

# Downregulation of *mdr1a* expression in the brain and liver during CNS inflammation alters the *in vivo* disposition of digoxin

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**1** Inflammation is a pathophysiological event that has relevance for altered drug disposition in humans. Two functions of P-glycoprotein (P-gp) are hepatic drug elimination and prevention of drug entry into the central nervous system (CNS). Our objective was to investigate if localized CNS inflammation induced by *Escherichia coli* lipopolysaccharide (LPS) would modify *mdr1a*/P-gp expression and function in the brain and liver.

**2** Our major finding was that the CNS inflammation in male rats produced a loss in the expression of *mdr1a* mRNA in the brain and liver that was maximal 6 h after intracranial ventricle (i.c.v.) administration of LPS. When <sup>3</sup>H-digoxin was used at discrete time points, as a probe for P-gp function *in vivo*, an increase in brain and liver <sup>3</sup>H-radioactivity and plasma level of parent digoxin was produced 6 and 24 h following LPS treatment compared to the saline controls. Digoxin disposition was similarly altered in *mdr1a*<sup>+/+</sup> mice but not in *mdr1a*<sup>-/-</sup> mice 24 h after administering LPS i.c.v.

**3** In male rats, the biliary elimination of parent digoxin was reduced at 24 h (60%) and 48 h (40%) after LPS treatment and was blocked by the P-gp substrate cyclosporin A. An observed loss in CYP3A1/2 protein and organic anion transporting polypeptide 2 mRNA in the liver may make a minor contribution to digoxin elimination in male rats after LPS treatment.

**4** Conditions which impose inflammation in the CNS produce dynamic changes in *mdr1a*/P-gp expression/function that may alter hepatic drug elimination and the movement of drugs between the brain and the periphery. The use of experimental models of brain inflammation may provide novel insight into the regulation of P-gp function in that organ.

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**Keywords:** P-glycoprotein; *mdr1a*; organic anion transporting polypeptide (*oatp2*); CNS inflammation; drug elimination; drug transport; digoxin.

**Abbreviations:** Cl, clearance; CNS, central nervous system; CSA, cyclosporin A; CYP, cytochrome P450, HSP25, heat shock protein 25; i.c.v., intracranial ventricle; i.p., intraperitoneal; LPS, lipopolysaccharide; *mdr*, multidrug resistance; *Oatp*, organic anion transporting polypeptide; P-gp, P-glycoprotein

## Introduction

P-glycoprotein (P-gp) encoded by the gene *MDR1* in humans and *mdr1a* and *mdr1b* in rodents is expressed normally in epithelial cells of several organs including brain, liver, intestine, kidney, adrenal gland and testes (Gottesman & Pastan, 1993). The protein products of *mdr1a* and *mdr1b* play a key role in disposition and excretion of a wide array of clinically used drugs and provides protection against exposure to toxic xenobiotics (Mayer *et al.*, 1996; Schinkel *et al.*, 1997; Johnson *et al.*, 2001). In rodents, *mdr1a* is the primary multidrug resistance (*mdr*) transcript expressed in the brain (Croop *et al.*, 1989). P-gp in the choroid plexus and microvessel endothelial cells contributes to the blood–cerebral spinal fluid and the blood–brain drug permeability barriers, respectively, and prevent accumulation of several drugs (e.g. digoxin, dexamethasone, cyclosporin A (CSA), loperamide and antineoplastics) in the central nervous system (CNS) (Schinkel *et al.*, 1994, 1995; Beaulieu *et al.*, 1997; Kawahara *et al.*, 1999; Rao *et al.*, 1999). In the periphery, P-gp expression in the intestinal epithelia reduces the systemic

absorption of several drugs (e.g. digoxin and CSA and vinblastine) and contributes to excretion of P-gp substrates from the blood (Mayer *et al.*, 1996; Schinkel *et al.*, 1997; van Asperen *et al.*, 2000). In the renal proximal tubule and in the canalicular membrane of hepatocytes, P-gp is important for the secretion of some drugs into the urine and bile, respectively (van Asperen *et al.*, 2000; Ando *et al.*, 2001; Tsuruoka *et al.*, 2001). Conditions that impair P-gp-mediated drug transfer in the peripheral organs and the blood–brain barrier or choroid plexus may lead to decreased elimination, or altered entry of a number of clinically important drugs into the CNS.

When host defense mechanisms are activated by the administration of lipopolysaccharide (LPS), inflammatory cytokines or turpentine to rodents, the expression of *mdr1a* mRNA in the liver is diminished and is followed by a reduction in the levels of P-gp expression (Piquette-Miller *et al.*, 1998; Hartmann *et al.*, 2001). *In vivo* functional studies have demonstrated that the systemic administration of *Klebsiella pneumoniae* endotoxin decreases hepatic elimination of the P-gp substrate rhodamine123 (Ando *et al.*, 2001). The effects of inflammation on hepatic *mdr1b* expression remain controversial. For instance, it has been shown that *mdr1b* mRNA

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is depressed by peripheral inflammation (Piquette-Miller *et al.*, 1998; Hartmann *et al.*, 2001), whereas other groups have shown an increase in hepatic *mdr1b* expression in response to inflammation (Vos *et al.*, 1998; Ando *et al.*, 2001). Nonetheless, the majority of findings support the idea that a depression of P-gp expression or function in the liver occurs in response to systemic inflammation. In addition to drug transport, it is well known that another hepatic drug-handling system, cytochrome P450 (CYP)-mediated biotransformation, is diminished during inflammatory responses (Renton, 2000; Morgan, 2001). Not only are CYP systems in the liver modulated during systemic inflammation, but they are also significantly changed when inflammation is confined to the brain (Renton & Nicholson, 2000; Nicholson & Renton, 2001). It remains to be determined if inflammation confined to the CNS modulates hepatic drug elimination through effects on *mdr1a*/P-gp expression in that organ.

Systemic inflammation caused a minor reduction in P-gp in mouse brain membrane preparations and increased brain/plasma ratio of the P-gp substrate doxorubicin without major histopathological changes to the blood – brain barrier (Zhao *et al.*, 2002). These findings suggest that P-gp function in the blood – brain barrier may be minimally impaired during systemic inflammation. However, it is possible that a localized inflammation in the brain may be required to induce more profound changes in brain expressed *mdr1a* and P-gp function. It is known that neuroinflammatory diseases can change the permeability characteristics of the blood – brain barrier (de Vries *et al.*, 1997). P-gp is an important determinant of the blood – brain barrier permeability to drugs, but the effects of neuroinflammation on the brain expression of *mdr1a* and P-gp function remain to be determined.

In the present study, we investigated if a localized CNS inflammation induced by *Escherichia coli* LPS will down-regulate P-gp function and/or *mdr1a* expression in the brain and liver. As the distribution of the heart glycoside, digoxin into the brain and several peripheral tissues is strongly affected by *mdr1a*/P-gp activity (Schinkel *et al.*, 1995; Mayer *et al.*, 1996; Kawahara *et al.*, 1999), we have chosen digoxin as a prototype drug to examine P-gp function during CNS inflammation. The use of experimental models of brain inflammation may provide insight into the regulation of P-gp function in that organ and may lead to novel approaches that facilitate drug delivery in the treatment of CNS disorders.

## Methods

### *Animal preparation and production of CNS inflammation*

All experimental procedures involving the use of animals have been approved by the Dalhousie University Committee on Laboratory Animals according to the guidelines established by the Canadian Council on Animal Care. Male or female Sprague – Dawley rats (175 – 200 g) obtained from Charles River Laboratories (Wilmington, MA, U.S.A.) or male FVB *mdr1a*<sup>+/+</sup> (wild-type) and *mdr1a*<sup>-/-</sup> mice (5 – 6 weeks age) obtained from Taconic Farms (Germantown, NY, U.S.A.) were housed in plastic cages, lined with clay chips with free access to Purina rat chow and water. Animals were allowed to acclimatize in our animal holding facilities for 1 – 2 weeks before use. On the starting day of the experiment, rats (250 –

300 g) and mice (25 – 30 g) were anaesthetized with enflurane (3 – 4%). CNS inflammation was generated by infusion of 25 µg in 5 µl of saline (rats) and 2.5 µg in 2.5 µl of saline (mice) of *E. coli* LPS (serotype 0127 : B8) into the left lateral cerebral ventricle at a rate of 2.5 µl min<sup>-1</sup> using a Harvard Apparatus syringe pump. Control rats and mice were injected with 5 and 2.5 µl of saline (0.9%), respectively. The coordinates (in mm) for the stereotaxic injection were –4.5 D/V, –1.5 L and –1.0 A/P from the bregma for the rat and –2.5 D/V, –1.0 L and –0.2 A/P from the bregma in mice (Paxinos & Watson, 1986; Franklin & Paxinos, 1997). There are several stereotypes of LPS and each may possess a different potency. We have titrated our intracranial ventricle (i.c.v.) dose of *E. coli* endotoxin (serotype 0127 : B8) to 25 µg in order to generate a reproducible nonlethal inflammatory response that is restricted to the CNS and reduces hepatic CYP metabolic activity (Nicholson & Renton, 2001; Renton & Nicholson, 2000). At 24 h after i.c.v. administration of LPS (25 µg) to rats, a loss (50%) in hepatic CYP1A1/2 metabolic activity is observed (Nicholson & Renton, 2001; Renton & Nicholson, 2000). The i.c.v. administration of LPS (2.5 µg) to wild-type FVB mice produced a similar reduction (50%) in the rate CYP1A1/2 metabolism in hepatic microsomes compared to rats and validated the use of this dosage of LPS for our studies in mice.

