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Induction of Cytochrome P450 2E1 Expression in Rat and Gerbil Astrocytes by Inflammatory Factors and Ischemic Injury

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SUMMARY

Hepatic cytochromes P450 are known to be down-regulated by cytokines, lipopolysaccharide, Gram-positive bacteria, and viruses. Little is known, however, about the regulation by inflammation of cytochromes P450 in other tissues. We have found that lipopolysaccharide and interleukin-1β stimulate the expression of catalytically active CYP2E1 (but not CYP1A1 or CYP2B) up to 7-fold in rat brain primary cortical glial cultures. The induction reached a maximum after 24 hr and was accompanied by an increase in CYP2E1 mRNA. Chlormethiazole, a specific inhibitor of hepatic CYP2E1 transcription, completely inhibited the induction of CYP2E1 at the mRNA and enzyme levels. Immunofluorescence studies showed CYP2E1 to be expressed in a subset of astrocytes in the lipopolysaccharide-stimulated cortical glial cultures. Using a model of global ischemic injury in the gerbil, we found CYP2E1 to be induced *in vivo* in astrocytes in the inflammatory phase, 1–3 weeks after the lesion. Likewise, CYP2E1 was induced in the rat cortex 1 week after a focal ischemic injury. Our results suggest tissue-specific regulation of CYP2E1 by inflammatory factors and that CYP2E1 may play a role in astrocytes during inflammation in the brain.

P450s, which are involved in drug- and steroid-metabolism as well as in activation of carcinogens, have been found in the central nervous system of at least nine different species, including the rat brain and human brain (1, 2). CYP2E1, the major ethanol-inducible isozyme in the liver, has been localized to, for example, astrocytes, hippocampal pyramid neurons, and cortical neurons of the rat brain (3, 4). The presence of CYP2E1 in the brain has been confirmed on the enzyme and mRNA levels in the rat hippocampus (4) as well as on the mRNA level in the human hippocampus (5). The level of CYP2E1 expression in the central nervous system is low; we have found CYP2E1 mRNA and catalytic activity in cortical glial cultures to be 1000-fold lower than in rat liver (4).

CYP2E1 accepts a broad range of substrates, with preference for small and hydrophobic molecules. These include a large number of neuroactive compounds such as general anesthetics (6), organic solvents (7), the centrally acting muscle relaxant CZN (8), as well as physiological substrates such as acetone and arachidonic acid (7, 9). Also, CYP2E1 activates precarcinogenic *N*-nitrosoamines and many other small carcinogenic compounds and is believed to play a role for the synergy of ethanol in the development of various forms of cancer (10). Furthermore, CYP2E1 easily autooxidizes and releases oxygen free radicals, which induce lipid peroxidation processes (11). Ethanol-dependent induction of this enzyme has therefore been implicated as an important factor in alcohol liver disease (12).

Astrocytes are macroglial cells of the nervous system that express a variety of immunological characteristics and can be activated by various agents (e.g., viruses or cytokines) (13). Accordingly, it has been shown that astrocytes secrete growth factors and cytokines and express such enzymes as iNOS and phospholipase A_2 (13–15) when stimulated by inflammatory agents or excitatory amino acids. After a transient forebrain ischemia induced in the Mongolian gerbil by a bilateral occlusion of the common carotid arteries, a characteristic pattern of neural degeneration is seen (16). This process, which takes place in the hippocampal CA1/CA2 area, is accompanied by an infiltration of microglia and astrocytes into the damaged area (16). The response of astrocytes to ischemic injury seems to be triggered by microglia that secrete IL-1β, in response to which astrocytes proliferate (17).

IL-1β, IL-6, tumor necrosis factor α, and interferon α/β cause down-regulation of hepatic P450s, including CYP2E1 (18, 19). These cytokines appear to act by inhibiting the transcription of P450 genes and may antagonize induction of P450s (19–21). During inflammation in the lung caused by

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ABBREVIATIONS: P450, cytochrome P450; CZN, chlorzoxazone; LPS, lipopolysaccharide; IL, interleukin; GFAP, glial fibrillary acidic protein; RT-PCR, reverse transcription-polymerase chain reaction; iNOS, inducible nitric oxide synthase; FITC, fluorescein isothiocyanate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

hyperoxia, however, CYP2E1 and total P450 levels are readily induced (11). Hence, the response of P450s to inflammation is not uniform. In the present study, we provide evidence that CYP2E1 expression is markedly enhanced in astrocytes by the inflammatory mediators LPS and IL-1β in cortical glial cultures and during inflammation after ischemic injury *in vivo*.

