LXR deficiency and cholesterol feeding affect the expression and phenobarbital-mediated induction of cytochromes P450 in mouse liver

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Abstract **Metabolic transformation by the superfamily of cytochromes P450 (CYPs) plays an important role in the detoxification of xenobiotics such as drugs, environmental pollutants, and food additives. Endogenous substrates of CYPs include fatty acids, sterols, steroids, and bile acids. Induction of CYPs via transcriptional activation by substrates and other xenobiotics is an important adaptive mechanism that increases the organism's defense capability against toxicity. Numerous in vivo and in vitro data have highlighted the concept that the molecular mechanism of hepatic drug induction is linked to endogenous regulatory pathways. In particular, in vitro data suggest that oxysterols via the liver X receptor (LXR) inhibit phenobarbital (PB)-mediated induction of CYPs. To study the link between LXR, cholesterol homeostasis, and drug induction in vivo, we designed experiments in wild-type, LXRα-, LXRβ-, and LXRα/β-deficient mice. Our data expose differential regulatory patterns for Cyp2b10 and Cyp3a11 dependent on the expression of LXR isoforms and on challenge of cholesterol homeostasis by excess dietary cholesterol. Our results suggest that, in the mouse, liver cholesterol status significantly alters the pattern of expression of Cyp3a11, whereas the absence of LXR leads to an increase in PB-mediated activation of Cyp2b10.**—Gnerre, C., G. U. Schuster, A. Roth, C. Handschin, L. Johansson, R. Looser, P. Parini, M. Podvinec, K. Robertsson, J-Å. Gustafsson, and U. A. Meyer. **LXR deficiency and cholesterol feeding affect the expression and phenobarbital-mediated induction of cytochromes P450 in mouse liver.** ***J. Lipid Res.*** **2005.** 46: **1633–1642.**

Supplementary key words liver X receptor • pregnane X receptor • constitutive androstane receptor • metabolism • cytochrome P450 3a11 • cytochrome P450 2b10

Manuscript received 15 November 2004 and in revised form 23 February 2005 and in re-revised form 5 May 2005.

Published, JLR Papers in Press, June 1, 2005.
DOI 10.1194/jlr.M400453-JLR200

The gene superfamily of cytochromes P450 (CYPs) encodes a large number of proteins that are typically involved in the metabolism of endogenous, lipid-soluble compounds. Among others, fatty acids, sterols, steroids, and bile acids have been identified as endogenous CYP substrates (1, 2). Furthermore, hepatic CYPs play a prominent role in the metabolic transformation of xenobiotics such as drugs, environmental pollutants, and food additives and thus in their detoxification and rapid elimination from the body (3).

Characteristically, xenobiotic-metabolizing members of the CYP superfamily are transcriptionally induced upon exposure to xenobiotics. This phenomenon increases the ability of the organism to adaptively defend itself against toxic foreign compounds. Since the discovery of CYP induction by the barbiturate phenobarbital (PB) >40 years ago (4), this molecule has served as a prototype for a large group of structurally and functionally diverse compounds that induce the expression of CYP2B genes (5), whereas the glucocorticoid dexamethasone and the antibiotic rifampicin represent drugs that increase CYP3A levels in humans (6). There is, however, considerable overlap between the different inducers and the patterns of genes that they induce. The induction of drug-metabolizing CYP has impor-

Abbreviations: CAR, constitutive androstane receptor; CYP, cytochrome P450; CYP7A1, cholesterol 7α-hydroxylase; DR4, direct repeat separated by four nucleotides; FXR, farnesoid X receptor; LXR, liver X receptor; MRP3, multidrug resistance-associated protein 3; PB, phenobarbital; PBRU, phenobarbital-responsive enhancer unit; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; SREBP-1c, sterol-regulatory element binding protein 1c; TG, triglyceride; XREM, xenobiotic-responsive enhancer module.

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tant clinical consequences: *1*) it affects the efficiency of drug treatment; *2*) it may cause drug-drug interactions; and *3*) it can influence endogenous regulatory pathways.

Numerous studies have established that members of the nuclear receptor superfamily of transcription factors are essential in mediating this drug response (reviewed in 7). Specifically, the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and the chicken xenobiotic receptor have been assigned key roles in the induction of CYP2B, CYP2C, CYP3A, and CYP2H1 genes in human, mouse, and chicken (8–13).

At the same time, CYP-catalyzed reactions are pivotal in the regulation of cholesterol balance and the synthesis and metabolism of steroids and vitamin D. Thus, although cholesterol homeostasis relies on multiple checkpoints, cholesterol 7α-hydroxylase (CYP7A1) is the first and rate-limiting enzyme in the conversion of cholesterol to bile acids (14, 15). Two nuclear receptors, the farnesoid X receptor (FXR) and the liver X receptor (LXR), are important regulators of CYP7A1. Thus, in the mouse, Cyp7a1 is induced by oxysterols via LXR (16, 17). The same gene can be repressed by bile acids via FXR. Here, FXR activates the small heterodimer partner, which in turn inhibits constitutive transcriptional activation by the liver receptor homolog-1 transcription factor on the CYP7A1 promoter (18, 19).

A large body of in vivo and in vitro data support the concept that the molecular mechanisms of hepatic drug induction are linked to endogenous regulatory pathways: observations made in epileptic patients treated with anticonvulsants and in rats treated with PB have revealed increased plasma cholesterol and lipoprotein levels (20–25). Moreover, a study of a 264 bp phenobarbital-responsive enhancer unit (PBRU) from the chicken CYP2H1 gene demonstrated that a DR4-type nuclear receptor response element (direct hexamer repeat separated by four nucleotides) contained within this PBRU was essential for conferring drug induction and that in the LMH chicken hepatoma cell line oxysterols can inhibit the PB induction mediated by this 264 bp PBRU through competitive binding of LXR to the DR4 element (26).

To study the link between LXR, cholesterol homeostasis, and drug induction in vivo, we designed experiments in mice lacking one or both LXR isoforms. Wild-type, LXRα-, LXRβ-, and LXRα/β-deficient mice were kept on standard laboratory chow or a 2% enriched cholesterol diet for 1 week and were given either vehicle (0.9% saline solution) or PB intraperitoneally. Subsequently, levels of Cyp7a1, Cyp2b10, and Cyp3a11, two murine drug-metabolizing enzymes, as well as cholesterol and bile acid transporters were quantified by real-time PCR. Cholesterol and triglyceride (TG) levels also were determined from the animals' livers.

In this report, we provide evidence for important cross-talk between xenobiotic- and oxysterol-sensing nuclear receptors in the regulation of enzymes involved in drug and cholesterol metabolism. Our data reveal differential regulatory patterns for Cyp2b10 and Cyp3a11 dependent on the expression of LXR isoforms and on challenge of cholesterol metabolism by excess dietary cholesterol. Our results suggest that in the mouse, liver cholesterol status significantly alters the pattern of expression of Cyp3a11, whereas the absence of LXR leads to an increase in PB-mediated activation of Cyp2b10. Finally, we demonstrate that under certain conditions, PB treatment not only affects drug-metabolizing enzymes but also influences hepatic cholesterol levels.

MATERIALS AND METHODS

Reagents

22(*R*)-Hydroxycholesterol, 22(*S*)-hydroxycholesterol, 25-hydroxycholesterol, and pregnenolone 16α-carbonitrile (PCN) were purchased from Sigma (distributed by Fluka AG). 24(*S*),25-Epoxycholesterol was a kind gift from Thomas A. Spencer (Department of Chemistry, Dartmouth College, Hanover, NH). 27-Hydroxycholesterol was obtained from Steraloids (Newport, RI).

