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Role of cytokines in the lipopolysaccharide-evoked depression of cytochrome P450 in the brain and liver☆

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Abstract

A role for cytokines as mediators of the depression in cytochrome P450 activity in brain and liver during CNS inflammation is proposed. Lipopolysaccharide (LPS) was given directly into the lateral ventricle of the brain to mimic a localized CNS infection. CYP1A activity and protein in both brain and liver were depressed in response to this treatment. The administration of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ) directly into the lateral ventricle emulated the effects of LPS on CYP1A activity only in the brain. In contrast, these centrally administered cytokines did not produce a concomitant loss of CYP1A activity in the liver. Significant levels of several cytokines (TNF-α, IL-1β, and IFN-γ) were produced in the serum of animals following intracerebroventricular (i.c.v.) administration of LPS. This production of peripheral cytokines by LPS could not be mimicked by the i.c.v. injection of IL-1β or TNF-α. These results suggest that induction of cytokines in the brain may play a direct role in the depression of CYP1A activity in the CNS following the administration of LPS into the lateral ventricle. The production of cytokines within the brain does not appear to participate in the signaling process in the brain that leads to the concomitant loss of CYP1A activity in the liver. The subsequent production of cytokines in peripheral tissues, however, does appear to play a role in the loss of cytochrome P450 in the liver.

Keywords: Cytochrome P450; CNS; Inflammation; Lipopolysaccharide; Cytokines; Liver

1. Introduction

Cytochrome P450 enzymes are known for their role in the metabolism of a vast array of endogenous and exogenous compounds. Although the majority of isoforms reside in the liver, they are known to occur in extrahepatic tissues such as the adrenal glands, lungs, kidneys, skin, and brain [1]. Within the CNS, expression of specific cytochrome P450 isoforms tends to be low, unequally distributed, and regionally localized [1–4]. The localization of enzymes in the brain parenchyma and in circumventricular organs is thought to contribute to a number of important functions, including the local synthesis of neurosteroids and participation in blood vessel integrity, and potentially could play a role in chemical toxicity [5,6].

Hepatic cytochrome P450 and drug biotransformation are susceptible to modulation by viral and/or bacterial infections via the activation of an immune or inflammatory response and subsequent generation of cytokines [7–9]. Recently, a similar response was described for the effects of inflammation on cytochrome P450 in the CNS [10,11]. Immune activation is a much more subtle process in the CNS than in the periphery. Immune activation caused by agents such as the bacterial endotoxin LPS results in microglial activation and the recruitment of monocytes from the periphery into the brain [12]. These macrophages, microglia, and astrocytes become activated and release a cascade of inflammatory cytokines including TNF-α, IL-1β, and IL-6, all of which mediate the subsequent immune response in the CNS [13]. In addition to the release of cytokines, the metabolism of arachidonic acid is stimulated

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Abbreviations: LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL, interleukin; IFN, interferon; i.c.v., intracereboventricular(ly); EROD, ethoxyresorufin *O*-deethylase; PFA, paraformaldehyde; hsp27, heat shock protein 27; PB, phosphate buffer; dLPS, detoxified LPS; HPA, hypothalamic-pituitary-adrenal axis; and iNOS, inducible nitric oxide synthase.

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and the expression of iNOS occurs [14,15]. We recently demonstrated that central inflammation induced by the i.c.v. administration of LPS not only depressed CYP1A activity in the brain but also produced a concomitant loss of the enzyme in the liver [10,11]. The mechanisms involved in the loss of cytochrome P450 in the brain and liver in response to CNS inflammation remain unclear.

Many neuro-degenerative disorders such as Parkinson's or Alzheimer's disease are thought to involve a chronic inflammatory component within the brain [16,17]. We have hypothesized that such conditions may produce cytokines in response to CNS inflammation and alter the level of cytochrome P450 activity and/or expression within the brain and liver. This may result in aberrant drug handling or alteration of the production or degradation of endogenous compounds. In this paper, we examine the role of cytokines in the loss of cytochrome P450 in brain and liver during an inflammatory response confined to the brain.

2. Materials and methods

2.1. Reagents

TNF-α and IL-1β were purchased from Cedarlane Laboratories Inc.; the remaining cytokines were purchased from R&D Systems. *Escherichia coli* LPS (serotype: 0127:B8) and all other reagents except those noted below were purchased from the Sigma-Aldrich Chemical Co.

2.2. Animals and treatment

Male Sprague–Dawley rats (125–150 g), obtained from Charles River Laboratories, were housed on clay chips in a 12-hr light/dark cycle, allowed free access to food and water, and acclimatized to the facility for a period of 5 days prior to use. Animals were anesthetized with enflurane (4%) throughout the surgical period. Intracerebroventricular injections were carried out using a KOPF stereotaxic frame with injection into the lateral ventricle using the coordinates 1.5 mm lateral from bregma and 4.7 mm below the skull surface. LPS or cytokines were dissolved in sterile endotoxin-free saline and injected in a 5 μL volume. The doses of cytokines utilized were based on those reported to cause effects in the CNS [18–20]. After recovering from the anesthetic, animals were allowed free access to water and food. A 24-hr treatment period was selected as it has been reported that LPS can induce an inflammatory response within this time and the blood–brain barrier is still relatively intact [21].