Experimental Procedures

Primary cortical glial cultures. Rat primary glial cultures were established from cortices of new-born (4–12-hr) Sprague-Dawley rats by a mechanical sieving technique. The cortical glial cultures produced with this method have been extensively characterized (22). The cells were seeded at a density of 7×10^4 cells/cm^2 and were grown in minimum essential medium supplemented with 20% fetal calf serum, D-glucose (final concentration, 5 mM), amino acids, vitamins, penicillin (250,000 IU/liter), and streptomycin (250 mg/liter) (all media were from GIBCO (Paisley, Scotland, UK). The cell cultures were stimulated with LPS, IL-1β, or IL-6 at confluence (after 7–9 days). In some experiments, cells were fixed in 2% paraformaldehyde and the total cell number in the cultures was measured using bisbenzimide H33258 (Calbiochem, San Diego, CA). Experiments with FITC-labeled secondary antibodies showed that 84 ± 11% (six experiments) of the cells stained for GFAP (Dakopatts A/S; Glostrup, Denmark), an astrocytic marker. The cell cultures were also stained with two different polyclonal antisera prepared from rabbits immunized with two different batches of purified rat liver CYP2E1 (3, 23). In double staining experiments, a secondary FITC-labeled antibody (Dakopatts A/S) that recognized rabbit anti-rat CYP2E1 and a secondary tetramethylrhodamine isothiocyanate-labeled antibody (Dakopatts A/S) that recognized mouse anti-rat GFAP (Boehringer-Mannheim, Mannheim, Germany) were used.

Animals and operative procedures. Male Mongolian gerbils were exposed to transient forebrain ischemia by bilateral occlusion of the common carotid arteries, as previously described (24). Briefly, gerbils weighing 60–80 g were anesthetized intraperitoneally with Saffan (alphaxalone 45 mg/kg and alphadalone 15 mg/kg). The gerbils were placed on electrically heated mats and were operated upon while under heating lamps, to maintain body temperature at 37° (continuously monitored by a rectal probe) (24). The carotid arteries were exposed by cervical midline incision, separated from the vagus nerve and occluded for 5 min with aneurysm clips. Sham operated animals had their arteries exposed but not occluded. The gerbils were killed on the 7th, 14th, or 21st postoperative day and the brains were subjected to paraformaldehyde fixation.

In male Lister rats (250–300 g), focal cortical ischemia was induced photochemically as previously described (25), under anesthesia with halothane (1.5–5.0% in O_2/N_2O). The skull was exposed and covered with black tape, with a 6-mm diameter hole over the right side behind the bregma. After injection of rose bengal (20 mg/kg) into the jugular vein, the skull was irradiated with green light for 7 min. One week after the ischemic injury, the animals were perfused and the brains immediately frozen in liquid nitrogen.

Preparation of microsomal fractions. After thawing, brains were carefully dissected at once and homogenized in 3–5 volumes of ice cold 10 mM Tris·HCl buffer, pH 7.4, with 20% glycerol, 1.14% KCl (w/v), 0.2 mM EDTA, 0.1 mM dithiothreitol, freshly dissolved 0.1 mM phenylmethylsulfonyl fluoride, and 20 μM butylated hydroxytoluene. Centrifugation for 5 min at 12,000 × *g* gave a nuclear pellet, which was washed once. A 100,000 × *g* centrifugation for 60 min of the 12,000-×-*g*-combined supernatants gave a microsomal pellet. All fractions were washed once and stored at −80°. For detection of CYP2E1 from rat cortices, a one-step *p*-chloroamphetamine chromatography purification based on hydrophobic interactions was carried out exactly as described previously (3). Microsomal fractions from the cortical glial cultures were isolated as described above, with the exception that cultured cells were sonicated rather than homogenized.

Western blot and RT-PCR procedures. SDS-PAGE was performed on a BioRad mini Protean II apparatus (Hercules, CA). Slab gels of an acrylamide concentration of 10% were used and 35 μg of glial-culture microsomal protein or 40 μg of partially purified cortical homogenate was applied in each well. Proteins were blotted over from the polyacrylamide gel to polyvinylidene difluoride membranes (DuPont, Bad Homburg, Germany). Enhanced chemiluminescence (ECL kit; Amersham, Buckinghamshire, UK) was used for visualization of the proteins. The same two different polyclonal antisera to CYP2E1 used in immunofluorescence experiments were used for Western blot, diluted 1/1000. Antiseras to CYP1A1/1A2 and CYP2B1/2B2 have been previously described (23, 26). An antisera specific for CYP1A1 was purchased from Genetest (Woburn, MA). Protein A-coupled horseradish peroxidase (BioRad) was used as secondary antibody.

