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REVIEW

Constitutive Androstane Receptor (CAR) Is a Xenosensor and Target for Therapy

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Abstract—Constitutive androstane receptor (CAR, NR1I3), which is under consideration in this review, is a member of the superfamily of nuclear receptors. However, certain features distinguish CAR from the variety of nuclear receptors. First, this receptor has structural features that allow it to display constitutive activity in the absence of a ligand and to interact in a species-specific manner with a huge number of ligands diverse in chemical structure and origin. Second, recently many researchers are focused on CAR because the significance is increasingly shown of its influence on a variety of physiological functions, such as gluconeogenesis, metabolism of xenobiotics, fatty acids, bilirubin, and bile acids, hormonal regulation, etc. In addition to the fundamental scientific interest, the study of CAR is of practical importance because changes in CAR activity can lead to disorders in physiological processes, which finally can result in changes in pathological states. However, despite intensive studies, many mechanisms are still unclear, which makes it difficult to understand the role of CAR in the overall picture of molecular regulation of physiological processes. This review analyzes the features and diversity of the functions of CAR.

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Key words: CAR, gene expression activation, ligands, xenobiotics, metabolism

Nuclear receptors constitute a superfamily of phylogenetically related proteins that in humans are encoded by 48 genes [1]. The superfamily is conventionally subdivided into three classes: endocrine receptors, orphan receptors, and the so-called adopted receptors [2]. The class of endocrine receptors includes receptors of steroid and thyroid hormones and of D and A vitamins. The second class includes true orphan receptors whose endogenous ligands are not yet found. Considering the small size of the ligand-binding pocket, their regulation is supposed to be not mediated through a ligand but through covalent modifications of the protein and interaction with coactivators [2]. The class of adopted receptors includes receptors whose ligands have been detected after the discovery of these receptors, which was the reason for their initial classification as orphan receptors. The class of adopted receptors also contains the so-called mystery orphan receptors. An endogenous ligand has been found for them, but the physiological significance of this interaction is not yet identified. This group includes receptors with activity modulated on the interaction with endogenous ligands. The constitutive androstane receptor (CAR, NR1I3), which is the subject of this review, is assigned to this class. The attention of researchers to this receptor is not accidental, but is reasoned by its specific features.

First, CAR is interesting because of its structural features that distinguish it from other nuclear receptors. Second, the constitutive androstane receptor (CAR) was initially characterized as a xenosensor [3]. But now it is shown to display pleiotropic effects on physiological and pathological processes in the organism. Activation of CAR changes lipid metabolism and glucose homeostasis [4, 5]. Moreover, CAR regulates detoxification and excretion of toxic endogenous metabolites, such as bilirubin and bile acids [6, 7]. CAR can be activated by synthetic exogenous compounds, and considering the diversity of effects of this receptor it seems to be an attractive thera-

Abbreviations: CAR, constitutive androstane receptor; DBD, DNA-binding domain; LBD, ligand-binding domain; NR, nuclear receptor; PB, phenobarbital; RXR, retinoic acid receptor; SRC, steroid receptor coactivator; XRE, xenobiotic-responsive element.

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peutic target. Thus, studies on CAR are important for both fundamental and applied purposes.

The cDNA of CAR was first isolated in 1993, and then it was termed MB67. In the absence of a ligand the heterodimer MB67/RXR (*9-cis retinoic acid receptor*) could only poorly activate expression of genes encoding the retinoic acid receptor β2 and alcohol dehydrogenase 3 [8]. Therefore, the new receptor was initially considered as an orphan one, and the abbreviation CAR was interpreted as *constitutive activated receptor*. However, soon endogenous ligands of CAR were found that were represented by testosterone metabolites 5α-androstan-3α-ol (androstanol) and 5α-androst-16en-3α-ol (androstenol). They were also shown to be inverse agonists of CAR preventing it interaction with a *steroid receptor coactivator* (SRC-1) [9]. Therefore, now it is most often called *constitutive androstane receptor* (CAR).

CAR belongs to subfamily NR1I of the nuclear receptors superfamily. CAR is predominantly expressed in the liver and kidneys, and a low basal expression of the genes encoding this receptor is also shown in brain, heart, and intestine tissues [10]. In humans this protein consists of 348 amino acid residues, and its molecular weight is 39 kDa [11]. Similarly to the majority of nuclear receptors, CAR has two main structural domains that determine its functions, the DNA-binding domain (DBD) and the ligand-binding domain (LBD) (Fig. 1) [12].

The presence of the DBD is responsible for the ability of all nuclear receptors to bind with specific regulatory sequences of DNA in the gene promoter region. In the case of nuclear receptors activated under the influence of xenobiotics these sequences are called *xenobiotic responsive element* (XRE). Usually these sequences are direct or inverse repeats of palindromic sequences consisting of six nucleotides. The specificity of the receptor binding with DNA is determined by the mutual orientation of the palindromes and the number of nucleotides between them [2]. The DBD is the most conservative domain of the nuclear receptor [13].

The LBD consists of 250 amino acid residues, the majority of which are hydrophobic [14]. Analysis of crystalline structure of many nuclear receptors reveals that the LBD usually contains 12 α-helices, which are packed as three antiparallel layers. A hydrophobic ligand-binding pocket is formed from the third, fourth, and fifth α-helices [14]. To provide for the movement between the nucleus and cytoplasm, the majority of nuclear receptors need signaling regions of the protein: NLS (*nuclear localization signal*) and NES (*nuclear export signal*). Therefore, the intracellular localization of the receptor is determined by a balance between NLS and NES [15]. Like the majority of nuclear receptors, CAR contains such sequences, but CAR is characterized by the crucial role in nuclear localization played by the XRS (*xenobiotic response sequence*), which is a leucine-enriched motif (LXXLXXL) located on the *C*-terminus of the ligand-binding domain [12] (Fig. 1).

