Induction of Drug Metabolism by Forskolin: The Role of the Pregnane X Receptor and the Protein Kinase A Signal Transduction Pathway

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ABSTRACT
An extract of the plant Coleus forskohlii has been used for centuries in Ayurvedic medicine to treat various diseases such as hypothyroidism, heart disease, and respiratory disorders. Additionally, complex herbal mixtures containing this extract are gaining popularity in the United States for their putative “fat-burning” properties. The active ingredient in C. forskohlii extract is the diterpene compound forskolin. Forskolin is a widely used biochemical tool that activates adenylyl cyclase, thereby increasing intracellular concentration of cAMP and thus activating the protein kinase A (PKA) signal transduction pathway. We show herein that both forskolin and its nonadenyl cyclase-activating drug-metabolizing enzymes in liver. Finally, our data suggest that herbal therapy with C. forskohlii extract should be approached cautiously due to the potential for herb-drug interactions in patients on combination therapy.

A large number of hormones and neurotransmitters use cAMP. As an intracellular second messenger, cAMP regulates a number of different cellular processes, such as cell growth and differentiation, ion channel conductivity, synaptic release of neurotransmitters, and gene transcription. The principal intracellular target for cAMP in mammalian cells is cAMP-dependent protein kinase (PKA). In the absence of cAMP, PKA is an enzymatically inactive tetrameric holoenzyme consisting of two catalytic subunits bound to a regulatory subunit dimer. Upon binding of cAMP, the PKA enzyme dissociates into a regulatory subunit dimer and two active catalytic subunits that phosphorylate serine and threonine residues on specific substrate proteins, including many transcription factors.

Several widely used pharmacological agents have been developed that activate PKA both directly and indirectly. For example, direct activation of PKA signaling can be achieved by using 8-bromo-cAMP (8-Br-cAMP), a cell-permeable cyclic nucleotide derivative that mimics the action of endogenous cAMP by binding to the PKA regulatory subunit. Indirect activation of PKA signaling can be achieved through pharmacological activation of adenylyl cyclase, the enzyme responsible for the production of intracellular cAMP. Forskolin (7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-en-11-one) is a diterpene derived from the Indian plant C. forskohlii. Forskolin is an efficacious activator of adenylyl cyclase (Seamon et al., 1981). This characteristic of forskolin led to its extensive use as a biochemical tool that increases intracellular cAMP concentration and activates the PKA signal transduction pathway in cells. C. forskohlii extract has been used for centuries in Ayurvedic medicine to treat various diseases such as hypothyroidism, heart disease, and respiratory disorders (for review, see Ammon and Muller, 1985). Moreover, complex herbal mixtures that contain C. forskohlii extract are gaining popularity in the United States for use in

ABBREVIATIONS: PKA, protein kinase A; 8-Br-cAMP, 8-bromo-cAMP; PXR, pregnane X receptor; PCR, polymerase chain reaction; XREM, xenobiotic responsive enhancer module; LUC, luciferase; DMSO, dimethyl sulfoxide; CARLA, coactivator receptor ligand-binding assay(s); SRC, steroid receptor coactivator; PBP, peroxisome proliferator-activated receptor-binding protein; NCoR, nuclear hormone receptor corepressor; TLC, thin layer chromatography; Q-PCR, quantitative PCR; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; CAR, constitutive androstane receptor; RIF, rifampicin; PCN, pregnenolone 16α-carbonitrile; HNF, hepatocyte nuclear factor.
weight loss regimens due to their putative fat-burning properties and over-the-counter availability.

Forskolin and the non-PKA-activating analog 1,9 dideoxyforskolin (7β-acetoxy-6β-hydroxy-8,13-epoxy-labd-14-en-11-one) have cAMP- and PKA-independent biological effects. One of the best characterized cAMP- and PKA-independent effects of these two compounds is the induction of CYP3A gene expression in cultured primary hepatocytes (Sidhu and Omiecinski, 1996); however, the molecular basis for this phenomenon is currently unknown. The pregnane X receptor (PXR, NR1I2) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. It is now well accepted that PXR is a key regulator of xenobiotic-inducible CYP3A gene expression. In addition, several studies have shown that PXR regulates other genes involved in the metabolism of xenobiotic and endobiotic compounds such as CYP2B10, CYP2C, glutathione S-transferases, sulfotransferases, and UDP-glucuronosyltransferases (Maglich et al., 2002; Sonoda et al., 2002; Wei et al., 2002; Chen et al., 2003). PXR also regulates the expression of the drug transporter genes Oatp2, Mrh1, Mrp2, and Mrp3 (Geick et al., 2001; Kast et al., 2002; Staudinger et al., 2003). Thus, we hypothesized that PXR activation by forskolin and 1,9 dideoxyforskolin represents the mechanism by which these two compounds induce CYP3A gene expression in hepatocytes.