### *Immunohistochemistry*

The methods for immunohistochemical detection of heat-shock protein (hsp25) in rat brain have been reported in detail (Renton & Nicholson, 2000). Briefly, following transcardial perfusion with ice-cold 4% paraformaldehyde, brains were dissected and cryopreserved in 30% sucrose. Primary rabbit anti-mouse hsp25 (1 : 2000 dilution) (Stressgene Biotechnologies, Victoria, Canada) was reacted with 30 µm coronal brain sections followed by incubation with biotinylated goat anti-rabbit IgG (1 : 5000), incubation with avidin – biotin solution and staining with DAB reagent. Biotinylated IgG, ABC and DAB reagents were obtained from Vector Laboratories (Burlingame, CA, U.S.A.).

### *RT-PCR analysis of mRNA*

Total RNA was extracted from livers and brain of male rats using the Amersham QuickPrep™ RNA extraction kit (Amersham, Piscataway, NJ, U.S.A.) following manufacturer's instructions. A semiquantitative RT-PCR assay was utilized based on previously published methods (Hartmann *et al.*, 2001). Reverse transcription (RT) of 0.5 µg RNA for rat liver or 2.5 µg RNA for rat brain was performed using the First-Strand cDNA Synthesis Kit from MBI Fermentas (Flamborough, ON, U.S.A.) in a total volume of 20 µl following manufacturer's instructions. Standard PCR curves with sequential dilutions of RT products were generated to establish linearity and to determine optimal template concentrations. A volume of 1 µl of RT product was then used for amplification of specific DNA sequences in the presence of 1 mM organic anion transporting polypeptide 2 (*oatp2*), 4 mM (*mdr1a*) and 1 mM (*mdr1b*) MgCl<sub>2</sub>, 200 µM dNTP and 50 pmol of each primer in a total volume of 100 µl using a GeneAmp™ 2400 Thermocycler (Perkin-Elmer, Mississauga, ON, Canada). The reaction was initiated by addition of 2.5 U of *Taq* polymerase (MBI Fermentas) and amplification proceeded

through 30 cycles (*mdr1a* and *mdr1b*) or 26 cycles (*oatp2*). The following primer sequences were used for amplification: *mdr1a*, forward primer 5'-GAC GGA ATT GAT AAT GTG GAC A-3', reverse primer 5'-AAG GAT CAG GAA CAA TAA A-3' (nt 2022–2372 in GenBank #AF286167). *Mdr1b*, forward primer 5'-GAA ATA ATG CTT ATG AAT CCC AAA G-3', reverse primer 5'-GGT TTC ATG GTC GTC GTC TCT TGA-3' (nt 2012–2337 in GenBank L16546). *Oatp2*, forward primer 5'-TTG GTG TTG GAT GTG CAG TT-3', reverse primer 5'-GCC AAT GGT CAT TCC TGT TT-3' (nt 484–833 in GenBank #U95011). The temperature steps for each PCR cycle were as follows: for *mdr1a* or *mdr1b*, 45 s at 94°C, 30 s at 55°C, 1 min 30 s at 72°C and for *oatp2*, 1 min at 94°C, 2 min at 55°C, 2 min at 72°C. The PCR products were separated by electrophoresis on 2% agarose gels, stained with SYBR Gold™ nucleic acid stain (Molecular Probes, Eugene, OR, U.S.A.) and visualized by UV and automatic detection. Size of DNA bands (approximately 350 bp for each gene product) was confirmed using Gene Ruler™ 100 bp DNA ladder from MBI Fermentas. The optical densities of PCR products were quantitated using a DC120 camera and DS1D Scientific Imaging software (Eastman Kodak) and were normalized to 18S ribosomal RNA (rRNA) band intensities. For 18S quantification 10 µg of total RNA were heated at 65°C for 10 min, then electrophoresed on denaturing gels, stained with SYBR Green™ II RNA stain (Molecular Probes, Eugene, OR, U.S.A.), visualized and quantified as above. Location of 18S rRNA was confirmed using high-range RNA ladder (0.24–9.5 kb) from Fermentas.

### *<sup>3</sup>H-digoxin tissue distribution*

<sup>3</sup>H-digoxin was used as a probe substrate to evaluate P-gp function *in vivo* 6, 24 or 48 h following the central administration of LPS. Animals were killed by decapitation at 2, 4, 8 or 16 h after intraperitoneal (i.p.) administration of <sup>3</sup>H-digoxin (0.5 mg kg<sup>-1</sup>) followed by collection of plasma and tissues for total <sup>3</sup>H-radioactivity measurements. In initial experiments, plasma radioactivity was maximally elevated in the LPS- versus the saline-treated rats at the 2 h time point (data not shown) and was chosen as the duration for our subsequent <sup>3</sup>H-digoxin disposition analyses. Tissues were weighed, rinsed and homogenized in phosphate-buffered saline (pH 7.4) with a Polytron homogenizer. Total volume of the homogenate was measured to determine mass of tissue/ml<sup>-1</sup> of suspension. A volume of 100 µl aliquots of each whole tissue homogenate or 200 µl of brain homogenate (in duplicate) were added to 6 ml of Beckman ready-safe scintillation cocktail and counted for total disintegrations per minute (DPM) using a Beckman LS5000TA scintillation counter (Beckman instruments, Fullerton, CA, U.S.A.). Similar procedures were used for digoxin disposition analysis that was performed in FVB wild-type or *mdr1a*<sup>-/-</sup> mice 24 h after i.c.v. injection of LPS.

### *Bile and intestinal elimination of <sup>3</sup>H-digoxin*

Saline- and LPS-treated male rats were pair fed for bile elimination studies based on the average amount of food normally eaten by LPS-treated rats. Rats were fed 2.5 and 12.5 g of food on the day of and day after i.c.v. LPS or saline treatment. At 24 or 48 h after i.c.v. injection of LPS, rats were anaesthetized with sodium pentobarbital (60 mg kg<sup>-1</sup> i.p.). A

tracheotomy was performed and animals were allowed to breath spontaneously. Body temperature was maintained at 37°C with a heating lamp. A heparinized arterial-venous loop was inserted from the internal carotid artery to the jugular vein to allow for i.v. <sup>3</sup>H-digoxin administration and arterial blood collection. The common bile duct was cannulated with PE10 polyethylene tubing. After surgical procedures animals were continuously infused with heparin (5 IU) in physiological saline (0.9% w/v) at 5 µl min<sup>-1</sup>. At 10 min after completion of surgery, 0.5 mg kg<sup>-1</sup> of <sup>3</sup>H-digoxin was administered at a rate of 100 µl min<sup>-1</sup> over approximately 2 min into venous side of the arterial-venous loop. At 5 min after completing the <sup>3</sup>H-digoxin infusion, the first of three successive 30 min bile collections was started. Arterial blood samples (120 µl) were drawn directly into capillary pipettes from the arterial-venous loop at the times corresponding to the beginning and end of each bile collection interval (5, 35, 65 and 95 min). Blood was then transferred to a microcentrifuge tube containing 1 IU of heparin and centrifuged immediately to separate the plasma. Aliquots of plasma (50 µl) and bile (20 µl) were counted for total <sup>3</sup>H-radioactivity as described above. At the end of the experiment, the small intestines were removed and flushed with 10 ml of phosphate-buffered saline (pH 7.4) to collect intestinal contents for total radioactivity determination. For the inhibition experiments <sup>3</sup>H-digoxin (0.5 mg kg<sup>-1</sup>) was administered as above followed by a single 30 min control bile collection interval. CSA (10 mg kg<sup>-1</sup>), estrone-3 sulfate (2.5 or 5.0 mg kg<sup>-1</sup>) or vehicle ethanol/saline (60/40%) was administered i.v. at a rate of 25 µl min over approximately 4 min at the start of the second bile collection period.

### *Thin layer chromatography <sup>3</sup>H-digoxin measurements*

Thin layer chromatography (TLC) was performed according to previously published methods that separate digoxin from its major metabolites (Carvalho & Figueira, 1973; Harrison & Gibaldi, 1976). Plasma samples, 750 µl were extracted three times in 2.5 ml of chloroform/methanol (9:1 v/v). The organic layer was evaporated under a stream of N<sub>2</sub> and sample was resuspended in 5 µl of 95% ethanol. Extracted plasma and crude bile samples were streaked on 10 × 2.5 cm<sup>2</sup> silica gel GF plates (Analtech Inc, Newark, DE, U.S.A.) and developed twice in an ethylacetate/chloroform/acetic acid (90:5:5) mobile phase. Controls of standard <sup>3</sup>H-digoxin, resuspended in blank plasma or bile samples were used for comparison. Sections of the plate (0.3–0.5 cm) from the origin to the solvent front were scraped and counted for <sup>3</sup>H-radioactivity. The percentage of DPM associated with parent digoxin was calculated as follows: % <sup>3</sup>H-digoxin = DPM associated with digoxin peak/total DPM recovered from TLC plate.

### *Western blot immunoassay*

Detailed methods for our Western blotting protocol have been previously reported (Renton & Nicholson, 2000). Briefly, 25 µg of hepatic microsomal protein was separated by electrophoresis under nonreducing conditions with a 7.5% SDS-polyacrylamide gel. Proteins were transferred to Immobilon-P membrane. Blots were blocked for 1 h in 5% skim milk followed by incubation with mouse anti-rat CYP3A1/2 monoclonal antibody 2-13-1 (National Institutes of Health, Bethesda, MD, U.S.A.) (1:5000) in 2% skim milk for 1 h at

room temperature and then anti-mouse horseradish peroxidase-conjugated secondary antibody (1:5000) in 5% skim milk for 1 h at room temperature. Immunoreactivity was detected by chemiluminescence (Amersham Pharmacia Biotech, U.K.) and exposure to X-ray film. Band intensities were assessed with Molecular Analyst (Bio-Rad) and expressed as a percentage of the saline control.