On the *N*-terminus of the nuclear receptor the *activation function* AF-1 domain is located, which is responsible for the tissue-corresponding specific activity of the receptor, and in the case of CAR this domain is constitutively active even in the absence of a ligand. Moreover, it is a target for protein kinases catalyzing posttranslational modification of the receptor [14].

The *hinge* region is responsible for DBD rotation relative to the LBD, which is essential on interaction of the receptor dimers with asymmetric sequences of DNA. This region also forms a surface for interaction with coregulators [12].

On the *C*-terminus of the nuclear receptor the AF-2 domain is located, which reacts to the LBD binding with the ligand by a conformational change. This domain is located on the 12th α-helix and has a dynamic structure [16]. Usually, on binding with the ligand the AF-2 domain changes conformation to promote dissociation of the complex with corepressors and the formation of a new complex with coactivators and histone-acetyl transferases [17]. However, in the case of CAR domain AF-2 is constantly fixed in an active conformation due to the presence in its structure of single-coil helix X. The interaction with the ligand only supports the active conformation of the receptor. CAR is regulated through localization of coactivators, the receptor itself, and also due to signaling modifications, such as phosphorylation and acetylation [15, 18]. These mechanisms are not sufficiently studied for CAR, but there is a general concept about molecular interactions associated with the activation of this receptor.

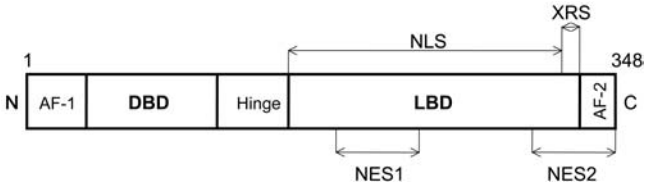

Fig. 1. Domain structure of human CAR. DBD, DNA-binding domain; LBD, ligand-binding domain; NLS, nuclear localization signal; NES, nuclear export signal; XRS, xenobiotic response sequence; Hinge region; AF, activation function.

ACTIVATION OF CAR

The role of CAR as a xenosensor was revealed in 1998 when phenobarbital (PB) was shown to cause not only an increase in the amount of mRNA of the *Cyp2b10* gene in mice, but also to induce the binding of CAR inside a RXR-containing heterodimer that had a sequence in the promoter region of the gene encoding Cyp2b10 [19]. CAR is known to regulate expression of genes encoding proteins involved in metabolism and

elimination of xenobiotics, including cytochromes P450, UDP-glucuronosyl transferase, sulfotransferases, and proteins of multiple drug resistance [20].

In the absence of ligand, CAR exists in the cytoplasm in complexes with chaperone proteins HSP90 (heat shock protein), CCRP (cytoplasmic CAR-retaining protein), and PPP1R16A (the membrane-associated subunit of protein phosphatase 1β). These proteins inhibit CAR transport across the nuclear membrane [21]. Under the influence of xenobiotics, CAR is dephosphorylated, dissociates from the cytoplasmic complex, and is translocated into the nucleus where it forms a complex with RXR. This translocation was revealed using receptors labeled with fluorescent protein [22]. On activation of the *CYP2B* gene by PB, the heterodimer CAR/RXR binds with a PB-responsive enhancer module (PBREM) in the gene *CYP2B* promoter at the distance of –2.2 kb in mice and rats and of –1.7 kb in humans from the site of transcription initiation. PBREM consists of 50 nucleotides and contains two DR4 sites (*direct repeat*) for binding nuclear receptors NR1 and NR2. This binding activates expression of the target gene [23] (Fig. 2). For the complete activation of the *CYP2B6* gene in humans, the site XREM located at the distance of –8.5 kb from the transcription start is also necessary [24]. By analogy with the earlier detected motifs NR1 and NR2, the binding sites in the XREM sequence were termed NR3-8. Due to this mechanism, the *CYP2B* gene can act as an inducer under the influence of chemically diverse xenobiotics including organic solvents, barbiturates, and pesticides [25, 26]. The interaction of the heterodimer CAR/RXR with regulatory sequences is promoted by coactivators GRIP-1 (*glucocorticoid receptor interacting protein*-1), PGC-1α (*proliferator-activated receptor gamma coactivator-1 alpha*), SRC-1 (*steroid receptor coactivator-1*), and Sp1 [27-29]. A corepressor of this interaction called SMILE (*small heterodimer partner interacting leucine zipper protein*) is also known. SMILE was shown to interact with the AF-2 domain of CAR and prevent its binding with coactivators [30].

On entrance into the nucleus, CAR does not immediately interact with the regulatory sequence PBREM, and phosphorylation/dephosphorylation processes seem to play an important role. This is confirmed by absence of PB-mediated induction in primary mouse hepatocytes treated by inhibitors of Ca^{2+}/calmodulin-dependent

