SXR, a novel steroid and xenobiotic-sensing nuclear receptor

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An important requirement for physiologic homeostasis is the detoxification and removal of endogenous hormones and xenobiotic compounds with biological activity. Much of the detoxification is performed by cytochrome P-450 enzymes, many of which have broad substrate specificity and are inducible by hundreds of different compounds, including steroids. The ingestion of dietary steroids and lipids induces the same enzymes; therefore, they would appear to be integrated into a coordinated metabolic pathway. Instead of possessing hundreds of receptors, one for each inducing compound, we propose the existence of a few broad specificity, low-affinity sensing receptors that would monitor aggregate levels of inducers to trigger production of metabolizing enzymes. In support of this model, we have isolated a novel nuclear receptor, termed the steroid and xenobiotic receptor (SXR), which activates transcription in response to a diversity of natural and synthetic compounds. SXR forms a heterodimer with RXR that can bind to and induce transcription from response elements present in steroid-inducible cytochrome P-450 genes and is expressed in tissues in which these catabolic enzymes are expressed. These results strongly support the steroid sensor hypothesis and suggest that broad specificity sensing receptors may represent a novel branch of the nuclear receptor superfamily.

[Key Words: Steroid; xenobiotic receptor; nuclear receptor; transcriptional activity]

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Lipophilic hormones, such as steroids, retinoic acid, thyroid hormone, and vitamin D3, control broad aspects of animal growth, development, and adult organ physiology. The effects of these hormones are mediated by a large superfamily of intracellular receptors that function as ligand-dependent and sequence-specific transcription factors. The nonsteroidal nuclear receptors for thyroid hormone [TR], vitamin D3 [VDR], all-trans retinoic acid [RAR], and fatty acids and eicosanoids [PPAR] form heterodimers with the 9-cis retinoic acid receptor [RXR] that bind bipartite hormone-response elements (HREs) composed of directly repeated half sites related to the sequence AGGTCA [Mangelsdorf and Evans 1995]. In contrast, the steroid receptors function as homodimers and bind to palindromic target sequences spaced by three nucleotides [Beato et al. 1995]. In addition to the known receptors, a large group of structurally related ‘orphan’ nuclear receptors has been described; that these receptors possess obvious DNA and ligand-binding domains but lack identified ligands [Mangelsdorf et al. 1995]. Each has the potential to regulate a distinct endocrine signaling pathway.

It is widely viewed that the hormone response is a consequence of the release from an endocrine gland of a ligand that circulates through the blood, and coordinately regulates responses in target tissues by acting through specific nuclear receptors. Hormone responsiveness is dependent on the ability to rapidly clear ligand from the blood and the body so that, in absence of a stimulus, target tissues return to a ground state. Hormonal homeostasis is thus achieved by the coordinated release and degradation of bioactive hormones. Steroid hormones and their many metabolites are primarily inactivated by reduction and oxidation in the liver. As there are >45 adrenal steroids identified [Norman and Litwack 1997], dozens of each of the sex steroids [androgens, estrogens, and progestins] [Norman and Litwack 1997], 25–35 vitamin D metabolites [Horst and Reinhardt 1997], and likely hundreds of fatty acids, eicosanoids, hydroxyfats, and related bioactive lipids, the problem of efficient ligand elimination is critical to physiologic homeostasis. In addition to a myriad of endogenous hormones, a similar diversity of ingested plant and animal steroids and bioactive xenobiotic compounds must also be degraded.

Selye (1971) first introduced the concept that endogenous steroids and pharmacologic substances may function to modulate the expression of enzymes that would protect against subsequent exposure to toxic xenobiotic
substances. These compounds, which Selye called cata-
toxic steroids, are typified by the synthetic glucocortic-
oid antagonist pregnenolone-16-carbonitrile (PCN).
PCN and a variety of xenobiotic steroids induce the pro-
leration of hepatic endoplasmic reticulum and the ex-
pression of cytochrome P-450 genes [Schuetz and Guzel-
lian 1984; Gonzalez et al. 1986; Burger et al. 1992]. One
consequence of PCN treatment is the induction of non-
specific ‘protection’ against subsequent exposure to such
diverse xenobiotics as digitoxin, indomethacin, barbitu-
rates, and steroids [Selye 1971]. Furthermore, it is known
that a variety of such compounds can activate P-450
genes responsible for their detoxification or degradation
[Denison and Whitlock 1995; Fernandez-Salgueiro and
Gonzalez 1995; Hankinson 1995; Rendic and Di Carlo
1997].