#### Data analysis and statistics

Radioactivity in each plasma, tissue and bile sample was recorded as disintegrations per minute (DPM). Background radioactivity (10–20 DPM) was subtracted to measure specific DPM. Tissue  $^3\text{H}$ -radioactivity was expressed as  $\text{DPM mg}^{-1}$  of tissue and plasma and bile  $^3\text{H}$ -radioactivity as  $\text{DPM ml}^{-1}$ . After correction for metabolism, the plasma or bile  $^3\text{H}$ -digoxin was expressed as  $\text{ng ml}^{-1}$ .  $\text{Cl}_{\text{bile}}$  of radioactivity equals the amount of total DPM eliminated in the bile over each time interval divided by the area under the plasma DPM *versus* time curve (AUC) for each time interval. AUC (linear trapezoidal rule) was calculated with the plasma DPM data at the beginning and end of each collection interval. Data are presented as mean  $\pm$  s.e.m. of at least three experiments. A two-tailed unpaired *t*-test or Wilcoxin's rank-sum test (non-parametric data) was used for statistical comparison between two groups. For multiple comparison procedures, an analysis of variance (ANOVA) or Kruskal–Wallis ANOVA on ranks (nonparametric data) was used followed by the Student–Newman–Keuls test for *post hoc* analysis. A difference in mean values with a value of  $P \leq 0.05$  was considered to be significant.

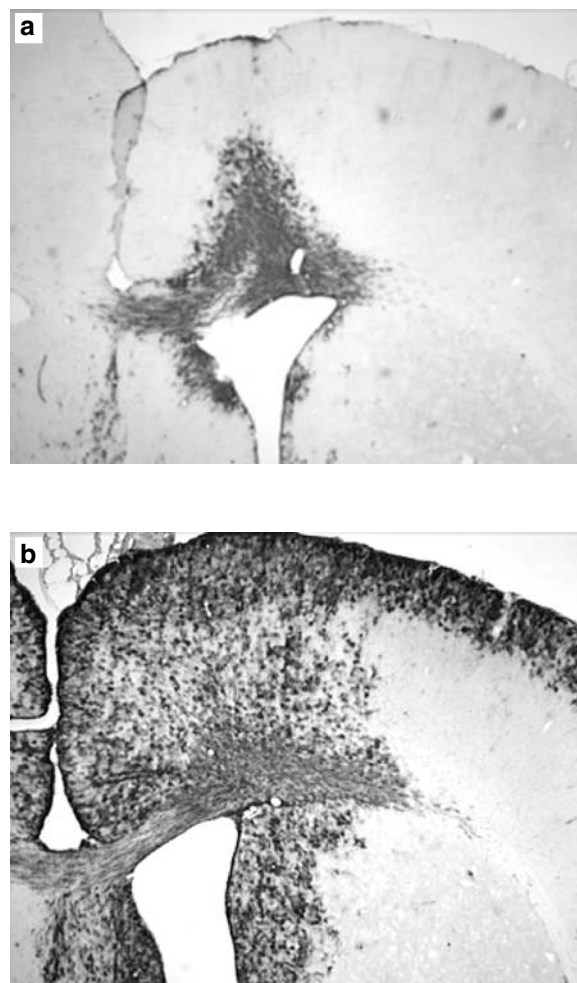
#### Chemicals and reagents

CYP3A1/2 mouse anti-rat monoclonal antibody (#2-13-1) was kindly provided by Dr. H. Gelboin, National Cancer Institute, NIH, (Bethesda, MD, U.S.A.). Unlabeled digoxin and *E. coli* LPS (serotype 0127:B8) were obtained from Sigma, (St Louis, MI, U.S.A.). Enflurane was obtained from Abbott Laboratories, Saint Laurent, Quebec, Canada.  $^3\text{H}$ -digoxin was obtained from Perkin-Elmer (Boston, MA, U.S.A.). CSA was obtained from Sandoz Canada Inc. (Dorval, Quebec, Canada). All other chemicals were of the highest grade available from commercial suppliers.

## Results

#### Induction of CNS inflammation by direct administration of LPS into the lateral ventricle

The characterization of localized CNS inflammation induced by i. c.v. administration of  $25 \mu\text{g}$  of LPS to rats has previously been reported by our laboratory (Nicholson & Renton, 2001; Renton & Nicholson, 2000). Hsp25 immunoreactivity in the brain was used as a marker in the current study to validate the LPS-evoked inflammatory response in the CNS of rats. As shown in Figure 1 hsp25 was extensively expressed throughout the brain 48 h after LPS ( $25 \mu\text{g}$ ) was administered i.c.v. In saline-treated rats only minor responses were detected in the region of the injection needle track. Similar expression was also observed 24 h after LPS administration (data not shown).



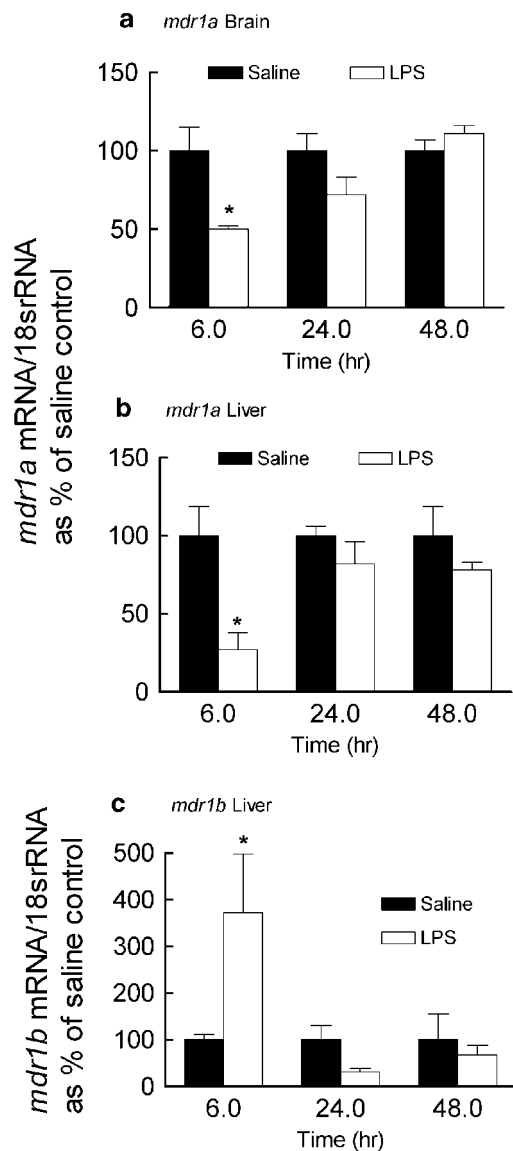
**Figure 1** Representative effect of LPS given directly into the lateral ventricle on the expression of hsp25 in male rat brain. Coronal brain sections ( $30 \mu\text{m}$ ) were exposed to anti-mouse/rat hsp25 antibody 48 h after  $5 \mu\text{l}$  saline (a) or  $25 \mu\text{g}$  of LPS (b) was injected directly into the left lateral ventricle. In all LPS-treated animals the sections demonstrated prolific hsp25 reactivity around the area of the lateral ventricle and the cerebral cortex, whereas in the saline-treated rats minor reactivity was observed only around the needle track and injected ventricle,  $n = 4$ .

#### Effect of CNS inflammation on *mdr1a*, *mdr1b* mRNA expression in the male rat brain and liver

In LPS-treated animals, brain and hepatic *mdr1a* mRNA were rapidly and maximally downregulated (50 and 70%, respectively) compared to saline controls at 6 h and were similar to the saline control at 24 and 48 h (Figure 2a and b). The expression of hepatic *mdr1b* mRNA was induced at 6 h (300% of control) and was not significantly different from the saline time controls at 24 or 48 h following LPS treatment (Figure 2c). We measured *mdr1b* mRNA only in the liver because it is minimally expressed in the brain (Croop *et al.*, 1989).

#### The disposition of $^3\text{H}$ -radioactivity after administration of $^3\text{H}$ -digoxin to rats

The disposition of the P-gp substrate digoxin at three discrete times (6, 24 and 48 h) following i.c.v. injection of LPS or saline is illustrated in Table 1. In rats given LPS for 6 or 24 h, the



**Figure 2** Effect of LPS given directly into the lateral ventricle of the brain on the expression of *mdr1a* (brain and the liver) and *mdr1b* (liver) mRNA. Brains and livers were collected from male rats at 6, 24 and 48 h following LPS (25  $\mu$ g) or saline (5  $\mu$ l) administration into the lateral ventricle. Semiquantitative RT-PCR analysis was performed to determine brain and liver *mdr1a* (a and b) and liver *mdr1b* (c) mRNA levels. For each time point the *mdr1a* or *mdr1b*/18s rRNA ratios are reported as a percentage of the saline control (mean  $\pm$  s.e.m. of three or four separate measurements). \* $P < 0.05$  compared to respective saline control, unpaired *t*-test or Wilcoxon's rank-sum test (*mdr1b* 6 h time point only).

total plasma radioactivity (2 h after the i.p. administration of  $^3\text{H}$ -digoxin) was increased by 300 and 150%, respectively, compared to the saline-treated controls. Significant increases in tissue  $^3\text{H}$ -radioactivity also occurred in the liver, kidney and brain of the LPS-treated (6 and 24 h) rats, but the tissue/plasma ratio of  $^3\text{H}$ -radioactivity for those organs was unchanged. No differences in intestinal content of  $^3\text{H}$ -radioactivity were observed and the intestine/plasma ratio of radioactivity significantly decreased for that organ in LPS-treated rats at 24 h. At 48 h after LPS treatment, total plasma  $^3\text{H}$ -radioactivity remained elevated (160%) but all tissue

$^3\text{H}$ -radioactivity levels were similar to controls. The elevated plasma  $^3\text{H}$ -radioactivity and nonchanging tissue  $^3\text{H}$ -radioactivity level were reflected as decreases in tissue/plasma  $^3\text{H}$ -radioactivity in the brain, liver, kidney and small intestine in LPS-treated relative to the saline control animals.

