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Perinatal Lipopolysaccharide Exposure Downregulates Pregnane X Receptor and Cyp3a11 Expression in Fetal Mouse Liver

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The pregnane X receptor (*PXR*) is a member of the nuclear receptor superfamily that regulates cytochrome P450 3A (CYP3A) gene transcription in a ligand-dependent manner. Lipopolysaccharide (LPS)-induced downregulation on *PXR* and *cyp3a11* in adult mouse liver has been well characterized. In this study, we investigated the effects of maternal LPS exposure on *PXR* and *cyp3a11* expression in fetal mouse liver. Pregnant ICR mice were injected intraperitoneally with different doses of LPS (0.1–0.5 mg/kg) on gestational day (GD) 17. *PXR* and *cyp3a11* mRNA levels were determined using RT-PCR. Erythromycin N-demethylase (ERND) activity was used as an indicator of CYP3A expression in this study. Results showed that LPS significantly downregulated *PXR* and *cyp3a11* mRNA levels and ERND activity in fetal liver in a dose-dependent manner. LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity was attenuated after pregnant mice were pretreated with alpha-phenyl-N-t-butylnitrone (PBN), a free radical spin trapping agent. Additional experiment revealed that LPS significantly increased lipid peroxidation in fetal liver, which was also attenuated by PBN pretreatment. Furthermore, LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity was prevented by maternal pretreatment with N-acetylcysteine (NAC). Maternal pretreatment with NAC also inhibited LPS-initiated lipid peroxidation and GSH depletion in fetal liver. However, maternal LPS treatment did not affect nitrite plus nitrate concentration in fetal liver. Correspondingly, aminoguanidine, a selective inhibitor of inducible nitric oxide synthase (iNOS), has no effect on LPS-induced downregulation of *PXR* and *cyp3a11* expression and ERND activity in fetal liver. These results indicated that maternal LPS exposure downregulates PXR and *cyp3a11* in fetal mouse liver. Reactive oxygen species (ROS) may be involved in LPS-induced downregulation of *PXR* and *cyp3a11* in fetal mouse liver.

Key Words: **lipopolysaccharide; fetal liver; pregnane X receptor; cytochrome P450 3A; reactive oxygen species.**

INTRODUCTION

The liver is the major organ of amino acid and lipid metabolism, gluconeogenesis, synthesis of serum proteins, and xenobiotic detoxification. The fetal liver functions as the major hematopoietic organ in the mid- to late fetal stage (Dzierzak and Medvinsky, 1995; Hardy and Hayakawa, 2001). With embryonic development, fetal hepatocytes gradually express various types of cytochromes P450 (CYPs) that play a key role in the detoxification of drug or other xenobiotics (de Wildt *et al.*, 1999; Hines and McCarver, 2002; Hulla and Juchau, 1989; Krauer and Dayer, 1991; Rich and Boobis, 1997).

The *cytochrome P450 3A* (*CYP3A*) is a member of the cytochrome P-450 monooxygenase superfamily. In humans, *CYP3A4* and *CYP3A5* gene products account for 30–40% of the total cytochrome P450 in the adult liver, which is responsible for the oxidative metabolism of numerous clinically used drugs and toxicants (Thummel and Wilkinson, 1998). Although *CYP3A4* and *CYP3A5* in fetal liver are not detectable, fetal hepatocytes express *CYP3A7* as early as 50 to 60 days of gestation, with continued significant levels of expression through the perinatal period (Stevens *et al.*, 2003). In mice, *cyp3a11* and *cyp3a13* are major members of *cyp3a* subfamily in the adult liver. In the developing mouse embryo, the amount of *cyp3a11* and *cyp3a13* expression gradually increases with the advancement of embryonic development (Choudhary *et al.*, 2003).

The pregnane X receptor (*PXR*) is a member of the nuclear receptor superfamily that regulates *CYP3A* gene transcription in a ligand-dependent manner (Bertilsson *et al.*, 1998; Kliewer *et al.*, 1998; Lehmann *et al.*, 1998). In addition, *PXR* plays pivotal roles in the regulation of genes contributing to hepatobiliary cholesterol and bile acid homeostasis. Several studies showed that fetal hepatocytes express *PXR* mRNA (Kamiya *et al.*, 2003; Li *et al.*, 2000; Miki *et al.*, 2005). Furthermore, *PXR* activity in liver nuclear extracts assayed by gel EMSA exhibit a strict correlation with mRNA levels. Protein levels for *PXR* also corresponded to the mRNA and functional activity (Balasubramaniyan *et al.*, 2005).