Although it appears that catatoxic compounds must
regulate the expression of cytochrome P-450s and other
detoxifying enzymes, two lines of evidence argue that
such regulation is independent of the classic steroid re-
cptors. First, many of the most potent compounds [e.g.,
PCN, spironolactone, cyproterone acetate] are steroid re-
cptor agonists, whereas others [e.g., dexamethasone]
are receptor antagonists [Burger et al. 1992]. Second, the
nonspecific protective response remains after bilateral
adrenalectomy [and presumably in the absence of adre-
nal steroids] but not after partial hepatectomy [Selye
1971]. Therefore, hepatic orphan nuclear receptors regu-
lated by these protective compounds would provide a
novel pathway for the induction of xenobiotic metabo-
limizing enzymes. Because such enzymes are induced by
high [pharmacological] doses of xenobiotics, natural and
synthetic steroids, and phytosteroids, we anticipate that
the sensor would be a broad specificity, low-affinity re-
cptor.

Here we describe the characterization of a novel hu-
man orphan nuclear receptor, termed the steroid and xe-
nobiotic receptor (SXR), that responds to an enormous
variety of natural and synthetic steroid hormones, in-
cluding antagonists as well as xenobiotic drugs such as
rifampicin and bioactive dietary compounds such as phy-
toestrogens. The ability of SXR to regulate expression of
catabolic enzymes in response to this diversity of natural
and pharmaceutical compounds is unprecedented for a
nuclear receptor and provides a novel mechanism for di-
rect regulation of metabolism.

Results

SXR is a novel human orphan nuclear receptor

SXR was isolated in a screen to identify potential human
homologs of the Xenopus benzoate X receptor (BXR)
[Blumberg et al. 1998]. The cDNA encodes a predicted
protein of 434 amino acids [Fig. 1A] that is 73% identical
to BXR in the DNA-binding domain [DBD] and 43% identical
in the ligand-binding domain [LBD] [Fig. 1B].

SXR is most closely related to the recently described
pregnane X receptor (PXR) [Kliewer et al. 1998] (95% identical in the DBD, 73% identical in the LBD). SXR is
related more distantly to the vitamin D3 receptor and
the orphan receptor CAR [constitutive androstane recep-
tor] [Baes et al. 1994] [Fig. 1B]. Other than these recep-
tors, SXR shows no more similarity to other nuclear re-
cptors than the different receptor subfamilies do to each
other [Fig. 1B]. Because true homologs among nuclear
receptors typically share considerable similarity, espe-
ially in the DBD, we conclude that SXR and PXR com-
prise a new branch of the nuclear receptor superfamily.

Screening of a mouse liver cDNA library at reduced
stringency resulted in the identification of 39 cDNAs, all
of which encoded PXR.1 [data not shown]. Because or-
thologous nuclear receptors typically share upward of
90% amino acid identity in the LBD when comparing
rodent and human receptors [e.g., RARα, 98% human/ mouse (h/m); PPARγ, 98% h/m; glucocorticoid receptor
[GR], 95% h/m; TRβ, 98% h/rat; estrogen receptorα
[ERα], 89% h/m], PXR and SXR may represent α and β subtypes of a new receptor family. Although this is sup-
ported by the distinct pharmacological properties of the
receptors [see below] further screening of mouse and hu-
man liver cDNA libraries has failed to identify other
family members. This suggests that PXR and SXR could
represent unusually divergent orthologous genes. If cor-
rect, this divergence may reflect receptor adaptation to
the different diets of rodents and primates and the re-
quirement to detoxify appropriate food-borne com-
ponds.