In the present report, we show that purified forskolin and 1,9 dideoxyforskolin induce CYP3A gene expression by functioning as PXR ligands. We show that activation of the PKA signal transduction pathway potentiates the PXR-mediated xenobiotic response. We also show that activation of PERK signaling strengthens the interaction between PXR and other xenobiotic response. We also show that activation of PKA signaling strengthens the interaction between PXR and xenobiotic response. We also show that activation of PERK signaling strengthens the interaction between PXR and xenobiotic response. We also show that activation of PERK signaling strengthens the interaction between PXR and xenobiotic response. We also show that activation of PERK signaling strengthens the interaction between PXR and xenobiotic response. We also show that activation of PERK signaling strengthens the interaction between PXR and xenobiotic response.

Materials and Methods

Animal Care. Generation of the PXR knockout mice was previously described (Staudinger et al., 2001b). All rodents were maintained on standard laboratory chow and allowed food and water ad libitum. The studies reported herein have been carried out in accordance with the Declaration of Helsinki and/or the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Compounds and Plasmids. Unless otherwise stated, all chemical compounds were purchased from Sigma-Aldrich (St. Louis, MO). 1,9 Dideoxyforskolin was purchased from Calbiochem (San Diego, CA), and taxol was a gift from Dr. Gunda Georg (University of Kansas). The pG5-hPXR and GST-hPXR ligand-binding domain fusion plasmids were previously described (Lehmann et al., 1998). The pG5-mPXR and GST-mPXR ligand-binding domain fusion plasmids were previously described (Kliwer et al., 1998). The pG5-mCAR plasmid was previously described (Moore et al., 2000). The GAL4-SRC1, GAL4-PBP, and GAL4-NCoR1 expression vectors were previously described (Synold et al., 2001). The GST-p75 neurotrophin receptor plasmid was previously described (Bilderback et al., 1997). The GST-hPXR DNA-binding domain fusion plasmid was generated using PCR primers that introduced EcoRI sites, and the plasmids were subcloned into pGEX4T-1 and encodes amino acid residues 1 to 107 of human PXR. The full-length human and mouse PXR cDNAs were isolated from the pG5-hPXR and pG5-mPXR plasmids and subcloned into pVP16 (BD Biosciences, San Jose, CA), in frame with EcoRI and BamHI strategy to create the full-length VP16-hPXR fusion protein. The pF-R-LUC reporter gene is commercially available (BD Biosciences).

Cell Culture and Transient Transfection Analysis. The XREM-LUC reporter gene assays were performed as previously described (Brobst et al., 2004). The mammalian two-hybrid system analysis was performed in 96-well plates using CV-1 cells at 7000 cells/well. Transfection mixtures contained pPR-LUC (20 ng), GAL4-cofactor (20 ng), VP16-hPXR (10 ng), pSV-ß-GAL (40 ng), and pBlue-script (20 ng). The next day, transfected cells were drug-treated for 24 h. All compounds were added to the culture medium at 1000× stocks dissolved in DMSO or ethanol. Luciferase and β-galactosidase activities were determined using the Dual-Light Kit per the manufacturer’s instructions (Applied Biosystems, Foster City, CA).

Coactivator Receptor Ligand-Binding Assays (CARLA). CARLA analysis was performed as described previously (Brobst et al., 2004).

Hepatocyte Cultures, RNA Isolation, and Northern Blot Analysis. Hepatocytes were isolated and cultured as previously described (LeCluyse et al., 1996). All compounds were added to the culture medium as 1000× stocks dissolved in DMSO, except forskolin and 1,9 dideoxyforskolin were dissolved in ethanol, and 8-Br-cAMP was dissolved in water. Control cells received DMSO and/or ethanol alone. Total RNA was isolated, and Northern blotting assays were performed as previously described (Staudinger et al., 2001). Blots were hybridized with the 32P-labeled cDNA corresponding to the cDNA sequences for mouse Cyp3a11 (bases 69–1609; GenBank NM 007818). The 18S ribosomal RNA probe was amplified from mouse liver cDNA by commercially available primers per the manufacturer’s instructions (Ambion, Austin, TX).

