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Toll-like receptor-4 regulation of hepatic Cyp3a11 metabolism in a mouse model of LPS-induced CNS inflammation

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The aim of this study was to examine the signaling mechanisms responsible for the loss in hepatic cytochrome P-450 3A in a model of gram-negative bacterial infection or inflammation of the mouse brain. Our novel findings indicated that, in this experimental model, the flux of LPS from brain to blood and the activation of a peripheral TLR4-mediated immune response lead to a reduction Cyp3a11 drug metabolism in the liver.

MATERIALS AND METHODS

Mice and production of CNS inflammation. FVB and C57BL/6 mouse strains were used initially in the study to develop methodology and to characterize the effects of CNS infection or inflammation on mouse cytochrome P-450 metabolism. Subsequently, the C3H/HeJ and closely related C3H/HeOuJ strains were used to specifically address the role of TLR4 in mediating changes in hepatic cytochrome P-450 expression and activity. Adult male mice (FVB strain) and adrenalectomized FVB mice were obtained from Taconic Laboratories (Germantown, NY). Male mice (6–8 wk old) containing either a spontaneous mutation in TLR4 gene (C3H/HeJ) and age-matched LPS-responsive (C3H/HeOuJ) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 mice were obtained from our in-house breeding colony. The mice were housed in cages lined with corncob bedding and had free access to water and Purina mouse chow. Drinking water for adrenalectomized mice was supplemented with 5% glucose. Mice were kept on a 12:12-h day-night cycle and were allowed to acclimatize in the animal holding facilities for a period of 1 wk before experimentation. The Dalhousie University Committee on Laboratory Animals approved all experimental procedures involving mice according to guidelines of the Canadian Council on Animal Care.

On the day of the experiments, mice were anesthetized with enflurane (3.0–4.0%) and were given an infusion of either endotoxin-LPS (serotype 0127:B8; 2.5 ng LPS was administered into the lateral ventricle in a total volume of 2.5 nl of the saline vehicle. LPS is the preferred preparation for in vivo studies of this nature. To prevent nonspecific responses in our model. For those reasons, chromatographically purified LPS, but not the gel-purified LPS, contains mi- neral oil of δ-1-14C]mannitol was administered peripherally to control mice and to mice 0, 1, 4, and 24 h after 2.5 μg LPS was administered into the lateral ventricle. Brain levels of total radioactivity were measured by storing at −80°C until utilization for cytokine measurements. Samples (100 mg) were obtained from the liver and frozen in liquid nitrogen for total RNA isolations. The remaining liver was removed and used to prepare microsomes according to previously published methods (10). We homogenized liver tissue in ice-cold 1.15% KCl for 15–20 s using a Polytron homogenizer. The homog-
Gapdh were obtained with a threshold of 3 SD above background. Relative TNF-α expression normalized to Gapdh was calculated using the ΔΔCt (where Ct is threshold cycle) method (see Ref. 22).

Cytokine measurements. We measured the levels of TNF-α and IL-1β in brain homogenates and serum 2 or 4 h after LPS administration ICV using a murine sandwich ELISA prepared as previously described (26). The results are expressed as nanograms of cytokine per gram of brain wet weight or nanograms of cytokine per milliliter of serum.

Serum LPS measurements. LPS (2.5 μg) was administered by the ICV or intraperitoneal route to endotoxin-responsive C3H/HeoJ mice. Mice were killed 15 min, 30 min, 2 h, or 24 h later, and trunk blood was collected. All groups were compared with noninjected control mice. We measured serum LPS levels using the kinetic chromogenic Limulus amebocyte lysate assay as per the manufacturer’s instructions (Associates of Cape Cod, Cape Cod, MA). The assay was linear from 0.395 pg/ml to 100 ng/ml LPS. We calculated the area under the curve (AUC0.25–2 h) from the mean serum LPS concentrations using the trapezoidal method.

Data analyses. Data are expressed as means ± SE of three or four separate animals. An unpaired t-test was used to compare means between two groups. ANOVA was used for multiple-comparison procedures. A Tukey’s test was used for post hoc analysis of the significant ANOVA. A difference in mean values with a value of P ≤ 0.05 was considered to be significant.