Northern blot analysis showed that SXR mRNA is ex-
pressed at high levels in liver and at more moderate lev-
els in the intestine [Fig. 1C]. Longer exposures did not
reveal expression in any other tissues on these blots.
Multiple mRNAs were detected, ranging from 3500
nucleotides to larger than 9000 nucleotides. Comparison
of the four cDNAs obtained suggests that these differ-
ences may be attributable to alternative polyadenylation
as they share the same protein coding and 5‘-untrans-
lated sequences, but each has a different 3′ end [data not
shown].

SXR DNA-binding specificity

Electrophoretic mobility shift assays were used to deter-
mine the ability of SXR to heterodimerize with RXR and
to analyze the selectivity and specificity of SXR DNA
binding. Receptors that heterodimerize with RXR typi-
ically bind to direct repeats of AGGTCA or closely re-
lated sequences [Mangelsdorf and Evans 1995]. We tested
SXR alone and in combination with RXR on a series of
‘testor’ elements differing in the spacing between half
sites from 0 to 15 nucleotides. No binding was seen on
classic steroid response elements [data not shown]. In
contrast, strong binding was selective to a DR-4 motif
with minimal binding to DR-3 and DR-5 and no binding
to other spacings [Fig. 2A; data not shown]. When the
variant AGTTCA (βDR) half site was used, strong bind-
ing was seen on βDR-4 and βDR-5 and significant, but
reduced, binding to βDR-3 [Fig. 2B]. These results dem-
onstrate that SXR binds DNA as a heterodimer with
RXR rather than as a homodimer like the classic steroid receptors (Beato et al. 1995).

**SXR is activated by steroids**

To determine whether the activity of SXR was ligand dependent, mixtures of natural and synthetic compounds were tested for their ability to activate SXR in transfection-based assays. A mixture containing dehydroepiandrosterone (DHEA) and pregnenolone was active, suggesting that SXR might be a new steroid receptor. To characterize its response properties, a large variety of steroids, including intermediate metabolites and major products of known steroid biosynthetic pathways were tested. Surprisingly, most of these compounds were active, although there were clear differences in potency (Fig. 3A). Of the >70 steroids tested most showed some activity at high doses (data not shown). Activation was dependent on the LBD of SXR, as both full-length receptors and GAL4-receptor LBD chimeras showed similar activity.
activity, whereas there was no activation of reporter gene expression in experiments with reporter alone or reporter plus GAL4 DNA-binding domain (Fig. 3A; data not shown). The most potent and efficacious activator of the numerous steroids tested is corticosterone (Fig. 3A). Estradiol and dihydrotestosterone are also remarkably effective activators, whereas aldosterone and 1,25-dihydroxy vitamin D3 are inactive, even at 50 µM (Fig. 3A; data not shown). Although ligands for the classic steroid receptors do show some overlap in receptor specificity, there is no example of a nuclear receptor that can be activated by so many different types of steroids. This broad ligand specificity of SXR parallels that of PPARα, which is activated by a very diverse group of dietary fatty acids at micromolar levels (Gottlicher et al. 1992; Forman et al. 1997; Kliewer et al. 1997).

The diversity of steroids showing activity on SXR led us to hypothesize that it might be able to sense cumulative, as well as individual steroid levels, predicting that combinations of activators might be more active than the individual components. As shown in Figure 3B, a cocktail containing 10 steroids each at 10 µM (for an overall concentration of 100 µM) was considerably more active than its individual components at 10 µM, a concentration at which most were inactive. These results

Figure 2. SXR DNA-binding specificity. (A) SXR:hRXRa heterodimers prefer DR-4 among a panel of AGGTCA-containing HREs. In vitro-transcribed and -translated SXR was incubated with 32P-labeled oligonucleotides and electrophoresed in native polyacrylamide gels. (B) AGTTCA is preferred to AGGTCA. SXR:hRXRa heterodimers were tested for their ability to bind half-sites of the sequence AGTTCA derived from the RARβ RA-responsive element [Sucov et al. 1990]. We found that in addition to a spacing motif of 4 βDR-4 they bind nearly as well to βDR-5 spacing and significantly to a βDR-3 motif. DR-4 and TREp are shown for reference.