Thin Layer Chromatography (TLC) Analysis of Forskohlii Ethanol Extract. Equal volumes of forskolin (10 mM), 1,9 dideoxyforskolin (10 mM), and forskohlii ethanol extract were loaded on a TLC plate. TLC was performed with a 4:1 ratio of hexane and ethyl acetate and visualized by standard blue stain.

Forskohlii Ethanol Extract Preparation. A commercial C. forskohlii root extract was obtained from the PhytoPharmica Inc. (Green Bay, WI). The content of two capsules (50 mg of powdered capsules) was extracted with 0.87 ml of absolute ethyl alcohol at 4°C overnight with gentle shaking. The extract was centrifuged at 16,000g for 5 min. The ethyl alcohol supernatant was decanted and kept at −20°C until use.

Real-Time Quantitative PCR (Q-PCR) Analysis for Cyp3a11 Gene Expression. The probe sets and procedures for the quantitative detection of β-actin and Cyp3a11 were previously described (Staudinger et al., 2003).

In Vitro Protein Kinase A Phosphorylation Assay. Purified GST fusion proteins (0.5 μg each) were incubated in 50 μl of PKA reaction buffer [10 mM Tris, pH 7.2; 6.25 mM MgCl2; 0.5 μg of catalytically active PKA (Promega, Madison, WI); 1 μl of [γ-32P]ATP; and 1 μl of 1 μM ATP] at 30°C for 30 min. An equal amount of 2× SDS loading buffer was added to each reaction, and the samples were heated at 100°C for 5 min. Samples were resolved on 10% SDS-PAGE. Gels were dried at 75°C for 1 h. Radiolabeled proteins were visualized by autoradiography.

Results

Because forskolin and 1,9 dideoxyforskolin induce expression of CYP3A independent of both cAMP concentration and
PKA signaling in hepatocytes (Sidhu and Omiecinski, 1996), we performed a standard cell-based reporter gene assay to investigate whether these compounds would activate human and mouse PXR (Fig. 1A). CV-1 cells were transfected with expression vectors encoding human PXR, mouse PXR, or mouse CAR nuclear receptor proteins together with the PXR-and CAR-responsive luciferase reporter gene XREM-LUC (Goodwin et al., 1999, 2001). Forskolin and 1,9 dideoxyforskolin activated the human and mouse PXR with an efficacy comparable to that achieved with the prototypical agonists of human and mouse PXR, rifampicin (RIF), and pregnenolone 16α-carbonitrile (PCN), respectively. In contrast, mouse CAR was not activated by forskolin or 1,9 dideoxyforskolin. The specific PKA activator 8-Br-cAMP was used as a negative control and had little effect on XREM-LUC reporter gene activity. Full concentration-response analysis revealed that forskolin activates human and mouse PXR with EC₅₀ values of approximately 400 and 900 nM, respectively (Fig. 1B), levels that are one order of magnitude below that required for activation of PKA.

We next tested whether forskolin and 1,9 dideoxyforskolin mediate their effects through direct binding to PXR. CARLA analysis (an in vitro coactivator receptor ligand-binding assay) was performed using the purified mouse PXR ligand-binding domain and the ³⁵S-radiolabeled nuclear receptor coactivator protein SRC1 (Fig. 2A). Treatment with forskolin and 1,9 dideoxyforskolin promoted the interaction between PXR ligand-binding domain and [³⁵S]SRC1 in vitro, suggesting that forskolin and 1,9 dideoxyforskolin interact directly with the PXR ligand-binding domain. PCN treatment was used as a positive control. Similar results were obtained with the human PXR protein (data not shown). To further investigate the molecular mechanism whereby forskolin and 1,9 dideoxyforskolin activate PXR in cells, we performed mam-