Plasmids

The coding regions of mouse CAR and mouse LXRα were amplified from mouse cDNA, followed by subcloning into the pSG5 expression vector (Stratagene, Basel, Switzerland). The expression vector for mouse PXR, pSG5-mPXR, was kindly provided by Steven A. Kliewer (University of Texas Southwestern Medical Center, Dallas, TX).

The pcDNA3 expression vector was obtained from Invitrogen AG (Basel, Switzerland). The pcDNA3/VP16 construct was provided by Dieter Kressler (Biozentrum, University of Basel, Switzerland). Vp16/mPXR was generated by PCR amplification of the mouse PXR from pSG5-mPXR and by insertion into the *Xba*I-digested pcDNA3/VP16 vector; the primers harboring *Xba*I restriction sites were chosen as follows: forward primer, 5′-GCTCTAGAAGCGGTAGCGGTAGACCTGAGGAGAGCTGGAGCC-3′; reverse primer, 5′-GCTCTAGATCAGCCATCTGTGCTGCTAAATAAC-3′. Vp16/mLXRα was generated by amplification of the mouse LXR from pSG5-mLXRα and by insertion into the digested *Eco*RI pcDNA3/VP16 vector. The primers harboring *Eco*RI restriction sites were chosen as follows: forward primer, 5′-CTGAATTCAGTGGTAGCGGTTCCTTGTGGCTG-3′, reverse primer 5′-CGGAATTCTCACTCGTGGACATCCCAGATCTC-3′. The pSV-βGal expression vector for β-galactosidase used for the normalization of transfection efficiencies was obtained from Promega (Promega Corp., Catalys AG, Wallisellen, Switzerland).

The pGL3-basic plasmid containing 13 kb of human CYP3A4 5′ flanking region was a gift from Christopher Liddle (University of Sidney at Westmead Hospital, Westmead, Australia). The xenobiotic-responsive enhancer module (XREM; bases −7,836 to −7,206) fragments were generated by PCR using primers specific to the sequence but tagged with a restriction endonuclease site to permit cloning into the (−362/+53)pGL3-basic luciferase reporter gene vector. Upstream primer 5′-ATGGTACCTCTAGAGAGATGGTTCATTCCT-3′ was used in combination with 5′-CATAGATCTTGAAACATGTTTCTTTCCTTGT-3′ downstream primer to generate the XREM.

The luciferase reporter plasmid containing two repeats of the mouse Cyp7a1 LXR response element (27), $(DR4)_2$tkluc, was produced using the oligonucleotides 5′-CTCTGGTCACCCAAGTTCAAGTTTCTGGTCACCCAAGTTCAAGTTC-3′ and 5′-TCGAGAACTTGAACTTGGGTGACCAGAAACTTGAACTTGGGTGACCAGAGGTAC-3′ synthesized by Microsynth (Balgach, Switzerland). The double-stranded oligonucleotide was digested with *Kpn*I/*Xho*I and ligated into the pGL3tk luciferase. The pGL3tk

TABLE 1. Taqman primer and probe sequences

Target Gene	Primer/Probe Sequence	Concentration
		nM
Cyp2b10	Forward: 5′-CAATGTTTAGTGGAGGAACTGCG-3′	900
	Reverse: 5′-CACTGGAAGAGGAACGTGGG-3′	900
	Probe: 5′-FAM-CCCAGGGAGCCCCCCTGGA-TAMRA-3′	300
Cyp3a11	Forward: 5′-AGAACTTCTCCTTCCAGCCTTGTA-3′	900
	Reverse: 5′-GAGGGAGACTCATGCTCCAGTTA-3′	900
	Probe: 5′-FAM-CTAAAGGTTGTGCCACGGGATGCAGT-TAMRA-3′	300
Cyp7a1	Forward: 5′-ACACCAAGTGTCCCCCTCTAGA-3′	900
	Reverse: 5′-CTCAATATCATTTAGTGGTGGCAAA-3′	900
	Probe: 5′-FAM-CAGTCCCGGGCAGGCTTGGG-TAMRA-3′	300
ABCA1	Forward: 5′-GGACATGCACAAGGTCCTGA-3′	900
	Reverse: 5′-CAGAAAATCCTGGAGCTTCAAA-3′	900
	Probe: 5′-FAM-AATGTTACGGCAGATCAAGCATCCCAAC-TAMRA-3′	300
ABCG5	Forward: 5′-TCAGGACCCCAAGGTTCATGAT-3′	900
	Reverse: 5′-AGGCTGGTGGATGGTGACAAT-3′	300
	Probe: 5′-FAM-CCACAGGACTGGACTGCATGACTGCA-TAMRA-3′	50
ABCG8	Forward: 5′-GACAGCTTCACAGCCCACAA-3′	900
	Reverse: 5′-GCCTGAAGATGTCAGAGCGA-3′	300
	Probe: 5′-FAM-CTGGTGCTCATCTCCCTCCACCAG-TAMRA-3′	50
GAPDH	Forward: 5′-CCAGAACATCATCCCTGCATC-3′	900
	Reverse: 5′-GGTCCTCAGTGTAGCCCAAGAT-3′	900
	Probe: 5′-FAM-CCGCCTGGAGAAACCTGCCAAGTATG-TAMRA-3′	300

FAM, 6-carboxyfluorescein; TAMRA, 6-carboxy-tetramethyl-rhodamine.

luciferase reporter gene vector was constructed by excising the thymidine kinase promoter from the pBLCAT5 reporter vector (28) with *Bam*HI and *Bgl*II and inserting it into the *Bgl*II site of the pGL3-basic promoterless reporter vector (Promega Corp., Catalys AG). All PCR products were verified by sequencing.

Animals

LXRα-, LXRβ-, and LXRα/β-deficient mice were generated as described previously (29, 30). All LXR knockout mice used in our study were between 12 and 16 weeks old and had a similar mixed genetic background based on 129/Sv and C57BL/6 strains. Mice with pure 129/Sv background were backcrossed into C57BL/6 mice for seven generations (29, 30). LXR-deficient mice and C57BL/6 wild-type control mice were bred by Taconic M&B. After delivery, all mice were allowed to adjust for 2 weeks and were housed with a regular 12 h light/12 h dark cycle. Mice were fed ad libitum either a low-fat standard rodent chow diet (R36; Lactamin AB, Vadstena, Sweden) or diet supplemented with 2% (w/w) cholesterol for 1 week. Female mice (n = 5–8) were kept on standard or cholesterol-supplemented chow and were injected one time intraperitoneally with vehicle alone (saline) or PB (100 mg/kg). After 16 h, animals were killed and livers excised and maintained in RNAlater (Ambion, Inc., Austin, TX) according to the manufacturer's protocol. Liver tissue samples were solubilized in 1 ml of TRIzol™ reagent (Invitrogen AG, Basel, Switzerland) and homogenized for 5 s in FastRNA tubes using a FastPrep FP120 homogenizer from Qbiogene (Carlsbad, CA), and total RNA was extracted according to the TRIzol protocol. Experiments were approved by the local ethics committee for animal experiments.

Chemical analysis of serum and tissue

Blood was drawn by cardiac puncture under light methoxyfluorane anesthesia before tissues were collected for further analyses. Total cholesterol and TGs were determined with a Monarch automated analyzer (ILS Laboratories Scandinavia AB, Sollentuna, Sweden). Lipids were extracted according to Folch, Lees, and Sloane Stanley (31). Total cholesterol and TG were analyzed using commercially available kits (Roche Molecular Biochemicals, Indianapolis, IN).