2.3. Tissue isolation and enzyme activity

Following the 24-hr recovery period, animals were killed by asphyxiation in a carbon dioxide chamber, and the brains were rapidly dissected out and rinsed in ice-cold KCl (1.15%). Each whole brain was homogenized in buffer (0.1 M Tris, pH 7.4, containing 0.1 mM dithiothreitol, 0.1 mM EDTA, 1.15% KCl, 0.1 mM phenylmethylsuphonyl fluoride, 22 μM butylated hydroxytoluene, and 10% glycerol) using a glass–teflon homogenizer and then centrifuged at 3000 *g* for 10 min at 4° [22]. The supernatant was removed and centrifuged at 100,000 *g* for 40 min at 4°, and the resulting pellet was resuspended in 400 μL of brain buffer supplemented with 20% glycerol. The preparation of these membrane fractions allowed the determination of enzymatic activity in individual rats. This fraction was comprised of mitochondria and microsomes, both of which contain CYP1A isoforms were homogenized using a mini-glass/PTFE homogenizer and then were stored at -80^{o}.

The livers also were removed, and microsomes were prepared as described elsewhere [23]. Briefly, the tissue was homogenized using a Polytron homogenizer and then centrifuged at 10,000 *g* for 10 min at 4°. The supernatant was removed and centrifuged at 100,000 *g* for 40 min at 4°. The pellet was resuspended (50%, w/v) in glycerol buffer (50 mM KH_2PO_4, 20% glycerol, and 0.4% KCl) and homogenized in a glass homogenizer. Aliquots of the liver microsomes were stored at -80^{o}.

Protein levels of tissue fractions were determined by the method of Lowry *et al.* [24]. CYP1A activity was assessed by the EROD assay, as described by Burke *et al.* [25]; this assay measures both CYP1A1 and CYP1A2 activity. In all cases, EROD activity was normalized to the amount of protein present in the sample. Control EROD activity levels tended to vary between experiments; therefore, separate control animals were used for each drug tested to ensure that the effects seen were due solely to the drug under investigation.

2.4. Western blot analysis

Each sample contained 37.5 μg of protein and was separated by electrophoresis on a 10% ready gel under non-reducing conditions [26]. The resulting bands were transferred electrophoretically to an Immobilon P membrane using a wet transfer running overnight at 40 V (Bio-Rad). Protein bands were detected immunologically according to standard procedures [27]. CYP1A1 and 1A2 protein bands were detected using a polyclonal anti-rat CYP1A1 antibody (1/1000 dilution) (Gentest) that cross-reacts with CYP1A2. Bands were visualized using an anti-goat IgG antibody conjugated to peroxidase (1/100 000) and Supersignal Ultra chemiluminescent substrate (Pierce). Band intensities were measured using the Molecular Analyst™ program (Bio-Rad).

2.5. Cytokine determination

Following injection with LPS, or cytokines, levels of TNF-α, IL-1β, and IFN-γ in the blood were measured using Quantikine® cytokine assay kits (R&D Systems). The pro-

cedure was followed as outlined in the instructions of the manufacturer. Serum was isolated from blood samples taken from treated rats at 2, 4, 6, 12, and 24 hr following i.c.v. injection. Samples were determined in duplicate, and the absorbance was read at 450 nm. Cytokine levels are reported as the average concentration (ng/mL) in two animals per treatment at each time point. The limit of detection for each cytokine assay was 5 pg/mL for TNF-α and IL-1β and 10 pg/mL for IFN-γ.

2.6. Immunohistochemistry

Following the 24-hr treatment period, animals were deeply anesthetized with pentobarbital (65 mg/mL) and then perfused transcardiacally with 120 mL of 0.9% saline followed by 60 mL of 4% PFA. Whole brains were removed and left in 4% PFA for 48 hr at 4° and then were cryopreserved in 30% sucrose at 4° until completely submerged Coronal sections (40 μm) were cut on a freezing microtome and stored in 1× Millonig's solution until processed.

Heat shock protein 27 (hsp27) staining was carried out according to Garcion *et al.* [28]. Sections were washed with PB (0.1 M, pH 7.4) and then were treated with 3% hydrogen peroxide for 30 min. The sections were washed again in PB and then incubated overnight at 4° with a rabbit anti-mouse hsp25 primary antibody (diluted 1/5000 in 2% goat serum) that cross-reacts with rat hsp27 (StressGen Biotechnologies). Following treatment with primary antibody, sections were washed and then were incubated with secondary antibody (biotinylated goat anti-rabbit IgG) diluted 1/400 in PB for 1 hr at room temperature. All sections were simultaneously washed, reacted with Advidin Biotin solution for 60 min at room temperature, and then reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB) simultaneously to ensure identical exposure times. Sections were washed with PB prior to mounting on glass slides. Omission of the primary antibody was used to check the specificity of the staining.

