Published in final edited form as:

Expert Opin Drug Metab Toxicol. 2010 March ; 6(3): 261–271. doi:10.1517/17425250903483215.

Nuclear Receptor-Mediated Regulation of Carboxylesterase Expression and Activity

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Abstract

i) Importance of the field—Emerging evidence demonstrates that several nuclear receptor (NR) family members regulate drug-inducible expression and activity of several important carboxylesterase (CES) enzymes in mammalian liver and intestine. Numerous clinically prescribed anticancer prodrugs, carbamate and pyrethroid insecticides, environmental toxicants, and procarcinogens are substrates for CES enzymes. Moreover, a key strategy employed in rational drug design frequently utilizes an ester linkage methodology to selectively target a prodrug, or to improve the water solubility of a novel compound.

ii) Areas covered in this review—This review will summarize the current state of knowledge regarding NR-mediated regulation of CES enzymes in mammals, and highlight their importance in drug metabolism, drug-drug interactions, and toxicology.

iii) What the reader will gain—New knowledge regarding the transcriptional regulation of CES enzymes by NR proteins pregnane x receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3) has recently come to light through the use of knockout and transgenic mouse models. Novel insights regarding the species-specific cross-regulation of glucocorticoid receptor (GR, NR3C1) and peroxisome proliferator activated receptor alpha (PPAR α , NR1C1) signaling and CES gene expression is discussed.

iv) Take home message—Elucidation of the role of NR-mediated regulation of CES enzymes in liver and intestine will have a significant impact on rational drug design and the development of novel prodrugs, especially for patients on combination therapy.

Keywords

carboxylesterase; drug metabolism; nuclear receptor; pregnane X receptor; constitutive androstane receptor; xenobiotic response

Introduction

1.1 Classification of Carboxylesterase

In 1953, Aldridge classified esterase enzymes in rabbit, rat, and horse serum based upon the nature of their interaction with organophosphates [1]. Esterases that were unaffected by organophosphates and degraded the compounds were classified as A-esterases, whereas

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Declaration of interest

The authors state no conflicts of interest and have received no payment in the preparation of this manuscript.

esterases that were inhibited by organophosphates were classified as B-esterases. Studies by Bergmann et al. revealed the presence of a third group of esterases (the C-esterases) that did not interact with organophosphates at all [2]. Using this classification scheme, the superfamily of carboxylesterase (CES) enzymes belong to the B-esterase group. Several attempts have been made to classify the CES enzymes. Walker and Mentlein et al., attempted to classify CES enzymes on the basis of their substrate specificity [3,4]. However, this classification scheme was ambiguous because of the broad and overlapping substrate specificity of CES enzymes. In 1998, Satoh and Hosokawa originally proposed a novel classification scheme of CES enzymes across species that was based upon the extent of amino acid homology and substrate selectivity [5]. This scheme classified the known CES enzymes into four main groups (CES 1–4), and several additional subgroups. More recently, the same authors have used this same scheme to show that there are five groups of CES enzymes (CES 1–5), and revealed that the majority of identified CES enzymes belong to either the CES 1 or CES 2 sub-family [6]. Importantly, it is now known that the CES-1 and CES-2 sub-families are the major source of carboxylesterase enzymatic activity in liver and intestine tissues that participate in the hydrolysis of drugs and xenobiotics in mammals [6].

1.2 Function of Carboxylesterase Enzymes

The CES family of enzymes is a key participant in the phase-I drug metabolism process, catalyzing the hydrolysis of a wide range of ester- and amide-containing compounds. Of particular clinical relevance, these enzymes participate in the biotransformation of numerous drugs and prodrugs including the anti-platelet drugs aspirin and clopidogrel [7], the angiotensin-converting enzyme inhibitors delapril, imidapril, and temocapril [8], the anti-tumor drugs irinotecan and pentyl PABC-doxaz [9,10], the narcotics cocaine and heroin [11], and the anti-influenza prodrug oseltamivir [12,13]. The CES family of enzymes is involved in the detoxification of environmental toxicants, such as pyrethroids, a major class of insecticides used world wide and extensively in the United States [14]. CES enzymes also play a role in the conversion of pro-carcinogens into carcinogens. For example, vinyl acetate, which is used in the paint, adhesive, and paper-board industry, is metabolized by CES enzymes into acetaldehyde in the liver. Acetaldehyde subsequently binds to DNA and proteins eventually leading to nasal tumor formation in rodents [15]. Numerous endogenous compounds are substrates for CES enzymes including palmitoyl-coenzyme A, short- and long-chain acylglycerols, as well as medium- and long-chain acylcarnitines [5,16]. Because a large number of clinically used drugs and prodrugs are metabolized by CES enzymes, it is important to clarify the structure, substrate selectivity, tissue distribution, and species specificity of CES enzymes.

