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# Mechanisms of xenobiotic receptor activation: Direct vs. indirect☆

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ABSTRACT

The so-called xenobiotic receptors (XRs) have functionally evolved into cellular sensors for both endogenous and exogenous stimuli by regulating the transcription of genes encoding drug-metabolizing enzymes and transporters, as well as those involving energy homeostasis, cell proliferation, and/or immune responses. Unlike prototypical steroid hormone receptors, XRs are activated through both direct ligand-binding and ligand-independent (indirect) mechanisms by a plethora of structurally unrelated chemicals. This review covers research literature that discusses direct vs. indirect activation of XRs. A particular focus is centered on the signaling control of the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and the aryl hydrocarbon receptor (AhR). We expect that this review will shed light on both the common and distinct mechanisms associated with activation of these three XRs. This article is part of a Special Issue entitled: Xenobiotic nuclear receptors: New Tricks for An Old Dog, edited by Dr. Wen Xie.



## 1. Introduction

Nuclear receptors (NRs) are important cellular proteins that govern the expression of numerous genes involved in a wide range of cellular processes including cell growth, differentiation, metabolism, and stress responses. Prototypical NRs exert their effects by acting as transcription factors, which sense both intracellular and extracellular signals and respond by inducing the transcription of their target genes [1]. Although the cellular impact of each NR is different, all NRs share a number of characteristic structures, functional domains, and sequence similarities. In general, NRs feature a variable N-terminal region with an activation function 1 (AF-1) domain, a highly conserved DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD) that contains the activation function 2 (AF-2) domain. The AF-1 domain mediates ligand-independent activation of the NR while AF-2 activation is ligand-dependent [2]. The hinge region connects the DBD to the LBD and upon ligand binding, helices in this region undergo a conformational change allowing coactivators to bind, ultimately leading to nuclear localization and activation [2,3]. These features differentiate NRs from many other regulatory proteins and allow them to be dynamic sensors that respond to various stimuli and regulate cellular responses.

NRs can be roughly divided into two main classes based on their ligand specificity — endocrine NRs and orphan NRs [4]. Endocrine NRs bind with nanomolar-affinity to specific endogenous ligands such as hormones and steroids that are present at low concentrations physiologically. Examples of endocrine NRs include, but are not limited to, the thyroid hormone receptor, the retinoic acid receptor, the androgen receptor, and the estrogen receptor [2,4]. On the other hand, orphan NRs usually have no identified high-affinity endogenous ligands and are instead activated by abundant and low-affinity endogenous metabolites or xenobiotics. However, it is important to note that some NRs previously designated as orphan NRs have been "adopted" after discovering an endogenous ligand, such as the farnesoid x receptor, which was "adopted" after bile acids were identified as high-affinity ligands [5]. A number of orphan NRs promiscuously bind to a wide range of both endogenous compounds and xenobiotics, often at micromolar concentrations, and are instrumental in mounting cellular responses to toxic compounds and their metabolites. This subset of orphan NRs, termed xenobiotic receptors (XRs), have become increasingly important in coordinating toxic or protective responses when humans are exposed to accumulated endotoxins or high concentrations of environmental chemicals [6]. XRs coordinate the gene transcription of numerous phase I and II drug-metabolizing enzymes and transporters in the liver and intestine, where XRs are also enriched [7]. To date, extensive investigations have been centered on three XRs; namely, the constitutive

*Abbreviations:* AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon nuclear translocator; CAR, constitutive androstane receptor; CCRP, cytoplasmic retention protein; CITCO, 6-(4-chlorophenyl)imidazo [2,1-beta][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime; DBD, DNA-binding domain; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FRET, fluorescence resonance energy transfer; GRIP1, glucocorticoid receptor interacting protein 1; HSP, heat-shock protein; LBD, ligand-binding domain; MEK, mitogen-activated protein kinase kinase; NcoR1, nuclear receptor co-repressor 1; NR, nuclear receptor; OA, okadaic acid; PB, phenobarbital; PKA, protein kinase A; PKC, protein kinase C; PP, protein phosphatase; PXR, pregnane x receptor; RXR, retinoid x receptor; SMRT, silencing mediator of retinoid and thyroid receptors; SRC-1, nuclear receptor coactivator 1; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene; TKI, tyrosine-kinase inhibitor; XR, xenobiotic receptors.