Chemicals. Purified E. coli LPS (serotype 0127:B8), phenacetin, α-hydroxytriazolam, ethoxyresorufin, resorufin, NADPH, and dichloromethane were obtained from Sigma. [32P]dCTP was obtained from Perkin-Elmer (Boston, MA). Triazolam was obtained from Roche Pharmaceuticals. All other reagents were of the finest grade available and were purchased from commercial suppliers.

RESULTS

Administration of LPS into the lateral ventricle reduces hepatic Cyp3a11 expression and activity. The initial experiments carried out in the two mouse strains (FVB and C57BL/6) were required to develop a mouse model of CNS infection and inflammation and to determine whether this model exhibited reductions in Cyp3a11 expression and activity. The dose of LPS was based on previous studies that demonstrated that 0.1 μg/kg LPS administered ICV was required for significant downregulation of hepatic cytochrome P-450 enzymes in rats (14, 31, 41). The expression of Cyp3a11 and Gapdh mRNA was measured in the liver by Northern blot analyses 4 and 24 h after the administration of E. coli LPS into the left lateral cerebral ventricle (Fig. 1A). Cyp3a11 mRNA was unchanged at 4 h and was decreased by 60% 24 h after LPS treatment compared with the respective saline controls (Fig. 1, B and D). Liver Gapdh expression was not altered by LPS treatment. Consistent with mRNA expression, the rate of α-hydroxylation of triazolam (Cyp3a11 activity) was unchanged at 4 h but was decreased by 60% at 24 h by LPS treatment compared with the respective saline control (Fig. 1, C and E). LPS (2.5 μg) administered ICV to C57BL/6 mice produced a similar reduction in Cyp3a11 mRNA (80%) and α-hydroxylation of triazolam (60%) and indicated that the effect of LPS was not restricted to the FVB strain of mice (Fig. 2).

Administration of LPS into the lateral cerebral ventricle produces CNS inflammation. Two hours after LPS was administered into the lateral ventricle of FVB mice, TNF-α and IL-1β levels were significantly increased (2-fold) compared with the saline controls (Fig. 3, A and B). The increased level of TNF-α and IL-1β are consistent with the production of CNS inflammation in response to LPS. A parallel increase (2-fold) in TNF-α and IL-1β in serum indicated a systemic response to ICV administered LPS (Fig. 3, C and D).

HPA axis does not mediate hepatic Cyp3a11 downregulation following ICV administration of LPS. The release of inflammatory cytokines after LPS injection into the brain is known to activate the HPA axis and increase circulating corticosteroids, which can affect the acute phase response and gene transcription in the liver (2, 9, 48). Thus it was important to determine the relevance of the HPA axis in contributing to the downregulation of Cyp3a11 mRNA and enzyme activity in our model (Fig. 4). Adrenalectomized and control FVB mice were left untreated or were injected with 2.5 μg or LPS or saline ICV. LPS reduced Cyp3a11 expression and activity to the same degree (70%) in both control and adrenalectomized

Fig. 1. Hepatic Cyp3a11 mRNA and activity were downregulated 24 h after intracerebroventricular (ICV) administration of LPS (2.5 μg) to male FVB mice. A: representative Northern blots of Cyp3a11 and Gapdh at 4 and 24 h after ICV administration of LPS or saline. Band intensities were determined by densitometry, and hepatic Cyp3a11 (B and D) mRNA was normalized to Gapdh mRNA. Cyp3a11 activity was measured 4 h (C) and 24 h (E) after LPS or saline administration by monitoring the formation of α-hydroxytriazolam (α-OH-triazolam) from triazolam in liver microsomal fractions. Each bar represents the mean ± SE of 4 mice. *Cyp3a11 mRNA and activity were lower compared with the respective saline-treated mice (P < 0.05, unpaired t-test).
mice (Fig. 4, A–C). In control mice and adrenalectomized mice, saline administration had no effect on hepatic Cyp3a11 mRNA or triazolam metabolism compared with the respective untreated animals. Similarly, basal Cyp3a11 expression and triazolam hydroxylation in adrenalectomized mice was not significantly different from controls. ICV LPS in control mice significantly increased serum corticosterone compared with saline-treated or untreated control mice, indicating activation of the HPA axis (Fig. 4D). Adrenalectomy completely prevented the elevation in serum corticosterone after ICV administration of LPS.