Lipopolysaccharide (LPS) is a toxic component of cell walls of gram-negative bacteria and is widely present in the digestive

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tracts of humans and animals. Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol drinking often increase permeability of LPS from gastrointestinal tract into blood (Mathurin *et al.*, 2000). Lipopolysaccharide has been associated with adverse developmental outcome, including intrauterine fetal death (IUFD) and intrauterine growth retardation (IUGR) in animals (Collins *et al.*, 1994; O'Sullivan *et al.*, 1988). On the other hand, numerous studies indicated that inflammation and infection reduce cytochrome P450 levels in various species including human, rat, and mouse (Morgan, 1997, 2000). Lipopolysaccharide-induced downregulation of *cyp3a11* has also been demonstrated in a mouse model (Li-Masters and Morgan, 2001; Sewer *et al.*, 1998). Moreover, LPS-induced downregulation of *cyp3a* is associated with a marked reduction in *PXR* mRNA and protein levels (Beigneux *et al.*, 2002; Sachdeva *et al.*, 2003). Therefore, the question of whether maternal LPS exposure downregulates *PXR* and *Cyp3a* in fetal mouse liver is especially interesting.

In the present study, we investigated the effects of maternal LPS administration on *PXR* and *cyp3a11* expressions in fetal mouse liver. Our results found that LPS downregulates *PXR* and c*yp3a11* in fetal mouse liver. Reactive oxygen species (ROS) may be involved in LPS-induced downregulation of *PXR* and *cyp3a11* in fetal mouse liver. Maternal NAC pretreatment prevents LPS-induced down-regulation of *PXR* and *cyp3a11* in fetal liver by counteracting LPS-induced oxidative stress.

MATERIALS AND METHODS

Chemicals. Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), alpha-phenyl-N-t-butylnitrone (PBN), aminoguanidine (AG), and N-acetylcysteine (NAC) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

Animals and treatments. The ICR mice (8 ~ 10 week-old; male mice: 28 ~ 30 g; female mice: 24 ~ 26 g) were purchased from Beijing Vital River, whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20°–25°C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, four females were housed overnight with two males starting at 9:00 P.M. Females were checked by 7:00 A.M. the next morning, and the presence of a vaginal plug was designated as gestational day (GD) 0. The present study included four separate experiments.

Experiment 1. To investigate the effects of LPS on *PXR* and *cyp3a11* gene expression in fetal liver, GD 17 ICR mice were injected with different doses of LPS (0.1, 0.2, and 0.5 mg/kg, i.p.). The saline-treated pregnant mice served as controls. Mice were sacrificed at 12 h after LPS treatment for measurement of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal livers.

Experiment 2. To investigate the effects of PBN on LPS-induced down-regulation of *PXR* and *cyp3a11* gene expression in fetal liver, pregnant mice were divided into four groups randomly. The pregnant mice in the LPS-treated group were injected with 0.2 mg/kg of LPS (i.p.) on GD 17. The pregnant mice in LPS + PBN group were injected with 100 mg/kg of PBN (i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) administration. Saline- and PBN-treated pregnant mice served as controls. Half of the mice were sacrificed at 6 h after LPS treatment for measurement of nitrite plus nitrate and thiobarbituric acid-reactive substance (TBARS) content in fetal livers. The other mice were sacrificed at 12 h after LPS treatment for measurement of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal livers.

Experiment 3. To investigate the effects of NAC on LPS-induced down-regulation of *PXR* and *cyp3a11* gene expression in fetal liver, pregnant mice were divided into four groups randomly. The pregnant mice in the LPS-treated group were injected with 0.2 mg/kg of LPS (i.p.) on GD 17. Pregnant mice in the LPS + NAC group were injected with NAC (100 mg/kg, i.p.) either at 30 min before or 3 h after LPS (0.2 mg/kg, i.p.) treatment. Saline- and NAC-treated pregnant mice served as controls. Half of the mice were sacrificed at 6 h after LPS treatment for measurements of TBARS and GSH content in fetal livers. The other mice were sacrificed at 12 h after LPS treatment for measurement of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal livers.

Experiment 4. To investigate the effects of AG on LPS-induced down-regulation of *PXR* and *cyp3a11* gene expression in fetal liver, pregnant mice were divided into four groups randomly. The pregnant mice in the LPS-treated group were injected with 0.2 mg/kg of LPS (i.p.) on GD 17. Pregnant mice in the LPS + AG group were injected with AG (100 mg/kg, i.p.) at 30 min before LPS (0.2 mg/kg, i.p.) treatment. Saline- and AG-treated pregnant mice served as controls. Half of the mice were sacrificed at 6 h after LPS treatment for measurement of nitrite plus nitrate concentration in fetal livers. The other mice were sacrificed at 12 h after LPS treatment for measurement of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal livers.