1.3 Structure of CES Enzymes

The crystal structure of human carboxylesterase 1 (hCE-1) was determined in 2003. The enzyme is comprised of three structural domains: a central catalytic domain, an α/β domain, and a regulatory domain. The central catalytic domain contains the serine hydrolase catalytic triad at the base of the active site gorge, whereas the regulatory domain contains the low-affinity surface ligand-binding Z-site [17,18]. The CES enzymes are localized in the endoplasmic reticulum and cytosol of many tissues, but are highly enriched in liver and intestine [6,23]. It has been determined that an 18-amino acid N-terminal hydrophobic signal peptide is responsible for the localization of these proteins to the endoplasmic reticulum [19], whereas enzymatic activity is lost by removing the N-terminal domain. The His-X-Glu-Leu (HXEL) sequence present at the C-terminal, which can bind with KDEL receptor, is essential for retention of the protein in the luminal site of the endoplasmic reticulum.

1.4 Substrate Selectivity of CES Enzymes

Amino acid sequence homology between human carboxylesterase 1 (hCE-1), a member of CES 1 family, and human carboxylesterase 2 (hCE-2), which belongs to CES 2 family is 48% [10]. However, the substrate selectivity of these two enzymes is different. The hCE-1 enzyme mainly hydrolyzes substrates with small alcohol groups and large acyl groups, such as cocaine (methyl ester), meperidine, and delapril. In contrast to hCE-1, the hCE-2 enzyme efficiently hydrolyzes compounds with large alcohol groups and relatively smaller carboxylate groups, such as 4-methylumbelliferyl acetate, heroin, and 6-acetylmorphine [20].

1.5 Tissue Distribution of CES Enzymes

The expression of CES enzymes is ubiquitous in mammals. Among various tissues of mammals, the highest hydrolase activity is present in liver [21]. In addition to liver, CES enzymes are also detected in small intestine, kidney, and lung [6]. The hCE-1 enzyme is highly expressed in the liver, and also detected in macrophages, human lung epithelia, heart, and testis [22]. The hCE-2 enzyme is found in the small intestine, colon, kidney, liver, heart, brain, and testis [23,24]. Although these two enzymes are present in various tissues, hCE-1 and hCE-2 contribute predominantly to the hydrolase activity of liver and small intestine, respectively. It has also been shown that CES enzymes exhibit species differences. For example, Li et al demonstrated that human plasma contains no CES enzyme activity, in contrast, the mouse, rat, rabbit, horse, cat, and tiger all have high levels of plasma CES enzymes [25]. However, in humans it is likely that serum butyrylcholinesterase and paraoxonase enzymes perform analogous functions to the CES enzymes found in serum from these other species.

2. PXR and CAR, Two Xenobiotic-Sensing NRs

As CES enzymes play very important roles in drug metabolism, their expression levels are tightly controlled by the NR proteins pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3). NR proteins comprise a large superfamily of ligand-activated transcription factors that are involved in diverse physiological, developmental, and metabolic processes. They are characterized by a conserved N-terminal zinc-finger type DNA-binding domain and a C-terminal ligand-binding domain [26]. The PXR and CAR proteins are two closely related members of this superfamily. Both of these proteins function as ligand-activated transcription factors by interacting with the retinoid-x-receptor- α (RXR α , NR2B1) on response elements located in the control regions of specific genes that they regulate.

PXR functions as a master-regulator of xenobiotic- and drug-inducible cytochrome P450 (CYP) gene expression in liver and it is now well established that PXR regulates the drug-inducible expression and activity of numerous genes that encode key members of the CYP3A, CYP2B and CYP2C subfamily of drug-metabolizing enzymes in humans and rodents [27, 28]. PXR also regulates the drug-inducible expression of other genes whose gene products are involved in the metabolism of xenobiotic compounds including glutathione *S*-transferase, sulfotransferase, and UDP-glucuronosyltransferase enzymes in liver [29-32]. Moreover, additional PXR-target genes encode key hepatic drug transporter proteins such as organic anion transporting polypeptide 2, multidrug resistance 1/P-glycoprotein, and multidrug resistance proteins 2 and 3 [33-35].