TLC was used to determine the percentage of radioactivity in the plasma that was associated with parent digoxin (Figure 3a). In male rats, plasma radioactivity was primarily (>70%) associated with the parent compound as identified by comigration with standard digoxin (rf 0.23–0.28) on TLC plates. The % radioactivity recovered as parent drug was identical in saline- and LPS-treated male rats 6, 24 and 48 h after treatment and suggested that altered metabolism was not primarily responsible for the observed increase in plasma radioactivity (Figure 3c). When corrected for metabolism,  $^3\text{H}$ -digoxin concentration (ng ml $^{-1}$ ) in the plasma was significantly elevated in male rats treated with LPS at 6 h (330%), 24 h (175%) and 48 h (180%) compared to the respective saline controls (Figure 3e). To further rule out metabolism as a major contributor to the increase in digoxin plasma concentration observed in LPS-treated male rats, we performed a single digoxin disposition experiment in female rats, which does not appreciably metabolize digoxin (Salphati & Benet, 1999) (Table 2). Changes in digoxin tissue disposition in female rats were similar to that observed in males. In female rats, however, the amount of parent digoxin recovered (percentage of total radioactivity) in the plasma was about 90% (Figure 3d), which is consistent with decreased digoxin metabolism in female rats. At 24 h after LPS treatment, plasma digoxin was elevated (183%) in female, which is similar to that observed in males (Figure 3f).

#### Effect of CNS inflammation on the biliary and intestinal elimination of $^3\text{H}$ -digoxin

The effect of CNS inflammation on  $^3\text{H}$ -digoxin elimination in the bile was determined in anaesthetized male rats 24 or 48 h after i.c.v. LPS or saline administration. Total  $^3\text{H}$ -radioactivity clearance (CI) was decreased 70% after 24 h and 50% after 48 h in the LPS-treated rats compared to the respective saline controls (Figure 4a and b). Representative thin-layer chromatograms for bile samples collected from saline- and LPS-treated male rats are shown in Figure 5a–d. The TLC analysis demonstrated that the majority of the radioactivity in the bile was resolved as digoxin and a primary metabolite peak. Cleavage of digoxin to the *bis*-digitoxide is the first step in metabolism of the parent compound (Carvalho & Figueira, 1973; Salphati & Benet, 1999). Although the precise identity of the metabolite peak was not determined, the major metabolite corresponded to the known rf value of digoxigenin-*bis*-digitoxide (0.4). When corrected for metabolism, the total biliary elimination of digoxin (percentage of original i.v. dose) was reduced by 60% after 24 h LPS-treated rats (Figure 5e).

After 48 h of LPS treatment the biliary elimination of digoxin was reduced by 40%. There was also a reduction (50%) in the major metabolite peak (Figure 5f) compared to the saline control and suggesting that CNS inflammation may also downregulate hepatic metabolism of digoxin. The microsomal levels of CYP3A1/2 protein at 6, 24 and 48 h after LPS or saline was administered i.c.v. was measured using a CYP3A1/2 monoclonal antibody, which produced a single band with a molecular weight of about 55 kDa on Western

Table 1

Effect of direct administration of LPS (25 µg) into the lateral ventricle on the tissue distribution of <sup>3</sup>H-radioactivity after <sup>3</sup>H-digoxin administration to male Sprague–Dawley rats

Tissue	Treatment	6 h	24 h	48 h
<i>DPM g<sup>-1</sup> tissue</i>				
Plasma <sup>a</sup>	Saline	2400 ± 400	1700 ± 300	1700 ± 500
	LPS	7300 ± 1000** (300)	2600 ± 400 (152)	2800 ± 400 (164)
Liver	Saline	18000 ± 3000	14000 ± 1000	12000 ± 3000
	LPS	37000 ± 6000* (206)	20000 ± 3000* (143)	13000 ± 1000 (108)
Brain	Saline	630 ± 60	650 ± 40	520 ± 70
	LPS	1000 ± 100* (160)	890 ± 90* (137)	590 ± 90 (113)
Kidney	Saline	4300 ± 600	3900 ± 400	5300 ± 1000
	LPS	12000 ± 1000** (280)	6100 ± 800* (156)	5900 ± 600 (111)
Small intestine	Saline	33000 ± 9000	15000 ± 2000	18000 ± 4000
	LPS	51000 ± 4000 (155)	14000 ± 1000 (93)	12000 ± 2000 (66)
<i>Tissue/plasma DPM ratio</i>				
Liver	Saline	6.6 ± 1.1	9.0 ± 1.0	8.8 ± 1.1
	LPS	4.3 ± 1.2	9.3 ± 0.8	5.3 ± 0.5**
Brain	Saline	0.22 ± 0.06	0.51 ± 0.1	0.68 ± 0.17
	LPS	0.12 ± 0.01	0.47 ± 0.1	0.25 ± 0.04*
Kidney	Saline	1.4 ± 0.3	2.6 ± 0.3	4.4 ± 1.0
	LPS	1.3 ± 0.2	2.7 ± 0.2	2.7 ± 0.2*
Small intestine	Saline	12.0 ± 4.0	9.2 ± 1.8	15.0 ± 3.6
	LPS	6.0 ± 1.0	4.7 ± 0.7*	5.2 ± 0.9*

LPS (25 µg) or saline (5 µl) was administered directly into the lateral ventricle of male rats and <sup>3</sup>H-digoxin (0.5 mg kg<sup>-1</sup>) was given i.p. 6, 24 or 48 h later. Animals were killed 2 h post digoxin administration for collection of tissues and plasma. <sup>a</sup>Plasma radioactivity is expressed as DPM ml<sup>-1</sup>. Tissue/plasma ratio represents the <sup>3</sup>H-radioactivity g<sup>-1</sup> of tissue divided by the <sup>3</sup>H-radioactivity in 1 ml of plasma. In parentheses is shown the percentage of difference in total radioactivity in LPS- versus saline-treated animals. Data are represented as the mean ± s.e.m. of 4–8 measurements. Each time point (6, 24 or 48 h) was performed as a discrete experiment on separate occasions; thus, within each time period an unpaired two-tailed *t*-test was used to compare LPS-treated rats to the respective saline time controls, \**P* < 0.05

blots (Figure 6a–c). In LPS-treated male rats, hepatic CYP3A1/2 expressed protein was similar at 6 h, and decreased at 24 h (32%) and 48 h (46%) compared to the respective saline controls (Figure 6d).

At the end of the 1.5 h bile CI experiment, the total small intestinal contents were collected and the percentage of intestinal <sup>3</sup>H-radioactivity was determined. Since the common bile duct was ligated all <sup>3</sup>H-radioactivity in the small intestine represents direct intestinal excretion. The total intestinal elimination of <sup>3</sup>H-radioactivity (% of the initial dose) was identical between saline and LPS-treated rats 24 h (5.9 ± 1.1 versus 6.0 ± 0.9) and 48 h (6.7 ± 0.3 versus 7.5 ± 1.0) after induction of CNS inflammation.

#### Effect of CSA on the biliary elimination of digoxin

CSA is an established P-gp substrate that can competitively inhibit the transport of other P-gp substrates (Ando *et al.*, 2001). We performed inhibition experiments with CSA to identify P-g-mediated digoxin transport into the bile. CSA administered at the start of the second bile collection interval significantly reduced the biliary CI of total radioactivity in both saline- and LPS-treated rats compared to the first bile collection period (Figure 7a). TLC analysis of the bile samples indicated that the presence of CSA resulted in a decrease in the percentage of radioactivity in the bile recovered as the parent drug digoxin (Figure 7b) and an increase in the percentage of radioactivity recovered as the metabolite (Figure 7c). When the vehicle (ethanol/saline, 60/40%) was administered no change