Staining for microglia was carried out in a manner similar to that described above with the following modifications. Sections were permeabilized in 0.3% hydrogen peroxide for 30 min and then were blocked with 2% horse serum in PB for 30 min at room temperature. The primary antibody, diluted 1/10,000, was an anti-rat CD11b/c (OX42) antibody made in mice (Cedarlane Laboratories Inc.). Brain sections were incubated with this antibody for 48 hr at 4° in PB with 2% horse serum and then were washed with PB. Sections were incubated with secondary antibody (biotinylated horse anti-mouse IgG, rat absorbed) (Vector Laboratories) diluted 1/400 with PB, overnight at 4°. The remainder of the processing was carried out as described above.

2.7. Statistical analysis

Data are reported as means ± SEM. Comparisons of two treatment groups were carried out using an unpaired Student's *t*-test. Statistical significance was defined as having a P value < 0.05.

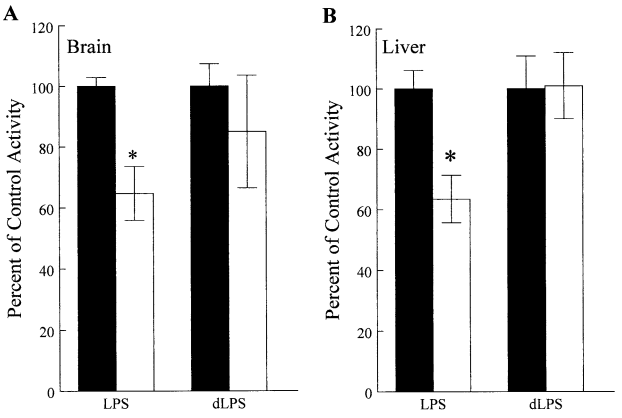

Fig. 1. Effects of LPS and dLPS on CYP1A activity. Twenty five micrograms of LPS or dLPS was given as a single dose, and the activities were examined 24 hr later. The solid bars represent the saline controls, and the open bars, the treated animals (LPS or dLPS). (A) EROD activity in brain for the saline controls = 0.34 ± 0.01 and 0.27 ± 0.02 pmol/mg protein/min in the LPS and dLPS experiments, respectively. (B) EROD activities in liver for the saline control = 57.6 3.5 and 96.1 ± 10.5 pmol/mg protein/min in the LPS and dLPS experiments, respectively. The comparisons of LPS to the saline control and of dLPS to the corresponding saline control were carried out in two separate experiments. Values are means ± SEM, N = 4. Key: (*) significantly different with respect to the corresponding saline control ($P < 0.05$).

3. Results

3.1. Effect of LPS on CYP1A activity in the brain and liver

The injection of LPS (25 μg) into the lateral ventricle evoked a significant decrease in CYP1A activity in whole brain membrane fractions, as shown in Fig. 1A. EROD activity, a specific measure of CYP1A activity, was depressed by 35% following a 24-hr exposure to the bacterial endotoxin. Western blot analysis indicated a significant decrease in CYP1A protein in the brains of treated animals compared with the controls (Fig. 2A). dLPS cannot elicit an immune response, as the lipid A portion of the molecule has been stripped off and thus cannot activate CD14 receptors on immunocompetent cells [29]. When administered into the lateral ventricle, dLPS had no significant effect on brain EROD activity (Fig. 1A).

Liver microsomes obtained from these animals were examined for alterations in the activity and expression of hepatic CYP1A2. EROD activity was decreased significantly (36%) 24 hr after the injection of LPS into the lateral ventricle (Fig. 1B). Western blot analysis of these microsomes demonstrated that hepatic CYP1A2 protein levels were decreased significantly (by 69%) in response to an i.c.v. injection of LPS (Fig. 2B). EROD activity in the liver