All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

Isolation of total RNA and reverse transcriptase (RT). For each sample, 50 mg of fetal liver tissue was collected. Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNase-free DNase (Promega, Madison, WI) was used to remove genomic DNA. The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels. Total RNA was stored at −80°C. For the synthesis of cDNA, 2.0 μg of total RNA from each sample was resuspended in a 20-μl final volume of reaction buffer, which contained 25 mM Tris · HCl, pH 8.3; 37.5 mM KCl; 10 mM dithiothreitol; 1.5 mM $MgCl_2$; 10 mM of each dNTP and 0.5 mg oligo$(dT)_{15}$ primer (Promega). After the reaction mixture reached 38°C, 400 units of RT (Promega) was added to each tube and the sample was incubated for 60 min at 38°C. Reverse transcription was stopped by denaturing the enzyme at 95°C.

Polymerase chain reaction (PCR) amplification. The final PCR mixture contained 2 μl of cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mixture, 2 U of Taq DNA polymerase, 1 μM sense and antisense primers, and sterile water to 25 μl. The reaction mixture was covered with mineral oil. Polymerase chain reaction for *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*) was performed on each individual sample as an internal positive-control standard. The following primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China), according to sequence designs previously described by others (Xu *et al.*, 2004). *Gapdh*, 5′-GAG GGG CCA TCC ACA GTC TTC-3′ and 5′-CAT CAC CAT CTT CCA GGA GCG-3′; *PXR*, 5′-GCG CGG AGA AGA CGG CAG CAT C-3′ and 5′-CCC AGG TTC CCG TTT CCG TGT C-3′□*cyp3a11*, 5′-CTC AAT GGT GTG TAT ATC CCC-3′ and 5′-CCG ATG TTC TTA GAC ACT GCC-3′. The sizes of amplified PCR products were 340 bp for *gapdh*, 254 for *PXR*, 423 bp for *cyp3a11*, respectively. Both number of cycles and annealing temperature were optimized for each primer pair. For *gapdh*, amplification was initiated by 3 min of denaturation at 94°C for 1 cycle, followed by 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. For *PXR*, amplification was initiated by 3 min of denaturation at 94°C for 1 cycle, followed by 45 cycles at 94°C for 30 s, 68°C for 30 s, and 72°C for 1 min. After the last cycle of amplification, samples were incubated for 10 min at 72°C. For *cyp3a11*, amplification was initiated by 3 min of denaturation at 94°C for 1 cycle, followed by 35 cycles at 94°C for

30 s, 56°C for 30 s, and 72°C for 1 min. A final extension of 72°C for 10 min was included. The amplified PCR products were subjected to electrophoresis at 75 V through 1.5% agarose gels (Sigma) for 45 min. The pBR322 DNA digested with Alu I was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 mg/ml ethidium bromide (Sigma,) TBE buffer.

Preparation of liver microsomes. Microsomes were isolated from livers by differential centrifugation (Haugen and Coon, 1976). All procedures were conducted at 4°C. Tissue was homogenized in four volumes of Tris/chloride buffer, pH 7.4, containing 150 mM potassium chloride and 1 mM EDTA, with a Polytron homogenizer and centrifuged at 10,000 × *g* for 20 min. The supernatant was collected and centrifuged at 211,000 × *g* for 40 min. The microsomal pellet was resuspended and washed in sodium pyrophosphate buffer, pH 7.4, containing 1 mM EDTA and centrifuged again at 211,000 × *g* for 40 min at 4°C. The washed microsomal pellet was resuspended in a trischloride buffer, pH 7.4, containing 20% glycerol, with a ground glass tissue grinder and stored at −80°C. Protein concentrations of microsome samples were measured according to the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

CYP3A catalytic activity. Erythromycin *N*-demethylase (ERND) was used as an indicator of CYP3A catalytic activity in this study. The ERND was measured according to the method of Werringloer (1978) with a 45-min incubation containing 4 mM erythromycin in the presence of 0.5 mM NADPH and 0.4 mg of microsomal protein in a total assay volume of 1 ml. The rate of formaldehyde formation was determined spectrophotometrically at 412 nm using the Nash reagent. Measurement for ERND catalytic activity was repeated twice for three separately prepared liver microsome samples.