Taqman analysis

One microgram of mouse liver RNA was reverse-transcribed with the Moloney murine leukemia virus reverse transcriptase (Promega Corp., Catalys AG) using oligo-(dT)15 N primers. PCR was performed with qPCR™ Mastermix Plus (Eurogentec GmbH, Köln, Germany). Transcript levels were quantified with an ABI Prism 7700 sequence detection system (PE Applied Biosystems) according to the manufacturer's protocol. Briefly, relative transcripts levels in induced livers and untreated controls were determined using the comparative Ct (cycle threshold) method. Multidrug resistance-associated protein 3 (MRP3) amplification products were detected using the Master Mix SYBR® Green 1 assay (Eurogentec GmbH), and the primers were optimized as follows: forward primer, 5′-GGCCTTTCTGTGTCCTATGCCTTA-3′ (500 nM); reverse primer, 5′-CCTTGACTCTCTCCACAGCTATGA-3′ (500 nM). Levels of GAPDH were used for normalization. All other primers and probes were chosen as indicated in **Table 1**.

Transcriptional activation assays

Transactivation assays were carried out in CV-1 green monkey kidney cells as described previously (32). Briefly, cells were expanded for 3 days in DMEM-F12 without phenol red supplemented with 10% charcoal-treated FBS. Subsequently, cells were plated onto 96-well dishes at a density of 25,000 cells/well and grown overnight. For transfection, cells were maintained in Opti-MemI (Invitrogen). Transfection mixes contained 20 ng of receptor expression vector, 8 ng of reporter vector, 60 ng of pSV-βGal, and carrier plasmid to a total of 100 ng of DNA per well. Cells were transiently transfected using the LipofectAMINE reagent (Invitrogen) according to the manufacturer's protocol. After 24 h of incubation, cells were exposed to drugs or vehicle for an additional 24 h. Transfections with vectors expressing constitutively active nuclear receptors were carried out for 48 h, and then cells were lysed. Cell extracts were prepared using 100 μl of passive lysis buffer (Promega Corp., Catalys AG). The supernatants were assayed for luciferase activities using the Luciferase Assay kit (Promega Corp., Catalys AG) and a Wallac 1420 Multilabel Counter. β-Galactosidase activities were measured as described previously (13). Luciferase levels were then normalized against

β-galactosidase values to compensate for the variation in transfection efficiencies and against untreated control cells to obtain relative luciferase activities.

RESULTS

LXR deficiency and its effect on the basal expression of drug-metabolizing enzymes

To investigate the effects of LXRα, LXRβ, or LXRα/β deficiency on the expression of Cyp2b10 and Cyp3a11, we measured the mRNA levels of these genes in the livers of mice receiving normal diet and treated with vehicle. As illustrated in **Fig. 1A**, Cyp2b10 and Cyp3a11 show different regulation patterns. Although the absence of one or both LXR isoforms did not significantly affect levels of Cyp2b10 transcript, the basal level of Cyp3a11 mRNA was significantly increased in LXRα/β-deficient mice. Hepatic cholesterol levels were measured in mice on normal diet (Fig. 1B), and in accordance with previously published data (30), total liver cholesterol was found to be increased significantly in LXRα/β-deficient mice.

Effect of dietary cholesterol on the expression of Cyp3a11 and Cyp2b10

Mice with different LXR genotypes were fed with a 2% cholesterol diet for 1 week, after which the expression of Cyp2b10 and Cyp3a11 (**Fig. 2**) was evaluated. The efficiency of the diet in activating LXR was tested by measuring Cyp7a1 mRNA levels under both normal and high-cholesterol dietary conditions (Fig. 2C). We observed a significant increase in Cyp7a1 expression in wild-type animals when challenged with the 2% cholesterol diet, whereas no significant effect was observed among the other genotypes. Interestingly, 2% cholesterol feeding affected Cyp2b10 and Cyp3a11 expression in a genotype-dependent manner. Expression of Cyp3a11 and Cyp2b10 mRNA is shown in Fig. 2A, B, respectively. In wild-type animals and LXRβ-deficient mice, dietary cholesterol did not significantly alter the expression of either gene. In LXRα- and LXRα/β-deficient mice, however, mRNA levels of Cyp3a11 were increased significantly in response to cholesterol; Cyp2b10 expression levels followed the same profile, but the changes were not statistically significant. This pattern of induction correlates with the liver cholesterol content profiles (Fig. 2D), in which cholesterol is strongly increased in LXRα- and LXRα/β-deficient mice. These results suggest that the influence of cholesterol feeding on Cyp2b10 and Cyp3a11 expression occurs when LXRα is absent and liver cholesterol content is high, partially as a result of the lack of Cyp7a1 induction by LXRα.

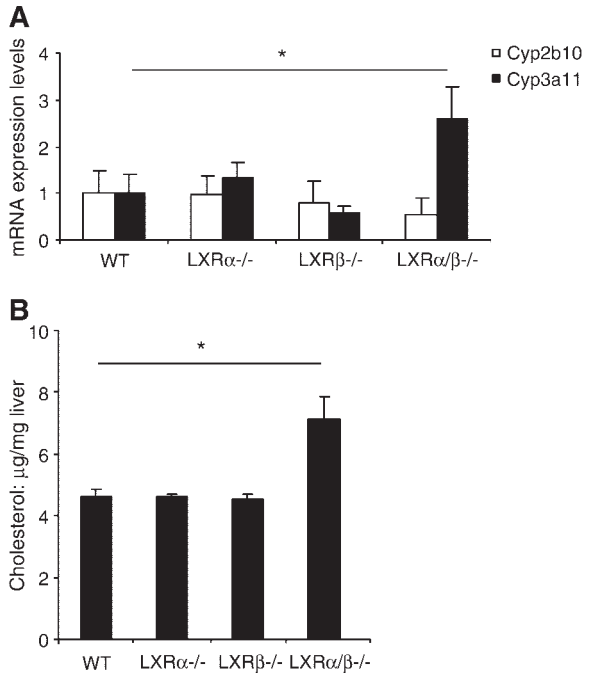

Fig. 1. Regulation of cytochrome P450 3a11 (Cyp3a11) and Cyp2b10 expression in liver X receptor (LXR)-deficient (−/−) mice. A: Wild-type (WT), LXRα-, LXRβ-, and LXRα/β-deficient mice (n = 5–8) were kept on standard laboratory chow and given vehicle intraperitoneally (saline) for 16 h. Hepatic Cyp2b10 and Cyp3a11 levels were determined by real-time PCR. Gene levels are expressed relative to the wild-type control. Error bars represent SD. B: Total cholesterol levels were determined in the livers of wild-type, LXRα-, LXRβ-, and LXRα/β-deficient mice. Error bars represent SEM. * $P < 0.05$ determined by Student's *t*-test.

Cholesterol feeding reduces PB-mediated increase of Cyp2b10 independently of LXR genotype

As shown previously in vivo (33), PB massively induces Cyp2b10 (140-fold) in wild-type mice under standard dietary conditions (**Fig. 3A**). In mice lacking LXRβ or both LXR isoforms, this strong PB-mediated induction was increased further (Fig. 3A), suggesting that the absence of LXR positively influences the capacity of Cyp2b10 to be induced by PB. When wild-type or LXR-deficient mice are treated with PB while fed a 2% cholesterol diet, decreased inducibility by PB was observed, irrespective of LXR genotype (Fig. 3A). This repression of PB induction by dietary cholesterol thus seems to be LXR-independent, which implies that the hepatic cholesterol status affects the regulation of the drug-metabolizing enzyme Cyp2b10.

Effect of cholesterol feeding on PB-mediated induction of Cyp3a11

To study the influence of dietary cholesterol on the PB-mediated increase of Cyp3a11, mRNA levels were determined in mice fed standard chow or a 2% cholesterol diet and treated with either vehicle or PB (Fig. 3B). In mice of all LXR genotypes, PB induced Cyp3a11 expression. However, this PB-mediated increase was smaller in LXRα/β-deficient mice, whereas the basal level of Cyp3a11 expression was higher than in the wild type (Fig. 1A). In contrast to Cyp2b10, cholesterol feeding did not significantly reduce PB-mediated induction of Cyp3a11 mRNA in any of the mouse genotypes. PB induction of Cyp3a11 is thus apparently independent of dietary cholesterol content.