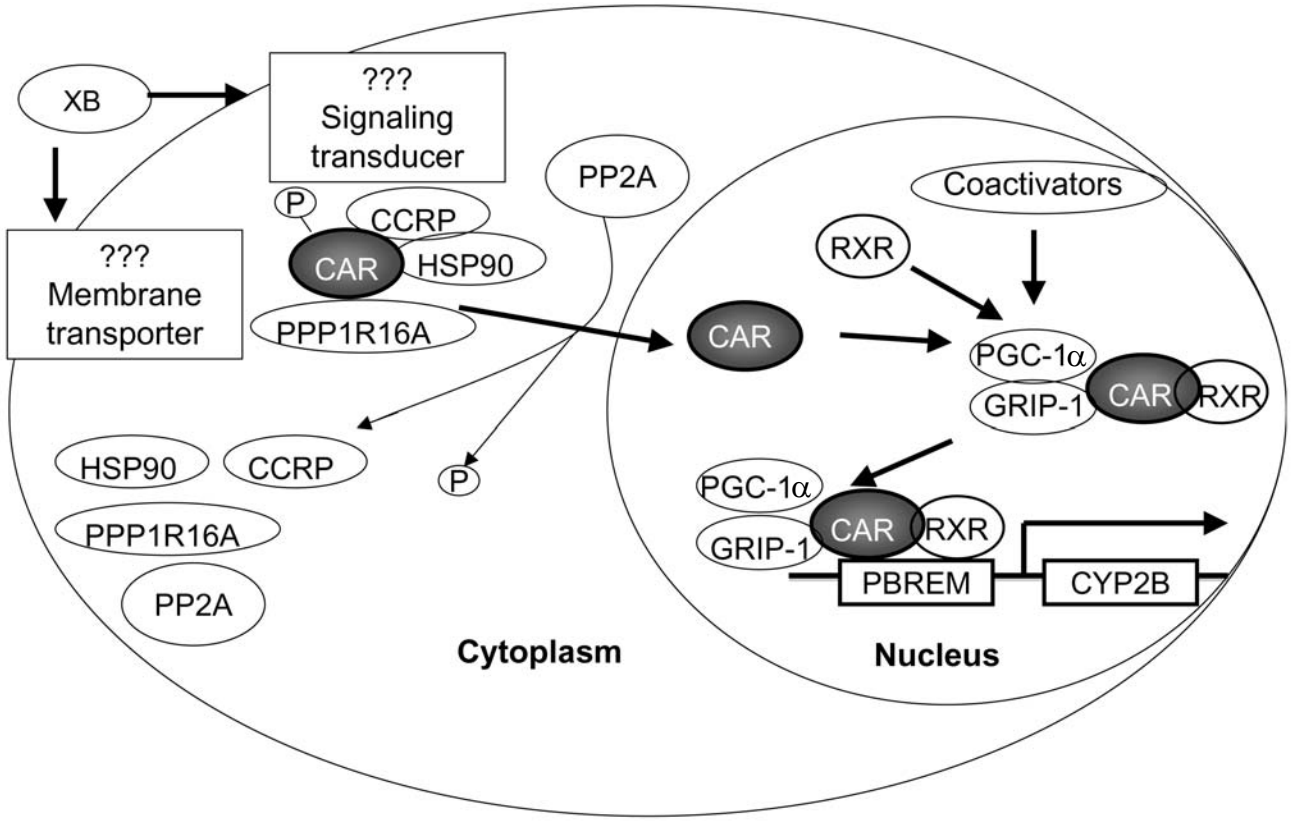

Fig. 2. Scheme of activation of gene *CYP2B* transcription under the influence of CAR. In the absence of xenobiotics, CAR is phosphorylated at Ser202 or Thr38 and is located in the cytoplasm as a complex with Hsp90, CCRP, and PPP1R16A. Upon activation, protein phosphatase PP2A dephosphorylates CAR, which results in the dissociation of the receptor from the cytoplasmic complex and its translocation into the nucleus. In the nucleus CAR forms a heterodimer with RXR, and the resulting heterodimeric complex interacts with the sequence PBREM of gene *CYP2B* distal promoter, which is accompanied by a significant increase in their transcription.

kinases (KN-62, KN-93), notwithstanding the translocation of CAR from the cytoplasm into the nucleus [31]. Thus, the receptor triggers the expression via at least two independently regulated stages: activation of the receptor translocation from the cytoplasm into the nucleus, and the receptor activation directly inside the nucleus [11]. Note that CAR is constantly present in the nucleus in immortalized cell lines, but not in the primary hepatocyte culture or in the liver *in vivo* [32].

Although PB failed to reveal the species specificity of the CAR effect in humans and rodents, the species-specific activation of CAR is still intriguing. Thus, the inducer TCPOBOP activated mouse CAR but did not activate it in humans [33], whereas CITCO species-specifically activated the human CAR [34]. The species specificity of CAR activation can be due to difference in the amino acid sequences. Thus, the substitution of human CAR Thr350 by Met in the mouse CAR results in the loss of the mouse CAR responsiveness to the action of TCPOBOP or steroid hormones [4].

Among species-specific highly effective activators of CAR the compound 2,4,6-triphenyldioxane-1,3 (TPD) should also be mentioned, which activates CAR and its target gene *CYP2B* in rats but not in mice [35, 36]. The TPD-caused induction is maximal at ab inducer dose that is an order of magnitude lower than in the case of the classic inducer PB [37].

In particular, CAR is characterized by its wide specificity to both endogenous and exogenous ligands with different chemical structure [38]. Because different amino acid residues can interact with different ligands and thus modulate the CAR activity, the broad spectrum of ligands is provided by the large size of the hydrophobic ligand-binding pocket [38]. Amino acids responsible for interaction with particular compounds have been detected in certain positions of the CAR ligand-binding domain [18, 38]. In mutant molecules this correlation is affected, and the resulting compound cannot cause an adequate activation of CAR and in some cases becomes its agonist [38].