TLR4 signaling regulates hepatic Cyp3a11 expression. TLR4-expressing (C3H/HeJ) and TLR4 mutant (C3H/HeJ) mice were utilized to determine whether the downregulation of hepatic Cyp3a11 by LPS was mediated through stimulation of the TLR4. Twenty-four hours after LPS (2.5 μg) administration to C3H/HeJ mice, Cyp3a11 mRNA was decreased by 70% compared with the saline control (Fig. 5, A and B). In sharp contrast, no loss in Cyp3a11 mRNA was evoked 24 h after LPS was administered directly into the lateral cerebral ventricle of TLR4 mutant (C3H/HeJ) mice. Liver Gapdh expression was similar in both mouse strains and was not altered by LPS treatment. Correspondingly, triazolam metabolism to α-hydroxytriazolam was reduced by 60% in the C3H/HeJ mice but not in the C3H/HeJ (wild-type) mice but was unchanged in the C3H/HeJ (TLR4 mutant) mice compared with the respective saline controls (Fig. 5C). Basal Cyp3a11 mRNA and triazolam hydroxylation were 40% lower in saline-treated C3H/HeJ vs. C3H/HeJ mice. After 4 h, LPS increased brain TNF-α protein in the C3H/HeJ mice but not in the C3H/HeJ mice compared with the respective saline controls (Fig. 5D). Liver TNF-α mRNA expression was increased 60-fold in the C3H/HeJ mice but only 5-fold in the C3H/HeJ mice 4 h after LPS treatment compared with the respective saline controls (Fig. 5E). These results demonstrate an inhibition of the brain and liver inflammatory responses to LPS in the TLR4-mutant C3H/HeJ mice and indicate that the reduction in hepatic Cyp3a11 expression and activity are linked to stimulation of TLR4 by LPS. Recombinant TNF-α (50 ng) administered ICV to the C3H/HeJ and C3H/HeJ mice did not produce a loss in Cyp3a11 expression in either mouse strain 24 h after they were dosed (Fig. 5F).

The dose of TNF-α was chosen to approximate the TNF-α level detected in the brain 2–4 h after the LPS dose.

LPS is rapidly transferred from the CNS to the periphery after administration into the lateral cerebral ventricle. During CNS infection, the leakage of endotoxin into the periphery could play a role in reducing hepatic cytochrome P-450 metabolism; however, the systemic distribution of LPS after its administration ICV has not been previously characterized. LPS (2.5 μg) administered by the ICV route was detected in the serum (1,000–10,000 pg/mL range) between 15 min and 2 h after mice were dosed. LPS was not detectable in the serum 24 h after ICV administration or in untreated control mice (Fig. 6A). When 2.5 μg of LPS were administered via the intraperitoneal route, the levels of endotoxin detected were very similar to those obtained after administration of LPS by the ICV route (Fig. 6B). The intraperitoneal administration of 2.5 μg LPS also produced a 60% reduction in hepatic Cyp3a11 mRNA and triazolam metabolism (Fig. 6, C–E). The AUC0.05–2 h results calculated for the serum LPS data were 11,817 and 8,847 pg·mL⁻¹·h⁻¹ after ICV and intraperitoneal injection of LPS, respectively. This result directly supports the idea that a peripheral inflammatory response produced by LPS reaching the circulation after ICV injection is sufficient to produce reductions in hepatic cytochrome P-450 metabolism. The uptake of peripherally administered [14C]mannitol was used as a marker of changes in blood-brain permeability induced by ICV administration of LPS (Fig. 6F). Increased blood-brain permeability was suggested by the significant increase in total brain [14C]mannitol 5 and 24 h after LPS administration compared with control mice.