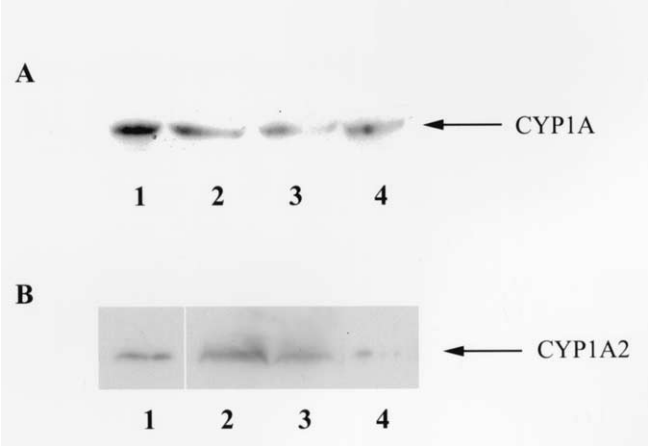

Fig. 2. Effect of LPS given by i.c.v. injection on CYP1A protein in brain and liver tissue. In these representative western blots, each lane contains 37.5 μg of tissue from each rat. (A) Lanes 1 and 2: brain tissue from control animals; lanes 3 and 4: brain tissue from animals treated with LPS (i.c.v.). CYP1A apoprotein levels in brain were decreased significantly in treated animals (642 $\pm$ 116 vs 293.8 $\pm$ 8.0 arbitrary units for saline and LPS, respectively). (B) Lanes 1 and 2: liver tissues from saline controls; lanes 3 and 4: liver tissues from LPS-treated animals. CYP1A2 apoprotein levels in liver were significantly different in animals that received LPS by i.c.v. injection (426.5 $\pm$ 51.0 vs 131.2 $\pm$ 57.6 arbitrary units for saline and LPS treated, respectively) (N = 4; $P < 0.05$).

of animals treated with dLPS remained unchanged following the 24-hr treatment period (Fig. 1B).

3.2. Effect of cytokines on CYP1A activity in brain and liver

The inflammatory cytokines TNF-α, IL-1β, and IFN-γ were examined as potential mediators of the LPS-induced depression of CYP1A activity. TNF-α (5 ng) injected i.c.v. decreased the level of EROD activity in brain by 27% but there was no concomitant effect on EROD activity in the liver (Fig. 3, A and B). Similarly, a 44% decrease in brain EROD activity occurred in response to 2.5 ng of IL-1β administered by i.c.v. injection with no concomitant effect on liver EROD activity (Fig. 3, A and B). In addition, no difference in EROD activity in the liver was seen when a dose of 50 ng IL-1β was injected into the lateral ventricle (data not shown). IFN-γ injected into the lateral ventricle resulted in an 88% decrease in brain EROD activity, again with no effect in liver (Fig. 3, A and B). CYP1A protein levels in brain were depressed by i.c.v. injection of IFN-γ, as measured by western blotting (Fig. 4). In contrast to the results obtained with the other three cytokines, IL-1α and IL-6, which are also released in response to LPS, had no effect on either brain or liver EROD activity when administered into the lateral ventricle (Table 1).

The sensitivity of the liver to cytokines was demonstrated by administering the cytokines by i.p. injection. Liver EROD activity was sensitive to exogenously administered cytokines. Animals treated i.p. with either 0.4 μg of

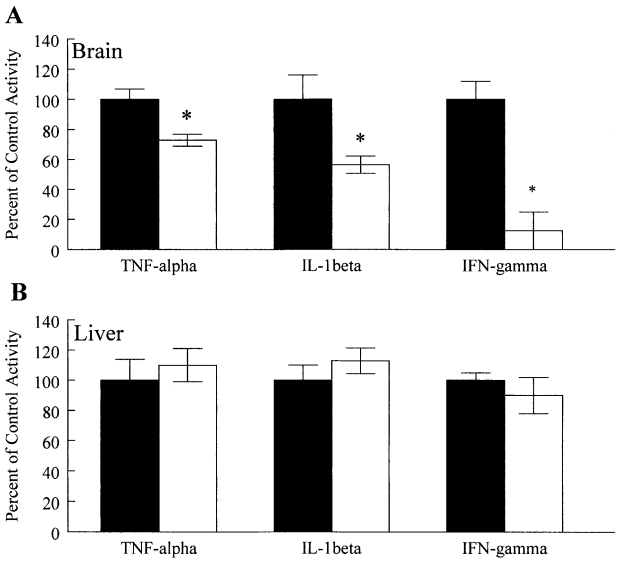

Fig. 3. Effect of cytokines administered by i.c.v. injection on CYP1A activity in the brain and liver. The solid bars represent the control animals, and the open bars, the treated animals. (A) The saline control value for EROD activity in the brain for the TNF-α (5 ng) experiment = 0.24 $\pm$ 0.02 pmol/mg protein/min. In animals treated with a 2.5 ng dose of IL-1β, the control value = 0.69 $\pm$ 0.11 pmol/mg protein/min. An i.c.v. injection of 5 ng of IFN-γ gave an EROD activity = 0.03 $\pm$ 0.004 pmol/mg protein/min for the saline control. (B) Hepatic EROD activity was 97.5 $\pm$ 9.6 pmol/mg protein/min for the TNF-α control, 84.9 $\pm$ 6.4 pmol/mg protein/min for the IL-1β control, and 43.7 $\pm$ 2.1 pmol/mg protein/min for the IFN-γ control. The activity data for TNF-α (i.c.v. injection) have been reported previously [11]. Values are means $\pm$ SEM, N = 4. Key: (*) significantly different with respect to the corresponding saline control ($P < 0.05$).