![Fig. 1. Forskolin and 1,9 dideoxyforskolin are PXR ligands. A, CV-1 cells transfected with expression vectors encoding human PXR, mouse PXR, and mouse CAR together with the XREM-LUC reporter gene. Cells were treated with vehicle (0.1% DMSO and 0.1% ethanol) or 10 μM of the indicated compounds [F, forskolin; F1,9, 1,9 dideoxyforskolin; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)benzene; PCN], except 8-Br-cAMP, which was used at 1 mM. The reporter gene activity of human PXR with RIF, mouse PXR with PCN, and mouse CAR with TCPOBOP is defined as 100%, respectively. B, CV-1 cells transfected with expression vectors encoding human PXR or mouse PXR together with the XREM-LUC reporter gene. Transfected cells were treated with increasing concentrations of forskolin. RLU, relative light unit (luciferase activity normalized to β-galactosidase activity).](image-url)
malian two-hybrid analysis (Fig. 2B). CV-1 cells were transfected with the expression vectors encoding the receptor-interacting domains from the corepressor protein NCoR1 or the coactivator protein SRC1 fused to GAL4, respectively, together with VP16-tagged human PXR and the GAL4-responsive luciferase reporter gene pFR-LUC. Both forskolin and 1,9 dideoxyforskolin reduced the interaction between PXR and the corepressor protein NCoR1 and strengthened the interaction between PXR and the coactivator protein SRC1 in cells. Forskolin and 1,9 dideoxyforskolin also strengthened the interaction between PXR and the coactivator proteins SRC2 and PBP (data not shown). The known human PXR ligands taxol and rifampicin were used as positive controls. Similar results were obtained using the mouse PXR protein in mammalian two-hybrid analysis (data not shown).

To determine whether the induction of CYP3A gene expression by forskolin and 1,9 dideoxyforskolin is mediated through PXR, we took advantage of our PXR knockout mouse model. Cultured hepatocytes isolated from wild-type mouse hepatocytes but not in PXR knockout hepatocytes. Treatment of hepatocytes with higher concentrations of forskolin (10 and 100 μM) produced very modest levels of CYP3A gene expression in PXR knockout hepatocytes when compared with that achieved in wild-type hepatocytes. In contrast to treatment with forskolin, treatment with PKA-inactive 1,9 dideoxyforskolin did not induce CYP3A gene expression at any concentration tested in PXR knockout hepatocytes, whereas in wild-type hepatocytes 10 and 100 μM concentrations induced CYP3A gene expression. When compared with forskolin, 1,9 dideoxyforskolin produced much lower levels of induction in wild-type hepatocytes. Interestingly, treatment with 8-Br-cAMP induced CYP3A gene expression in both wild-type and PXR knockout hepatocytes, suggesting the induction of CYP3A in PXR knockout hepatocytes by high concentrations of forskolin is likely mediated through activation of the PKA signal transduction pathway.

Because significant quantities of both forskolin and 1,9 dideoxyforskolin are contained in the herb C. forskohlii (Fig. 3B), we investigated whether an extract isolated from this herbal remedy induces CYP3A gene expression in a PXR-dependent manner. Wild-type and PXR knockout hepatocytes

**Fig. 2.** Forskolin and 1,9 dideoxyforskolin modulate PXR-coactivator interaction. A, CARLA analysis performed with vehicle (0.1% ethanol and 0.1% DMSO), 100 μM forskolin (F), 100 μM 1,9 dideoxyforskolin (F1,9), and 10 μM PCN together with mouse GST-PXR ligand-binding domain and 35S-radiolabeled steroid receptor coactivator 1 protein. B, CV-1 cells transfected with the GAL4-responsive pFR-LUC reporter gene and the expression vectors encoding GAL4 cofactors and VP16-hPXR and treated with vehicle or 10 μM of the indicated compounds. Data points in reporter gene assays represent the mean ± S.E.M. (n = 4).
cytes were treated with *C. forskohlii* extract, forskolin, and 8-Br-cAMP. Northern blot analysis was performed using a radiolabeled cDNA probe for Cyp3a11 (Fig. 3C). As expected, *C. forskohlii* extract induced CYP3A gene expression in a concentration-dependent manner in wild-type mouse hepatocytes. In contrast, induction of CYP3A gene expression by *C. forskohlii* extract in PXR knockout hepatocytes was absent at low doses and minimal at higher doses.