Species-specific action of compounds on CAR*

Classification	Compound	Activation/deactivation
Activator of hCAR	acetaminophen	?
	artemisinin	?
	atorvastatin	?
	diazepam	?
	carbamazepine	?
	sulfanilamides	?
	tri-*p*-methylphenyl phosphate	direct
	triphenyl phosphate	direct
	phenytoin	indirect
	phenobarbital	indirect
	chloropromazine	?
	6,7-dimethylesculetin	?
	CITCO	direct
Activator of mCAR	acetaminophen	indirect
	atorvastatin	?
	bilirubin	indirect
	meclizine	direct
	phenobarbital	indirect
	chloropromazine	?
	17β-estradiol	direct
	TCPOBOP	direct
Deactivator of hCAR	clotrimazole	direct
	17β-estradiol	direct
	meclizine	?
	PK11195	direct
Deactivator of mCAR	okadaic acid	indirect
	progesterone	direct
	testosterone	direct
	5α-androstan-3α-ol	direct
	5α-androst-16en-3α-ol	direct
	KN-62	indirect
	KN-93	indirect

* Taken with changes from [11, 15, 38].

Note that many CAR activators fail to directly bind with it (table). Thus, PB did not bind with mouse or human CAR [33, 39]. Nevertheless, the PB-treated receptor was translocated into the nucleus. This suggested that for the CAR translocation into the nucleus additional mechanisms regulating this translocation should be activated. The dephosphorylation of CAR is supposed to be a crucial stage. This hypothesis is supported by a decrease in the inducing effect of PB caused by okadaic acid (an inhibitor of protein phosphatases PP1A and PP2A). Yoshinari et al. showed in 2003 that under the influence of PB a complex of protein phosphatase 2A and dimer CAR/Hsp90 is formed in the hepatocyte cytoplasm [40]. Later the CAR dephosphorylation sites were identified. For the mouse CAR a dephosphorylation site on PB-caused induction is represented by Ser202 [41] and for the human CAR this site is represented by Thr38 [42]. However, it is unknown whether some signaling molecules are dephosphorylated and how the signal transmission occurs under conditions of an indirect influence. Therefore, the mechanism of the CAR translocation from the cytoplasm into the nucleus remains unclear.

CAR AND PHARMACEUTICAL PREPARATIONS

As mentioned, CAR plays an important role in regulation of a great number of genes involved in metabolism of xenobiotics and pharmaceutic preparations. The involvement of CAR in regulation of the *CYPs* genes, which participate in the first phase of xenobiotic metabolism, is studied in most detail [43]. However, activation of these receptors can induce an increase in expression of genes encoding enzymes of a second phase, such as UDP-glucuronosyl transferase (UGT), sulfotransferase (SULT), and glutathione-S-transferase (GST) [43]. These enzymes catalyze the attachment of hydrophilic groups to increase the solubility of exogenous compounds. Moreover, activated CAR can increase expression of genes of membrane transporters BSEP, NTCP, OATP2, MRP3, and MDR2, which promotes the entrance of xenobiotics into the liver and the elimination of their metabolites through kidneys or with bile [44, 45].

Very often effective doses of a drug are different in different subjects. This can be due to different activity of enzymes responsible for drug metabolism due to individual genetic features [46]. This can be also due to taking together of some drugs or even food products containing substances that are agonists or antagonists of these enzymes [46]. In particular, such antiepileptic drugs as PB and phenytoin are CAR activators [26]. In turn, CAR can directly activate CYP2B, which is involved in drug metabolism. Thus, on taking such drugs together with other drugs, it must be taken into account that their effective doses can be significantly changed.

CAR regulates the expression of genes encoding enzymes of metabolism of xenobiotics including drugs, and this makes this receptor an important member of the organism's defense system against toxic compounds. But sometimes this metabolism is associated with production of chemically active agents that can interact with macromolecules of liver cells and as a result cause different forms of hepatitis [47]. Thus, acetaminophen, which is used for headache, for decreasing body temperature in fever, and for myalgia, neuralgia, and arthralgia is an example of such compounds. Therapeutic doses of acetaminophen rarely induce side effects. But the toxic dose of acetaminophen is only thrice higher than its therapeutic dose [48]. Overdosing the drug is accompanied by a hepatotoxic effect and by inducing necrosis of liver cells due to production of a toxic acetaminophen metabolite *N*-acetyl-*p*-benzoquinone imine. This metabolite produced under the influence of enzymes CYP1A2, CYP2E1, and CYP3A is covalently bound with the cellular macromolecules and causes generation of reactive oxygen species [49]. A pretreatment with inducers of CYPs increased the toxic effect of acetaminophen in both rodents and humans. CAR activators in wild type mice are shown to induce the expression of enzymes metabolizing acetaminophen (CYP1A2, CYP2A11, GST*Pi*), whereas this does not occur in $CAR^{-/-}$ mice [50]. Therefore, the $CAR^{-/-}$ mice were resistant to the action of acetaminophen. Thus, new agents for protection against the toxic effect of acetaminophen and other hepatotoxic compounds can be based on strong specific antagonists of CAR.

CAR AND METABOLITES OF ENDOGENOUS MOLECULES

Due to activation of enzymes of the first and second phases of metabolism and of transporters, CAR can influence catabolism and elimination not only of exogenous but also of endogenous compounds, such as bilirubin and bile acids [20]. Hemoglobin disintegration results in production of bilirubin, which is toxic and can cause neurologic disorders and jaundice [51]. During effective elimination of bilirubin from the organism, it penetrates into liver cells with a membrane transporter OATP2, and then under the influence of the enzyme UGT1A1 the nonconjugated bilirubin is conjugated and with a membrane transporter MRP2 (*multidrug resistance protein*) is transported into the bile duct and partially into the sinusoidal space with MRP3. CAR induces the expression of genes encoding the above-mentioned transporters and UGT1A1 and thus can control the level of bilirubin [20].