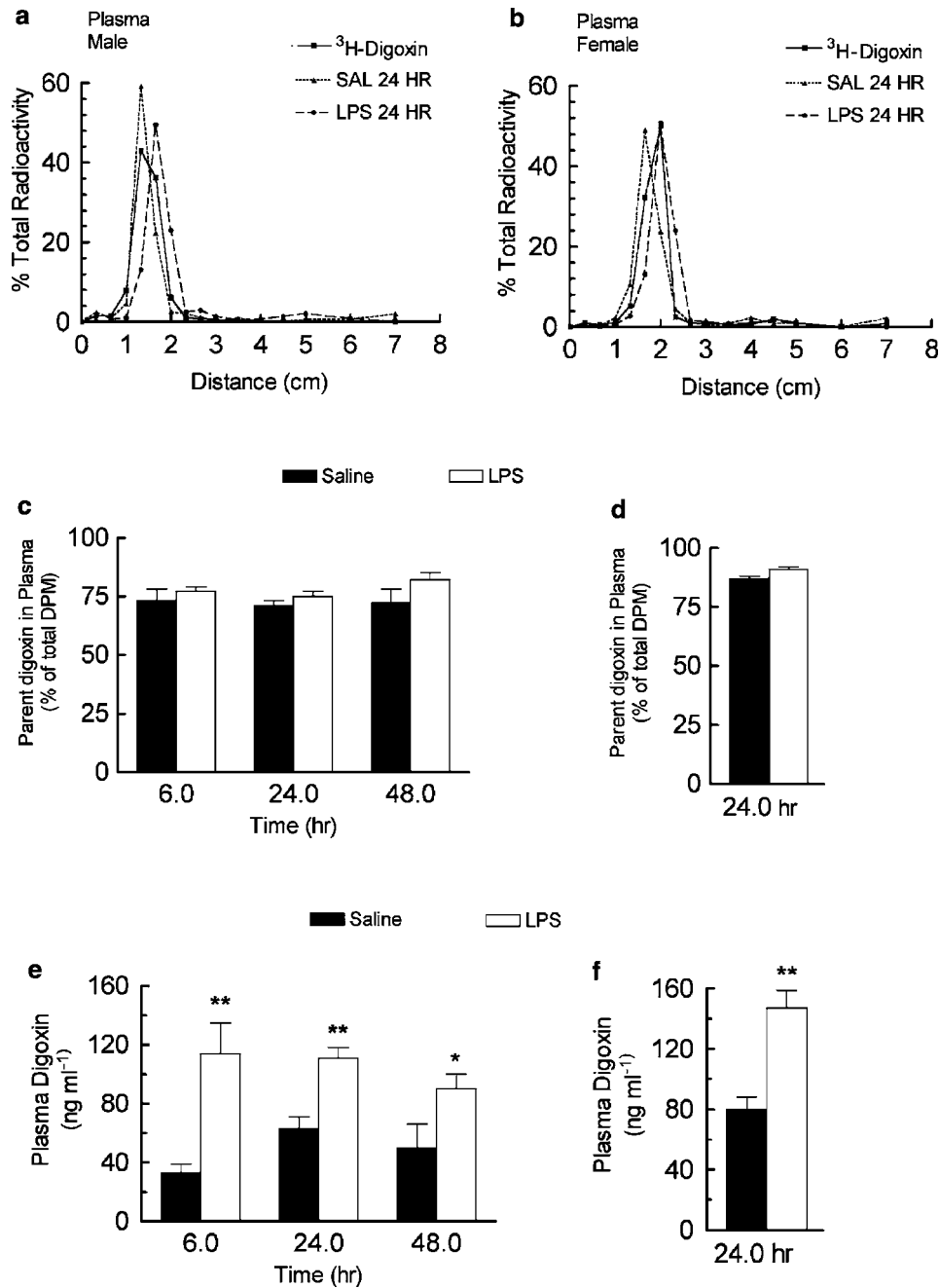
in biliary CI of total <sup>3</sup>H-radioactivity was observed (data not shown).

#### Depression of *oatp2* mRNA levels in response to CNS inflammation

The rat *oatp2* transports digoxin with high affinity *in vitro* ( $K_m = 0.24 \mu\text{M}$ ), mediates digoxin uptake into isolated hepatocytes and it has been hypothesized that *oatp2* may contribute to the first step in hepatic elimination of digoxin and its uptake into the CNS (Noe *et al.*, 1997; Gao *et al.*, 1999; Reichel *et al.*, 1999; Kodawara *et al.*, 2002). Brain *oatp2* mRNA was almost completely abolished 6 h after the induction of inflammation (Figure 8a). At 24 h, brain *oatp2* mRNA partially returned to normal levels and at 48 h was significantly elevated (140%) compared to saline control. In LPS-treated rats, hepatic *oatp2* mRNA was decreased at 6 h and 24 h but was similar to control level at 48 h (Figure 8b). These results suggested that a depression in *oatp2* expression might contribute to altered digoxin elimination in the bile. However, when the *oatp2* substrate estrone 3-sulfate (2.5 or 5.0 mg kg<sup>-1</sup>) was administered instead of CSA no change in biliary CI of total <sup>3</sup>H-radioactivity was observed (Figure 9).

#### Tissue distribution of digoxin in *mdr1a*<sup>-/-</sup> mice

When LPS (2.5 µg) was administered i.c.v. to *mdr1a*<sup>+/+</sup> mice, total radioactivity was significantly increased in plasma (200%), brain (160%) and kidney (230%) 2 h after i.p.



**Figure 3** Effect of LPS given directly into the lateral ventricle of the brain on the plasma concentration of digoxin. LPS (25  $\mu$ g) or saline (5  $\mu$ l) was administered directly into the lateral ventricle of rats and  $^3\text{H}$ -digoxin (0.5 mg kg<sup>-1</sup>) was given i.p. 6, 24 or 48 h (male) or 24 h (female) later. Blood samples were collected 2 h later for determination of plasma radioactivity level. Representative TLC detection of  $^3\text{H}$ -digoxin in plasma extracts, the parent digoxin recovered in the plasma (as a percentage of total radioactivity) and plasma digoxin (ng ml<sup>-1</sup>) are shown, respectively, in (a, c, e) for male and (b, d, f) for female rats. Data are represented as the mean  $\pm$  s.e.m. of four to eight separate measurements.  $^3\text{H}$ -digoxin recovered in standards were  $>95\%$ . \* $P < 0.05$ , \*\* $P < 0.01$  plasma  $^3\text{H}$ -digoxin was significantly higher in LPS compared to the corresponding saline control, unpaired  $t$ -test (male 48 h). Wilcoxon's rank-sum test (male 6, 24 h and female 24 h).

administration of  $^3\text{H}$ -digoxin (0.5 mg kg<sup>-1</sup>) compared to the saline-treated *mdr1a*<sup>+/+</sup> mice (Figure 10). Hepatic total radioactivity was increased (170%) in LPS- versus saline-treated *mdr1a*<sup>+/+</sup> mice. A power calculation indicated that with the observed average standard deviation (11,500 DPM g<sup>-1</sup>), 8 animals per group would be required to detect a statistical difference ( $P < 0.05$ ) between the means of these two groups when the power of the performed test = 80%. In *mdr1a*<sup>-/-</sup> mice

LPS had no effect on plasma, liver, kidney or intestinal radioactivity compared to the saline-treated animals. Interestingly, there was a lower accumulation of radioactivity in the brain of *mdr1a*<sup>-/-</sup> mice treated with LPS compared to the saline controls. The increased tissue and plasma radioactivity observed in *mdr1a*<sup>-/-</sup> mice compared to *mdr1a*<sup>+/+</sup> mice confirms the validity of digoxin as a probe for *mdr1a*-expressed P-gp function *in vivo*.

**Table 2** Effect of direct administration of LPS (25 µg) into the lateral ventricle on the tissue distribution of <sup>3</sup>H-radioactivity after <sup>3</sup>H-digoxin administration to female Sprague–Dawley rats

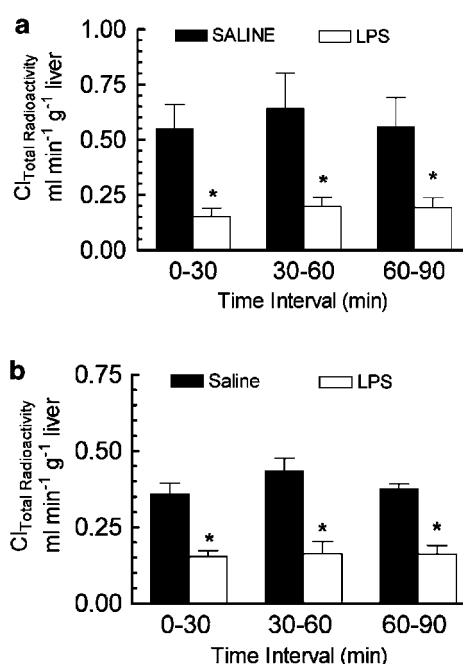
	24 h after LPS administration			
	Saline	Tissue radioactivity (DPM g <sup>-1</sup> tissue) <sup>a</sup> LPS	Tissue/Plasma radioactivity ratio	
Plasma <sup>a</sup>	4400 ± 400	7600 ± 700** (1.73)		
Brain	990 ± 50	1300 ± 90*(1.31)	0.23 ± 0.03	0.18 ± 0.02
Liver	25000 ± 1800	37000 ± 2300**(1.48)	5.8 ± 0.4	5.1 ± 0.6
Kidney	7000 ± 700	12000 ± 500*** (1.71)	1.6 ± 0.1	1.5 ± 0.1
Small intestine	29700 ± 3600	31900 ± 7000 (1.07)	7.1 ± 1.2	4.5 ± 1.4

Female rats were treated as indicated in Table 1 <sup>a</sup>Plasma radioactivity is expressed as DPM ml<sup>-1</sup>. In parentheses are shown the % difference in total radioactivity in LPS *versus* saline-treated animals. Tissue/plasma ratio represents the radioactivity g<sup>-1</sup> of tissue divided by the radioactivity in 1 ml of plasma. Data are represented as the mean ± s.e.m. of 4–5 measurements. \**P* < 0.05 and \*\**P* < 0.01 and \*\*\**P* < 0.001 different compared to the saline-treated rats, unpaired *t*-test.