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androstane receptor (CAR, NR1I3), the pregnane x receptor (PXR, NR1I2), and the aryl hydrocarbon receptor (AhR), due majorly to their predominance in regulating hepatic responses to drugs and environmental chemicals. Although AhR, a member of the Per-ARNT-Sim (PAS) family of proteins, is not typically classified as a nuclear receptor, it has similar functionality and follows the same overall paradigm as other XRs from a pharmacological or toxicological perspective [8]. While all of these XRs are capable of altering cellular metabolism and homeostasis individually, significant cross-talk exists between these receptors leading to multifaceted regulation of xenobiotic detoxification. For example, previous studies have shown that PXR activation in human primary hepatocytes increases the conversion of AhR antagonist omeprazole-sulfide to a prototypical AhR activator, omeprazole [9]. In addition, all three XRs are known to regulate the important drug-metabolizing enzyme UDP glucuronosyltransferase 1A1 and the transporter breast cancer resistance protein (ABCG2) at the transcriptional level [10–15]. Also, shared ligands such as non-coplanar polychlorinated biphenyls and flavonoids derived from *Ginkgo biloba* extract activate CAR, PXR, and AhR, which can lead to complicated effects in the liver [16,17]. As these XRs exhibit complex interplay that markedly alters cellular metabolism and homeostasis, understanding the types of compounds that activate these XRs and the underlying mechanical bases of XR activation are essential.

Traditionally, ligand-binding has been thought of as an essential component of XR activation and has been studied with methods such as mammalian two-hybrid assays, luciferase reporter assays, and more recently, fluorescence resonance energy transfer (FRET) assays [18]. However, recent evidence has shown that many compounds activate XRs in lieu of direct ligand binding; rather, they activate XRs via ligand-independent (indirect) mechanisms that have yet to be fully elucidated. This paradigm of XR activation mediated through both direct and indirect mechanisms raises new challenges in our understanding of how XR signaling is controlled when exposed to various cellular stresses and has profound implications on how XR modulators will be identified in the future. As such, this review will highlight the recent research advances regarding mechanisms of direct and indirect activation for XRs with the focus on CAR, PXR, and AhR.

## 2. Constitutive androstane receptor

Screening of a human liver library with a degenerate oligonucleotide based on the sequence of the conserved DNA binding domain of NRs led to the cloning of CAR, originally named MB67, in 1994 [19]. CAR heterodimerizes with the retinoid x receptor (RXR) and transactivates genes that contain the retinoic acid response element "constitutively" in the absence of a ligand, leading to its early name as the constitutive activated receptor [20]. The mouse and rat orthologs of CAR were cloned soon thereafter, exhibiting the same heterodimerization and constitutive activation features as human CAR (hCAR) [20,21]. Under normal physiological conditions, CAR is sequestered in the cytoplasm in a complex with heat-shock protein (HSP) 90 and CAR cytoplasmic retention protein (CCRP); and HSP70 has recently been shown to stabilize this complex in the inactive state [22–24]. Interestingly, although CAR is retained in the cytoplasm in the inactive state and translocates to the nucleus upon activation in physiologically-relevant primary hepatocytes, CAR is localized to the nucleus and constitutively active in immortalized cell lines [25,26].