Determination of glutathione content. The glutathione (GSH) level was determined by the method of Griffith (1980). Proteins of 0.4 ml tissue homogenates were precipitated by the addition of 0.4 ml of a metaphosphoric acid solution. After 40 min, the protein precipitate was separated from the remaining solution by centrifugation at 5000 rpm at 4°C for 5 min. Then 400 µl of the supernatant was combined with 0.4 ml of 300 mM Na_2HPO_4, and the absorbance at 412 nm was read against a blank consisting of 0.4 ml supernatant plus 0.4 ml H_2O. Then, 100 µl DTNB (0.02%, w/v; 20 mg DTNB in 100 ml of 1% sodium citrate) was added to the blank and sample, and absorbance of the sample was read against the blank at 412 nm. The GSH content was determined using a calibration curve prepared with an authentic sample. Glutathione values were expressed as nanomoles per milligram of (nm · mg^{-1} protein. Protein content was measured according to the method of Lowry *et al.* (1951).

Lipid peroxidation assay. Lipid peroxidation was quantified by measuring TBARS as described previously (Ohkawa *et al.*, 1979). Tissue was homogenized in 9 volumes of 50 mmol/l Tris-HCl buffer (pH 7.4) containing 180 mmol/l KCl, 10 mmol/l EDTA, and 0.02% butylated hydroxytoluene. To 0.2 ml of the tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, 1.5 ml of 0.9% thiobarbituric acid, and 0.6 ml of distilled water were added and the solution was vortexed. The reaction mixture was placed in a water bath at 95°C for 1 h. After cooling on ice, 1.0 ml of distilled water and 5.0 ml of butanol/pyridine mixture (15:1, v/v) were added and vortexed. After centrifugation at 10,000 × *g* for 10 min, absorbance of the resulting lower phase was determined at 532 nm. The TBARS concentration was calculated using 1,1,5,5-tetraethoxypropane as standard.

Analysis of nitrite plus nitrate concentration in fetal liver. The stable end products of L-arginine–dependent nitric oxide synthesis, nitrate plus nitrite, were measured in fetal liver using a colorimetric method based on the Griess reaction (Tracey *et al.*, 1995). Briefly, aliquots of homogenates were added to 35% sulfosalicylic acid and vortexed every 5 min for 30 min to deproteinize samples. The samples were then centrifuged at 10, 000 × *g* at 4°C for 15 min. An aliquot of the supernatant was taken for nitrite and nitrate analysis. Then 20 µl of plasma sample was mixed with 20 µl of 0.31 M phosphate buffer, pH 7.5, 10 µl of 0.1 mM FAD, 10 µl of 1 mM NADPH, 10 ml of nitrate reductase (10 units/ml), and 30 µl of water in a 96-well plate. The reaction was allowed to proceed for 1 h in the dark. The percent conversion of nitrate to nitrite was 98%. To each sample, 1 µl of lactate dehydrogenase (1500 units/ml) and 10 µl of 100 mM pyruvic acid were added and incubated for 15 min at 37°C. The samples were then mixed with an equivalent volume of Griess reagent and incubated for an additional 10 min at room temperature. Nitrite levels were determined colorimetrically at 550 nm with a Universal microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) and a sodium nitrite standard curve.

Statistical analysis. The *PXR* and *cyp3a11* mRNA levels were normalized to the Gapdh mRNA level in the same samples. The *PXR* and *cyp3a11* mRNA levels of the control were assigned as 100%. Quantified data from analysis of GSH, TBARS and nitrate plus nitrite concentrations, RT-PCR, and ERND assay were expressed as means ± S.E.M. at each point. Analysis of variance (ANOVA) and the Student-Newmann-Keuls *post hoc* test were used to determine differences between the treated animals and the control and statistical significance.

RESULTS

Effects of LPS on PXR and Cyp3a11 mRNA Expression and ERND Activity in Fetal Mouse Liver

The *PXR* and *cyp3a11* mRNA levels in fetal liver were determined at 12 h after LPS treatment using RT-PCR. The effects of LPS on *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal mouse liver are presented in Figure 1. Results showed that LPS significantly downregulated *PXR* and *cyp3a11* mRNA levels in a dose-dependent manner. Consistent with downregulation of *cyp3a11* mRNA, LPS significantly inhibited ERND activity in fetal liver microsomes.

Effects of PBN on LPS-Induced Downregulation of PXR and Cyp3a11 mRNA and ERND Activity in Fetal Liver

The effects of PBN on LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity are presented in Figure 2. Results showed that PBN pretreatment and posttreatments significantly attenuated LPS-induced downregulation of *PXR* and *cyp3a11* mRNA levels in fetal liver. In addition, PBN pretreatment and posttreatment also significantly attenuated LPS-induced downregulation of ERND activity in fetal liver.