Similar to PXR, the NR superfamily member CAR is also recognized as a xenobiotic-sensing NR mainly expressed in hepatic tissue. It was originally demonstrated to regulate the phenobarbital-inducible expression of several genes encoding important members of the CYP2B subfamily of enzymes [36]. CAR has since been shown to regulate the expression and activity of a number of phase-I and phase-II metabolic enzymes, as well as the expression and activity of numerous important membrane transporter proteins involved in the metabolism and

elimination of xenobiotics [37]. It has been demonstrated that PXR and CAR share distinct but overlapping sets of target genes involved in drug and xenobiotic metabolism, often through shared NR-response elements. For instance, PXR can regulate CYP2B genes through recognition of the Phenobarbital-response element (PBREM), whereas CAR is also found to activate gene expression through the xenobiotic response element (XREM) in the upstream promoter of the CYP3A4 gene in humans [29,38,39]. Because PXR and CAR are activated by a myriad of xenobiotic compounds and regulate the expression of numerous genes involved in drug and xenobiotic metabolism, the activation of these two receptors serves as a principal defense mechanism defending the body from toxic insult. In this way, activation of PXR and CAR by xenobiotic compounds and drugs coordinately regulates the expression and activity of functionally linked metabolic enzymes and membrane-bound transporter proteins to increase the elimination of potentially toxic compounds from the body [27,32,39-41]. Additionally, these two transcription factors form the molecular basis of an important class of drug-drug interactions in the clinical setting. PXR and CAR-mediated gene activation by one drug increases the metabolism and elimination of a myriad of other co-administered drugs from the body.

3. Regulation of CES Enzymes by PXR and CAR

3.1 Regulation by PXR

Studies by Zhu et al. show the involvement of PXR and the glucocorticoid receptor (GR, NR3C1) in regulating the species-specific expression and activity of the homologous enzymes in both rat and human model systems [42]. The hCE-1 and hCE-2 genes encode the two major forms of human liver microsomal carboxylesterase enzymes. Exposure of primary cultures of human hepatocytes to micromolar concentrations of dexamethasone induces hCE-1 and hCE-2 protein expression in a concentration-dependent manner. In contrast, exposure of rat hepatocytes to nanomolar concentrations of dexamethasone represses the expression of carboxylesterase genes [43]. The GR serves as a master-regulator of glucocorticoid-regulated gene expression in response to nanomolar concentrations of glucocorticoids. It is interesting to note that the promoter sequences of hydrolase B and S that contribute to the repression of CES enzymes lack any canonical GR-response elements. This suggests that the GR-mediated suppression of CES gene expression in rats is achieved via a non-classical mechanism. In contrast to rats, treatment of human hepatocytes with ten micromolar rifampicin, the prototypical human PXR-activating compound, causes moderate induction of hCE-1 and hCE-2 gene expression [43]. Interestingly, treatment of cultured human hepatocytes with 8-methoxypsoralen, which is a prototypical photochemotherapeutic drug, increases hCE-2 gene expression [13]. Moreover, knockdown of PXR using si-RNA technology decreases hCE-2 mRNA levels, whereas over-expression of the PXR protein significantly increases hCE-2 expression at both the messenger RNA and protein levels.

PXR also exerts a positive effect on liver-enriched expression of CES enzymes in rodents. In rats, the best characterized carboxylesterase enzymes include hydrolase A, B and S (HA, HB, HS). Co-transfection of PXR stimulates the promoter activity of HB and HS in response to dexamethasone at micromolar concentrations [43]. Tully et al. characterized the effects of triazole fungicides in SD rats using microarray analysis [31]. Gene expression profiling of liver shows induction of *Ces2* is produced by four triazole fungicides, and is likely dependent on PXR/CAR-mediated gene activation pathways. A similar study by Goetz et al., utilized gene expression profiling of the liver of CD-1 mice treated with four triazole fungicides. Expression of the *Ces2* gene is induced by three triazole fungicides, suggesting involvement of PXR/CAR-regulated pathways in triazole metabolism and perhaps toxicity [44]. Earlier research by Rosenfeld et al., indicates that over-expression of a constitutively active form of human PXR in mouse liver has a positive effect on the expression of mouse genes encoding *Ces2* and *Ces3* enzymes in liver [45].

The mouse *Ces6* gene was first identified in 2004 and encodes a protein of 558 amino acid residues in length that functions to hydrolyze select pyrethroid compounds [46]. Recently, our lab has demonstrated that the *Ces6* gene represents a likely PXR-target gene in mouse liver and small intestine [47]. By exploiting the PXR knockout mouse model, we reveal that induction of *Ces6* messenger RNA and protein by pregnenalone 16 α -carbonitrile (PCN), a well known rodent PXR activator, is PXR-dependent in both mouse liver and intestine.