PB treatment affects hepatic TG and cholesterol levels

To evaluate the effect of PB on cholesterol homeostasis and lipogenesis, hepatic cholesterol and TG levels were

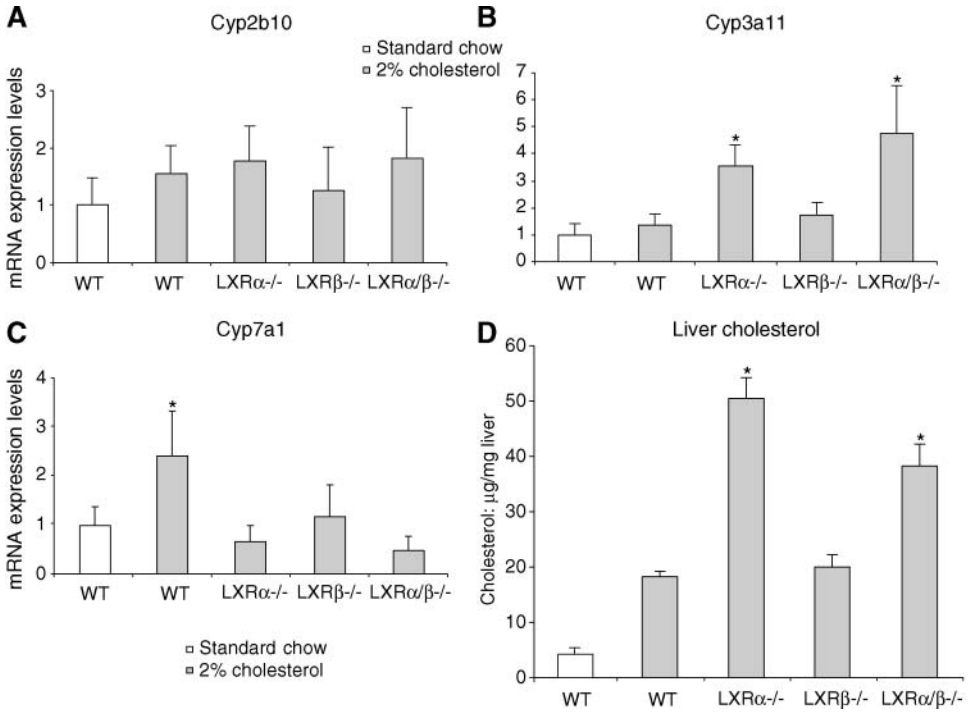

Fig. 2. Effect of 1 week of 2% cholesterol feeding on cholesterol 7α-hydroxylase (Cyp7a1), Cyp2b10, and Cyp3a11 expression in LXR-deficient (−/−) mice. Wild-type (WT), LXRα-, LXRβ-, and LXRα/β-deficient mice (n = 5–8) were fed standard laboratory chow or a diet supplemented with 2% cholesterol for 1 week. Real-time PCR analysis was performed with probes specific for Cyp2b10 (A), Cyp3a11 (B), or Cyp7a1 (C). Hepatic gene levels are expressed relative to those in wild-type vehicle-treated control animals on a standard diet. Error bars represent SD. D: Total cholesterol levels were determined in the livers of wild-type, LXRα-, LXRβ-, and LXRα/β-deficient mice. Error bars represent SEM. * $P < 0.001$ determined by Student's *t*-test.

measured in mice fed standard laboratory chow or the 2% cholesterol diet (**Fig. 4**). Compared with wild-type animals, LXRα/β-deficient mice displayed significantly decreased levels of hepatic TG (Fig. 4A) in the absence of drug treatment, consistent with previous observations (30). When mice were fed standard chow, PB treatment had no significant effect on hepatic TG. In contrast, PB significantly decreased hepatic TG levels in wild-type, LXRα-deficient, and LXRβ-deficient animals but not in LXRα/β-deficient mice (Fig. 4B) on the 2% cholesterol diet. Furthermore, hepatic cholesterol levels of LXRα/β-deficient mice were increased significantly compared with the other genotypes when mice were fed standard chow (Fig. 4C), in agreement with previously published data (30). Most interestingly, PB treatment was found to decrease hepatic cholesterol levels in mice on the high-cholesterol diet, and this

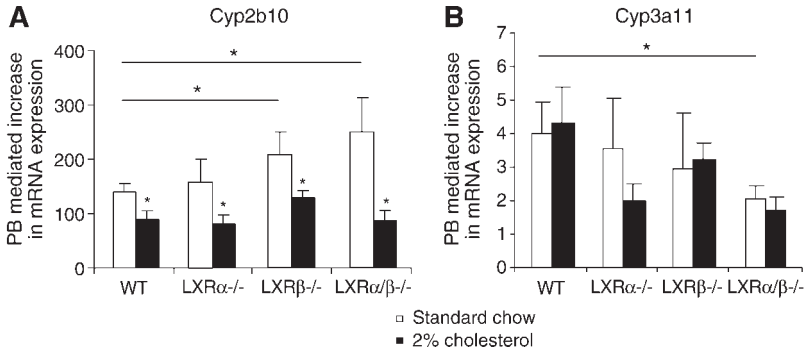

Fig. 3. Effect of cholesterol feeding on phenobarbital (PB)-mediated increase of Cyp2b10 and Cyp3a11. Wild-type (WT), LXRα-, LXRβ-, and LXRα/β-deficient (−/−) mice (n = 5–8), fed standard laboratory chow or a 2% cholesterol-supplemented diet for 1 week, were administered either vehicle (saline) or PB (100 mg/kg) for 16 h. Cyp2b10 (A) and Cyp3a11 (B) levels were quantified by real-time PCR. The PB-mediated increase in gene expression is calculated for each genotype and feeding condition and expressed as fold increase relative to that of the corresponding vehicle-treated control. Error bars represent SD. * $P < 0.02$ determined by Student's *t*-test.