Total RNA was isolated from rat liver and primary glial cultures according to Chomczynski and Sacchi (27). cDNA synthesis was performed using Clontech's "First strand" synthesis kit (Palo Alto, CA). A *CYP2E1* 5′ primer corresponding to position −31 to −12 upstream of the initiation codon (5′-TACAAGTTTACAGTGGAGCC-3′) and a *CYP2E1* 3′ primer (5′-CGGAGAATGCTTAGGGAAAA-3′) complementary to bases 394–413 was used for amplification of CYP2E1 cDNA. A β-actin 5′primer corresponding to position 566–585 (5′-CCTGCGTCTGGACCTGGCTG-3′) and a β-actin 3′ primer complementary to bases 1024–1043 (5′-CTCAGGAGGAGCAATGA TCT-3′) was used for β-actin PCR. PCR samples were amplified with thermostable DNA polymerase (Advanced Biotechnologies, London, UK) in cycles of melting of DNA strands for 90 sec at 94°, annealing for 90 sec at 60°, and extension for 90 sec at 72°. Astrocyte cDNA was amplified for 20 to 35 cycles (CYP2E1 primers) or 12 to 25 cycles (β-actin primers) with 2 μCi of [α-^{32}P]dCTP (Amersham) added per sample (28). RT-PCR products were separated on a 5% polyacrylamide gel, which was subsequently exposed to film.

CZN 6-hydroxylation assay. The 6-hydroxylation of CZN was monitored to detect CYP2E1-dependent catalytic activity (8). One milligram of microsomal protein was incubated with 500 μM CZN and 0.5 mM NADPH in 50 mM potassium phosphate buffer for 120 min. NADPH was added every 30 min, and the reaction was linear up to at least 150 min. After termination of the incubations with 43% *o*-phosphoric acid, internal standard (1 μg of acetaminophen or 5 μg of 5-fluor-benzoxazoline-2-on) was added to the incubations. After extraction twice with dichloromethane and evaporation to dryness under N_2, the precipitate was dissolved in 50 μliters of mobile phase. The products of CZN oxidation were analyzed on a Millipore/Waters (Bedford, MA) 510 high performance liquid chromatography system and on a Hibar prepacked column RT 250–4 (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile:0.5% phosphoric acid (22:78, v/v) at a flow rate of 1.5 ml/min. The effluent was detected by a Millipore/Waters 480 liquid chromatography spectrophotometer (at 287 nm for 6-OH-CZN detection and at 250 nm for acetaminophen detection) and simultaneously by a LC-4A amperometric detector (Bioanalytical Systems, West Lafayette, IN), with a potential of 1.3 V over the electrochemical detector cell. The sensitivity for detection of 6-OH-CZN was determined to 0.5 pmol.

Immunohistochemistry. Sagittal 25-μm sections of gerbil brain were prepared on a cryostat and stained using the avidin-biotin-complex method as previously described (3). The same CYP2E1 antisera used for immunofluorescence (see above) were used for immunohistochemistry, diluted 1/5,000 to 1/10,000. In some experiments, a monoclonal antibody to GFAP (Dakopatts) was used. Control experiments included staining with control sera and antisera to CYP2B1/2 (23) or CYP2D1 (29) and preadsorption of the antiserum with 1 μM purified antigen for 24 hr at 4° (3). Sections were stained with cresyl violet in control experiments, which verified the effects of ischemia (24).

Materials. LPS, cresyl violet, and acetaminophen reference standard were purchased from Sigma (St. Louis, MO). Recombinant human IL-1β and mouse IL-6 were from Boehringer Mannheim. CZN was purchased from Aldrich Chemical (, Germany) and 6-OH-CZN was obtained from McNeil Chemical Company (Fort Washington, PA). 5-fluor-benzoxazoline-2-one was a kind gift from Dr. Raimund Peter, University of Erlangen, Germany.

Statistical analysis. Statistical analysis was carried out using the Student *t* test.

Results

Induction of the rate of 6-hydroxylation of CZN in rat cortical glial cultures. Rat cortical glial cultures (84% astrocytes) were established to study the expression of CYP2E1. It was found that bacterial LPS (100 ng/ml) caused a more than 7-fold stimulation of the rate of microsomal 6-hydroxylation of CZN 24 hr after the addition to the cells (Fig. 1, Table 1). The time course of induction showed an increase after 6 hr and a maximal effect at 24 hr; thereafter the activity declined (Fig. 1).

IL-1β and IL-6, cytokines known to be released during inflammation in the central nervous system (17, 30), were tested for their ability to induce CZN 6-hydroxylation. Addition of IL-1β (1000 units/ml) to the cultures resulted in a near 5-fold increase in the hydroxylation rate of CZN, whereas IL-6 (1000 units/ml) increased this catalytic activity only 2-fold (Table 1).

Identification of CYP2E1 as the enzyme induced by LPS. The rate of CZN 6-hydroxylation in microsomes from the cortical glial cultures was monitored in the absence or in the presence of inhibitors. A polyclonal CYP2E1 anti-IgG fraction (4 mg of IgG/mg of microsomal protein) inhibited the reaction by about 85% (Fig. 2A), whereas IgG from control sera was essentially without effect. Potent inhibition of the reaction was also achieved using ethanol (200 mM), a major substrate for CYP2E1 (Fig. 2A).