Effects of PBN on LPS-Induced Lipid Peroxidation in Fetal Liver

Lipid peroxidation was quantified by measuring TBARS levels at 6 h after LPS treatment. As shown in Figure 3, LPS significantly increased TBARS level in fetal liver. Furthermore, PBN pretreatment and posttreatment significantly attenuated LPS-induced increases in TBARS level.

Effects of NAC on LPS-Induced Downregulation of PXR and Cyp3a11 in Fetal Mouse Liver

To evaluate the effects of NAC on LPS-induced downregulation of *PXR* and ***cyp3a11*** in fetal liver, *PXR* and ***cyp3a11*** mRNA expression and ERND activity were determined at 12 h after LPS treatment. Results showed that NAC pretreatment significantly attenuated LPS-induced downregulation of *PXR*

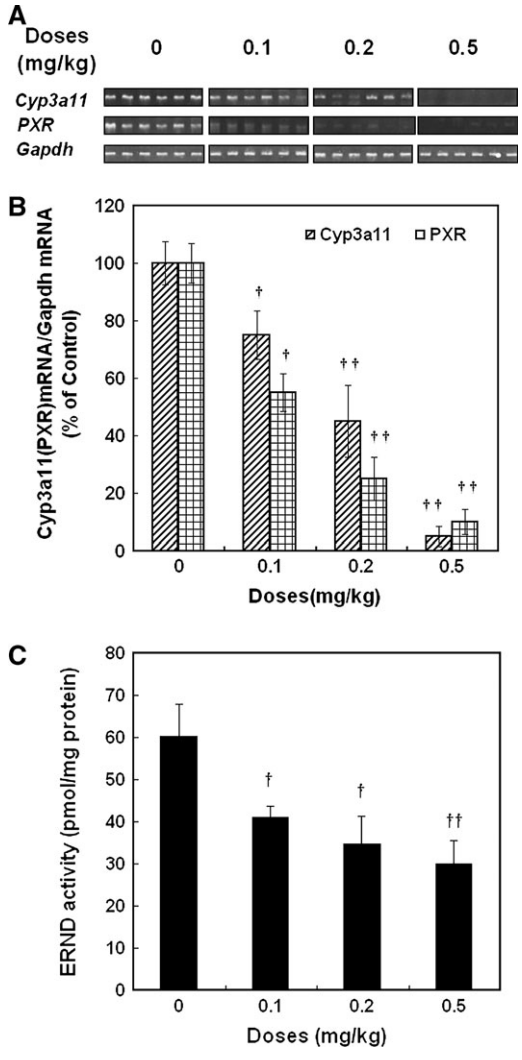

FIG. 1. The effects of LPS on *PXR* and *Cyp3a11* mRNA expression and ERND activity in fetal liver. Pregnant mice were injected with different doses of LPS (0.1 ~ 0.5 mg/kg, i.p) on GD 17. Fetal livers were excised at 12 h after LPS treatment. Total RNA was extracted and microsomes were isolated from liver tissue. A. *PXR* and *cyp3a11* mRNA levels were determined using RT-PCR. B. Quantitative analysis of *PXR* and *Cyp3a11* mRNA levels was performed on 12 fetal mouse livers from three dams at each time point. The *PXR* and *cyp3a11* mRNA levels were normalized to *Gapdh* mRNA level in the same samples. The *PXR* and *cyp3a11* mRNA levels of the control was set at 100%. †$p < 0.05$, ††$p < 0.01$ as compared with control group. C. ERND activity was measured as described in *Materials and Methods*. Data were expressed as means ± S.E.M. of 12 fetal livers from three dams. †$p < 0.05$, ††$p < 0.01$ as compared with the control.