CAR is involved in regulation of secondary bile acid metabolism through inhibition of expression of the gene encoding the key enzyme CYP7A of bile acid synthesis in response to increase in the lithocholic acid concentration

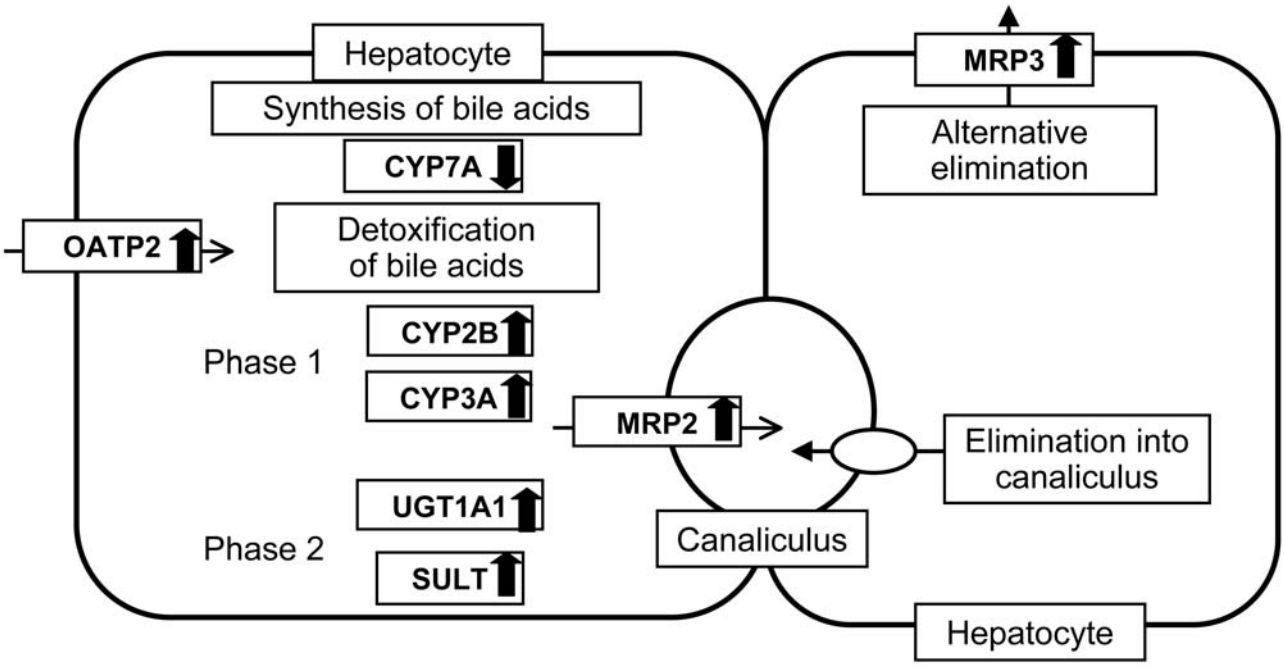

Fig. 3. Role of CAR in detoxification of potentially dangerous endogenous metabolites. CAR regulates the expression of genes encoding enzymes of the first and second phases of metabolism of xenobiotics and of membrane transporters; on activation CAR accelerates the entrance of bilirubin and bile acids into the liver and promotes elimination of their metabolites through the kidneys or with bile.

in blood [51]. Moreover, the elimination of bile acids from the organism is also contributed by membrane transporters of organic anions and enzymes of the first and second phases of xenobiotic metabolism (UGT1A1, CYP3A11, MRP3 and SULT2A1), some of which are regulated by CAR [52] (Fig. 3).

Considering the role of CAR in catabolism of dangerous endogenous compounds, it has been proposed to treat this receptor during therapy of liver and bile ducts diseases, such as cholestatic liver disease, nonhemolytic hyperbilirubinemia, neonatal jaundice, and primary bile cirrhosis.

CAR AND ENDOCRINE REGULATION

Thyroid hormone in the relatively low activity form of thyroxin (3,5,3′,5′-tetraiodothyronine, T4) is synthesized in the thyroid gland and then is converted into other forms under the influence of deiodinases in peripheral tissues, e.g. in the liver and kidneys [53]. Three types of deiodinases are known: D1 (*Dio1*), D2 (*Dio2*), and D3 (*Dio3*) [53]. D1 is a key enzyme responsible for transformation in the liver of T4 into the active form T3 (3,3′,5-triiodothyronine). D2 converts T4 into T3 outside the liver. D3 catalyzes the formation from T4 of the least active form rT3 (3,3′,5′-triiodothyronine) and also the formation from T3 of the inactive form T2. D1 also transforms rT3 into T2 to eliminate it from the circulation [53].

It is known that the chronic use of PB causes thyroid gland hypertrophy in rats and humans [54]. This observation was the reason for determination of blood levels of T4, T3, and rT3 in $CAR^{+/+}$ and $CAR^{-/-}$ mice that were injected with PB after partial hepatectomy [54]. An increased level of rT3 was recorded, which on injections of PB became normal only in the $CAR^{+/+}$ mice. Moreover, an increase in the rT3 level relative to that of T3 weakened the thyroid hormone effect manifested by a decreased expression of the T3 target genes, e.g. the tyrosine aminotransferase gene after partial hepatectomy in the liver of wild type and $CAR^{-/-}$ mice. But the mechanism underlying this effect is unknown. A decrease in activity due to competitive interaction with thyroid hormone receptor (TR) is not likely, because EC_{50} of the active T3 is some orders of magnitude lower than EC_{50} of rT3 [55]. Consequently, rT3 is a weak agonist of TR. Nevertheless, the induction of *Dio1* in response to PB injection only in the $CAR^{+/+}$ mice was accompanied by a decrease in the rT3 level and an increase in the expression of T3 target genes to the normal level [54]. Thus, CAR via *Dio1* gene regulation can modulate the thyroid hormone effect in the regenerating liver [54].