CYP2E1 has been shown to be the major enzyme responsible for CZN 6-hydroxylation in liver (8, 31). CYP1A1 and CYP1A2, however, have also been shown to be able to catalyze the same reaction, although at lower efficacy (32, 33). Western-blot analysis of glial culture microsomal fractions using antisera reactive toward CYP1A1/CYP1A2 (Genetest; see Western blot and RT-PCR procedures) (26) revealed expression of CYP1A1 in the cultures. Both LPS and IL-1β, however, caused pronounced down-regulation of immunodetectable CYP1A1 (Fig. 2B), which implicates CYP2E1 as the major catalyst of the inducible reaction. In contrast to both CYP2E1 and CYP1A1, the expression of CYP2B1/2 was not changed by LPS or IL-1β treatment for 24 hr (data not shown).

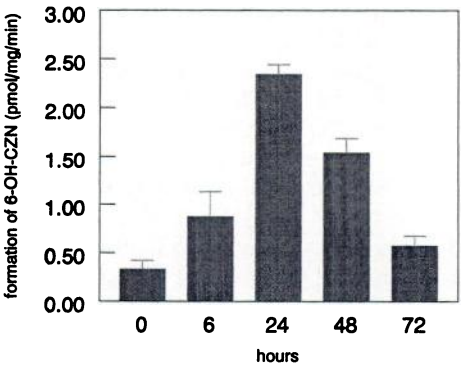

Fig. 1. Time course of LPS-dependent induction of 6-OH-CZN formation in cortical glial cultures. Catalytic activity was measured in microsomal fractions isolated from cortical glial cultures, stimulated with LPS for the indicated number of hours. Microsomes (1 mg) were incubated with 500 μM CZN for 120 min. The results are shown as mean ± standard deviation (three or more experiments).

TABLE 1

CZN 6-hydroxylation in microsomal fractions from cortical glial cultures incubated under various conditions for 24 hr.

Statistical analysis using Student's *t* test showed that the rate of 6-hydroxylation of CZN was significantly increased in cultures treated with LPS, IL-1β, or IL-6, compared with control.

Conditions	6-hydroxylation of CZN	
	pmol/mg/min	
Control	0.33 ± 0.09	(n = 9)
LPS (100 ng/ml)	2.34 ± 0.20	(n = 7, $p < 0.0001$)
IL-1β (1000 units/ml)	1.60 ± 0.19	(n = 3, $p < 0.0001$)
IL-6 (1000 units/ml)	0.77 ± 0.09	(n = 3, $p = 0.0001$)
LPS + Chlormethiazole (150 μM)	0.42 ± 0.16	(n = 5)
LPS + Actinomycin D (5 mg/l)	0.46 ± 0.02	(n = 2)

CYP2E1 itself, as well as CYP1A2, was not detectable in the Western blot experiments from glial cultures, despite the high sensitivity of enhanced chemiluminescence. As shown below, a partial purification with *p*-chloro-amphetamine is necessary for Western blot detection of CYP2E1 in nervous tissue fractions.

CYP2E1 is expressed in astrocytes in cortical glial cultures. Immunofluorescence studies of cortical glial cultures double-stained with antibodies directed to CYP2E1 and GFAP showed that CYP2E1 was expressed in $GFAP^+$ cells (Fig. 3) in LPS-treated cultures. Because the intensity of staining for CYP2E1 was rather low, surrounding cells inevitably showed a general background staining (Fig. 3A; compare $CYP2E1^+$ cells in the center with background stained cells on the periphery). Although all $CYP2E1^+$ cells in cortical glial cultures were $GFAP^+$, all $GFAP^+$ cells were thus not $CYP2E1^+$: it was estimated that 8.1 ± 4.6% (n = 10) of $GFAP^+$ cells were $CYP2E1^+$ in LPS-treated cultures. $CYP2E1^+$ $GFAP^+$ cells were present also in control cultures, but the staining was very faint.

Evaluation of LPS-dependent regulation of CYP2E1 mRNA. The level of CYP2E1 mRNA in the primary glial cultures was determined using a semiquantitative RT-PCR assay (28). LPS-treatment resulted in CYP2E1 mRNA levels 5.6-fold higher than controls (Fig. 4A). This value was calculated from an amplification efficiency of 84.4% (i.e., under exponential conditions), and a difference in PCR cycle numbers of 2.83 ± 0.47 (n = 4) for the same intensity of the signal between samples obtained from control and LPS-treated cell cultures. β-Actin was amplified using the same cDNA, which yielded similar efficiency and amounts in control and LPS-stimulated glial cells (data not shown).