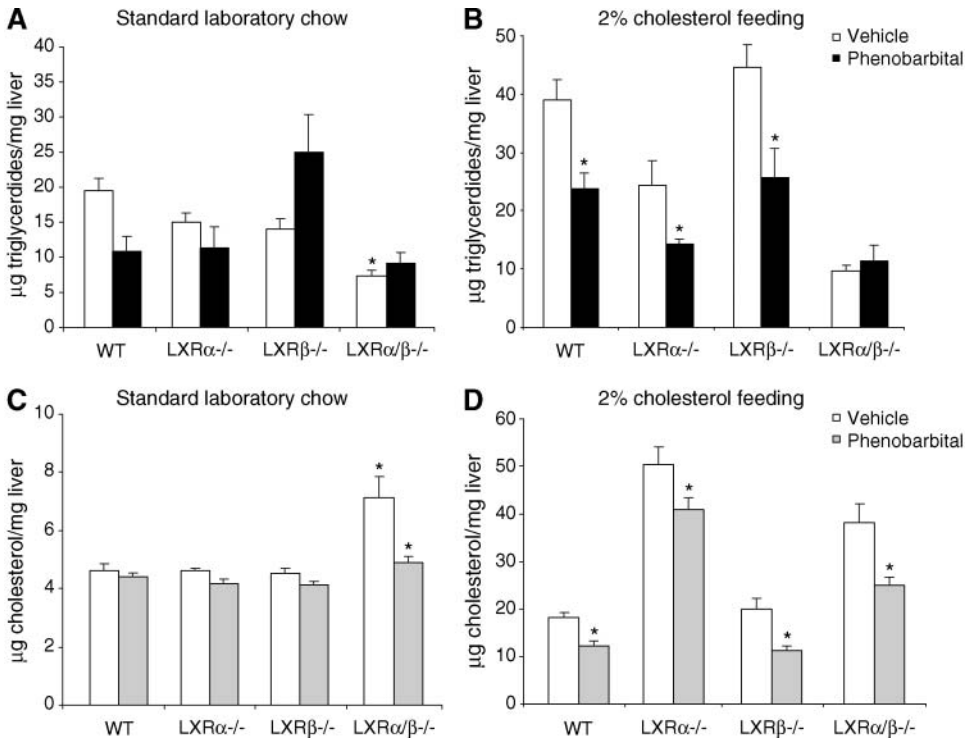

Fig. 4. PB treatment affects triglyceride (TG) and cholesterol levels. Wild-type (WT), LXRα-, LXRβ-, and LXRα/β-deficient (−/−) mice (n = 5–8), fed standard laboratory chow or a 2% cholesterol-supplemented diet for 1 week, were administered either vehicle (saline) or PB (100 mg/kg) for 16 h. TG contents in livers from animals fed standard chow (A) or 2% cholesterol (B) as well as total cholesterol contents in animals fed standard chow (C) or 2% cholesterol (D) and treated with vehicle or PB were determined. Error bars represent SEM. * $P < 0.05$ determined by Student's *t*-test.

effect was independent of their LXR genotype (Fig. 4D). In mice kept on the normal diet, PB did not further decrease hepatic cholesterol levels, with the exception of LXRα/β-deficient mice (Fig. 4D), which exhibited increased cholesterol levels already under normal dietary conditions (Fig. 4C).

The occurrence of this effect in LXRα/β-deficient mice suggests that the capacity of PB to decrease hepatic cholesterol levels is independent of LXR. Interestingly, the effect of PB occurs only when hepatic cholesterol and TG levels are increased.

PB affects hepatic cholesterol levels by acting on Cyp7a1 and MRP3 expression

To gain insight into the pathways by which PB modifies hepatic cholesterol levels, Cyp7a1 mRNA levels were quantified by Taqman analysis (**Fig. 5A**). In addition to the increase observed in wild-type vehicle-treated mice upon cholesterol feeding, a PB-mediated increase in Cyp7a1 expression was measured in LXRα/β-deficient mice under both feeding conditions. PB treatment did not lead to significant changes in Cyp7a1 expression in the other genotypes (data not shown), suggesting that this effect occurs predominantly in the total absence of LXR.

To examine the molecular details underlying the activation of Cyp7a1, we cloned a tandem repeat of a previously identified LXR response element from the Cyp7a1 gene (27) into a luciferase reporter vector under the control of the thymidine kinase promoter. This reporter construct, $(DR4)_2$tkluc, was then used in transcriptional activation assays in CV-1 cells (Fig. 5B, C). As expected, reporter activity was increased when cells were cotransfected with a VP16mLXRα construct expressing a constitutively active derivative of the mouse LXRα isoform (Fig. 5B). It is well established that PB mediates the activation of drug-metabolizing CYP3A and CYP2B enzymes via PXR and CAR (5). For this reason, we tested whether these two nuclear receptors interact with the known regulatory sequence in the mouse Cyp7a1 gene in vitro. Interestingly, $(DR4)_2$tkluc reporter activity was strongly increased when cells were cotransfected with a plasmid expressing a constitutively active mouse PXR-VP16 fusion protein (Fig. 5B).

When cells were cotransfected with an expression vector for mouse CAR (Fig. 5C), a 3-fold increase of basal activation was observed. The activity of mouse CAR can be modulated by androstanol, an inverse agonist, and by the inducer compound TCPOBOP [1,4-bis(3,5-dichloropyridyloxy) benzene], as has been reported (34). Using these compounds, we could modulate the expression of the $(DR4)_2$tkluc reporter (Fig. 5C) to a comparable extent as reported for bona fide CAR target genes. These data implicate PXR and CAR in the regulation of mouse Cyp7a1, particularly in the absence of LXRs, and suggest that the cholesterol-lowering effect observed in the pres-

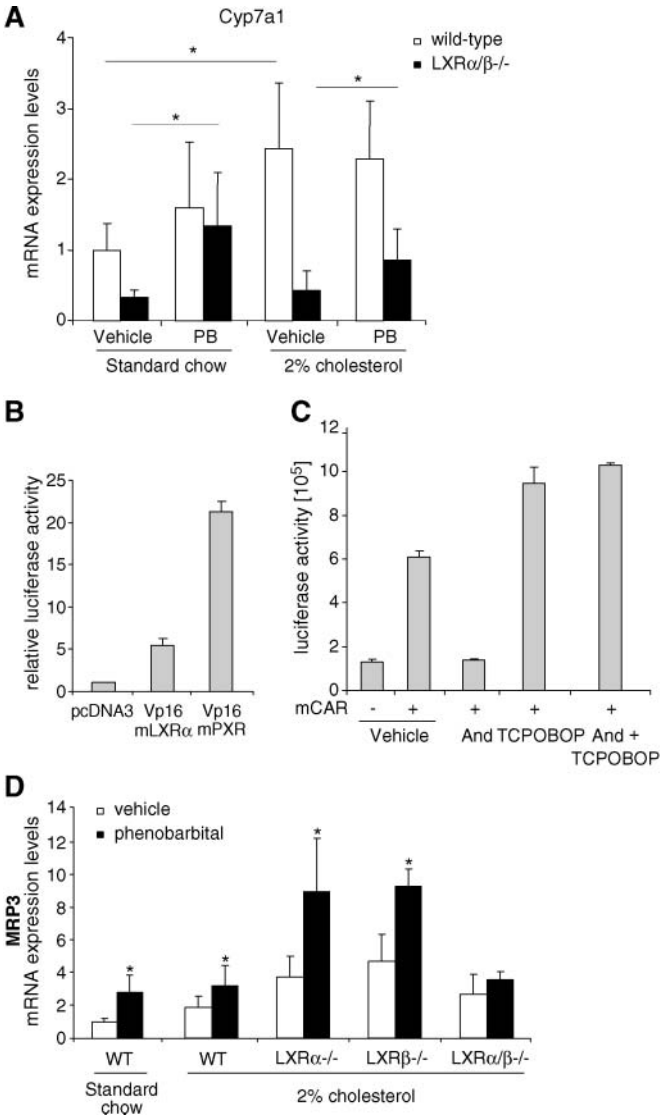

Fig. 5. PB treatment affects the hepatic expression of Cyp7a1 and multidrug resistance-associated protein 3 (MRP3). A: Wild-type and LXRα/β-deficient mice (n = 5–8), fed standard laboratory chow or a 2% cholesterol-supplemented diet for 1 week, were administered either vehicle (saline) or PB (100 mg/kg) for 16 h. Hepatic Cyp7a1 expression was determined by real-time PCR. Error bars represent SD. * $P < 0.05$ determined by Student's *t*-test. B: A luciferase reporter vector containing two copies of the mouse Cyp7a1 LXR response element, a direct-repeat separated by four nucleotides, $(DR4)_2$tkluc, was cotransfected in CV-1 cells together with expression vectors for VP16mLXRα, VP16mPXR, or a control vector. Cell extracts were analyzed for luciferase activities, which then were normalized against β-galactosidase activities. Luciferase activities relative to cells cotransfected with empty vector are shown. Values represent means of at least three independent experiments + SD. C: $(DR4)_2$tkluc was transfected in CV-1 cells with or without an expression vector for mouse constitutive androstane receptor (mCAR). Cells were treated for 24 h with vehicle (0.1% DMSO), androstanol (And; 5 μM), and/or TCPOBOP [1,4-bis(3,5-dichloropyridyloxy)benzene] (10 μM). Cell extracts were analyzed as described above. Absolute luciferase activities are shown. Values represent means of at least three independent experiments + SD. D: MRP3 levels were determined by real-time PCR in the indicated mouse livers. Gene levels are expressed relative to the wild-type (WT) vehicle-treated control. Error bars represent SD. * $P < 0.05$ determined by Student's *t*-test.