Fig. 2. Hepatic Cyp3a11 mRNA and activity were downregulated 24 h after ICV administration of LPS (2.5 μg) to male C57BL/6 mice. A: hepatic Cyp3a11 mRNA expression normalized to Gapdh expression as determined by Northern blot analysis. Band intensities were determined by densitometry. Cyp3a11 activity was measured by monitoring the formation of α-OH-triazolam from triazolam in liver microsomal fractions (B). Each bar represents the mean ± SE of 4 mice. *P < 0.05 compared with the respective saline-treated mice (unpaired t-test).

Fig. 3. Brain and serum inflammatory cytokines were elevated after ICV administration of LPS. Male FVB mice were injected with 2.5 μg of LPS or 2.5 μL of saline into the lateral cerebral ventricle. Levels of TNF-α and IL-1β proteins in brain (A and B) and serum (C and D) were measured 2 h after ICV administration of LPS or saline. Each bar represents the mean ± SE of 4 mice. *Mean cytokine levels were higher compared with the respective saline-treated control (P < 0.05, unpaired t-test).
Systemic administration of LPS mediates a reduction in hepatic Cyp3a11 expression and activity through TLR4 signaling. The importance of TLR4 in mediating hepatic Cyp3a11 downregulation after the administration of LPS by the intra-

Fig. 5. Toll-like receptor-4 (TLR4) mutant mice (C3H/HeJ) are resistant to downregulation of Cyp3a11 mRNA and activity after ICV administration of LPS. TLR4 mutant (C3H/HeJ) and wild-type (C3H/HeouJ) mice were injected with 2.5 μg of LPS or 2.5 μl of saline into the lateral cerebral ventricle. Analyses were performed 24 h after the ICV injections. A: representative Northern blots of Cyp3a11 and Gapdh. B: hepatic Cyp3a11/Gapdh expression as determined by densitometry. C: hepatic Cyp3a11 activity (α-OH-triazolam formation). D: serum corticosterone level. For the corticosterone measurement, the LPS-ADX (adrenalectomy) bar represents the average of 2 mice. All other bars represent means ± SE of 3 or 4 mice. *Different compared with the respective saline-treated control or the untreated control (P < 0.05, ANOVA followed by Tukey’s post hoc test). Statistical comparison of serum corticosterone in the ADX mice was not performed because of insufficient sample size (n = 2) in the LPS-ADX group.

Fig. 4. Hypothalamic-adrenal-pituitary (HPA) axis does not contribute to hepatic Cyp3a11 downregulation after ICV administration of LPS. Experiment was performed 2 wk after adrenalectomy. Control (Con) mice and adrenalectomized (ADX) mice were injected with 2.5 μg of LPS or 2.5 μl of saline into the lateral cerebral ventricle. Analyses were performed 24 h after the ICV injections. A: representative Northern blots for hepatic Cyp3a11 and Gapdh. B: hepatic Cyp3a11/Gapdh expression as determined by densitometry. C: hepatic Cyp3a11 activity (α-OH-triazolam formation). D: serum corticosterone level. For the corticosterone measurement, the LPS-ADX (adrenalectomy) bar represents the average of 2 mice. All other bars represent means ± SE of 3 or 4 mice. *Different compared with the respective saline-treated control or the untreated control (P < 0.05, ANOVA followed by Tukey’s post hoc test). Statistical comparison of serum corticosterone in the ADX mice was not performed because of insufficient sample size (n = 2) in the LPS-ADX group.
peritoneal route was examined with TLR4-expressing and TLR4 mutant mice. In C3H/HeouJ mice, a high dose (5 mg/kg) of LPS produced a 90% reduction in Cyp3a11 mRNA and a corresponding 60% loss of α-hydroxytriazolam formation (Fig. 7). In contrast, TLR4 mutant (C3H/HeJ) mice were completely resistant to the loss in Cyp3a11 mRNA and activity produced by the high dose of LPS administered intraperitoneally. These data identify that peripheral TLR4 stimulation is a requirement for the downregulation of the major drug-metabolizing hepatic cytochrome P-450 enzyme by systemic E. coli LPS.