There are also data on a decreased blood level of T4 in mice on injection of PB or TCPOBOP and also on an increased level of T4 in $CAR^{-/-}$ mice relative to wild type animals. This effect is supposed to be caused by changes in the regulation of genes encoding enzymes UGT1A1 and SULT1A1 of the second phase of metabolism of

xenobiotics under the influence of CAR [56]. Thus, the influence of CAR activators on endocrine signaling molecules is mediated by an increased activity not only of enzymes of xenobiotic metabolism, but also of enzymes of hormone metabolism.

Sex hormones. CAR can be activated by estrogens although to a lesser degree than by exogenous ligands of the receptor [57]. The activation of CAR by estrogens promotes its translocation into the nucleus. This effect is physiologically suppressed by the action of androgens. Moreover, GRIP-1 is a coactivator for CAR and the estrogen receptor (ER). CAR is more effective in competition for binding with the coactivator, and as a result GRIP-1 dissociates from the complex with ER, decreasing the estrogen activity [57]. Progesterone derivatives and androgens are known to be inverse agonists of mouse CAR. The CAR activity is the most strongly suppressed by androgen derivatives androstanes. The crystal structure of mouse CAR suggests that on binding with an inverse agonist in the hydrophobic pocket the receptor conformation changes, in particular the orientation of the H12-α helix containing the AF-2 domain is changed [58]. However, as discriminated from other receptors, the mCAR inverse agonist fails to directly interact with H12-α/AF-2, but acts through H11-α. On one hand, in such form CAR is already unable to bind with coactivators because it loses the active conformation, but, on the other hand, the interaction of the receptor with corepressors increases.

Phenytoin is shown to influence testosterone metabolism in the brain of mice [59]. This hormone is metabolized by hydroxylation with involvement of enzymes CYP2B, CYP2C, and CYP3A. Phenytoin activates expression of the *CYP2B* genes regulated through CAR, and this changes the ratio between different products of testosterone hydroxylation [26, 59].

CAR AND CARCINOGENESIS

CAR activators can cause the liver hyperplasia and hepatomegaly [60]. The crucial role of CAR in inducing tumor formation of the liver was first shown by Yamamoto et al. in experiments on $CAR^{-/-}$ and wild type mice. No tumor developed in the PB-treated $CAR^{-/-}$ mice, whereas hepatocellular carcinoma (HCC) and/or adenoma developed in the $CAR^{+/+}$ mice [61].

CAR regulates some genes involved in cell division and growth, in particular, it increases expression of the cell cycle genes encoding cyclin D1 and cdk2 [62]. The factor Gadd45β contributing to repression of apoptosis is also activated on injection of CAR activators. Under usual conditions Gadd45β is regulated by tumor necrosis factor α (TNF)/nuclear factor-κB (NF-κB). However, TCPOBOP can activate Gadd45β in mice also in the absence of TNF receptors. In this case CAR acts as a reserve signaling pathway independent of TNF and NF-κB [63]. Moreover, the protein Mdm2 inhibiting the apoptosis factor p53 also seems to act as a mediator of the CAR-dependent development of HCC [60]. In specimens of human HCC tissue the region 1q21-23 of chromosome 1 was amplified more often than in half. And the CAR locus is just in this region in both humans and mice [64].

Thus, CAR plays a significant role in inhibition of apoptosis, and this can be very essential for development of HCC. It seems that CAR enhances tumorigenesis in the liver and therefore can be a promising object for antitumor therapy.

CAR AND ENERGY EXCHANGE IN LIVER

Glucose homeostasis and CAR. Gluconeogenesis is a very important metabolic pathway of glucose synthesis from non-carbohydrate precursors, which occurs in living cells. An increase in the glucose concentration in blood of patients with *diabetes mellitus* can cause many complications associated with damages of the heart, blood vessels, eyes, kidneys, and nerves. Inhibition of gluconeogenesis in the liver is shown to normalize the glucose level in blood and to decrease its biosynthesis in patients with type 2 diabetes [65].

A hypothesis about a interrelationship between the metabolism of drugs (xenobiotics) and diabetes is based on observations of a decrease in blood glucose level and increase in the responsiveness to insulin of animals repeatedly injected with PB, which is a classic CAR activator. Moreover, similarly to insulin, CAR agonists cause repression of gluconeogenesis enzymes including phosphoenol pyruvate carboxykinase (PEPCK1) and glucose-6-phosphatase (G6Pase) [66, 67]. Thus, CAR activators can be used for development of drugs correcting metabolic disorders, including those in glucose homeostasis, especially in patients with type 2 diabetes.

Insulin and glucagon control the production of glucose via regulation of expression of genes encoding key enzymes of gluconeogenesis and glycogenolysis, G6Pase and phosphoenol pyruvate carboxykinase (PEPCK). Insulin decreases the glucose level suppressing expression of the *G6Pase* and *PEPCK* genes. In the absence of insulin the *fork-head transcription factor* 1 (FoxO1) binds with the insulin response sequence (IRS) in the promoter and thus activates IRS-containing genes, such as *G6Pase* and *PEPCK*. Insulin activates the phosphatidylinositol-3 kinase pathway (PI3K-Akt) that results in phosphorylation of FoxO1. The phosphorylated FoxO1 leaves the nucleus, and the target genes are not activated [68].