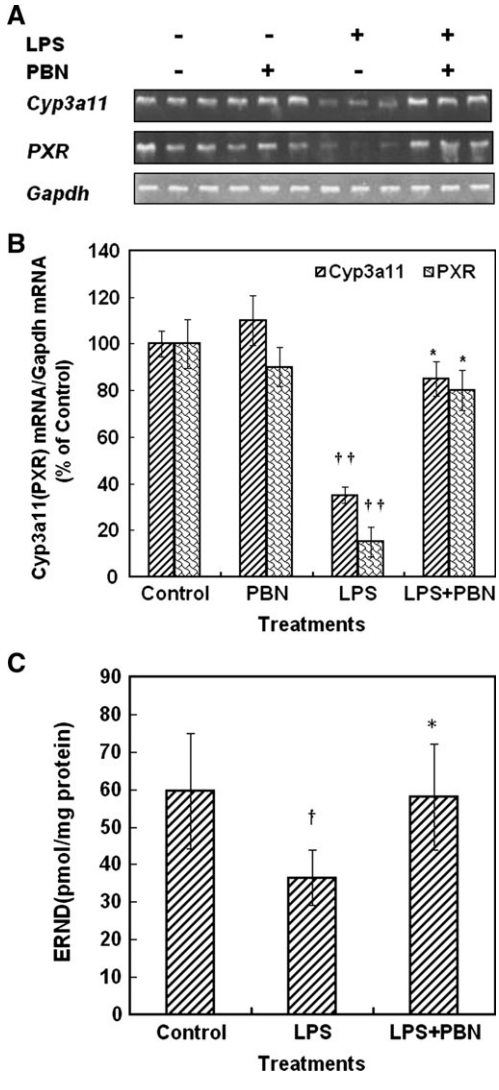

FIG. 2. The effects of PBN on LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal liver. Pregnant mice were injected with PBN (100 mg/kg, i.p.) 30 min before and 3 h after LPS (0.2 mg/kg, i.p) treatment. Fetal livers were excised at 12 h after LPS treatment. Total RNA was extracted and microsomes were isolated from liver tissue. A. *PXR* and *cyp3a11* mRNA levels were determined using RT-PCR. B. Quantitative analysis of *PXR* and *Cyp3a11* mRNA expression was performed on 12 fetal mouse livers from three dams at each time point. The *PXR* and *cyp3a11* mRNA levels were normalized to the *Gapdh* mRNA level in the same samples. The *PXR* and *Cyp3a11* mRNA level of control was set at 100%. **$p < 0.01$ as compared with the LPS-treated group. C. ERND activity was measured as described in *Materials and Methods*. Data were expressed as means ± S.E.M. of 12 fetal livers of three dams. †$p < 0.05$ as compared with the control. *$p < 0.05$ as compared with the LPS-treated group.

and ***cyp3a11*** mRNA levels in fetal liver (Figs. 4A and 4B). In addition, maternal NAC pretreatment significantly attenuated LPS-induced decrease in ERND activity in fetal liver (Fig. 4C).

Effects of NAC on LPS-Induced GSH Depletion and Lipid Peroxidation in Fetal Liver

The effects of NAC on LPS-induced GSH depletion and lipid peroxidation are shown in Figure 5. As expected, maternal LPS exposure significantly decreased GSH content in fetal liver. On the one hand, NAC pretreatment attenuated LPS-induced GSH depletion in fetal liver. On the other hand, maternal LPS exposure significantly increased TBARS levels in fetal liver. The NAC pretreatment attenuated LPS-induced lipid peroxidation in fetal liver.

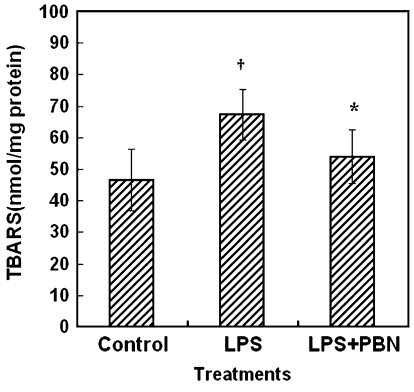

FIG. 3. The effects of PBN on LPS-induced lipid peroxidation in fetal liver. Pregnant mice were injected with PBN (100 mg/kg, i.p.) at 30 min before LPS (0.2 mg/kg, i.p) treatment. Fetal livers were excised and homogenized at 6 h after LPS treatment. The TBARS content was measured as described in *Materials and Methods*. Data were expressed as means ± S.E.M. ($n = 12$). †$p < 0.05$ as compared with control group. *$p < 0.05$ as compared with the LPS-treated group.

DISCUSSION

CYP3A is a member of the cytochrome P-450 monooxygenase superfamily, which is responsible for the oxidative metabolism of numerous clinically used drugs. The pregnan X receptor is the key transactivator of the *CYP3A* gene. Several studies have demonstrated that LPS downregulates *PXR* and *cyp3a* in adult mouse liver (Beigneux *et al.*, 2002; Sachdeva *et al.*, 2003). In this study, we investigated the effects of maternal LPS exposure on *PXR* and *cyp3a11* expression in fetal mouse liver. Results indicated that LPS significantly inhibited the constitutive expression of *PXR* and *cyp3a11* mRNA in fetal liver in a dose-respondent manner. It has been demonstrated that CYP3A is the major enzyme catalyzing erythromycin N-demethylation in mouse liver (Lan *et al.*, 2000). Thus, ERND activity was used as an indicator of CYP3A expression in this study. The present results indicate that maternal LPS exposure significantly represses ERND activity in fetal liver microsomes.