ence of PB (Fig. 4C, D) is, at least in part, mediated by PXR or CAR and may be the result of increased cholesterol catabolism. Because PB did not lead to a significant upregulation of Cyp7a1 when wild-type mice received a standard diet (Fig. 5A) and when LXRα- or LXRβ-deficient mice were fed cholesterol (data not shown), PB may, in these cases, decrease hepatic cholesterol levels by increasing the export of cholesterol and/or bile acids. Therefore, we quantified the expression levels of several genes implicated in cholesterol and bile acid transport in mouse liver. None of the cholesterol transporters ABCA1 (35), ABCG1 (36), or ABCG5/ABCG8 (37) was induced by PB, either in LXRα/β-deficient mice under standard dietary conditions or in any of the four mouse genotypes when challenged with cholesterol feeding; therefore, the regulation pattern of these transporters cannot explain the effect of PB observed on liver cholesterol (data not shown). Interestingly, however, MRP3 expression was increased in all genotypes by PB when mice were fed cholesterol (Fig. 5D), suggesting that bile salt export from the liver is increased in those animals in which liver cholesterol was increased. PB treatment also increased MRP3 expression in wild-type mouse liver, confirming previous observations (38).

Cholesterol derivatives are activators of mouse PXR

To assess the potential of cholesterol and several of its derivatives to activate mouse PXR, transcriptional activation assays were performed in CV-1 cells (**Fig. 6**). PCN, a known potent activator of murine PXR used as a positive control, led to 18-fold activation of the reporter gene activity over vehicle-treated cells. In addition, hydroxylated derivatives of cholesterol, like the potent LXR agonist 24(*S*),25-epoxycholesterol, also could activate PXR, confirming previous observations in mouse hepatocytes (39). 22(*R*)-Hydroxycholesterol displayed only weak activation of PXR, whereas 22(*S*)-hydroxycholesterol was inactive. Interestingly, the 25- and 27-hydroxylated derivatives of cholesterol proved to be good mouse PXR activators in vitro, suggesting that fluctuation in the hepatic concentration of these substances could affect PXR target gene regulation.

DISCUSSION

In this report, we describe the influence of LXRα and/or LXRβ deficiency and the effect of cholesterol feeding on the expression and PB-mediated induction of two murine drug-metabolizing enzymes, Cyp2b10 and Cyp3a11. In addition, we describe the consequences of a short-term PB treatment on hepatic cholesterol and TG content in mouse livers.

Our data clearly show that LXR deficiency differentially affects not only the basal expression levels of Cyp2b10 and Cyp3a11 but also the capacity of these genes to be induced by PB. Upon exposure to dietary cholesterol, Cyp2b10 and Cyp3a11 exhibited a gene-specific response. Although no significant effect of LXR deficiency was observed on the

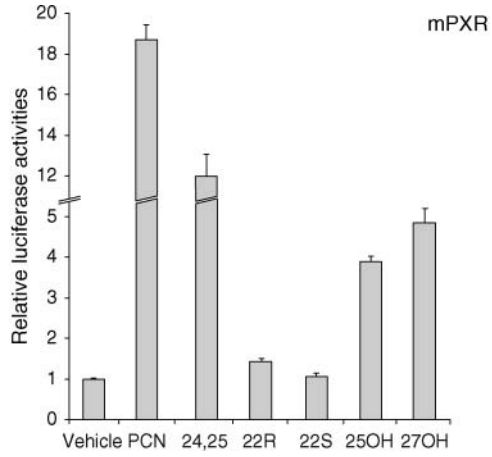

Fig. 6. 24(*S*),25-Epoxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol activate mouse pregnane X receptor (mPXR) in vitro in cell culture. A luciferase reporter construct containing the human CYP3A xenobiotic-responsive enhancer module was cotransfected in CV-1 cells with an expression vector for mouse PXR. Cells were treated for 24 h as indicated with vehicle (0.1% DMSO), pregnenolone 16α-carbonitrile (PCN; 10 μM), 24(*S*),25-epoxycholesterol (24,25; 10 μM), 22(*R*)-hydroxycholesterol (22R; 10 μM), 22(*S*)-hydroxycholesterol (22S; 10 μM), 25-hydroxycholesterol (25OH; 10 μM), or 27-hydroxycholesterol (27OH; 10 μM). Cell extracts were analyzed as described above. Relative luciferase activities were calculated over vehicle-treated cells. Values represent means of at least three independent experiments + SD.

expression of Cyp2b10, Cyp3a11 was increased significantly in mice lacking both LXR isoforms. This increase may be either a direct consequence of the lack of LXRα and LXRβ (derepression of gene activation) or a response to secondary events occurring in mouse liver linked to LXR deficiency. Therefore, we determined hepatic cholesterol levels and found them to be significantly higher in LXRα/β-deficient mice compared with wild-type animals. Cyp3a11 expression was increased in mice fed cholesterol and displaying higher hepatic cholesterol content, whereas no increase was seen in Cyp2b10 expression. Studies in vitro have shown that CYP3A4 can metabolize cholesterol to 4β-hydroxycholesterol and that this metabolite is present at relatively high concentrations in human plasma (40). This metabolic conversion was shown to occur only for CYP3A4 and not for CYP1A1, CYP2C9, or CYP2B6. Although it is currently unknown whether the mouse homolog Cyp3a11 metabolizes cholesterol like CYP3A4, a possible interpretation of our findings is that cholesterol could act as a likely substrate for Cyp3a11 and may increase its own metabolism when present in the liver in large amounts. Indeed, in wild-type mice, cholesterol feeding leads to an activation of LXR and its target gene Cyp7a1, which leads to an increase in cholesterol metabolism, decreases cholesterol levels, and presumably does not increase Cyp3a11. By contrast, in LXR-deficient mice, cholesterol levels are not controlled by LXR and Cyp7a1; therefore, they are excessive and may induce Cyp3a11.

As murine PXR is well known as a key regulator of Cyp3a11 expression both in vitro and in vivo (41), we suspect that the induction observed can be at least partially attributable to the activation of this nuclear receptor. In addition to cholesterol, the levels of several of its hydroxylated derivatives are increased in mice when one or both LXRs are ablated or when mice are fed cholesterol (42). Thus, it is known that 24(*S*),25-epoxycholesterol accumulates in the liver after cholesterol feeding (43). Moreover, wild-type mice and mice lacking LXRα have been described to have increased circulating levels of 27-hydroxycholesterol when fed 2% cholesterol (29). Rats, when kept on an atherogenic diet, show a considerable increase in 25-hydroxycholesterol in liver homogenates, whereas 27-hydroxycholesterol levels remain unaffected (44). Based on these observations, and considering the remarkable correlation between hepatic cholesterol levels and Cyp3a11 expression, we evaluated the potential of several hydroxylated derivatives of cholesterol to activate mouse PXR in cell culture. 24(*S*),25-Epoxycholesterol was a good activator of mouse PXR in our system, in accordance with previous observations in mouse hepatocytes (39). Interestingly, 25- and 27-hydroxycholesterol efficiently activated murine PXR as well. These data suggest that in mice, not only bile acid precursors (45) and bile acids (41) but also other endogenous cholesterol derivatives can regulate Cyp3a11 expression via PXR.

