerize with the same protein (RXR) may indicate that the metabolism of exogenous compounds interferes with endogenous signal transduction [55]. Study of molecular mechanisms that control these relationships will let us determine (1) to what extent the activation of monooxygenases can accelerate the metabolism of endogenous ligands for orphan receptors and (2) to what extent endogenous ligands (steroid hormones and fatty acids) can modify the metabolism of drugs, toxins, and mutagens (Fig. 6).

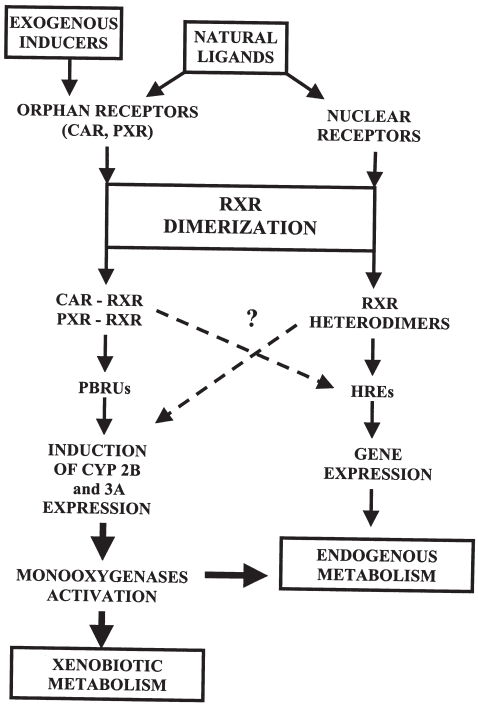

Figure 6. Interactions between PB-dependent induction of monooxygenases, xenobiotic metabolism, and metabolism of endogenous compounds.

Endogenous and exogenous ligands may utilize the same orphan receptors. RXR heterodimerizes with orphan receptors for both exogenous and endogenous ligands and with nuclear receptors. Natural ligands for nuclear receptors can be metabolized by CYP enzymes. Other relations are also possible. Thus, the metabolism of exogenous compounds interferes with endogenous signal transduction. HRE, hormone response element.

REGULATION OF PBRU

Based on experimental data, Kemper [27] proposed two models of the regulation of *CYP2B2* expression by PB in which changes in the configuration of chromatin accompa-

nied by histone acetylation are a major activating factor. It is known that the acetylation of lysine residues is more abundant in the amino-terminal regions of histones H3 and H4 [56]. The role of the relationship between histone acetylation and transcription remains obscure, though histone acetylation may be associated with activation or recruitment of major transcription factors and the RNA polymerase II complex to the RNA initiation site.

The more probable model of promotor regulation is based on the presence of DNase I hypersensitivity of PBRU in both untreated and PB-treated animals, which reflects binding of regulatory proteins and disruption of the chromatin structure [27]. In this model the chromatin, which is maintained in its transcriptionally inactive state by negative coregulators, is bound to regulatory factors and the putative PB receptor in the absence of the ligand, and either suppressors or negative cofactors inhibit transcriptional activity while positive coregulators stimulate it. In this model, PB would alter the activity of regulatory factors, thus leading to changes in the chromatin structure, recruitment of major transcription factors and gene expression. In this case, PB would abolish gene repression and would activate positive factors without uncoupling proteins from the promoter and PBRU. There is increasing evidence that phosphorylation of transcription factors may be of crucial importance for DNA binding in mammalian *CYP2B* genes because it has been shown that increased cellular cAMP levels inhibited PB induction in primary hepatocyte cultures [57].

In the other model [27], constitutive positive factors cannot bind to the proximal and distal regions of 5′-end because of the chromatin structure maintained by repressor factors or deacetylation of the histones. In this model, PB would cause the binding of a factor that alters the chromatin structure, thus enabling the binding of positive factors to DNA. A change in the chromatin structure at the PBRU would facilitate the binding of positive factors and would alter the chromatin structure at the proximal promoter, making it possible for positive factors to bind to this region.

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