Figure 3. SXR is activated by many steroids. (A) Chimeric receptors composed of the GAL4 DNA-binding domain and the SXR-ligand binding domain were cotransfected into CV-1 cells with the reporter gene tk(MH100)4–luc [Forman et al. 1995]. DHEA and pregnenolone activated this chimeric receptor; therefore, other steroids were tested for activation. Results are shown as fold induction over solvent (DMSO) control for 50 µM steroid and represent the averages and standard error from triplicate assays. Reporter alone or reporter plus GAL4–DBD was not activated by any of these compounds [data not shown]. Similar results were obtained using full-length receptors and appropriate reporters (see below). (B) The ability of steroidal activators to act additively was tested using full-length SXR and the reporter tk[LXRE]3–luc (Willy et al. 1995). The cocktail contained 10 µM of each steroid for an overall concentration of 100 µM total steroid. The cocktail and its individual components were tested at 100, 10, and 1 µM; results are shown for 100 µM cocktail and 10 µM component steroids. Similar results were obtained using GAL–SXR [not shown].
support the proposal that SXR is a broad specificity, low-affinity, steroid-activated receptor.

**SXR may regulate the activity of steroid-inducible P-450s**

A search of the GenBank database for genes expressed in liver containing potential SXR response elements identified the steroid hydroxylases CYP2A1, CYP2A2, CYP2C1, CYP2C6, CYP3A1, CYP3A2, P-450 oxidoreductase, and UDP-glucuronosyltransferase as candidate target genes [Fig. 4A]. The data shown in Figure 4B verify that SXR can activate DR-3, DR-4, and DR-5 elements that are present in these genes. In this series of transfections, corticosterone along with pregnenolone, progesterone, dihydrotestosterone (DHT), estradiol, and PCN are consistently among the best activators. Dexamethasone, cortisone, and DHEA are in the intermediate group with little response from either aldosterone or cortisol [Fig. 4B]. Consistent with the DNA-binding data, maximal activities are achieved on BDR-3, βDR-4, and βDR-5 elements [Fig. 4B].

The inducibility of SXR by PCN and other steroids led us to consider whether P-450s known to be inducible by these compounds could be SXR targets. The primary human steroid-inducible P-450 is the CYP3A4 gene [Beaune et al. 1986; Molowa et al. 1986]. Unlike the rat and mouse CYP3A genes, all of which contain a DR-3 element that SXR can activate [Fig. 4B], the human and rabbit promoters do not contain such an element. Steroid and xenobiotic inducibility of CYP3A4 has been localized to an 19-bp element that is functional in transient transfection assays [Barwick et al. 1996]. This element contains an IR-6 motif [TGAAC/ TcaagcAGGTCA] and similar elements are also present in the human CYP3A5, CYP3A7, and the rabbit CYP3A6 genes [Fig. 4C; Barwick et al. 1996]. We tested the ability of SXR to bind a series of inverted repeat elements with spacings from 0 to 6 nucleotides and found that only an IR-6 showed significant binding that, as with the direct repeats, was RXR dependent [Fig. 4D, data not shown]. Competition binding experiments demonstrated little difference in the apparent affinity of SXR:RXR heterodimers for the βDR-4 or CYP3A4 IR-6 response elements [Fig. 4E]. In accord with the known inducibility of the parent promoters, SXR could activate reporter constructs containing the CYP3A4, but not the CYP3A5 or CYP3A7 motifs [Fig. 4F].