Induction of Cyp2b10 and Cyp3a11 by PB was studied in LXR-deficient and cholesterol-fed mice. Compared with wild-type animals, LXRβ- or LXRα/β-deficient mice showed significantly higher induction of Cyp2b10 mRNA levels, suggesting that LXRs can repress PB-mediated activation of Cyp2b10 in vivo in mice. Moreover, when mice were fed cholesterol, the PB-mediated increase in expression was reduced independently of the genotype, suggesting that this dietary effect on induction is not LXR-dependent. For Cyp3a11, the situation is probably different, because whereas dietary cholesterol had no effect on PB induction in wild-type and single-LXR-deficient mice, a significant decrease of induction occurred in LXRα/β-deficient mice. However, the reduced capacity of Cyp3a11 to respond to PB may be a consequence of the high basal expression of the gene in the absence of LXRs, preventing further response. Moreover, when mice were fed cholesterol, no significant difference was observed in the PB-mediated response of Cyp3a11 compared with standard chow feeding, in contrast to our observations with Cyp2b10.

In addition to the effect of PB on murine drug-metabolizing enzymes, we were also interested in studying the effect of short-term PB treatment on hepatic TG and cholesterol levels, especially given that cholesterol and several of its derivatives can act as important signaling molecules. Hepatic TG production is determined mainly by the fatty acid synthesis rate, which is controlled to a large extent at the transcriptional level by peroxisome proliferator-activated receptor α, which stimulates fatty acid β-oxidation (46), and by sterol-regulatory element binding protein 1c (SREBP-1c) (47), which controls fatty acid synthesis. In addition to cholesterol transport genes, LXR has been identified as an important regulator of SREBP-1c and de novo lipogenesis (48). The TG-lowering effect of PB occurred

only when wild-type, LXRα-deficient, or LXRβ-deficient mice were fed cholesterol and had increased TG before PB treatment. Our data show that PB treatment significantly decreases hepatic cholesterol levels, but only when they are high. The main pathway for the elimination of cholesterol is its conversion to bile acids, which is dependent on Cyp7a1. In mice, positive regulation of Cyp7a1 is mediated by LXRα (17), whereas negative feedback is exerted by bile acids via FXR and the small heterodimer partner (19). Moreover, it has been shown that PCN treatment represses Cyp7a1 expression in vivo in mice and that this effect is PXR-dependent (49). Interestingly, we observed that, in LXRα/β-deficient mice, PB acts as a positive regulator of Cyp7a1 under both dietary conditions, thus potentially explaining the observed decrease in cholesterol levels under these circumstances. A similar observation was made in earlier studies in rats, in which PB treatment led to a decreased content of cholesterol in liver microsomes in a strain that responded with increased Cyp7a1 activity (50). In addition, we showed in vitro that not only PXR but also CAR can interact with the DR4 element present in the 5′ flanking region of the murine Cyp7a1, providing a mechanism for this particular effect of PB. An additional mechanism to reduce cholesterol levels by PB could be the activation of MRP3 and the consequent increased excretion of bile salts.

In conclusion, our data demonstrate that basal and PB-induced expression of Cyp2b10 and Cyp3a11 is differentially and specifically influenced by the absence of LXR as well as the status of cholesterol in the liver. These observations suggest that cholesterol and its hydroxylated derivatives are endogenous signaling molecules, normally serving as ligands to LXR, but under extraordinary conditions, such as stringent diet or the absence of functional LXR, they may also serve as ligands for other nuclear receptors such as PXR or CAR and therefore affect the expression of drug-metabolizing enzymes. Indeed, recent gene expression data in cholesterol-fed mice support the concept that cholesterol or its products increase a number of PXR/CAR target genes (A. Roth, R. Looser, and U. A. Meyer, unpublished observation). The intricate nuclear receptor signaling network allows a single gene to be a target for several nuclear receptors, sensing different markers of endogenous or exogenous origin. Our findings suggest that processes that influence hepatic cholesterol levels, such as defects in cholesterol biosynthesis pathways or hepatic accumulation of lipids, can ultimately alter drug metabolism, affecting the efficiency of drug treatment.

The authors thank Steven A. Kliewer, Dieter Kressler, Christopher Liddle, and Thomas A. Spencer for kindly providing substances and plasmids. This work was supported by a grant from the Swiss National Science Foundation.

REFERENCES

1. Nebert, D. W., and M. Z. Dieter. 2000. The evolution of drug metabolism. *Pharmacology.* **61:** 124–135.
2. Araya, Z., and K. Wikvall. 1999. 6α-Hydroxylation of taurochenodeoxycholic acid and lithocholic acid by CYP3A4 in human liver microsomes. *Biochim. Biophys. Acta.* **1438:** 47–54.
3. Waxman, D. J. 1999. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.* **369:** 11–23.
4. Remmer, H., and H. Merker. 1963. Drug-induced changes in the endoplasmic reticulum: association with drug-metabolizing enzyme. *Science.* **142:** 1657–1658.
5. Sueyoshi, T., and M. Negishi. 2001. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu. Rev. Pharmacol. Toxicol.* **41:** 123–143.
6. Gonzalez, F. J., S. Y. Liu, and M. Yano. 1993. Regulation of cytochrome P450 genes: molecular mechanisms. *Pharmacogenetics.* **3:** 51–57.
7. Handschin, C., and U. A. Meyer. 2003. Induction of drug metabolism: the role of nuclear receptors. *Pharmacol. Rev.* **55:** 649–673.
8. Honkakoski, P., and M. Negishi. 2000. Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem. J.* **347:** 321–337.
9. Wang, H., and M. Negishi. 2003. Transcriptional regulation of cytochrome P450 2B genes by nuclear receptors. *Curr. Drug Metab.* **4:** 515–525.
10. Ferguson, S. S., E. L. LeCluyse, M. Negishi, and J. A. Goldstein. 2002. Regulation of human CYP2C9 by the constitutive androstane receptor: discovery of a new distal binding site. *Mol. Pharmacol.* **62:** 737–746.
11. Baader, M., C. Gnerre, J. J. Stegeman, and U. A. Meyer. 2002. Transcriptional activation of cytochrome P450 CYP2C45 by drugs is mediated by the chicken xenobiotic receptor (CXR) interacting with a phenobarbital response enhancer unit. *J. Biol. Chem.* **277:** 15647–15653.
12. Xie, W., J. L. Barwick, C. M. Simon, A. M. Pierce, S. Safe, B. Blumberg, P. S. Guzelian, and R. M. Evans. 2000. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev.* **14:** 3014–3023.
13. Handschin, C., M. Podvinec, and U. A. Meyer. 2000. CXR, a chicken xenobiotic-sensing orphan nuclear receptor, is related to both mammalian pregnane X receptor (PXR) and constitutive androstane receptor (CAR). *Proc. Natl. Acad. Sci. USA.* **97:** 10769–10774.
14. Russell, D. W., and K. D. Setchell. 1992. Bile acid biosynthesis. *Biochemistry.* **31:** 4737–4749.
15. Chiang, J. Y. 2002. Bile acid regulation of gene expression: roles of nuclear hormone receptors. *Endocr. Rev.* **23:** 443–463.
16. Lehmann, J. M., S. A. Kliewer, L. B. Moore, T. A. Smith-Oliver, B. B. Oliver, J. L. Su, S. S. Sundseth, D. A. Winegar, D. E. Blanchard, T. A. Spencer, et al. 1997. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **272:** 3137–3140.
17. Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J. M. Lobaccaro, R. E. Hammer, and D. J. Mangelsdorf. 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell.* **93:** 693–704.
18. Goodwin, B., S. A. Jones, R. R. Price, M. A. Watson, D. D. McKee, L. B. Moore, C. Galardi, J. G. Wilson, M. C. Lewis, M. E. Roth, et al. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell.* **6:** 517–526.
19. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell.* **6:** 507–515.
20. Thomas, K. D. 1984. Plasma cholesterol levels in adult male and female rats after chronic treatment with phenobarbitone. *Isr. J. Med. Sci.* **20:** 240–241.
21. Eiris, J. M., S. Lojo, M. C. Del Rio, I. Novo, M. Bravo, P. Pavon, and M. Castro-Gago. 1995. Effects of long-term treatment with antiepileptic drugs on serum lipid levels in children with epilepsy. *Neurology.* **45:** 1155–1157.
22. Aynaci, F. M., F. Orhan, A. Orem, S. Yildirmis, and Y. Gedik. 2001. Effect of antiepileptic drugs on plasma lipoprotein (a) and other lipid levels in childhood. *J. Child Neurol.* **16:** 367–369.
23. Schwaninger, M., P. Ringleb, A. Annecke, R. Winter, B. Kohl, E. Werle, W. Fiehn, P. A. Rieser, and I. Walter-Sack. 2000. Elevated plasma concentrations of lipoprotein(a) in medicated epileptic patients. *J. Neurol.* **247:** 687–690.
24. Verrotti, A., S. Domizio, B. Angelozzi, G. Sabatino, G. Morgese, and F. Chiarelli. 1997. Changes in serum lipids and lipoproteins in