As shown in Fig. 3A, the specific and potent PKA activator 8-Br-cAMP (1 mM) induced modest levels of CYP3A gene expression in mouse hepatocytes. We therefore asked whether PKA activation modulated the induction of CYP3A gene expression by PXR agonists. We performed a comparison of the induction of CYP3A expression following coadministration of increasing concentrations of 8-Br-cAMP (0.01, 0.1, and 1.0 mM) in the presence of a fixed concentration of the PXR agonist taxol (10 μM) (Fig. 4A). Strikingly, treatment with 8-Br-cAMP greatly increased the induction of CYP3A gene expression by taxol in mouse hepatocytes. Moreover, cotreatment with 8-Br-cAMP (1 mM) increased the induction of CYP3A gene expression by taxol in mouse hepatocytes. Furthermore, cotreatment with 8-Br-cAMP (1 mM) increased the induction of CYP3A gene expression by both forskolin and 1,9 dideoxyforskolin. As expected, treatment with 1 mM 8-Br-cAMP alone produced only modest induction of CYP3A gene expression. To quantify the increase in CYP3A gene expression produced by 8-Br-cAMP, we used probes specific for mouse Cyp3a11 and β-actin in real-time Q-PCR analysis (Fig. 4B). Treatment with 8-Br-cAMP induced Cyp3a11 gene expression levels by 19.7 ± 1.5-fold. Treatment of cultured mouse hepatocytes with taxol produced 45.2 ± 5.8-fold induction of Cyp3a11 gene expression, whereas cotreatment with taxol and 8-Br-cAMP produced 1202.1 ± 166.3-fold induction of Cyp3a11 gene expression. Treatment with the PKA-

activating forskolin produced 482.9 ± 70.3-fold induction of Cyp3a11 gene expression, whereas cotreatment with forskolin and 8-Br-cAMP produced 3651.2 ± 285.0-fold induction of Cyp3a11 gene expression. Treatment with the non-PKA-activating 1,9 dideoxyforskolin produced 22.4 ± 3.0-fold induction of Cyp3a11 gene expression, whereas cotreatment with 1,9 dideoxyforskolin and 8-Br-cAMP produced 868.1 ± 43.5-fold induction of Cyp3a11 gene expression. Thus, treatment of mouse hepatocytes with the strong PKA activator 8-Br-cAMP potentiates the induction of Cyp3a11 gene expression by PXR agonists.

To determine whether the potentiation of PXR-mediated induction of CYP3A gene expression is due to the modulation of PXR-coactivator protein-protein interaction, we used the mammalian two-hybrid system reporter gene assay (Fig. 5). Treatment of transfected cells with either taxol or 8-Br-cAMP alone strengthened the interaction between PXR and the receptor-interacting domains of the known PXR-interacting coactivator proteins SRC1 and PBP. Coadministration of taxol and 8-Br-cAMP further strengthened the interaction between PXR and these coactivator proteins.

The potentiation of CYP3A gene expression produced by cotreatment with 8-Br-cAMP in mouse hepatocytes prompted us to determine whether PXR can serve as a direct substrate for PKA. In vitro kinase assays were performed using a catalytically active purified PKA preparation and purified human GST-PXR fusion proteins (Fig. 6). GST alone and GST-p75 neurotrophin were used as negative and positive controls, respectively. Both the PXR DNA-binding domain and ligand-binding domain were phosphorylated by PKA in vitro. The GST alone showed a weak band corresponding to
autophosphorylated PKA. The smaller band that appears in the GST-LBD lane occurs through degradation or proteolysis of the GST-LBD protein during the incubation period. Similar results were obtained using purified mouse PXR proteins (data not shown).

Discussion

Although most of the biological effects of forskolin depend on its ability to increase levels of cAMP, several cAMP-independent effects have been identified through the use of adenyl cyclase-inactive forskolin analogs such as 1,9 dideoxyforskolin. One of the best-characterized cAMP- and PKA-independent effects of forskolin is the induction of CYP3A gene expression in cultured hepatocytes (Sidhu and Omiecinski, 1996). To date, the molecular mechanism of the ability of forskolin and 1,9 dideoxyforskolin to induce CYP3A gene expression has remained obscure. We show herein that both forskolin and 1,9 dideoxyforskolin, two ingredients in the herbal remedy *C. forskohlii*, activate PXR by functioning as ligands for this receptor, thereby inducing the expression of CYP3A genes in hepatocytes.

In India, practitioners of traditional Ayurvedic medicine have long used an extract of *C. forskohlii* to treat various disorders (Ammon and Muller, 1985). Available without a prescription in supplement form and containing up to 18% forskolin, *C. forskohlii* extract is commonly recommended for treating hypothyroidism. Additional conditions, including excess weight, asthma, heart disease, high blood pressure, glaucoma, eczema, and psoriasis, may respond to *C. forskohlii* therapy. Human clinical trial information regarding the effectiveness of forskolin therapy is currently limited. However, treatment with *C. forskohlii* extract, although common, is contraindicated in combination with antihypertensives and anticoagulants due to the high potential for herb-
drug interactions. Our data further strengthen the notion that herbal remedies should be routinely screened for their ability to interact with prescription medications.