TNF-α or 50 ng of IFN-γ displayed a 43 and 36% decrease in liver EROD, respectively. The enzyme activity in the brains of these animals remained unchanged (Table 2). In contrast, IL-1β had no effect on EROD activity in either liver or brain at the dose examined (Table 2).

3.3. Expression of hsp27 in the brain following LPS treatment

The occurrence of an immune/inflammatory response in the CNS during these experiments was demonstrated by examining brain sections for markers of inflammation. Robust staining for hsp27 was observed in the hippocampus

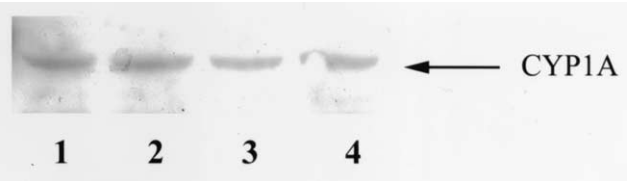

Fig. 4. Effect of cytokines given by i.c.v. injection on CYP1A protein in the brain. Lanes 1 and 2: saline-treated animals; lanes 3 and 4: animals that received 5 ng of IFN-γ. These representative samples (of several experiments) suggest a decrease in brain CYP1A protein following injection of IFN-γ.

Table 1
Effects of other acute phase cytokines given i.c.v. on CYP1A activity in brain and liver

Treatment	Tissue	CYP1A activity (pmol/mg protein/min)
Saline, i.c.v.	Brain	0.3 ± 0.07
5 ng IL-1α, i.c.v.		0.2 ± 0.01
Saline, i.c.v.	Liver	161 ± 5.0
5 ng IL-1α, i.c.v.		211 ± 17.8
PBS, i.c.v.	Brain	0.1 ± 0.02
20 ng IL-6, i.c.v.		0.1 ± 0.02
PBS, i.c.v.	Liver	117 ± 12.3
20 ng IL-6, i.c.v.		111 ± 7.7

Values are means ± SEM, N = 4.

and surrounding the ventricles on both the ipsilateral and contralateral sides of LPS-treated animals (Fig. 5, C and D). In contrast, no expression of hsp27 was observed in saline-treated animals (Fig. 5, A and B). In a similar manner, animals treated with an i.c.v. injection of TNF-α had no detectable levels of hsp27 staining (Fig. 5, E and F). Minimal patterns of staining were observed in animals treated with i.c.v. injections of IL-1α, IL-1β, or IL-6 (data not shown). The negative controls (absence of the primary antibody) did not contain any staining.

3.4. Activation of microglia

Robust staining for microglia was observed throughout the thalamic area, adjacent to the ventricles, and in the hippocampus of LPS-treated animals (Fig. 6, C and D). In the saline-treated controls (Fig. 6, A and B), some staining could be observed around the ventricles and in the hippocampus, but to a much lesser extent than observed in LPS-treated animals. In response to an i.c.v. injection of TNF-α, microglia were observed in the thalamic area, surrounding the ventricles and in the hippocampus, although to a much lesser extent than seen in LPS-treated animals (Fig. 6, E and F). In contrast, animals treated with IL-1α, IL-1β, or IL-6 demonstrated little or no staining (data not shown). The negative control did not contain any detectable staining.

Table 2
Effects of cytokines on levels of CYP1A activity in brain and liver

Treatment	Tissue	CYP1A activity (pmol/mg protein/min)
Saline, i.p.	Brain	0.4 ± 0.06
0.4 μg TNF-α, i.p.[a]		0.3 ± 0.03
Saline, i.p.	Liver	129 ± 11.2
0.4 μg TNF-α, i.p.[a]		75 ± 11.7*
Saline, i.p.	Brain	0.4 ± 0.03
0.1 μg IL-1β, i.p.		0.4 ± 0.06
Saline, i.p.	Liver	159 ± 21.4
0.1 μg IL-1β, i.p.		162 ± 18.9
Saline, i.p.	Brain	0.3 ± 0.03
50 ng IFN-γ, i.p.		0.3 ± 0.02
Saline, i.p.	Liver	103 ± 3.8
50 ng IFN-γ, i.p.		66 ± 7.4*

Values are means ± SEM, N = 4.
[a] TNF-α results were taken from Renton and Nicholson [11].
* Significant with respect to the saline control ($p < 0.05$).