Effect of the CYP2E1 inhibitor chlormethiazole. Chlormethiazole is a specific inhibitor of hepatic *CYP2E1* gene transcription (34). When added to the cell cultures 1 hr before LPS, it caused an efficient inhibition of the induction of the 6-hydroxylation of CZN, with an IC_{50} value of 5 μM (Fig. 4B). Chlormethiazole also potently inhibited (by 85%) the LPS-dependent increase in CYP2E1 mRNA in the cortical glial cultures (Fig. 4A). In addition, actinomycin D, a general inhibitor of transcription added to the cell medium 1

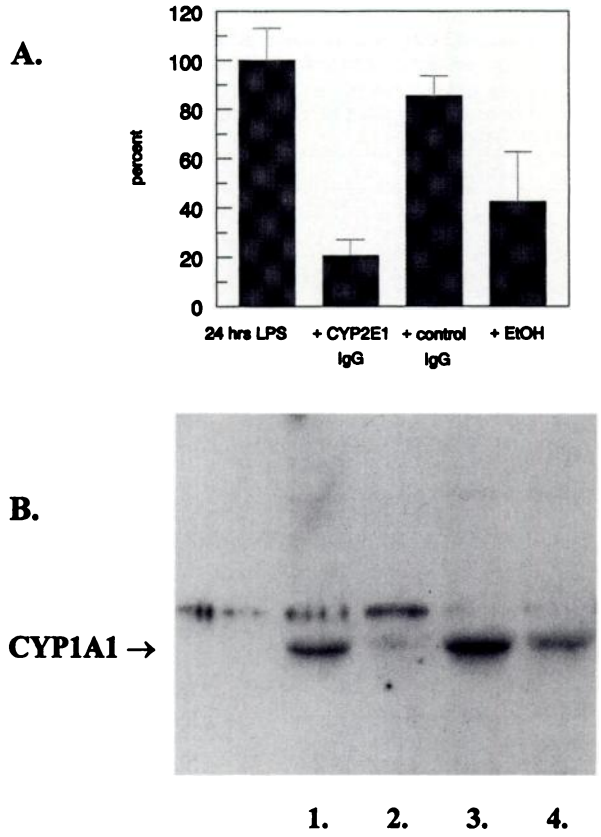

Fig. 2. Effect of ethanol or anti-CYP2E1 IgG on 6-hydroxylation of CZN, and effect of LPS and IL-1β on CYP1A1 expression. A, Effect of ethanol or anti- CYP2E1 IgG on the rate of 6-hydroxylation of CZN in microsomal preparations from LPS treated cortical glial cultures. Microsomes (1 mg) in 1 ml of 50 mM potassium phosphate buffer, pH 7.4, with 500 μM CZN, were either pretreated with 4 mg of CYP2E1 or control IgG or were incubated in the presence of 200 mM ethanol. The results are shown as percentage of maximal activity [mean ± standard deviation (three experiments)]. B, Effect of LPS on the expression of CYP1A1 in cortical glial cultures as monitored by Western blot analysis. Microsomes corresponding to 35 μg of protein were loaded into each well. The filters were incubated with a polyclonal serum raised against purified CYP1A1/CYP1A2 (26). *Lanes 1 and 3,* microsomes from control cultures; *lane 2,* microsomes from cultures treated with LPS (100 ng/ml) for 24 hr; *lane 4,* microsomes from cultures treated with IL-1β (1000 units/m) for 24 hr. The indicated band was identified as CYP1A1 by comparison with β-naphthoflavone treated rat liver microsomes, as well as by using an antiserum specific for CYP1A1 (see Experimental Procedures). The bands present in some lanes, with lower mobility than CYP1A1, represent unspecific staining; it was present also in lanes with only SDS-PAGE loading buffer (see the lane to the left of *lane 1*).

hr before LPS-addition, completely blocked the induction of 6-OH-CZN formation (Table 1).

CYP2E1 expression after global ischemic injury in the gerbil. A transient, bilateral occlusion of the carotid arteries of the gerbil for 5 min resulted in neuronal degeneration in the hippocampus, as judged by cresyl violet staining. A complete degeneration of the pyramid cells of the CA1/CA2 area was present in all ischemic animals (n = 12) (Fig. 5A and 5B), and in a few animals, it extended into the CA3 area. Neurons of the dentate gyrus, however, appeared intact in all cases. Sham operated animals appeared normal.

Immunohistochemical analysis of the distribution of CYP2E1 revealed limited staining in control gerbils; the enzyme was only seen in the choroid plexa, ependymal cells lining ventricles, and in part of the olfactory nerve but not in