Reactive oxygen species (ROS) are known to influence the expressions of a number of genes and signal transduction pathways (Allen and Tresini, 2000). Lipopolysaccharide, a potent activator for macrophages, stimulates Kupffer cells to generate ROS, such as O_2^- (Bautista *et al.*, 1990). In addition, several studies found that LPS increased nitrotyrosine, a marker for O_2^-, NO and $ONOO^-$ formation in macrophage-rich organs, such as liver (Bian and Murad, 2001; Ottesen *et al.*, 2001). Our earlier studies showed that ROS are involved in LPS-induced downregulation of *PXR* and *cyp3a11* in adult mouse liver (Xu *et al.*, 2004, 2005). To determine the role of ROS on LPS-induced downregulation of *PXR* and *cyp3a11* in fetal liver, alpha-phenyl-N-t-butylnitrone (PBN), a free radical spin trapping agent, was used to decrease LPS-induced ROS production. As expected, maternal PBN pretreatment and posttreatment significantly inhibited LPS-induced lipid peroxidation in fetal liver, suggesting that PBN was effective in the *in vivo* trapping of free radicals. Interestingly, LPS-induced downregulation of *PXR* and *cyp3a11* mRNA in fetal liver was significantly attenuated in mice pretreated with PBN. In addition, PBN pretreatment also significantly attenuated LPS-induced downregulation on ERND activity in fetal liver. These

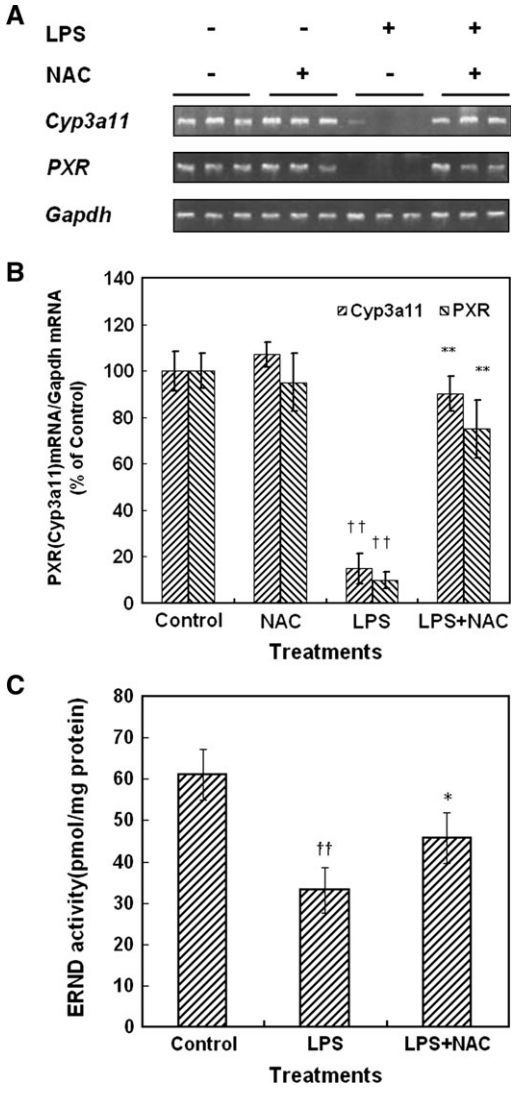

FIG. 4. The effects of NAC on LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal liver. Pregnant mice were injected with NAC (100 mg/kg, i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p) treatment. Fetal livers were excised at 12 h after LPS treatment. Total RNA was extracted, and microsomes were isolated from liver tissue. A. *PXR* and *cyp3a11* mRNA levels were determined using RT-PCR. B. Quantitative analysis of *PXR* and *cyp3a11* mRNA levels was performed on 12 fetal mouse livers from three dams at each time point. The *PXR* and *cyp3a11* mRNA levels were normalized to the *gapdh* mRNA level in the same samples. The *PXR* and *cyp3a11* mRNA level of control was set at 100%. **$p < 0.01$ as compared with the LPS-treated group. C. ERND activity was measured as described in *Materials and Methods*. Data were expressed as means ± S.E.M. of 12 fetal livers of three dams. ††$p < 0.01$ as compared with control group. *$p < 0.05$ as compared with the LPS-treated group.