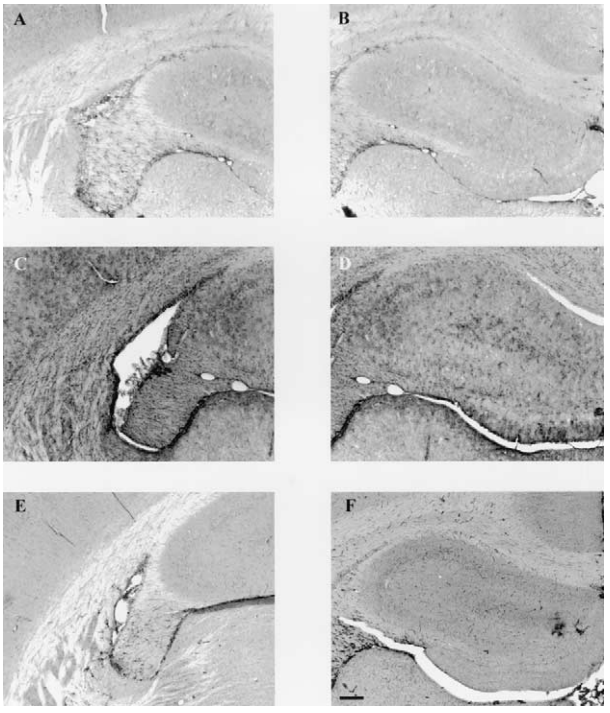

Fig. 5. Effect of LPS or cytokines on hsp27 expression in the brain. Coronal sections were stained with a primary antibody targeted against murine hsp25. Robust staining was seen in the hippocampus and surrounding the lateral ventricles of LPS-treated animals (C and D, respectively), whereas saline controls had virtually no staining in either area (A and B). Animals treated i.c.v. with 5 ng of TNF-α had no detectable staining in the cortex and the hippocampus (E and F). Scale bar = 200 μm.

3.5. Peripheral cytokine levels following i.c.v. LPS administration

Cytokine levels in serum at various times following the i.c.v. administration of saline, LPS, TNF-α, and IL-1β are shown in Fig. 7. The levels of TNF-α, IL-1β, IFN-γ, and IL-6 were minimal over a 24-hr time period in animals receiving an i.c.v. injection of saline. Substantial levels of all four cytokines occurred in blood in response to an i.c.v. injection of LPS. The profiles of cytokine release generally showed a peak in release between 2 and 4 hr, with levels dropping off to near undetectable levels by 12 hr. The levels of these four cytokines in animals injected i.c.v. with either TNF-α or IL-1β were similar to those observed in saline-treated animals.

4. Discussion

Systemic administration of LPS differentially depresses the activity of a variety of hepatic cytochrome P450 iso-

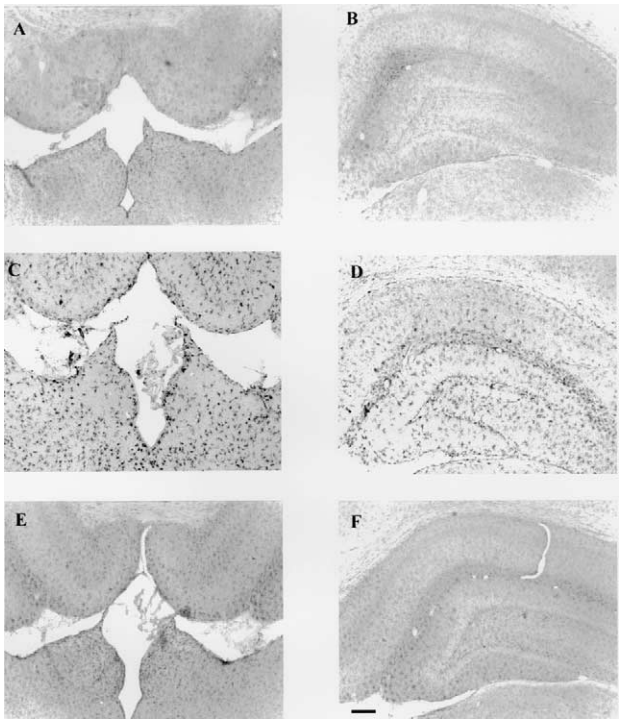

Fig. 6. Effect of a 24-hr exposure to LPS or cytokines on microglial expression throughout the brain. Saline controls showed some staining surrounding the ventricles and through the hippocampus (A and B, respectively). In contrast, LPS-treated animals demonstrated a high level of microglial activity throughout the hippocampus, and around the ventricles (C and D, respectively). TNF-α showed a modest amount of microglial staining in the hippocampus and surrounding the ventricles (E and F). Scale bar = 200 μm.

forms [30–37]. In 1986, Ghezzi *et al.* [8] used an LPS-resistant mouse strain (C3H/HeJ) to show that serum from LPS-responsive animals depresses hepatic ethoxycoumarin deethylase activity in these C3H/HeJ animals. This provided strong evidence that a serum factor was mediating the effects of LPS on hepatic cytochrome P450 activity. In addition, the injection of IL-1 could depress hepatic cytochrome P450 activity in these LPS-resistant mice. Subsequently, others have demonstrated that systemic administration of cytokines such as TNF-α, IL-1β, IL-1α, IFNs, and to some extent IL-6 could differentially depress levels of hepatic cytochrome P450 activity [8,32–36]. Since one of the primary actions of LPS *in vivo* is the release of cytokines via the activation of immunocompetent cells, these effects are not surprising.