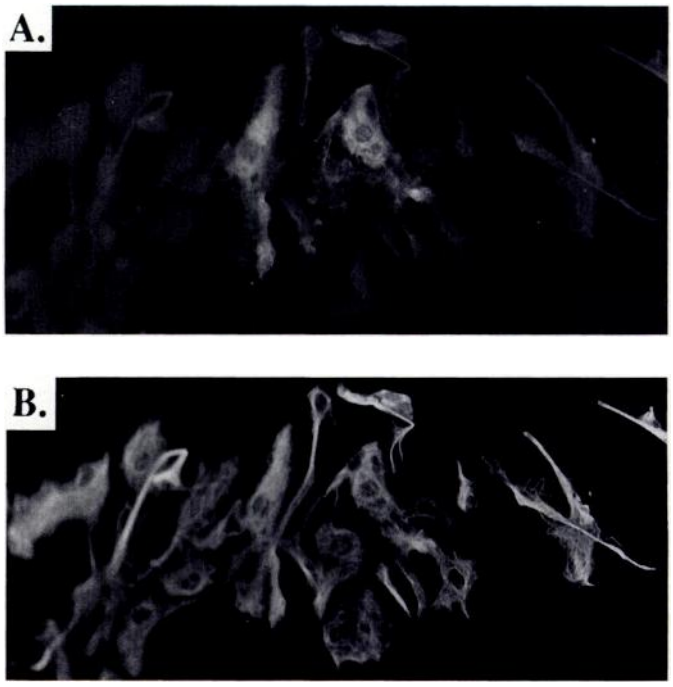

Fig. 3. Expression of CYP2E1 and glial fibrillary acidic protein (GFAP) in cortical glial cultures. Cultured cortical glial cells stimulated with LPS (100 ng/ml for 24 hr) were doublestained with a polyclonal antiserum directed against CYP2E1 (diluted 1/100) with a FITC-labeled secondary antibody (A) and a monoclonal antibody (5 μg of protein/ml) directed to GFAP with a tetramethylrhodamine isothiocyanate-labeled secondary antibody (B). Note the two CYP2E1$^+$ cells in the center of A that are also GFAP$^+$ (B). The faint staining of cells in the periphery (A) was not above background.

the hippocampus, striate body, or the cerebral cortex. Seven days after the ischemic injury, however, CYP2E1 immunoreactive cells were present in hippocampal areas, mainly in the CA1 area. There was a clear time-dependence of the induction process with an increasing number of stained cells up to 21 days after the ischemia (Fig. 5E to 5H). Staining with antibodies to GFAP revealed the same type of time dependence on the proliferation of astrocytes as for CYP2E1 (Fig. 5C and 5D). CYP2E1$^+$ cells were identified as astrocytes on the basis of their appearance under phase, as well as the similarity to GFAP$^+$ cells *in vivo*. The number of GFAP$^+$ cells was always larger than the number of CYP2E1$^+$ cells, which is consistent with the finding from rat brain cortical glial cultures that not all GFAP$^+$ cells were CYP2E1$^+$ (see above).

No staining of astrocytes appeared when we used control antisera, 2E1-antisera preadsorbed with purified CYP2E1, or antisera against CYP2B1/2 or CYP2D1 (not shown). Identical patterns of staining were seen using two different CYP2E1 antisera raised against two different antigen preparations.

CYP2E1 expression after focal ischemic injury in the rat. Cerebral cortical homogenates were prepared from rats exposed to photochemically induced focal ischemia and controls, 7 days after the insult. The rat brain homogenates were partially purified with respect to CYP2E1 using *p*-chloroamphetamine-coupled Sepharose columns. Western blot analysis of these fractions using a CYP2E1 primary antibody revealed induction of a band with the expected mobility on SDS-PAGE (Fig. 6). Densitometric quantification of fractions from ischemic and control rats indicated about 2-fold higher