The long-term use of PB is known to decrease the glucose level in blood and increase responsiveness to insulin of patients with diabetes. Moreover, such CAR activators as PB and TCPOBOP decrease the *G6Pase* and

PEPCK expression in mouse liver [66] and in rat hepatocytes [67]. Studies on $CAR^{-/-}$ mice revealed the involvement of this receptor in glucose synthesis that was manifested by a decrease in *PEPCK1* gene mRNA level in $CAR^{+/+}$ mice but not in $CAR^{-/-}$ mice [69]. Moreover, the significance of the CAR-mediated inhibition of gluconeogenesis was shown *in vivo*. The activation of CAR decreased hyperglycemia and increased the sensitivity to insulin in *ob/ob* and wild type mice fed a lipid-enriched diet [70, 71]. CAR was shown to directly bind with FoxO1 and to inhibit the interaction of the latter with the regulatory sequence IRS (Fig. 4). Thus, the interaction of CAR with FoxO1 can determine the decrease in *G6Pase* and *PEPCK* expression in response to the action of xenobiotics [69].

Another pathway of gluconeogenesis regulation includes the competition of CAR with hepatic nuclear factor 4α (HNF4α) for binding with the DR1 site inside the promoter of the *PEPCK* and *G6Pase* genes and for binding with common coactivators GRIP-1 and PGC-1α [72] (Fig. 4).

The xenobiotic-induced suppression of gluconeogenesis can have physiological significance as follows. A decreased activity of G6Pase in the liver is associated with an increase in the level of glucose-6-phosphate, which is not converted into glucose. This can result in conversion of glucose-6-phosphate into pentose phosphate via the hexose monophosphate shunt. This is associated with formation of NADPH, which is necessary for the P450 monooxygenase system and glutathione cycle. It seems that inhibition of gluconeogenesis helps liver cells to save a sufficient amount of NADPH required for metabolism of xenobiotics [73].

The feedback also exists because insulin can decrease the PB-mediated induction of CYP2B in rat primary hepatocytes. In rats and mice with experimental diabetes the level of CYP2B is increased, and insulin injections decrease it to normal. FoxO1 is shown to act as a CAR coactivator increasing the expression of its target genes. Removing FoxO1 from the nucleus, insulin effectively decreases the activation of CAR and inhibits expression of the corresponding genes [69].

Lipid metabolism. Activation of CAR by various compounds can change lipid metabolism due to decrease in the expression of genes encoding enzymes enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase, which are involved in peroxisomal β-oxidation of fatty acids [74]. Expression of these genes is activated on binding of the transcription factor PPARα (peroxisome proliferator-activated receptor α, which is an important activator of fatty acid metabolism) with regulatory regions of peroxisome proliferator response elements (PPREs)

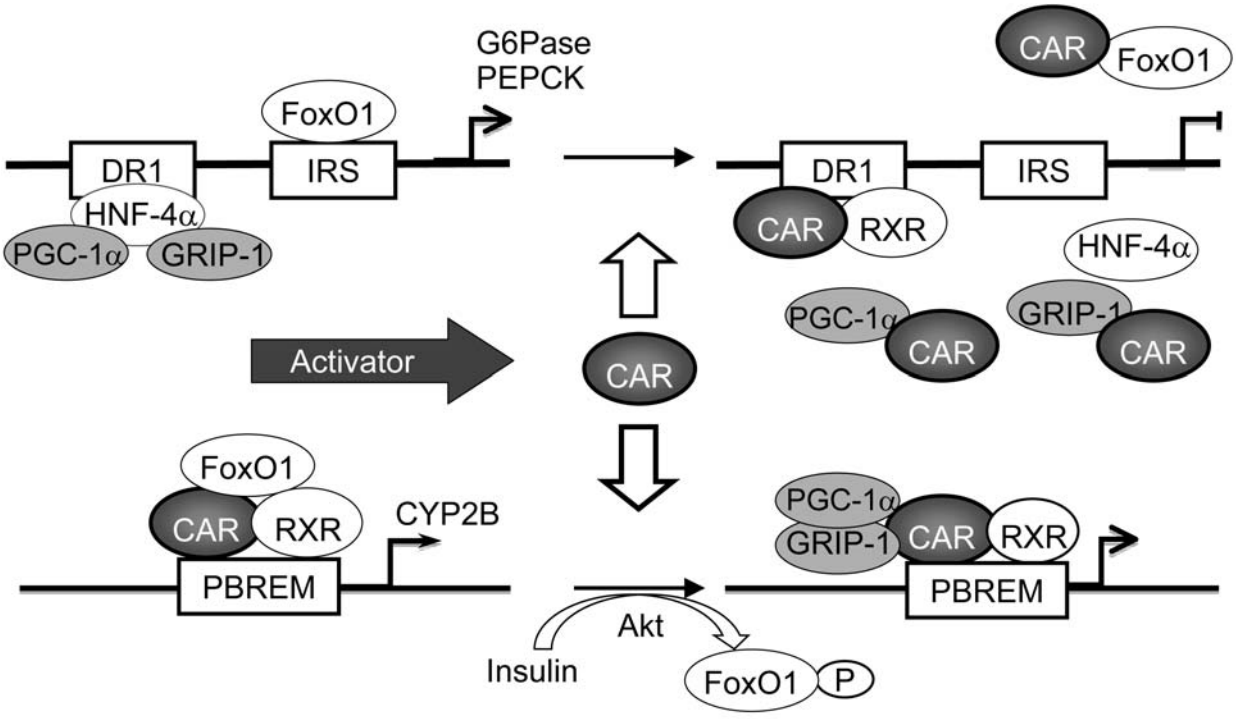

Fig. 4. Involvement of CAR in regulation of gluconeogenesis. Activated CAR inhibits expression of genes encoding the gluconeogenesis enzymes PEPCK and G6Pase by two pathways: 1) preventing the interaction of FoxO1 with the regulatory sequence IRS; 2) preventing the interaction of HNF4α with coactivators PGC-1 and GRIP-1 and with the regulatory sequence DR4 (upper part of figure). FoxO1, fork-head transcription factor 1; HNF4α, hepatic nuclear factor 4α. The influence of coactivators on the CAR target genes (lower part of figure). The CAR coactivator FoxO1 increases expression of target genes. Insulin decreases this effect, eliminating FoxO1 from the nucleus.