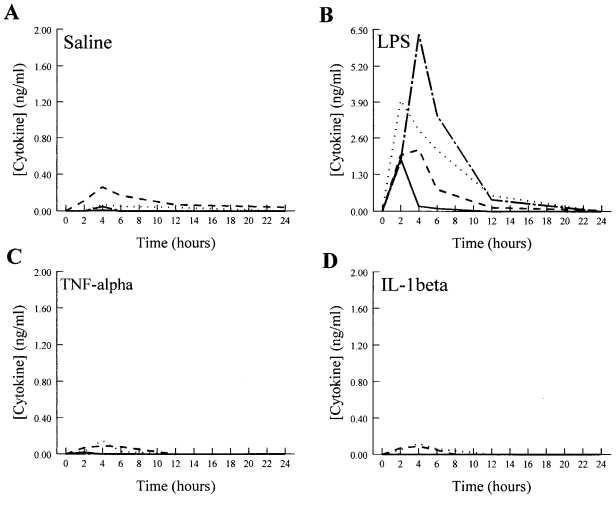

Fig. 7. Time course for the appearance of TNF-α, IL-1β, IFN-γ, and IL-6 in serum samples of animals treated with an i.c.v. injection of saline, LPS, TNF-α, or IL-1β. Each time period represents the mean value from two animals. Key: (——) TNF-α; (—-) IL-1β; (-·-·-) IFN-γ; and (·····) IL-6.

Over the 24-hr study period used in the present experiments, LPS injected into the lateral ventricle of the brain significantly depressed CYP1A activity in the CNS. CYP1A protein levels in the brain were decreased by the i.c.v. injection of LPS, implicating a pre-translational mechanism of enzymatic inhibition. These observations are in agreement with earlier reports from this laboratory where a decrease in CYP1A staining was seen in brain sections of animals treated with an i.c.v. injection of LPS [10]. The experiments described here attempted, in part, to determine whether cytokines could mediate this LPS-induced decrease in CYP1A activity within the CNS. When injected into the lateral ventricle, LPS activates immunocompetent cells within the brain parenchyma to release pro-inflammatory cytokines [38]. LPS induces the cytokine cascade by promoting the synthesis and release of TNF-α, which activates the synthesis of IL-1 by binding to its cellular receptor. IL-1, in turn, binds to its cell surface receptor and stimulates the release of IL-6 [39]. These cytokines have a diverse number of actions *in vivo,* including the activation of immune cells, the stimulation of the HPA, and the induction of fever and sickness behaviours [40]. To investigate the possibility that cytokines are responsible for mediating the effects of centrally administered LPS on CYP1A activity, recombinant cytokines were administered by i.c.v. injection, and the effects on CYP1A were assessed. The pro-inflammatory cytokines TNF-α and IL-1β both significantly decreased CYP1A activity in the brain, indicating that they are likely candidates for mediating the effects of LPS. This was not uniformly observed with all acute phase or pro-inflammatory cytokines as neither IL-1α nor IL-6 had any effect on brain CYP1A activity when administered by i.c.v. injection. Previous work using *in vitro* models has shown that IL-6 is capable of depressing the activity of various cytochrome P450 isoforms in hepatocytes [41–43]. However, when administered *in vivo,* IL-6 has a variety of different effects, depending on the isoform examined [33,44]. In our experiments, IL-6 did not affect brain CYP1A activity when injected i.c.v., indicating that this cytokine is not directly involved in the effects of LPS observed in this model. Somewhat surprising was the observation that IL-1α did not have any effect on CNS CYP1A activity, as the biological activities of both IL-1 isoforms are mediated through the same receptor and Il-1β depresses CYP1A activity significantly [45]. However, it has been reported previously that

the relative potencies of these two isoforms of IL-1 are not the same but depend upon various factors such as the end-point measured.

IFN-γ, an anti-viral cytokine, is released in response to LPS and has effects on hepatic cytochrome P450 when administered systemically [46,47]. Injection of this cytokine into the lateral ventricle also resulted in a substantial loss of CYP1A activity in the brain and a reduction in protein expression. IFN-γ is one of the primary mediators released in response to viral infections, and thus could alter local drug handling during a CNS infection.