It is interesting to note that forskolin and 1,9 dideoxyforskolin contain a three-ring structure that closely resembles the basic steroid ring. Structurally diverse molecules, including certain naturally occurring and synthetic steroids, activate PXR (Moore et al., 2003). Additionally, molecules that bear little structural resemblance to steroids activate PXR, including the antibiotic rifampicin and the antifungal agent clotrimazole (Lehmann et al., 1998). Our studies add forskolin and 1,9 dideoxyforskolin, two compounds found in *C. forskohlii*, to the growing list of exogenous PXR ligands and further support the notion that PXR evolved partly as a xenobiotic sensor that functions to promote the biotransformation and subsequent excretion of potentially toxic compounds humans ingest. It is worth noting that forskolin activates PXR with EC\textsubscript{50} values that are approximately one order of magnitude below that required for activation of PKA. This is important because the therapeutic effect of *C. forskohlii* depends on its ability to activate PKA signaling, and therefore, significant PXR-mediated herb-drug interactions are likely to occur even before therapeutic levels of forskolin are achieved in patients using this powerful herbal remedy.

Although induction of CYP3A gene expression by both forskolin and *C. forskohlii* extract is detectable in cultured hepatocytes isolated from PXR knockout hepatocytes, it is severely diminished. Moreover, higher concentrations of forskolin are required in the cell-free in vitro CARLA analysis to produce modulation of PXR-coactivator interaction when compared with cell-based assays. These data highlight the dual nature of forskolin as both a PXR agonist and an activator of PKA signaling. Moreover, these data indicate that PKA signaling has a profound impact upon the induction of PXR-target gene expression, likely through phosphorylation of the PXR protein. Additionally, we note that activation of PKA signaling by treatment with 8-Br-cAMP produced relatively modest, though roughly equivalent, levels of CYP3A induction in both wild-type and PXR null hepatocytes when compared with that produced by forskolin in wild-type hepatocytes. These data suggest that PKA signaling also contributes to PXR-independent pathways that produce induction of
CYP3A gene expression, perhaps involving the constitutive androstane receptor or the glucocorticoid receptor. These data represent stimulating issues that warrant further analysis in the future.

Activation of PKA signaling with 8-Br-cAMP is known to modulate the interaction of the nuclear receptor corepressor proteins NCoR1 and NCoR2 with the progesterone receptor (Wagner et al., 1998). In this report, we show that when subjected to in vitro kinase assays, the purified PXR protein was phosphorylated by catalytically active PKA. Moreover, we show that activation of PKA signaling with 8-Br-cAMP increases the strength of PXR-coactivator protein-protein interaction in cell-based assays. Furthermore, we have shown that activation of the PKA signaling pathway potentiates the induction of CYP3A by PXR ligands in cultured hepatocytes. Together, these data suggest that phosphorylation of PXR by PKA may play a key role in regulating the induction of CYP3A gene expression in hepatocytes, in part, through its ability to modulate PXR-protein cofactor interaction.

The PKA catalytic subunit has been identified in the nucleus and phosphorylates a number of nuclear receptor superfamily members, thereby modulating their trans-acting properties (Shao and Lazar, 1999). The activity of the liver-enriched hepatocyte nuclear factor 4 (HNF4, NR2A1) is modulated by PKA-mediated phosphorylation in cell-based assays (Violett et al., 1997). Because HNF4 is required for maximal induction of CYP3A gene expression (Tirona et al., 2003) and is a known substrate for PKA, it likely represents another important determinant of the PKA-dependent effects observed in this study, although it is not currently known how phosphorylation of HNF4 affects CYP3A gene expression.

Our data show that, in addition to the evolution of the ligand-binding pocket of PXR, the activation of the PKA signal transduction pathway likely plays a pivotal role in the induction of CYP3A gene expression by PXR agonists. As a master regulator of xenobiotic-inducible CYP3A gene expression, PXR is well positioned to integrate external cues via phosphorylation together with ligand-dependent induction of target gene expression. Future studies should focus on the identification of sites of PXR phosphorylation by PKA and determining the molecular basis of the modulation of PXR-target gene expression following the activation of the PKA signal transduction pathway.

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References


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