epileptic children treated with anticonvulsants. *J. Paediatr. Child Health.* **33:** 242–245.
25. Yilmaz, E., Y. Dosan, M. K. Gurgoze, and S. Gungor. 2001. Serum lipid changes during anticonvulsive treatment serum lipids in epileptic children. *Acta Neurol. Belg.* **101:** 217–220.
26. Handschin, C., M. Podvinec, R. Amherd, R. Looser, J. C. Ourlin, and U. A. Meyer. 2002. Cholesterol and bile acids regulate xenosensor signaling in drug-mediated induction of cytochromes P450. *J. Biol. Chem.* **277:** 29561–29567.
27. Chiang, J. Y., R. Kimmel, and D. Stroup. 2001. Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). *Gene.* **262:** 257–265.
28. Boshart, M., M. Kluppel, A. Schmidt, G. Schutz, and B. Luckow. 1992. Reporter constructs with low background activity utilizing the cat gene. *Gene.* **110:** 129–130.
29. Alberti, S., G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudling, B. Angelin, I. Bjorkhem, S. Pettersson, and J. A. Gustafsson. 2001. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. *J. Clin. Invest.* **107:** 565–573.
30. Schuster, G. U., P. Parini, L. Wang, S. Alberti, K. R. Steffensen, G. K. Hansson, B. Angelin, and J. A. Gustafsson. 2002. Accumulation of foam cells in liver X receptor-deficient mice. *Circulation.* **106:** 1147–1153.
31. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
32. Handschin, C., M. Podvinec, R. Looser, R. Amherd, and U. A. Meyer. 2001. Multiple enhancer units mediate drug induction of CYP2H1 by the xenobiotic-sensing orphan nuclear receptor chicken xenobiotic receptor. *Mol. Pharmacol.* **60:** 681–689.
33. Ueda, A., H. K. Hamadeh, H. K. Webb, Y. Yamamoto, T. Sueyoshi, C. A. Afshari, J. M. Lehmann, and M. Negishi. 2002. Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. *Mol. Pharmacol.* **61:** 1–6.
34. Tzameli, I., P. Pissios, E. G. Schuetz, and D. D. Moore. 2000. The xenobiotic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. *Mol. Cell. Biol.* **20:** 2951–2958.
35. Brooks-Wilson, A., M. Marcil, S. M. Clee, L. H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Collins, H. O. Molhuizen, et al. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* **22:** 336–345.
36. Klucken, J., C. Buchler, E. Orso, W. E. Kaminski, M. Porsch-Ozcurumez, G. Liebisch, M. Kapinsky, W. Diederich, W. Drobnik, M. Dean, et al. 2000. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. *Proc. Natl. Acad. Sci. USA.* **97:** 817–822.
37. Kosters, A., R. J. Frijters, F. G. Schaap, E. Vink, T. Plosch, R. Ottenhoff, M. Jirsa, I. M. De Cuyper, F. Kuipers, and A. K. Groen. 2003. Relation between hepatic expression of ATP-binding cassette transporters G5 and G8 and biliary cholesterol secretion in mice. *J. Hepatol.* **38:** 710–716.
38. Cherrington, N. J., A. L. Slitt, J. M. Maher, X. X. Zhang, J. Zhang, W. Huang, Y. J. Wan, D. D. Moore, and C. D. Klaassen. 2003. Induction of multidrug resistance protein 3 (mrp3) in vivo is independent of constitutive androstane receptor. *Drug Metab. Dispos.* **31:** 1315–1319.
39. Shenoy, S. D., T. A. Spencer, N. A. Mercer-Haines, M. Alipour, M. D. Gargano, M. Runge-Morris, and T. A. Kocarek. 2004. CYP3A induction by liver X receptor ligands in primary cultured rat and mouse hepatocytes is mediated by the pregnane X receptor. *Drug Metab. Dispos.* **32:** 66–71.
40. Bodin, K., L. Bretillon, Y. Aden, L. Bertilsson, U. Broome, C. Einarsson, and U. Diczfalusy. 2001. Antiepileptic drugs increase plasma levels of 4beta-hydroxycholesterol in humans: evidence for involvement of cytochrome P450 3A4. *J. Biol. Chem.* **276:** 38685–38689.
41. Staudinger, J. L., B. Goodwin, S. A. Jones, D. Hawkins-Brown, K. I. MacKenzie, A. LaTour, Y. Liu, C. D. Klaassen, K. K. Brown, J. Reinhard, et al. 2001. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci. USA.* **98:** 3369–3374.
42. Saucier, S. E., A. A. Kandutsch, A. K. Gayen, D. K. Swahn, and T. A. Spencer. 1989. Oxysterol regulators of 3-hydroxy-3-methylglutaryl-CoA reductase in liver. Effect of dietary cholesterol. *J. Biol. Chem.* **264:** 6863–6869.
43. Francis, G. A., E. Fayard, F. Picard, and J. Auwerx. 2003. Nuclear receptors and the control of metabolism. *Annu. Rev. Physiol.* **65:** 261–311.
44. Zhang, Z., D. Li, D. E. Blanchard, S. R. Lear, S. K. Erickson, and T. A. Spencer. 2001. Key regulatory oxysterols in liver: analysis as delta4-3-ketone derivatives by HPLC and response to physiological perturbations. *J. Lipid Res.* **42:** 649–658.
45. Goodwin, B., K. C. Gauthier, M. Umetani, M. A. Watson, M. I. Lochansky, J. L. Collins, E. Leitersdorf, D. J. Mangelsdorf, S. A. Kliewer, and J. J. Repa. 2003. Identification of bile acid precursors as endogenous ligands for the nuclear xenobiotic pregnane X receptor. *Proc. Natl. Acad. Sci. USA.* **100:** 223–228.
46. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37:** 907–925.
47. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109:** 1125–1131.
48. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev.* **14:** 2819–2830.
49. Staudinger, J., Y. Liu, A. Madan, S. Habeebu, and C. D. Klaassen. 2001. Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. *Drug Metab. Dispos.* **29:** 1467–1472.
50. Sudjana-Sugiaman, E., G. Eggertsen, P. Sjoblom, Y. Maeda, M. Rudling, K. Okuda, and I. Bjorkhem. 1994. Presence of cholesterol 7 alpha-hydroxylase enzyme protein in COS-cells leads to increased HMG CoA reductase activity. *Biochem. Biophys. Res. Commun.* **202:** 896–901.