We then asked whether compounds known to induce CYP3A4 could activate SXR, as would be predicted from our model. Compounds tested included drugs such as rifampicin and nifedipine, steroid antagonists such as tamoxifen, spironolactone, and PCN, natural and synthetic steroids such as dexamethasone [DEX], diethylstilbestrol [DES], estradiol, DHT, corticosterone, and cortisone, and phytoestrogens such as coumestrol, equol, and genistein. Of these compounds, rifampicin, nifedipine, corticosterone, estradiol, DES, and coumestrol were the most potent activators. We note that SXR response to PCN is variable between experiments, typically ranging from low to modest [cf. Figs. 4B and 5A]. The CYP3A4 promoter responds to PCN with similar variability in cultured hepatocytes [Barwick et al. 1996]. Remarkably, PXR responded poorly to these inducers, showing preferential activation by PCN, a weak activator of SXR [Fig. 5B]. Interestingly, although PXR is reported to prefer pregnes [C21 steroids such as DEX and pregnenolone; Kliewer et al. 1998] we find that it is similarly activated by C19 androstanes like testosterone, and C18 estrones like estradiol [Fig. 5B]. Similar results were obtained with other natural steroids, including progesterone, pregnenolone, and DHEA [data not shown]. To demonstrate that the activation of SXR and PXR by high steroid concentrations is not a general property of all steroid receptors, we tested the human estrogen receptor for its response to the same panel of compounds. Among steroids, only DHT and estradiol were activators of ER, along with the synthetic ER agonist, DES, and the phytoestrogens, including coumestrol [Fig. 5C].

To evaluate the efficacy of SXR activation by various compounds, we determined EC_{50} (50% effective concentration) values in dose-response experiments. The chemical structures of compounds are shown in Figure 5D and the dose responsiveness in Figure 5E. In contrast to the rank order of potency [coumestrol > rifampicin > corticosterone > nifedipine, estradiol, DES shown in Fig. 5A], the most efficacious activator of SXR was rifampicin [EC_{50} of 3 µM] and the order was rifampicin > corticosterone > estradiol > coumestrol.

Despite continued effort, we have been unable to demonstrate specific binding of any of these activators to baculovirus-expressed, full-length SXR:RXR heterodimers, using protease protection, corepressor dissociation, and coactivator association. Unfortunately, the most efficacious activator rifampicin is not available in radiolabeled form; we did test radiolabeled corticosterone without success. It is possible that all of our activators have too little affinity for SXR to demonstrate binding above background and this could be taken as evidence that a high-affinity, endogenous ligand remains to be identified as has been postulated for PXR [Kliewer et al. 1998]. However, we believe that the number of SXR activators that are also CYP3A4 inducers is too large to be coincidental and conclude it is more likely that SXR is acting as a broad specificity, low-affinity sensor that regulates catabolism through CYP3A4 and other steroid and xenobiotic inducible P-450 enzymes.

**Partially metabolized steroids activate SXR**

The localization of apparent SXR-responsive elements in genes encoding steroid hydroxylases led us to consider whether products of steroid catabolism, such as reduced or hydroxylated corticosterone derivatives, could also activate SXR. Figure 5F shows that both 5a- and 5β-reduced forms of corticosterone are effective SXR activators, whereas 5α is slightly active and 5β is completely inactive on GR. Although a few 5α-reduced steroids remain active [e.g., DHT], 5β-reduced steroids fail to activate classic steroid receptors [Russell and Wilson 1994].
Therefore, the activation of SXR by 5β-reduced steroids may reflect a previously undetected regulatory pathway for these compounds. Interestingly, 6β-hydroxy corticosterone is virtually inactive on SXR [Fig. 5F], suggesting

![Figure 4](image-url)
that CYP3A4 catalyzed hydroxylation is a potential definitive regulatory step in steroid metabolism.

**Discussion**

We have proposed a novel model, termed the steroid sensor hypothesis, in which the induction of some xenobiotic-metabolizing enzymes by pharmacological levels of steroids, drugs, and xenobiotic compounds is regulated by a broad specificity sensor, rather than numerous specific receptors. In support of our hypothesis we show SXR is a novel member of the nuclear receptor superfamily that is activated by a diverse group of steroids and their metabolites. These include molecules that have high-affinity receptors such as progesterone, testosterone, estrogen, and corticosterone as well as their reduced catabolites that are, for the most part, inactive on the high-affinity receptors. In addition to the natural steroids, SXR is activated by synthetic steroids including PCN and DEX as well as xenobiotic drugs and phytoestrogens. Direct regulation by a broad specificity sensor is biologically economical as much of the detoxification...
and catabolism of such compounds is mediated by cytochrome P-450 enzymes, particularly members of the CYP3A family, which both metabolize and are induced by a wide spectrum of diverse compounds, including steroids.