## Discussion

In experimental models, the central administration of LPS produces a robust inflammatory response in the brain and is characterized by: activation of microglia, astrocytes and proinflammatory cytokine pathways, the release of inflammatory cytokines (TNF- $\alpha$ , IL- $\beta$  and IL-6) and the infiltration of leukocytes into that organ (Perry *et al.*, 1993; Nadeau & Rivest, 2002). Bacterial LPS is the major stimuli for such inflammatory responses in CNS infections caused by Gram-negative meningeal pathogens (Scheld *et al.*, 2002). In addition, the overproduction and release of inflammatory cytokines may contribute to the pathogenesis of traumatic head injury or diseases of the CNS such as multiple sclerosis, HIV dementia, Alzheimer's and Parkinson's disease (de Vries *et al.*, 1997; Gonzalez-Scarano & Baltuch, 1999). During those CNS disorders with an associated inflammatory component, a reduction in brain and hepatic drug P-gp function could greatly increase the risk for harmful drug interactions or toxicity.

We have demonstrated for the first time that inflammation localized to the CNS can alter the hepatic elimination of a known P-gp substrate digoxin. Inhibition of transport-mediated elimination of digoxin by CNS inflammation was supported by our observations that biliary elimination of parent digoxin was depressed in LPS- *versus* saline-treated rats. In the presence of the P-gp inhibitor CSA, the ratio of parent digoxin to metabolite detected in the bile was reduced and provided evidence of P-gp-mediated transport of digoxin into the bile. These results are consistent with the loss of hepatic *mdr1a* mRNA expression as detected by RT-PCR and support the hypothesis that reduced P-gp expression/function contributes to reduced biliary elimination and increased plasma digoxin in rats with CNS inflammation. The induction of hepatic *mdr1b* mRNA does not correlate with decreased digoxin elimination and indicated that this change did not appreciably contribute to the biliary elimination of digoxin. Other studies have also shown that the distribution and elimination of digoxin is not substantially altered in the absence of *mdr1b* (Mayer *et al.*, 1996; Schinkel *et al.*, 1997). Interestingly, CSA reduced <sup>3</sup>H-radioactivity CI to lower levels in rats with CNS inflammation. Thus, drug interactions involving P-gp may be more profound during episodes of inflamma-

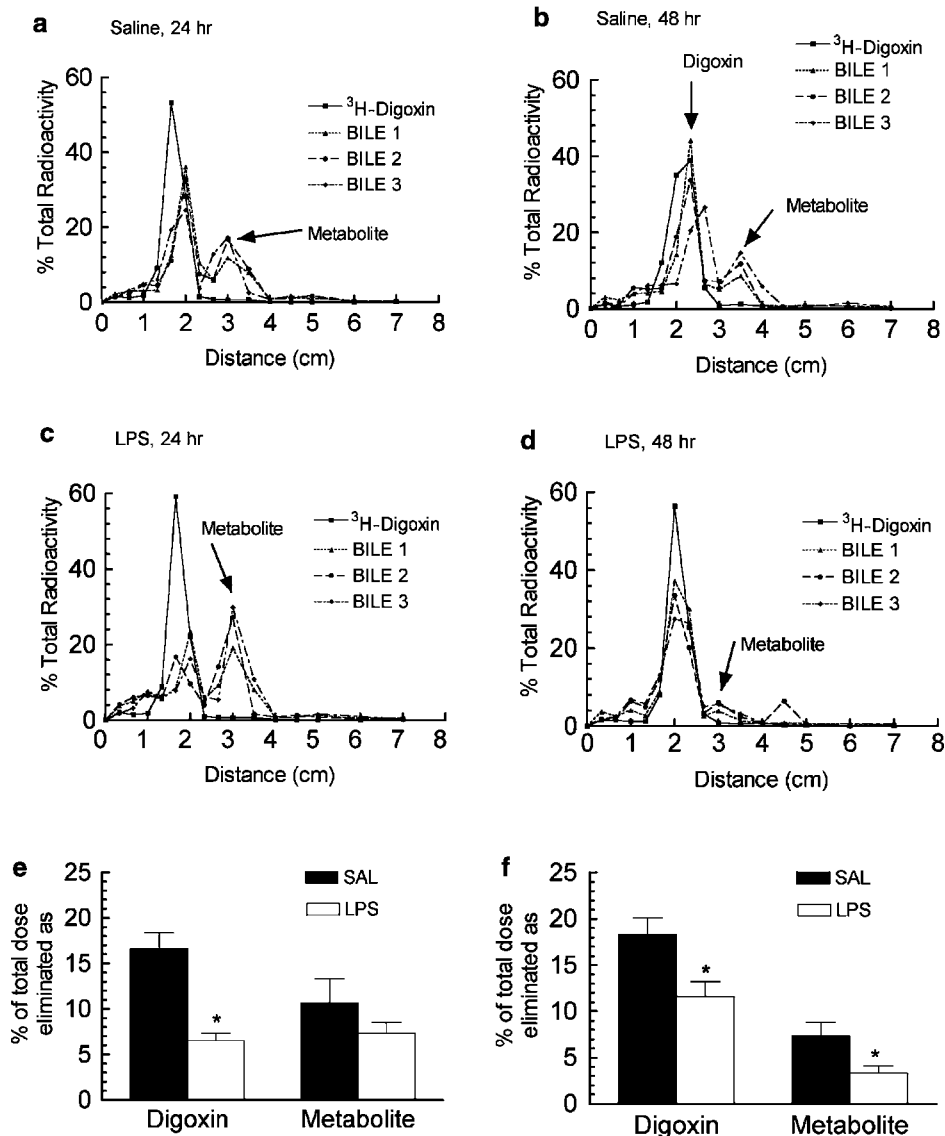


**Figure 4** Effect of LPS administered directly into the lateral ventricle on the biliary CI of <sup>3</sup>H-radioactivity after <sup>3</sup>H-digoxin administration. Male rats were given a single i.c.v. injection of LPS (25 µg) or saline (5 µl). <sup>3</sup>H-digoxin (0.5 mg kg<sup>-1</sup>) was administered i.v. 24 h (a) or 48 h (b) later. Biliary CI was measured over three consecutive 30 min intervals starting 5 min after completing the injection of <sup>3</sup>H-digoxin. Each bar represents the mean biliary CI of total <sup>3</sup>H-radioactivity ± s.e.m. of three to four separate measurements. \**P* < 0.05, significantly different compared saline control, ANOVA followed by Student – Newman – Keuls *post hoc* test.

tion when the basal capacity to eliminate drugs is already reduced.

An impairment of luminal (P-gp) transport of digoxin in the organs of drug elimination (liver, kidney and intestine) during CNS inflammation would be predicted to result in decreased digoxin elimination, and increased intracellular and plasma concentrations of digoxin. Impairment of P-gp in the blood – brain barrier would be predicted to result in increased brain uptake of digoxin (Schinkel *et al.*, 1995). The maximal percentage increases in plasma digoxin and, brain and liver



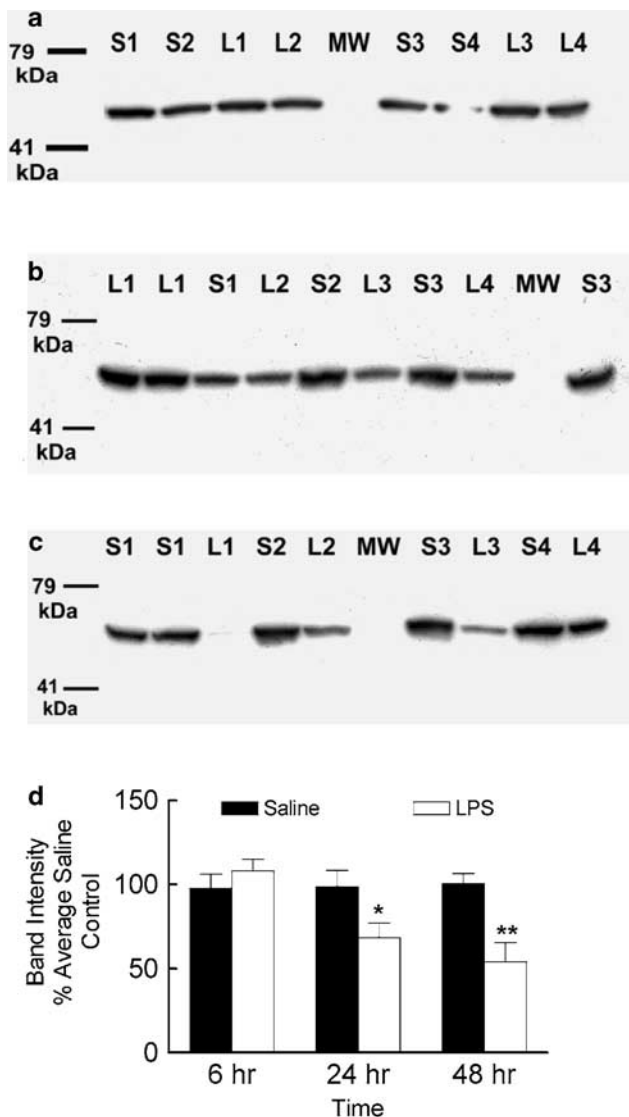


**Figure 5** Effect of LPS administered directly into the lateral ventricle on the biliary elimination  $^3\text{H}$ -digoxin. Male rats were treated as indicated in the legend of Figure 4. Representative TLC detection of digoxin is shown for 24 h and 48 h saline (a, b) and LPS- (c, d) treated rats. Bile samples 1, 2, and 3 represent the three collections from an individual experiment. Digoxin ( $r_f = 0.23 - 0.28$ ) was identified as the peak that comigrated with the standard  $^3\text{H}$ -digoxin. The amount of digoxin and metabolite eliminated in the bile (as percentage of the digoxin dose) are shown for the 24 h (e) and 48 h (f) treatment groups. Each bar represents the mean  $^3\text{H}$ -digoxin or metabolite eliminated in the bile over the 1.5 h experimental duration  $\pm$  s.e.m. of three to four separate measurements. \* $P < 0.05$  significantly different compared saline control, unpaired  $t$ -test.