3.2 Regulation by CAR

Compared to PXR, relatively little is known about the regulation of drug-inducible CES gene expression by CAR activation in any species or tissue. Historical reports indicate that treatment of rats with phenobarbital (PB) increases CES expression in liver tissue [48]. Studies from Xu et al. show that *Ces6* represents a CAR-target gene in mouse liver and small intestine [47]. It is interesting to note that in small intestine, the expression of *Ces6* is exclusively regulated by 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) but not by PB, both of which are CAR activators. These data suggest that there may be differences in the bioavailability of PB and TCPOBOP, or perhaps the differences in the mode of CAR activation by these two ligands in small intestine are responsible for the absence of *Ces6* gene activation by PB. Moreover, TCPOBOP is a much more potent and efficacious activator of rodent CAR, thus is a much more effective chemical to use in rodent studies.

3.3 Coordinate Regulation of Gene Expression by PXR and CAR

Using ChIP-sequencing analysis of control and PCN-treated mouse livers, we observed constitutive PXR-binding to three enhancer elements located in the upstream region of the mouse *Ces6* gene under physiological conditions (figure 1, top panel), which are approximately 84bp (site 1), 1796bp (site 2), and 2340bp (site 3) upstream of the transcription start site of *Ces6*. Most interestingly, treatment with the mouse PXR agonist PCN produces an approximately 2-fold overall increase in PXR binding to all the three sites, particularly to the second site (site 2), which binds to PXR with the highest affinity. In addition, a new PXR binding site occurs further upstream (–2772bp) with moderate fold-enrichment (average value = 40) (figure 1, bottom panel). Close examination of the DNA sequences that constitute site 2 reveals a cluster of likely NR-response elements located within 70 base pairs of each other and these are depicted in figure 2. Using two oligonucleotides, designated as ‘long’ and ‘short’, derived from this DNA sequence we performed electrophoretic mobility-shift analysis and show that both CAR/RXR α and PXR/RXR α protein complexes bind directly to these putative response elements (figure 3). Importantly, competition-binding using an oligonucleotide that comprises the prototypical shared PXR/CAR response element, an everted repeat spaced by 6 nucleotides (ER6) derived from the well-characterized promoter of the CYP3A4 gene, shows that binding to the putative *Ces6* response elements is specific. Conversely, a mutant form of the same oligonucleotide (mtER6) did not compete for binding, whereas the homologous oligonucleotides comprising the ‘long’ and ‘short’ experimental oligonucleotides compete well for binding of both the CAR/RXR α and PXR/RXR α protein complexes. Hence, the PXR and CAR NR superfamily members play direct and competitive roles in regulating the drug-inducible expression and activity of an important liver- and intestine-enriched mouse CES enzyme. Together with numerous other drug-metabolizing enzymes and drug transporter proteins in liver and intestine, PXR and CAR regulate the expression and activity of key CES enzymes that coordinately determine the pharmacokinetic and pharmacodynamic properties of numerous clinically prescribed and xenobiotic compounds in vivo in liver and intestine. Taken together, the data lead to a model in which drug-inducible activation of intestinal CES activity in intestine would be expected to increase the conversion of prodrugs to the active form of the drug, thereby increasing transport to portal vein and liver (figure 4). In the liver, high levels of cytochrome P450 and CES activity would be expected to further increase metabolism of co-administered drugs, thereby leading to increased prospects for drug-drug interaction in

patients on combination therapy. Moreover, activation of these pathways by PXR and CAR would be expected to increase the conversion of pro-carcinogens into carcinogenic compounds in these tissues.

4. Other NRs Regulate CES Enzymes

In addition to PXR and CAR, CES enzymes are also regulated by other NR proteins, such as hepatocyte nuclear factor-4 α (HNF-4 α , NR2A1), peroxisome proliferator-activated receptor α (PPAR α , NR1C1), and glucocorticoid receptor (GR, NR3C1). HNF-4 α is mainly expressed in liver, intestine, pancreas and kidney, and is critical for transcriptional regulation of many genes in liver, such as Cyp7a1, CAR, and genes involved in the control of lipid homeostasis, glucose transport and glycolysis [49-52]. HNF-4 α has also been implicated in the regulation of mouse *Ces2* gene transcription. In the same study, bile acids are shown to repress expression of mCES2 by inhibiting the HNF-4 α -mediated transactivation of the mCES2 gene promoter [53].