DISCUSSION

In experimental models of CNS infection and/or inflammation, the central administration of LPS produces an inflammatory response in the brain characterized by the activation of microglia, astrocytes, and proinflammatory cytokine pathways; the release of inflammatory cytokines (TNF-α, IL-1β, and IL-6); and leukocyte infiltration (30, 35). The CNS inflammatory response in our mouse model was confirmed by the presence of elevated inflammatory cytokines (TNF-α and IL-1β) in the brains of LPS-treated FVB or C3H/HeouJ mice. We have observed a significant downregulation of expression and metabolic activity of hepatic Cyp3a11 in FVB, C57BL/6, and C3H/HeouJ mice but not in C3H/HeJ mice. A large reduction in hepatic CYP3A metabolism produced by an inflammatory response would have implications for drug toxicity because the cytochrome P-450 subfamily metabolizes a large percentage of 7). In contrast, TLR4 mutant (C3H/HeJ) mice were completely resistant to the loss in Cyp3a11 mRNA and activity produced by the high dose of LPS administered intraperitoneally. These data identify that peripheral TLR4 stimulation is a requirement for the downregulation of the major drug-metabolizing hepatic cytochrome P-450 enzyme by systemic E. coli LPS.

Fig. 7. Regulation of hepatic Cyp3a11 metabolism by systemic LPS is mediated via TLR4 signaling pathways. TLR4 mutant (C3H/HeJ) and wild-type (C3H/HeouJ) mice were injected with 125 μg (5 mg/kg) of LPS or saline into the peritoneal cavity. A: hepatic Cyp3a11/Gapdh mRNA expression as measured by densitometry. B: Cyp3a11 enzyme activity 24 h after IP injection of LPS. Each bar represents the mean ± SE of 4 mice. *Cyp3a11 expression or activity was lower compared with the saline-treated C3H/HeouJ mice (P < 0.05). †Cyp3a11 expression in the C3H/HeJ mice was different compared with the respective saline-treated or LPS-treated C3H/HeouJ mice (P < 0.05, ANOVA followed by Tukey’s post hoc test).
The existence of a pathway to activate a peripheral acute phase response during infections of the CNS was supported by our laboratory's previously established rat model of CNS infection or inflammation. We observed that centrally administered TNF-α response in the brain and liver.

The lack of the TNF-α response in C3H/HeJ mice after the ICV injection of LPS. First, it was clear from the peripheral inflammation study that LPS could reduce Cyp3a11 mRNA and triazolam hydroxylation in C3H/HeouJ mice below basal levels in the C3H/HeJ mice. Second, basal Cyp1a1 expression was similar in C3H/HeouJ and C3H/HeJ (Fig. 8) mice but was only depressed by LPS in the C3H/HeouJ mice. Finally, hyporesponsiveness to LPS in the TLR4-mutant mice was confirmed by the lack of a TNF-α response in the brain and liver.

The lack of the TNF-α response in C3H/HeJ mice after the ICV administration of LPS indicated the absence of a CNS inflammatory response. However, we could not conclude that clinically used drugs in humans (1). The present results were not restricted to Cyp3a11 expression as similar losses in hepatic Cyp1a1 expression were generated by the imposed experimental conditions (Fig. 8). The extent (50–70%) of hepatic cytochrome P-450 downregulation in the mouse was similar to the loss in hepatic cytochromes P-450 that occurred in our laboratory's previously established rat model of CNS infection/inflammation (14, 31, 41).

The existence of a pathway to activate a peripheral acute phase response during infections of the CNS was supported by the previous observations that inflammatory cytokine levels in the periphery were elevated and the hepatic acute phase response was stimulated (8, 15, 48, 51). This undetermined signaling pathway has been proposed to regulate drug metabolism in the liver during CNS infections or inflammation (41, 45). The HPA axis is activated by cytokines during the acute phase response and may have a suppressive role against the reduction of ethoxysresorufin O-deethylase (Cyp1a1), pen- toxysresorufin O-depentylyase (Cyp2b), imipramine N-demethylase (Cyp2d), and erythromycin N-demethylase (Cyp3a11) metabolism after ICV injection of LPS to rats (9, 45, 48). In the present study, adrenalectomy prevented the elevation of plasma corticosterone but did not protect or enhance the LPS-evoked depression in hepatic Cyp3a11 expression and activity after LPS administration. This confirms that the HPA axis plays no role in hepatic Cyp3a11 downregulation in the mouse model of CNS infection or inflammation. Others have examined the stimulation of the sympathetic nervous system or transduction of TNF-α, IL-1β, and IFN-γ from the brain to the periphery, but neither of these pathways could explain the link between CNS infection/inflammation and the loss in hepatic cytochrome P-450 enzyme activity in rats (31, 39, 45). Consistent with the findings of Nicholson et al. (31), in the present study, centrally administered TNF-α did not reduce Cyp3a11 expression in C3H/HeouJ or C3H/HeJ mice, suggesting that CNS-derived TNF-α is not the major factor involved in down-regulation of hepatic Cyp3a11 after the ICV injection of LPS. IL-6 could also contribute to Cyp3a11 downregulation. However, LPS decreased hepatic Cyp3a11 expression by 80% in both IL-6-expressing and IL-6-null mice and suggested against a major role for that cytokine for the downregulation of Cyp3a11 expression by LPS (46).