A more surprising result from this work and that of others [11,48] is that LPS given i.c.v. can depress levels of CYP1A2 activity in peripheral organs such as the liver. Examination of protein levels revealed that the depression of CYP1A2 activity seen in liver resulted from a down-regulation of CYP1A2 protein. Similar results obtained by Shimamoto *et al.* [48] found that hepatic CYP1A2 activity and protein are decreased following LPS administration in the brain. There are several possible mechanisms by which centrally administered LPS can depress CYP1A2 activity in the liver: through centrally produced cytokines, activation of the HPA, sympathetic nerve stimulation, or peripherally produced cytokines. It was postulated that the effects of centrally administered LPS on hepatic CYP1A activity might result from the local induction of cytokine synthesis and release. However, we have now shown that when hepatic CYP1A2 activity was assessed in animals that received centrally administered cytokines, no alterations in enzymatic activity were seen for any of the cytokines examined at the dosages used. It was initially thought that higher doses of cytokines may be needed to mimic the effects of LPS on CYP1A2 activity in the liver. However, when a high dose of IL-1β (50 ng) was injected into the lateral ventricle, no effect was seen on CYP1A2 activity in the liver. The inability of these pro-inflammatory cytokines to have an effect on hepatic CYP1A2 activity when administered into the CNS suggests that local production of cytokines within the brain does not trigger a signaling pathway involved in the down-regulation of CYP1A2 activity, as seen with LPS.

Shimamoto *et al.* [49] demonstrated that activation of the HPA does not mediate the effects of an i.c.v. injection of LPS on cytochrome P450 in the liver as adrenalectomy failed to prevent this effect. In fact, they found that adrenalectomy depressed levels of cytochrome P450 even further than seen with sham-operated animals that were given LPS by i.c.v. injection. This led them to suggest that perhaps it was peripherally released cytokines that may be involved in this effect. It has also been proposed that activation of the sympathetic nervous system may mediate the effect of centrally administered LPS on cytochrome P450 activity in liver. However, experiments by Shimamoto *et al.* [49] showed an independence of this effect from sympathetic stimulation, as surgical or chemical sympathectomized animals responded to centrally administered LPS by decreasing hepatic cytochrome P450 activity in a manner similar to that of the controls. In addition, our laboratory has shown that the loss of hepatic CYP1A activity resulting from an i.c.v. injection of LPS is not reversed by blocking β-receptors with propranolol [11]. Work by De Luigi *et al.* [50] suggests that the sympathetic nervous system tonically inhibits the peripheral release of cytokines, as sympathectomy increased the production of cytokines in response to centrally administered LPS. This increase in peripheral cytokine production following sympathectomy may account for the inability to prevent the decrease in CYP1A2 activity in the liver. These reports, along with the results presented here, support the idea that peripherally derived cytokines might be crucial for mediating the effects of an i.c.v. injection of LPS on CYP1A2 activity in the liver.

Animals treated with LPS (i.c.v.) had high concentrations of the pro-inflammatory cytokines in peripheral blood. Blood levels of TNF-α, IL-1β, IFN-γ, and IL-6 were observed in the first few hours after LPS administration. We demonstrated that peripherally derived cytokines are capable of altering hepatic CYP1A2 activity by showing that exogenously administered cytokines, given at doses lower than those measured in blood samples from animals treated with LPS (i.c.v.), can depress hepatic CYP1A2 activity. This confirms observations first made by others [8,30,35, 51]. From these results, it appears that the induction of peripheral cytokine release is consistent with the idea that cytokines may mediate the effects of centrally administered LPS on hepatic CYP1A activity.

The induction of an immune response has classically been considered a requirement for the LPS-induced decrease of cytochrome P450 activity [8,52]. Immunohistochemical studies reported here demonstrate that i.c.v. LPS induces a robust expression of hsp27 in the brain, a classical marker of stress [53]. In addition, LPS induced the activation of numerous microglia in the hippocampus, ventricles, and thalamus. These results did not seem to be mediated through the production of cytokines TNF-α, IL-1β, IL-1α, and IL-6 as the injection of these cytokines neither induced the expression of hsp27 nor activated microglia. A previous report [54] concerning the effects of cytokines on hsp27 indicated that they do not induce levels of hsp27 but alter its phosphorylation state, which is consistent with the results described here. It is possible that the global immune response mediated by LPS may be necessary for the decrease in CYP1A activity in liver. This is supported by results from these experiments as cytokines injected i.c.v. could not mimic the immune activation in brain as assessed by immunohistochemistry, nor could they decrease CYP1A activity in the liver. In addition to cytokines, LPS is known to activate other immunological mediators such as the arachidonic acid metabolites and iNOS, both of which could potentially participate in mediating this brain-to-liver response [14,15]. Although the induction of cytokines in the periphery seems to play an important role in this effect, it

should be recognized that they are not the only mediators of this effect.

In conclusion, we have demonstrated that cytokines participate in the down-regulation of CYP1A activity in the brain in response to inflammation within the CNS. In contrast, the concomitant modulation of cytochrome P450 in the liver during a CNS inflammatory response is not mediated by a pathway involving centrally produced cytokines. However, the subsequent induction of cytokines outside the brain likely participates in the depression of this enzyme in peripheral organs. It is most likely that the effects on CYP1A activity in liver are not mediated by one system alone but result from a complex interplay between the HPA, the nervous system, and generation of peripheral immune mediators.

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