radioactivity observed in LPS-treated rats coincided with the time (6 h) of maximal loss in brain or hepatic *mdr1a* expression and is consistent with a loss in P-gp function in those organs. Although hepatic and brain *mdr1a* mRNA returned to control levels by 24 h, brain, hepatic and plasma radioactivity remained higher and bile elimination of digoxin was reduced in LPS- versus saline-treated rats. Functionally, this would be expected since the half-life of P-gp is approximately 20 hr (Zhang & Ling, 2000). The elevated plasma, brain, kidney and liver radioactivity in *mdr1a*<sup>+/+</sup> but not in *mdr1a*<sup>-/-</sup> LPS-treated mice further support that changes to *mdr1a*/P-gp expression or function continue to affect the disposition of digoxin up to 24 h after the administration of LPS into the CNS. The observation that LPS-treated *mdr1a*<sup>-/-</sup> mice displayed a decreased accumulation of brain digoxin compared to the saline-treated

*mdr1a*<sup>-/-</sup> mice supports the idea that CNS inflammation may also have reduced a transport mechanism for digoxin uptake into the brain. If a process for digoxin uptake into the brain is reduced, it may explain why administering LPS directly into the lateral ventricle of wild-type mice or rats only produced modest increases in digoxin accumulation in the brain as compared to the larger increases in plasma digoxin concentration.

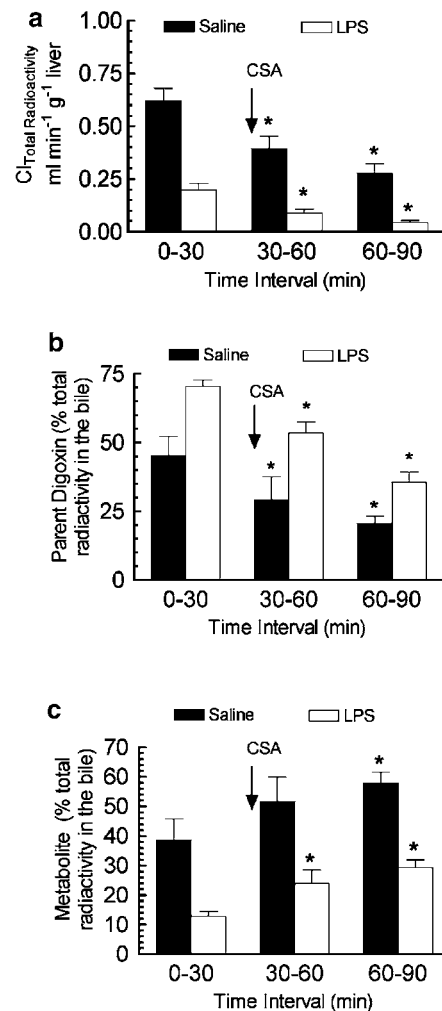
At variance to *mdr1a*, brain and liver *oatp2* mRNA levels in LPS-treated rats recovered more slowly to control levels. One may envision that if basolateral transport process for digoxin was depressed by CNS inflammation and recovered more slowly than luminal efflux transport processes (P-gp) an increased plasma digoxin concentration would be maintained but tissue levels would decrease (i.e. decrease tissue/plasma



**Figure 6** Representative Western blots demonstrating the effect of LPS administered directly into the lateral ventricle of male rats on the hepatic microsomal expression of CYP3A1/2 protein 6 h (a), 24 h (b) or 48 h (c) after treatment. Lanes S1–S4 and L1–L4 represent individual saline or LPS-treated animals, respectively. The results of densitometry analysis (d) are expressed as the mean density  $\pm$  s.e.m. of four individual samples repeated in duplicate or triplicate. For the individual Western blots, the band intensities were normalized to the average band intensity of the saline-treated animals and allowed for pooling of the replicated data. \* $P < 0.05$ , \*\* $P < 0.01$ , compared to the saline time control, unpaired *t*-test.

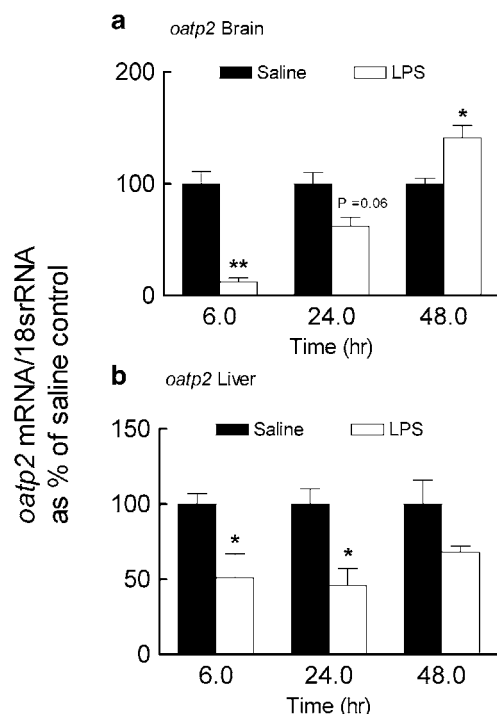
digoxin). The decrease in *oatp2* expression may in part explain the reduced hepatic elimination of digoxin and reduction in brain and liver radioactivity compared to plasma radioactivity 48 h after the administration of LPS. However, estrone 3-sulfate, a substrate of *oatp2* (Cattori *et al.*, 2001) did not block the biliary Cl of  $^3\text{H}$ -radioactivity after  $^3\text{H}$ -digoxin administration. This result suggested that blocking P-gp has more relevance for altered digoxin elimination in the bile but may also indicate that other basolateral uptake processes for digoxin exist in the liver.

The activities of several hepatic P450 enzymes are down-regulated after CNS inflammation (Nicholson & Renton,

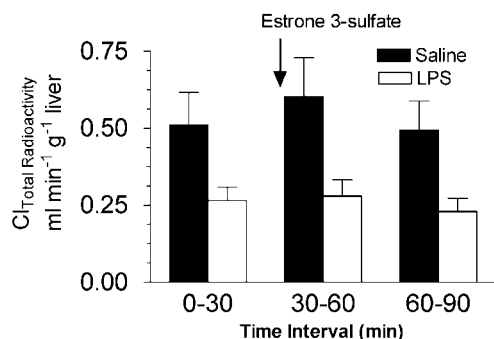


**Figure 7** Effect of administration of the P-gp inhibitor CSA on the biliary Cl of total  $^3\text{H}$ -radioactivity (a) and the percentage of radioactivity recovered in the bile as digoxin (b) and metabolite (c). Inhibition experiments were carried out 48 h after i.c.v. LPS or saline treatment.  $^3\text{H}$ -digoxin ( $0.5 \text{ mg kg}^{-1}$ ) was administered 5 min prior to the start of the first bile collection period (control collection) and a single i.v. injection of CSA ( $10 \text{ mg kg}^{-1}$ ) was given at the start of the second bile collection interval. Each bar represents the mean  $\pm$  s.e.m. of three to four separate measurements. \* $P < 0.05$ , within treatment group, significantly different compared to the first bile collection interval, ANOVA followed by Student–Newman–Keuls *post hoc* test.

2001; Renton & Nicholson, 2000). Digoxin has been reported to undergo significant metabolism in male rats via CYP3A1/2 in liver microsomes (Harrison & Gibaldi, 1976; Salphati & Benet, 1999). In the present study, we have demonstrated for the first time that hepatic CYP3A1/2 protein is depressed following the i.c.v. injection of LPS. This reduced hepatic CYP3A level likely did not greatly contribute to the increases in plasma digoxin and tissue radioactivity that were observed at 6 and 24 h as this enzyme was minimally altered at those times. However, at 48 h after induction of inflammation the digoxin metabolite profile in the bile indicated that an alteration in metabolism contributed in a minor way to the overall loss in digoxin elimination by the liver at this time. A simultaneous depression of CYP3A and P-gp during episodes of inflammation could have severe clinical implications for



**Figure 8** Effect of LPS given directly into the lateral ventricle of the brain on the expression of *oatp2* mRNA in the brain (a) and the liver (b). Brains and livers were collected 6, 24 and 48 h following LPS (25  $\mu$ g) or saline (5  $\mu$ l) administration into the lateral ventricle. *Oatp2* mRNA was determined by semiquantitative RT-PCR analysis. For each time point the *oatp2/18s* rRNA ratios are reported as percentage of the saline control (mean  $\pm$  s.e.m. of three or four separate measurements). \* $P < 0.05$ , \*\* $P < 0.01$  compared to respective saline control, unpaired *t*-test.