Our hypothesis leads to several predictions concerning the relationship among target genes, the sensor, and its activators. First, the sensor should be expressed in tissues that catabolize steroids and xenobiotics. SXR is highly expressed in liver, the major expression site of steroid and xenobiotic-metabolizing enzymes (Fig. 1C). Prominent expression of SXR mRNA is also found in the intestine [Fig. 1C]. Although less is known about the role of this tissue in steroid metabolism, the gut is known to play an important role in first-pass metabolism of dietary and orally administered compounds [Kolars et al. 1991; Holtbecker et al. 1996] and CYP3A4 is highly expressed in enterocytes [Kolars et al. 1992]. Thus, SXR is expressed at high levels in two key tissues for steroid and xenobiotic catabolism. Second, catabolic enzymes expressed in these tissues should be induced by the sensor. Putative SXR response elements are found in the well-characterized, CYP3A4 promoter as well as those of P-450 oxidoreductase CYP2A, CYP2C, CYP2E, and glucuronosyl transferase, all known to be involved in steroid and xenobiotic catabolism [Fig. 4A; Gonzalez 1992]. Third, compounds known to induce catabolic enzymes should activate the sensor. SXR is activated by a variety of xenobiotic compounds, including drugs such as rifampicin and nifedipine, steroid receptor agonists and antagonists such as estrogen and tamoxifen, and bioactive dietary compounds such as phytoestrogens [Figs. 4 and 5]. In particular, CYP3A4 has been shown to be inducible by virtually all known SXR activators [Figs. 4 and 5; Rendic and Di Carlo 1997]. Last, because some partially metabolized [reduced] steroids retain biological activity, it would be desirable that these continue to activate the sensor thereby ensuring their complete inactivation and elimination. As expected, products of earlier catabolic steps, such as reduced steroids, are activators of SXR but not classic steroid receptors [Fig. 5D; data not shown]. Taken together, these observations provide strong support for the sensor hypothesis.

The observation that SXR can be activated by drugs and xenobiotic compounds suggests the possibility that these compounds could affect endogenous steroid metabolism indirectly. However, because steroid levels are tightly regulated, increased catabolism will be compensated by the pituitary (in healthy individuals) leading to adrenocorticotropic (ACTH) release, increased biosynthesis, and maintenance of plasma steroid levels. The increased catabolism will, however, be reflected by elevated urinary levels of steroid metabolites. Indeed, treatment with rifampicin, a strong SXR activator and CYP3A4 inducer, increases urinary metabolites such as 6β-hydroxy cortisol [Ohnhaus et al. 1989; Watkins et al. 1989], and bile acid metabolites such as 6α-hydroxy cholic and 6α-hydroxycholic acids [Wietholz et al. 1996], whereas the plasma levels of many circulating steroids increase slightly as a result of increased synthesis [Edwards et al. 1974; Lonning et al. 1989; Bammel et al. 1992]. When synthetic steroids, such as prednisolone [McAllister et al. 1983; Lee et al. 1993] or 17α-ethynyl estradiol [Guengerich 1990] are administered together with rifampicin, plasma levels are rapidly decreased due to enhanced urinary clearance. In some patients undergoing rifampicin therapy for tuberculosis, the increase in urinary steroid levels has led to misdiagnosis of Cush- ing’s syndrome [Kyriazopoulou and Vagenakis 1992; Terzolo et al. 1995; Zawawi et al. 1996]. Steroid production and clearance normalized when rifampicin was withdrawn. In patients with Addison’s disease, who mostly lack the ability to synthesize adrenal steroids, rifampicin treatment leads to rapid depletion of endogenous and administered steroids, confirming that induction of CYP3A4 causes increased steroid catabolism as predicted by the model [Edwards et al. 1974; Kyriazopoulou et al. 1984].