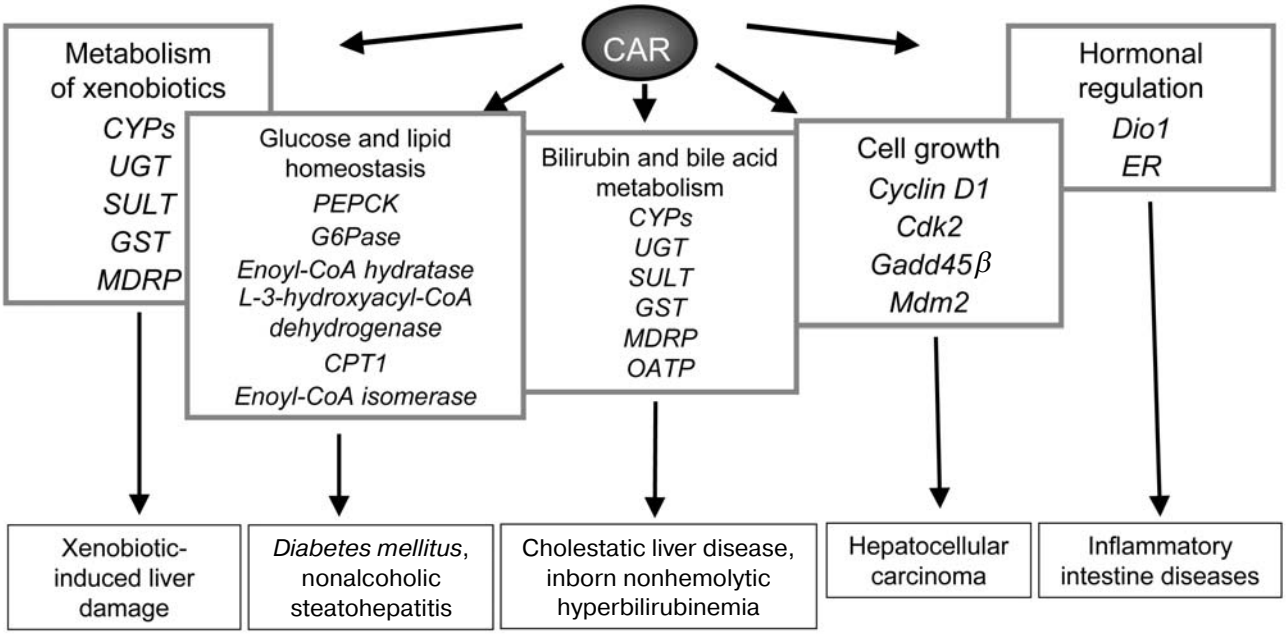

Fig. 5. Pathophysiological role of CAR.

located in promoters of these genes. The heterodimer CAR/RXR is also capable of binding with the regulatory regions of PPREs [5]. Moreover, in wild type mice PB lowers the level of mRNA of mitochondrial carnitine palmitoyl transferase 1 (CPT1) and of enoyl-CoA isomerase, which are also enzymes of β-oxidation. However, such changes are not observed in $CAR^{-/-}$ mice [4].

Both HNFα and thyroid hormone receptor (TR) can activate the gene *CPT1* through binding with the nuclear receptor motif. PGC1α is also required for complete activation of *CPT1* through HNFα and TR [5]. However, PGC1α is required for a great number of nuclear receptors, including CAR. Therefore, PGC1α is an intersection point of xenobiotic signaling pathways with those of other compounds. The amount of PGC1α in hepatocytes was recently shown to be limited; therefore, an increase in production of CAR/PGC1α complexes results in a decrease in production of HNFα/PGC1α complexes, and this affects the expression of genes regulated with involvement of these transcription factors [73].

As in the case of glucose metabolism, an inverse influence is also observed in lipid metabolism. The expression of the *CYPs* genes is decreased in obesity, steatosis, and nonalcoholic steatohepatitis. Polyunsaturated and free fatty acids inhibit the PB-mediated activation of CAR, and the crucial transcription factor of lipogenesis SREBP-1 decreases its transcriptional activity, suppressing the interaction of CAR with coactivators [74].

CAR is involved in regulation of lipid metabolism genes, and, therefore, it is a promising therapeutic target for treatment of such diseases as fatty nonalcoholic liver disease and steatohepatitis. Initially CAR was thought to play in the organism the role of "xenosensor". But now the receptor is shown to be involved in many processes. In addition to gluconeogenesis and lipid metabolism, CAR is involved in the regulation of hormonal signals and in cell growth and apoptosis (Fig. 5).

CAR contributes to pathogenesis of many diseases. It can be used as a therapeutic target for prevention and treatment of obesity and type 2 *diabetes mellitus* [70]. Therefore, understanding the mechanisms of action of CAR, studies of its molecular targets, and searches for effective agonists/antagonists are extremely important for solution of both scientific and applied problems in medicine.

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