LPS has been identified as a specific ligand of TLR4 (6, 36). Signaling through the LPS receptor (TLR4) is an additional pathway that could initiate the reduction in hepatic drug metabolism in the model of CNS inflammation. We observed that the TLR4 mutants (C3H/HeJ mice) were totally resistant to the LPS-mediated loss in hepatic Cyp3a11 expression and activity that was observed in mice that express functional TLR4. Basal levels of Cyp3a11 mRNA expression and activity in C3H/HeouJ or C3H/HeJ mice, suggesting that C3H/HeouJ mice from that of a fast metabolizer to that of a slow metabolizer of triazolam. Several of our findings also support that C3H/HeJ mice are resistant to LPS-mediated reductions in Cyp3a11 expression and activity because of inhibition of signal transduction through the TLR4 and not because of reduced basal expression of that cytochrome P-450 gene. First, it was clear from the peripheral inflammation study that LPS could reduce Cyp3a11 mRNA and triazolam hydroxylation in C3H/HeouJ mice below basal levels in the C3H/HeJ mice. Second, basal Cyp1a1 expression was similar in C3H/HeouJ and C3H/HeJ (Fig. 8) mice but was only depressed by LPS in the C3H/HeouJ mice. Finally, hyporesponsiveness to LPS in the TLR4-mutant mice was confirmed by the lack of a TNF-α response in the brain and liver.

The lack of the TNF-α response in C3H/HeJ mice after the ICV administration of LPS indicated the absence of a CNS inflammatory response. However, we could not conclude that...
blockade of the CNS inflammatory response was the only factor that protected the TLR4 mutant mice from the loss in hepatic Cyp3a11 because TLR4 are also present on peripheral macrophages and hepatocytes and could still elicit an immune response to E. coli LPS (4, 21). This idea of a peripheral inflammatory response is supported by the observation that liver TNF-α mRNA and serum levels of TNF-α and IL-1β were elevated by ICV administered LPS. If LPS was secreted into the blood from the cerebral spinal fluid, then a resulting peripheral inflammatory response could explain the decrease in hepatic Cyp3a11 metabolism.

Several studies have suggested that LPS was restricted to the CNS when it was injected into the lateral ventricle of the brain (41, 45). In contrast, the present observations provide convincing evidence that LPS is not restricted to the CNS after central administration and is transferred to the blood in significant quantities. Similar quantities of LPS were detected in serum, and a similar loss in hepatic Cyp3a11 expression and activity was observed after the intraperitoneal administration of LPS (2.5 µg). The systemic bioavailability, as approximated by AUC, was similar after both routes of administration. These results indicated that, after ICV administration, the amount of LPS reaching the systemic circulation is large enough to alter hepatic cytochrome P-450 solely by actions in peripheral tissues. Thus a transfer of LPS from the CNS may have lead to the previous observation of reduced hepatic cytochrome P-450 after ICV administration of LPS to rats (41, 45).