The induction of CYP3A4 by SXR activators has implications for drug interactions. In principle, strong SXR activators should lead to higher levels of CYP3A4, which is involved in the clearance of 60% of clinically relevant drugs [Cholerton et al. 1992]. For example, rifampicin leads to increased clearance of calcium channel blockers such as nifedipine [Holtbecker et al. 1996; Ndanusa et al. 1997] and verapamil [Barbarash et al. 1988], anti-arrhythmics such as pimelom [Stringer et al. 1988], and β-block- ers such as propranolol [Herman et al. 1983], in addition to the steroid interactions mentioned above. It should be noted that, although most CYP3A4 inducers are SXR activators, a few such as cyclosporine A fail to activate SXR. This could be the result of the presence of additional pathways for CYP3A4 regulation. However, the ability of a particular compound to induce catabolic P-450s by activating SXR places it as a candidate for drug-drug interactions. Thus, screening against SXR provides a potential in vitro molecular test for such drug interactions.

Activation of SXR also provides a molecular explanation for the paradoxical induction of the CYP3A genes [a.k.a. P-450PCN] by both glucocorticoid receptor agonists and antagonists and the differential response of orthologous enzymes in different species. The inducible CYP3A genes harbor a SXR activatable response element in their promoters that has been shown to be responsible for PCN and glucocorticoid induction [see Fig. 4A,C] (Schuetz and Guzelian 1984; Gonzalez et al. 1986; Burger et al. 1992; Barwick et al. 1996; Kliewer et al. 1998). Despite their common role in steroid and xenobiotic catabolism, CYP3A genes from different species, and particularly the glucocorticoid-responsive promoter elements, show considerable differences in the pharmacology of their inducers [Barwick et al. 1996]. For example, PCN is a strong inducer of rat CYP3A2 and CYP3A23, but a weak inducer of human CYP3A4 and rabbit CYP3A6, whereas rifampicin is a strong inducer of the human and rabbit but not the rat genes [Barwick et al. 1996]. However, when these elements are tested by transient transfection into primary hepatocytes from rats or rabbits the responsiveness changes to that of the host...
Materials and methods

cDNA identification

SXR was identified from a human genomic library (Clontech) hybridized with a full-length cDNA encoding *Xenopus* BXR [Blumberg et al. 1998] under reduced stringency conditions by hybridization in 0.5 M NaPO₄ (pH 7.0), 7% SDS, 5% dextran sulfate at 65°C overnight, washing three times for 20 min in 2× SSC, 0.1% SDS at 37°C. Restriction mapping and Southern analysis showed that three exons were contained within the 9-kb EcoRI hybridizing fragment. This fragment was used to probe a human multiple tissue Northern blot (Clontech) at high stringency (hybridization as above, washing twice for 20 min in 0.1× SSC, 0.1% SDS at 50°C) and hybridization was detected in liver. A human liver cDNA library (Stratagene) was screened subsequently using the same conditions and four independent clones identified. Each of these was sequenced on both strands within the protein-coding region. DNA sequences were compiled and aligned using the programs of Staden (1986), University of Wisconsin Genetics Computer Group (Devereaux et al. 1984). Database searching was performed using the BLAST network server at the National Center for Biotechnology Information (Altschul et al. 1990). PXR was isolated from a mouse liver cDNA library (Stratagene) by screening with the SXR protein-coding region at reduced stringency (5× SSC, 43% formamide, 5× Denhardt’s, 0.1% SDS, 0.1 mg/ml denatured, sonicated salmon sperm DNA at 37°C). Three, 20-min washes were performed in 0.5× SSC, 0.1% SDS at 50°C.