Peroxisome proliferator-activated receptors (PPARs) are mainly involved in lipid and glucose homeostasis, control of inflammation and wound healing, and regulation of food intake and body weight [54,55]. However, there appears to be a connection between PPARs and hepatic CES gene expression in rodents as well. The PPAR α protein, one of the three subtypes of PPARs, is predominantly expressed in tissues with a high oxidative capacity such as heart and liver. Research by Poole et al. showed that exposure to peroxisome proliferators, strong activators of PPAR α in liver, leads to down-regulation of the expression of CES family members. The alteration in CES expression is dependent on the PPAR α protein in mouse [56].

5. Conclusions

NRs are key regulators of many drug metabolizing enzymes that play diverse roles in xenobiotic and endobiotic metabolism. This review summarizes the evidence that several key NR proteins, including PXR, CAR, HNF-4 α , PPAR α , and GR, are involved in the regulation of CES enzymes. Because CES enzymes are one of the major determinants of the metabolism and disposition of numerous prodrugs through their actions in liver and small intestine, elucidating the mechanism governing the regulation of CES enzyme expression and activity by NR proteins will have a significant impact on rational drug design and the future development of prodrugs.

6. Expert Opinion

It is well known that activation of NRs, such as PXR and CAR, coordinately regulates the expression and activity of numerous drug-metabolizing enzymes as well as multiple drug transporter proteins. Not only can this coordinated regulation protect cells from toxic insult, but it also represents the molecular basis for an important class of drug-drug interactions in clinical settings. For example in multi-drug therapy, if one drug activates PXR and/or CAR, and the other is administered as a prodrug that is metabolized and eliminated by PXR/CAR-target genes, the resulting increased biotransformation of the prodrug into an active drug would probably lead to markedly discrepant pharmacological activities and pharmacokinetic behavior, or even serious and toxic side effects. As is the case with several anticancer drugs, there is an emerging role for PXR and CAR in regulating CES enzymes that exert an important effect on the hydrolytic biotransformation of a number of clinically used drugs and prodrugs. Additional evidence is emerging which points to a key role for PXR in regulating blood-brain barrier permeability in response to drug treatment [57,58], and also in modulation of multi-drug resistance and estrogen sensitivity in certain breast cancers [59,60]. Further elucidation of the role of the PXR protein in these clinically significant areas will likely produce important information that could be exploited as novel targets for cancer treatments.

Numerous classes of xenobiotic compounds activate either PXR or CAR including numerous clinically prescribed drugs, active compounds in popular herbal remedies, several prodrugs that are anti-cancer agents, drug metabolites, and the list is growing. Activation of these receptors by anti-cancer drugs would be expected to have profound impact on the pharmacokinetics of drug metabolism in patients taking prodrugs activated by CES and eliminated by the action of the CYP3A4 enzyme in liver. Moreover, both PXR and CAR activities appear to be modulated through alterations in post-translational modifications such as phosphorylation [61,62,63,64]. It is likely that these and other key signaling pathways are altered in patients experiencing disease states such as inflammation or diabetes. There is increasing recognition that key drug metabolism pathways are under metabolic control, and are altered in patients who are administered drugs while fasting or are cachectic. Because the activity of PXR and CAR proteins also appear to be under metabolic control [62,64], these two transcription factors are likely to be, in part, responsible for such alterations. If true, this would have enormous implications in the field of drug-drug interactions in the most ill cancer patients that are undergoing polytherapy with simultaneous pharmacological interventions who are experiencing cachexia. More research needs to be conducted into the possible metabolic control of PXR and CAR activity.

The observation that treatment with GR and PPAR α agonists produces repression of CES gene expression in rodent models could also have a significant impact on patient care. If the same is true for humans, it would be expected that the numerous clinically prescribed medications and newly discovered drug candidates that work through GR and PPAR α would suppress the biotransformation of the anticancer prodrugs that are targeted for biotransformation by CES enzymes. Obviously, more research is necessary to clearly elucidate differences in the regulation of drug-inducible expression and activity of CES enzymes in liver and intestine across species. This is particularly important because of the use of rodent models to determine drug efficacy and drug toxicity screening by the pharmaceutical industry. Thus, continued research using knockout mice, “humanized” mouse models, and human cell-based model systems will undoubtedly contribute significant knowledge that will elucidate the molecular mechanisms governing the regulation of CES enzyme expression and drug metabolism activity by these important metabolic enzymes. This thrust of research highlights the importance of monitoring the ratio and efficacy of the conversion of prodrug into active drug in patients receiving novel combination therapies.

Acknowledgments

We apologize for many valuable publications that cannot be included in this review due to space limitations. Funding provided by NIH grant DK068443 to J.L.S.

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Figure 1.

Site 2 Contains Several NR-response Elements

Figure 2.

Figure 3.

Figure 4.