As a xenobiotic receptor, CAR governs the inductive expression of many phase I and phase II drug-metabolizing enzymes and transporter proteins, coordinating a defensive network against xenobiotic challenges in the liver [27,28]. Although the well-established xeno-sensing role of CAR continues to be important in predicting potential drug–drug interactions (DDIs) and pharmacokinetic profiles of drugs, emerging evidence reveals that CAR also affects physiological and pathophysiological conditions including obesity, diabetes, and tumor development by modulating hepatic energy homeostasis, insulin signaling, and cell proliferation [29–32]. For instance, in contrast to the up-regulation of genes encoding drug-metabolizing enzymes and transporters, activation of CAR by the selective mouse CAR (mCAR) activator 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) significantly represses a cluster of genes associated with gluconeogenesis and lipogenesis, and attenuates high-fat diet (HFD)-induced obesity and diabetes in wild-type but not CAR-knockout mice [29–31]. Assuming that such beneficial effects also occur in humans, CAR may function as a novel therapeutic target for metabolic disorders in addition to its known role as a xenobiotic sensor. Therefore, understanding of the mechanistic basis of CAR activation is pivotal, and these recent developments have stimulated much interest in identifying selective and potent activators of human CAR.

### 2.1. Activation of CAR

The pharmacological importance of CAR was first appreciated when CAR activation was linked to the induction of CYP2b10 expression by phenobarbital (PB) in mouse liver [33]. Subsequent studies revealed that numerous structurally unrelated PB-like compounds induce CYP2B genes in different species through the activation of CAR [25,27,34]. The initial step of CAR activation involves cellular translocation of the receptor from the cytoplasm to the nucleus, where it interacts with its heterodimer partner RXR and other transcriptional proteins to stimulate the expression of target genes [25,35]. Further analysis of the 5′ upstream regions of CAR target genes revealed key promoter elements often containing direct repeats of the hexamer AGGTCA separated by 3–5 nucleotides which directly interact with the CAR/RXR heterodimer [36]. These findings led to the establishment of specific cell-based luciferase reporter assays, by which pharmacological modulation of CAR activity could be monitored efficiently. Initial ligands identified for CAR include two endogenous testosterone metabolites, 5α-androst-16-en-3α-ol (androstenol) and 5α-androstan-3α-ol (androstanol) [37]. Both compounds repressed the constitutive activity of mCAR in vitro and disrupted its interactions with coregulatory proteins such as the nuclear receptor coactivator 1 (SRC-1) [37]. Notably, although this finding leads to the current name of CAR as the constitutive androstane receptor, these steroid metabolites are not likely to be "real" endogenous ligands of CAR *in vivo* because the concentrations needed to antagonize CAR *in vitro* are several magnitudes higher than their physiological levels. Subsequently, TCPOBOP was identified as a potent and selective agonist of mCAR with the ability to reverse the antagonism conferred by androstanes while promoting CAR interaction with coactivator SRC-1 [38,39]. Interestingly, although CAR demonstrates promiscuity in ligand binding, it also exhibits divergent activation profiles across species. For instance, TCPOBOP activates mouse but not human CAR while androstanol represses mouse but not human CAR [34,38]. The later discovery of a potent and selective hCAR agonist, 6-(4-chlorophenyl)imidazo[2,1-beta][1,3]-thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime (CITCO), has been instrumental in studying the effects of CAR in human systems and conferring physiological relevance to many studies carried out in mice only [40].

CAR displays unique activation mechanisms compared with typical nuclear receptors, requiring both nuclear translocation and nuclear activation. In HepG2 cells, transfected CAR spontaneously accumulates in the nucleus in the absence of an inducer and exhibits constitutive activation of its target genes [20,25]. This would suggest that nuclear translocation alone is sufficient to confer CAR activation. However, several lines of evidence support nuclear activation as a distinct step in CAR-mediated gene regulation in primary hepatocytes and intact liver *in vivo*. For example, pretreatment of primary hepatocytes with the protein phosphatase inhibitor okadaic acid (OA) inhibits PB-induced CAR nuclear translocation but does not repress CAR-mediated activation of reporter genes in HepG2 cells since CAR is constitutively localized in the nuclei of HepG2 cells [25,41]. Additionally, pretreatment of primary mouse hepatocytes with KN-62, a calcium/calmodulin-dependent