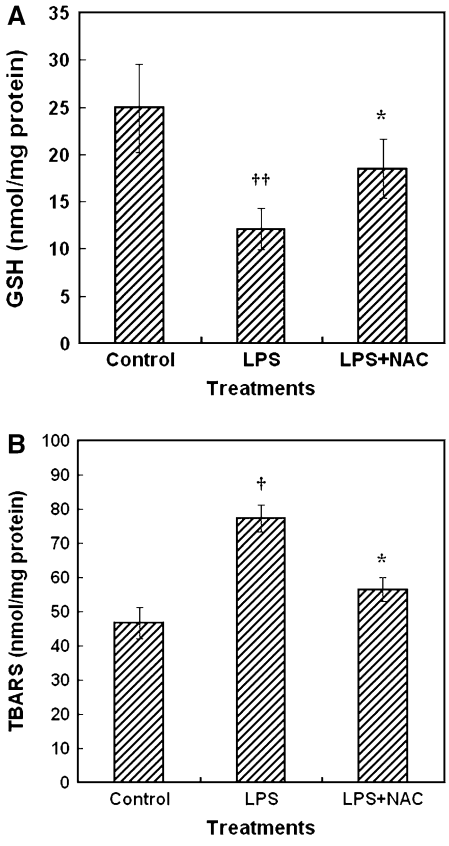

FIG. 5. The effects of NAC on LPS-induced GSH depletion and lipid peroxidation in fetal liver. Pregnant mice were injected with NAC (100 mg/kg, i.p.) at 30 min before LPS (0.2 mg/kg, i.p) treatment, followed by an additional dose of NAC after LPS treatment. Fetal livers were excised and homogenized at 6 h after LPS treatment. Both GSH and TBARS content was measured as described in *Materials and Methods*. Data were expressed as means ± S.E.M. ($n = 12$). ††$p < 0.01$ as compared with the control group. *$p < 0.05$ as compared with the LPS-treated group.

results indicate that ROS mediate LPS-induced downregulation of *PXR* and *cyp3a11* in fetal liver.

N-acetylcysteine (NAC) is a GSH precursor and direct antioxidant. Recent study has indicated that NAC protected against fetal death and preterm labor induced by maternal inflammation (Buhimschi *et al.*, 2003). The present study showed that NAC prevented LPS-induced GSH depletion and lipid peroxidation in fetal liver. To investigate the effect of NAC on LPS-induced downregulation of *PXR* and *cyp3a11* in fetal liver, pregnant mice were pretreated with NAC at 30 min before LPS administration, followed by additional dose of NAC at 3 h after LPS treatment. Consistent with its antioxidative effect, NAC significantly attenuated LPS-induced downregulation of *PXR* and *cyp3a11* mRNA levels. This antioxidant also prevented the repressive effect of LPS on ERND activity in fetal liver.

Several studies indicated that nitric oxide (NO) mediates LPS-induced inhibition of cytochrome P450 in adult liver (Kitaichi *et al.*, 1999; Muller *et al.*, 1996). A lot of studies have demonstrated that LPS induced NO production in adult liver and maternal serum (Athanassakis *et al.*, 1999; Hida *et al.*, 2003; Zhang *et al.*, 2000). However, the present study showed that there was no difference in nitrate plus nitrite concentration in fetal liver between the LPS-treated group and the control group (data not shown). This result is in agreement with the earlier work by Casado *et al.* (1997), in which treatment of rats on GD 21 with lipopolysaccharide (LPS) induced inducible nitric oxide synthase (iNOS) expression in maternal liver but completely failed to elicit this response in the corresponding fetal tissue. Furthermore, the present study found that aminoguanidine, a selective inhibitor of iNOS, has no effect on LPS-induced downregulation of *PXR* and *cyp3a11* mRNA expression and ERND activity in fetal liver (data not shown). These results suggest that LPS-induced downregulation of *PXR*and *cyp3a11* in fetal liver is also independent of NO production.

Numerous studies have demonstrated that pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are involved in LPS-induced downregulation of cytochrome P450s (Monshouwer *et al.*, 1996; Sewer and Morgan, 1997). Several studies found that LPS induced TNF-α, IL-1β, and IL-6 in the maternal serum, the amniotic fluid, and the placenta (Bell *et al.*, 2004; Gayle *et al.*, 2004). Therefore, our results do not exclude the involvement of pro-inflammatory cytokines.

In summary, the present results allow us to reach several conclusions. First, maternal LPS exposure downregulates *PXR* and *cyp3a11* in fetal mouse liver; second, ROS may be involved in LPS-induced downregulation of *PXR* and *cyp3a11* in fetal mouse liver; and third, maternal NAC pretreatment prevents LPS-induced downregulation of *PXR* and *cyp3a11* in fetal liver by counteracting LPS-induced oxidative stress in fetal liver.

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