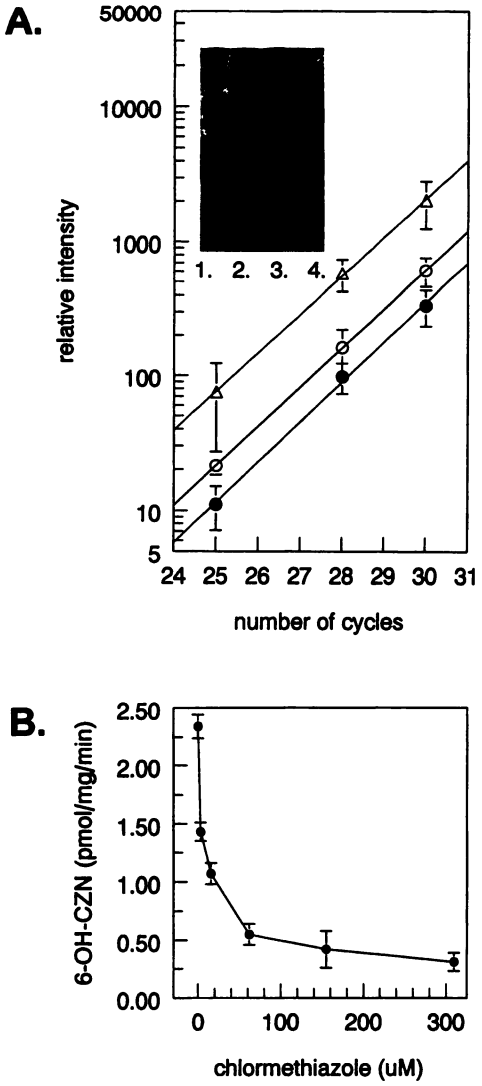

Fig. 4. CYP2E1 mRNA expression analyzed with semiquantitative RT-PCR, and effect of chlormethiazole on induction of CYP2E1 mRNA and 6-hydroxylation of CZN by LPS. A, Results of RT-PCR analysis of control astrocyte cultures (●), cell cultures treated with LPS for 24 hr (△) and of cultures pretreated with chlormethiazole for 1 hr before addition of LPS (○). *Parallel lines,* similar efficiency of amplification in each case. The results shown are mean values from four independent experiments. *Inset,* autoradiogram of [α-^{32}P]-dCTP labeled products of RT-PCR, amplified with primers to *CYP2E1. Lane 1,* RT-PCR products of control astrocyte RNA; *lane 2,* products from LPS-treated astrocytes (24 hr); *lane 3,* products from LPS + chlormethiazole treated astrocytes (24 hr); *lane 4,* products from control liver RNA. *Lanes 1–3,* RT-PCR products after 28 cycles of amplification; *lane 4,* results after 20 cycles of amplification. Concentrations of LPS were 100 ng/ml and of chlormethiazole, 150 μM. Statistical analysis using Student's *t* test showed that at 28 cycles, levels of RT-PCR products from LPS-treated cultures were significantly higher than RT-PCR products from control cultures (p = 0.006), and levels of RT-PCR products from LPS and chlormethiazole treated cultures were significantly lower than RT-PCR products from LPS-treated cultures (p = 0.029). B, Effect of chlormethiazole on the rate of 6-hydroxylation of CZN in microsomes of LPS-treated cortical glial cultures. Chlormethiazole was added to the cultures 1 hr before addition of LPS (100 ng/ml), after which the cells were cultured for an additional period of 24 hr.

amounts of immunodetectable CYP2E1 in the fractions obtained from the cortices of ischemic animals compared with controls [187 ± 47 compared with 100 ± 41 (arbitrary units, mean ± standard deviation) n = 4, p = 0.033]. No staining of filters occurred when probed with control sera.

Discussion

The present study describes enhanced CYP2E1 expression in astrocytes in rat cortical glial cultures in response to inflammatory factors and in rat and gerbil brain *in vivo* during the inflammatory response to ischemic injury. The identity of the induced CYP2E1 is supported by these findings: 1) CZN 6-hydroxylation was detectable in microsomes from stimulated cultured glial cells and was inhibited *in vitro* by the competitive substrate ethanol and with CYP2E1 antisera, but not with control sera; 2) CYP2E1 mRNA levels were induced in response to LPS; 3) chlormethiazole, a specific inhibitor of hepatic CYP2E1 transcription, was a potent inhibitor of induction of both CYP2E1-dependent catalytic activity and CYP2E1 mRNA in cortical glial cultures in response to LPS; 4) incubation of the CYP2E1 antisera with purified CYP2E1 from rat liver blocked all immunoreactivity in the histochemistry experiments; and 5) Western blot experiments indicated the induction of an anti-CYP2E1 immunoreactive protein in ischemic rat cortex.

Our demonstration of induction of CYP2E1 in astrocytes in response to inflammatory factors and postischemic inflammation is in contrast to the well-characterized depression of P450s in liver by cytokines, bacteria, and viruses (19, 20, 21, 35). Specifically, IL-1β decreases CYP2E1 mRNA to less than 40% of control levels in hepatocytes (18). In addition, the 2-fold induction of the rate of 6-hydroxylation of CZN by IL-6 seen in cortical glial cultures is in contrast with the situation in hepatocytes since IL-6 depresses hepatic CYP2E1 expressed (18). Furthermore, Muntane *et al.* (36) have recently shown that inflammation associated with carrageenan-induced granuloma potently down-regulates hepatic CYP2E1 mRNA. Thus, differential regulation of CYP2E1 expression between liver and brain during inflammation is evident. Interestingly, three other drug-metabolizing P450s, CYP1A1, CYP2B, and CYP2D, were either unaltered or down-regulated by ischemia and/or the inflammatory factors, indicating specificity of the induction of CYP2E1 during inflammation in the central nervous system.

Only a minority of GFAP$^+$ cells appeared to be CYP2E1$^+$ in LPS-treated cortical glial cultures (Fig. 3). Also, many more GFAP$^+$ cells were present *in vivo* in the hippocampus than CYP2E1$^+$ cells (compare Fig. 5D with Fig. 5H) 3 weeks after an ischemic episode. It seems, then, that a subset of astrocytes express CYP2E1 after induction by inflammation. This is interesting because even though the total level of CYP2E1 expression in cortical glial cultures is 1000-fold lower than in rat liver (4), the level of expression in the individual CYP2E1$^+$ astrocyte may therefore be higher than 0.1% of hepatocyte levels.