DNA-binding analysis

Electrophoretic mobility shift assays were performed using in vitro transcribed, translated proteins (TNT, Promega). Proteins [1 µl each] were incubated for 20 min at room temperature with 100,000 cpm of Klenow-labeled probes in 10 mM Tris (pH 8), 100 mM KCl, 6% glycerol, 0.05% NP-40, 1 mM DTT, 100 ng/µl poly[dI-C] (Pharmacia) and then electrophoresed through a 5% polyacrylamide gel in 0.5× TBE (45 mM Tris-base, 45 mM boric acid, 1 mM EDTA) at room temperature. For competition binding, protein plus unlabeled oligonucleotides at 5 or 50-fold molar excess were preincubated for 10 min on ice, labeled probes added, and incubated for 20 min at room temperature. Electrophoresis was as above. βD-series oligonucleotides were described previously [Perlmann et al. 1993]. DR0-15 oligonucleotides had the following sequences [DR-0, catagtcAGGTCAAGGTCAgatcaac; DR-1, catagtcAGGTCAAggGTCAgatcaac; DR-2, catagtcAGGTCAaAGGTCAgatcaac; DR-3, catagtcAGGTCAatatAGGTCAgatcaac; DR-4, catagtcAGGTCAatatAGGTCAgatcaac; DR-5, catagtcAGGTCAatatAGGTCAgatcaac; DR-6, catagtcAGGTCAatatAGGTCAgatcaac; DR-7, catagtcAGGTCAatatAGGTCAgatcaac; DR-10, catagtcAGGTCAatatataAGGTCAgatcaac; DR-15, catagtcAGGTCAatatataAGGTCAgatcaac]. IR series oligonucleotides had the following sequences [IR-0, agcttATGTACATATAGGTCAgatcaac; IR-1, catagtcAGGTCAgatcaac; IR-2, catagtcAGGTCAgatcaac; IR-3, catagtcAGGTCAgatcaac; IR-4, catagtcAGGTCAgatcaac; IR-5, catagtcAGGTCAgatcaac; IR-6, catagtcAGGTCAgatcaac; IR-7, catagtcAGGTCAgatcaac; IR-8, catagtcAGGTCAgatcaac; IR-9, catagtcAGGTCAgatcaac; IR-10, catagtcAGGTCAgatcaac; IR-11, catagtcAGGTCAgatcaac; IR-12, catagtcAGGTCAgatcaac; IR-13, catagtcAGGTCAgatcaac; IR-14, catagtcAGGTCAgatcaac; IR-15, catagtcAGGTCAgatcaac]. CYPA oligonucleotides were the following (CYPA4, tagaataTGAACTcaaaagAGGTCAgatcaac; CYPA5, tagaataTGAACTcaaaagAGGTCAgatcaac; CYPA7, tagaataTGAACTcaaaagAGGTCAgatcaac).

Plasmid construction and transfection

The protein-coding region of SXR was PCR amplified and subcloned into NcoI and BamHI sites of the vector pCDG1 [Blumberg et al. 1998] using ExoII-mediated ligation independent cloning [Li and Evans 1997]. During this process the putative initiator Leu was converted to Met with a Kozak consensus sequence CCATGG. GAL4-SXR was constructed by subcloning acidic I07–343 into pCMX–GAL4 (Perlmann et al. 1993). Similarly, the PXR.1 protein-coding region was PCR amplified and subcloned into NcoI–BamHI cut pCDG1, whereas acidic I04–431 were subcloned into CMX–GAL4. Reporter plasmids were constructed by synthesizing three-copy response elements and subcloning into HindIII–BamHI cut pTk-luc [Hollenberg et al. 1987].
CV-1 cells were maintained in DMEM containing 10% resin charcoal stripped calf bovine serum. Liposome-mediated transient transfections were performed using DOTAP reagent (Boehringer Mannheim) at a concentration of 5 μg/ml in DMEM containing 10% resin charcoal stripped fetal bovine serum in 96-well format using a Beckman Biomax 1000 laboratory workstation as described (Blumberg et al. 1996). Ligands were added the next day in DMEM containing 10% delipidated FBS. After 18–24 hr incubation, the cells were lysed and luciferase reporter gene assays and β-galactosidase transfection control assays performed as described (Blumberg et al. 1996). Reporter gene expression was normalized to the β-galactosidase transfection control and expressed as relative light units per OD per minute of β-galactosidase activity or fold induction over solvent control. Each data point represents the average of triplicate experiments ± S.E. and was replicated in independent experiments.

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References