E. coli LPS (0127:B8, 200 µg) injected ICV to rats increased blood-brain barrier permeability between 4 and 16 h after the injection (18). Changes in blood-brain barrier permeability could result in LPS leakage into the peripheral circulation. However, our experimental results suggested that peak LPS transfer (between 1 and 2 h) from the ventricles occurred on a time scale that preceded the opening of the blood-brain barrier. The majority of cerebral spinal fluid is resorbed by bulk flow into the systemic circulation via transfer from the subarachnoid vili into the superior sagittal venous sinus (7). The bulk flow mechanism begins to return other blood-brain barrier-impermeable substances (albumin and leptin) to the systemic circulation within minutes of their injection by the ICV route (7, 24). Thus the bulk flow reabsorption of cerebral spinal fluid is likely the more important mechanism for LPS transfer to the peripheral circulation as opposed to passage across the blood-brain barrier.

The decrease in hepatic Cyp3a11 expression and activity in response to circulating levels of LPS also appears to involve TLR4, as the intraperitoneal injection of LPS had no effect in TLR4-mutant mice. Others have shown that C3H/HeJ mice were resistant to LPS downregulation of hepatic ethoxycoumarin dealkylase (Cyp2a) and ethoxyresorufin dealkylase (Cyp1a2) enzyme activities 24 h after LPS was administered intraperitoneally, but this work was conducted before the identification of TLR4 as a critical component of the LPS signal transduction pathway (13, 44). Also, in a model of indomethacin-induced intestinal injury, TLR4 activation by LPS released from intestinal flora may contribute to downregulation of hepatic Cyp3a11, Cyp1a1/2, and Cyp2d9 enzyme activities (25). Together, these results directly support the idea that peripheral TLR4 are involved in the regulation of hepatic cytochrome P-450 enzymes by peripherally administered LPS. TLR4-mediated signaling occurs through two intracellular pathways (47). The MyD88-dependent pathway results in the direct induction of inflammatory cytokines (e.g., TNF, IL-1β, and IL-6). The MyD88-independent pathway activates NF-kB with delayed kinetics as well as the interferon response factor-3, which upregulates the expression of interferon-inducible genes. Although not determined in the present study, experiments in MyD88-deficient and interferon response factor-3-deficient mice should reveal which of these two pathways is most important for Cyp3a11 downregulation by LPS.

Our findings have led to a putative model of how CNS infection could lead to the loss of cytochrome P-450 expression and activity in the liver, as shown in Fig. 9. LPS injected within the lateral ventricle produces TLR4-dependent CNS inflammation. The localized inflammation is likely important for the loss in cytochrome P-450 that occurs in the brain (41) but is not the major factor that depresses cytochrome P-450.
metabolism in the liver. We propose that centrally administered LPS is rapidly transferred from the cerebral spinal fluid to the blood. Through a TLR4-dependent mechanism, circulating LPS then reduces hepatic Cyp3a11 mRNA expression and enzyme activity. The loss of hepatic cytochrome P-450 produced by systemic LPS could occur indirectly through the action of circulating cytokines such as TNF-α, IL-1β, and IL-6 that are released from a peripheral source (e.g., macrophages or Kupffer cells) (12, 13, 28, 31, 39, 44). Those cytokines activate nuclear transcription factors within the hepatocyte, which regulate cytochrome P-450 expression in the liver (2, 3, 17, 19, 29, 33, 38). Despite the direct link between cytokines and cytochrome P-450 expression, large doses of LPS (1–5 mg/kg) were able to mediate hepatic Cyp3a11 downregulation in vivo in mice with targeted disruptions in IL-6 and the TNF-α receptor (46, 50). This could be explained by the observations that LPS stimulates NF-κB signaling in isolated hepatocytes through TLR4 present on those cells (21, 49). Thus the direct action of LPS on the hepatocyte represents a potential mechanism for Cyp3a11 downregulation in the liver.

In summary, in the presence of a CNS infection, the transfer of endotoxin from the brain to the periphery could stimulate a peripheral immune response that produces a loss in cytochrome P-450-mediated drug metabolism in the liver. We have also identified that stimulation of TLR4 is responsible for linking the immune response to E. coli endotoxin with a major drug metabolizing cytochrome P-450 in the mouse liver. The reduction in cytochrome P-450 enzymes during inflammatory responses or infections of the CNS may reduce the metabolism and elimination of several clinically used drugs and increase the potential for adverse drug interactions.

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