**Figure 9** Effect of administration of the *oatp2* substrate estrone 3-sulfate on the biliary Cl of total  $^3\text{H}$ -radioactivity. Inhibition experiments were carried out 48 h after i.c.v. LPS or saline treatment.  $^3\text{H}$ -digoxin (0.5 mg  $\text{kg}^{-1}$ ) was administered 5 min prior to the start of the first bile collection period (control collection) and a single i.v. injection of estrone 3-sulfate (2.5 or 5 mg  $\text{kg}^{-1}$ ) was given at the start of the second bile collection interval. Each bar represents the mean  $\pm$  s.e.m. of three to four separate measurements.

impaired elimination of the many drugs that are both metabolized by CYP3A and transported by P-gp.

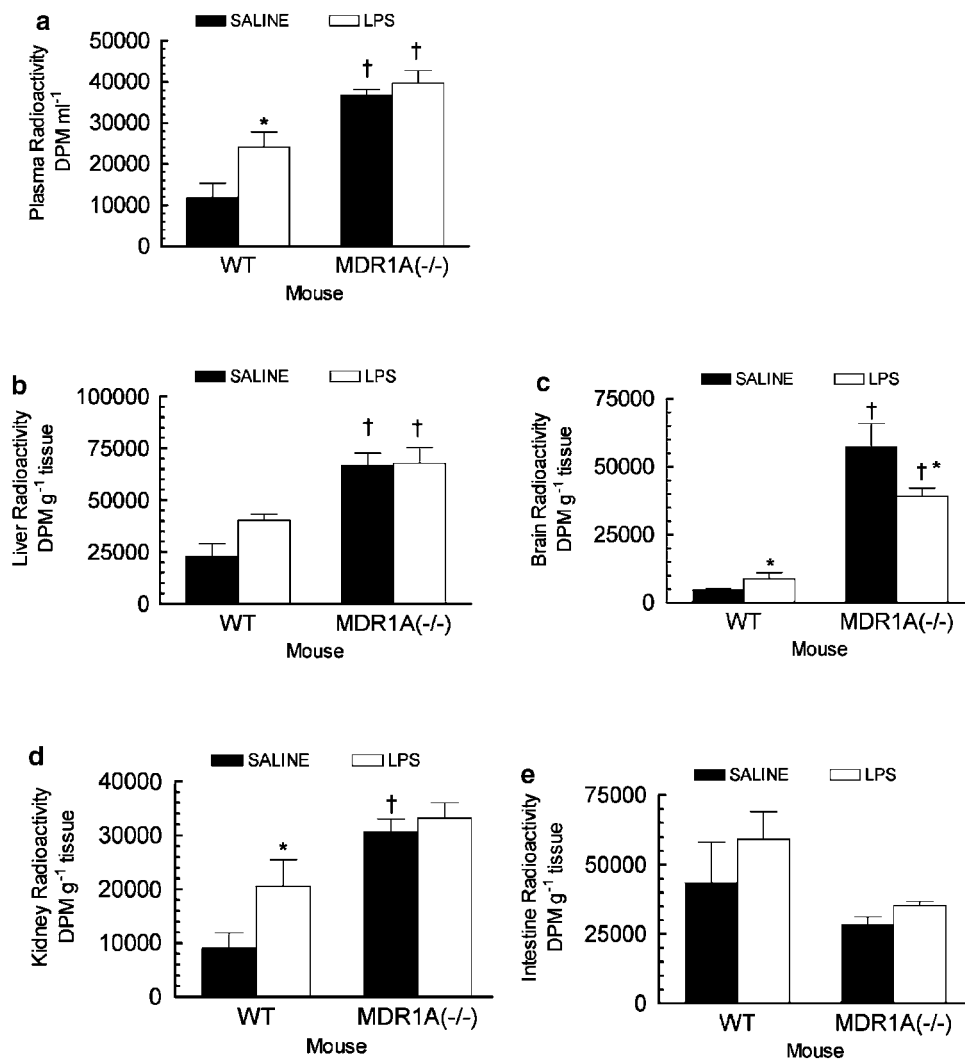
In our studies, the observed changes in intestinal to plasma distribution of radioactivity likely occur only as a consequence of CNS inflammation-induced alterations in hepatic digoxin elimination. Decreased biliary secretion of digoxin into the

small intestine is confirmed by the decreased intestinal/plasma radioactivity in the LPS- versus saline-treated rats. Moreover, saline- and LPS-treated rats with ligated bile ducts did not demonstrate differences in intestinal content of radioactivity. Systemic inflammation induced by *K. pneumoniae* decreased renal *mdr1a* levels and elimination of rhodamine123 by that organ (Ando *et al.*, 2001). The observed increases in renal radioactivity in rats (6 and 24 h) and *mdr1a*<sup>+/+</sup> mice (24 h) treated with LPS also suggested that CNS inflammation might reduce digoxin elimination by the kidney.

During systemic inflammation, Ando *et al.* (2001) and Zhao *et al.* (2002) have demonstrated a decrease in the biliary Cl of the P-gp substrate rhodamine123, a 30% reduction of P-gp detected in the brain and a corresponding increase in the brain uptake of doxorubicin 6 h after but not 24 h after administration of *K. pneumoniae* endotoxin. In contrast, our data obtained during inflammation localized in the CNS produced a more prolonged effect on the disposition of the P-gp substrate digoxin into the brain and liver. In the present study, hepatic *mdr1a* mRNA was reduced as early as 6 h after i.c.v. administration of *E. coli* LPS (25  $\mu$ g), which is similar to that found generated by peripheral administration of *K. pneumoniae* endotoxin (Ando *et al.*, 2001). Piquette-Miller *et al.* (1998) demonstrated a more prolonged reduction in hepatic *mdr1a* mRNA and P-gp up to 48 h after the peripheral administration of *E. coli* LPS (5 mg  $\text{kg}^{-1}$ ). The differences observed in these studies may indicate that the mechanisms that lead to a loss in *mdr1a* expression and P-gp function after CNS inflammation are different from that of peripheral inflammation. The type of endotoxin (*E. coli* versus *K. pneumoniae*), the dose, the P-gp substrate (digoxin, doxorubicin and rhodamine123) studied and the site of endotoxin administration (peripheral versus central) may also contribute to the observed differences in the different models of inflammation.

Several lines of evidence support the idea that peripheral release of cytokines modulates hepatic *mdr1a* levels and digoxin elimination as a result of inflammation in the CNS. LPS administered i.c.v. (25  $\mu$ g) causes inflammation that is localized to the CNS but also triggers an elevation in serum inflammatory cytokine (TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$  and IL-6) levels (Nicholson & Renton, 2001; Renton & Nicholson, 2000). In rodents, *mdr1a* is downregulated in the liver or hepatocytes 24 h following acute inflammation in the periphery induced by i.p. administration of cytokines IL-6 and IL-1 $\beta$  (Hartmann *et al.*, 2001; Sukhai *et al.*, 2001). Ando *et al.* (2001) demonstrated that the pentoxifylline (an inhibitor of TNF- $\alpha$  production) prevented the loss in biliary elimination of rhodamine123 after administration of *K. pneumoniae* endotoxin. Alternatively, endogenous compounds that are substrates for P-gp including inflammatory cytokines and corticosterone (Wolf & Horwitz, 1992; Drach *et al.*, 1996) that are released after administration of LPS may inhibit P-gp-mediated drug transport, especially at the earlier time points. Lastly, LPS may also decrease P-gp transport activity through protein kinase-mediated phosphorylation of P-gp (Miller *et al.*, 1998; Tepperman *et al.*, 2000).

It has been suggested that the expression of P-gp may be important in the immune response (Panwala *et al.*, 1998; Johnstone *et al.*, 2000). P-gp and members of the *oatp* family transport endogenous molecules, which are involved in inflammation including steroids, leukotrienes, prostaglandins and cytokines (Schinkel *et al.*, 1995; Drach *et al.*, 1996; Meijer



**Figure 10** Effect of administration of 2.5  $\mu\text{g}$  of LPS directly into the lateral ventricle of *mdr1a* expressing (*mdr1a*<sup>+/+</sup>) and deficient mice (*mdr1a*<sup>-/-</sup>) on the plasma (a), liver (b), brain (c), kidney (d) and small intestine (e) levels of total radioactivity. <sup>3</sup>H-digoxin (0.5 mg kg<sup>-1</sup>) was administered 24 h following the i.c.v. injection of LPS or saline and animals were killed 2 hr later for collection of tissues and plasma. Each bar represents the mean  $\pm$  s.e.m. of four separate measurements. \* $P < 0.05$ , compared to the *mdr1a*<sup>+/+</sup> saline treated animals, † $P < 0.05$ , compared to the saline or LPS *mdr1a*<sup>+/+</sup> treated animals, ANOVA (plasma, kidney and intestine) or Kruskal–Wallis ANOVA on ranks (brain and liver) followed by Student–Newman–Kuels *post hoc* test.

et al., 1998). It should be a priority to investigate if the loss in expression of these transporters in the brain and liver are important for regulating the body disposition of inflammatory/antiinflammatory mediators during episodes of inflammation.

In summary, our findings indicate that in addition to modulating drug biotransformation in the CNS and periphery (Renton & Nicholson, 2000), inflammation localized in the CNS may also modulate drug disposition through effects on drug transport proteins in the liver and brain. In the liver, a depression of P-gp could alter the transport and elimination of a number of important drugs and lead to drug toxicity. Most importantly, the loss of *mdr1a*

P-gp expression in the brain could have consequences for the passage of endogenous/exogenous molecules between the brain and the periphery during inflammation. Conversely, inflammatory mechanisms that block P-gp expression in the brain may be useful to facilitate drug delivery to that organ during the treatment of CNS disorders.

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