Chlormethiazole has previously been characterized as a selective inhibitor of *CYP2E1* gene transcription (34). Chlormethiazole potently inhibited LPS dependent induction of CYP2E1 mRNA in the cortical glial cultures. Also, because both chlormethiazole and actinomycin D were effective inhibitors of the induction of the rate of 6-hydroxylation of CZN, it is likely that LPS acts by stimulating *CYP2E1* transcription in astrocytes. Because IL-1β has been shown to down-regulate CYP1A1, CYP1A2, and CYP2C11 expression in hepato-

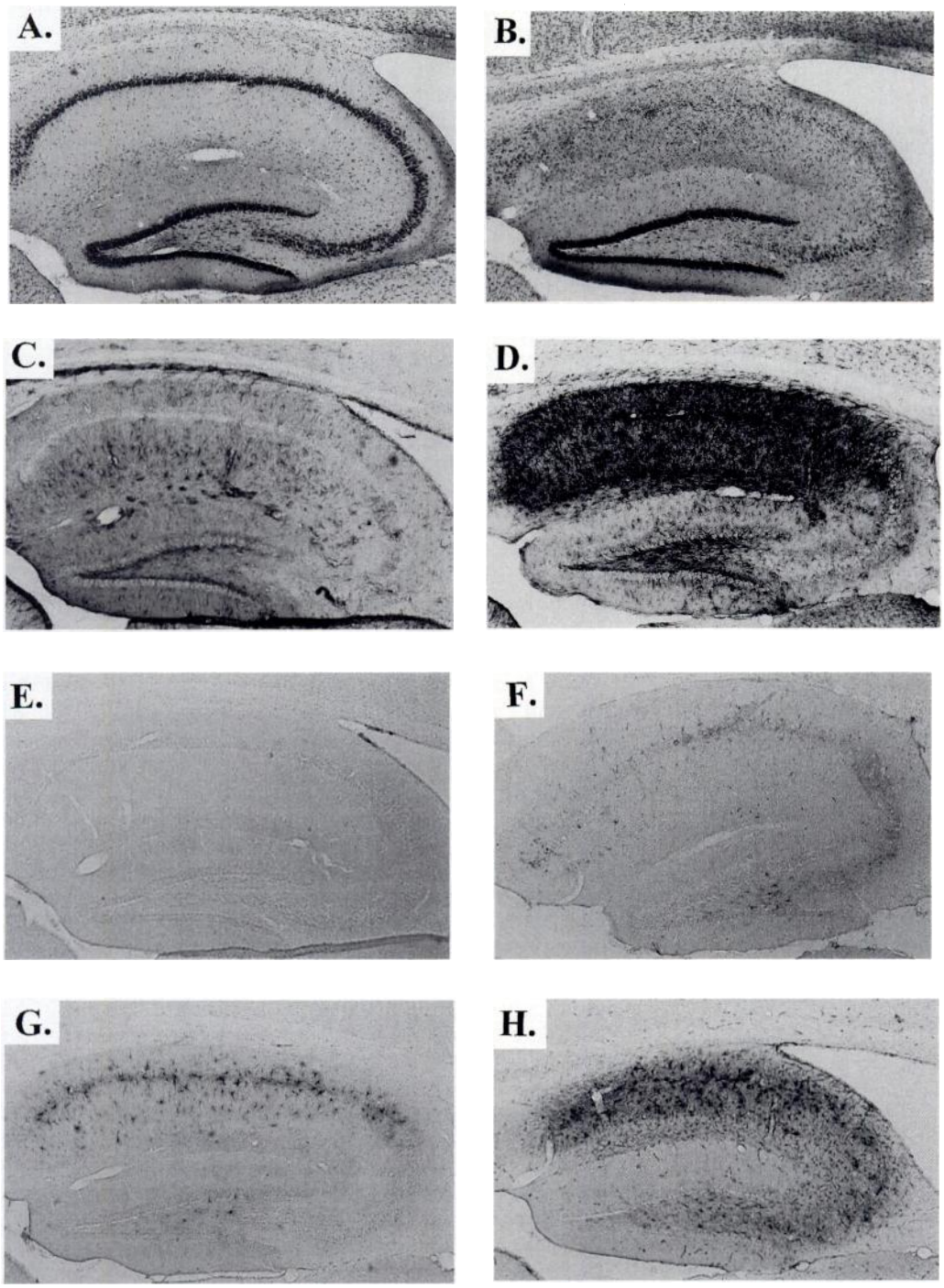

Fig. 5. Effect of transient global ischemia or sham operation on expression of CYP2E1 in the gerbil hippocampus. Sections from gerbil were stained with cresyl violet (A and B), with monoclonal antibodies to glial fibrillary acidic protein (GFAP) (C and D) or with polyclonal antibodies to CYP2E1 (E–H). A, C, and E show sham-operated animals. Micrographs of animals subjected to 5 min of transient global ischemia were killed after 1 week (F), 2 weeks (G) or 3 weeks (B, D, and H). Magnification: 40×; photographed with a Nikon Microphot-fx.

cytes by inhibiting gene transcription (20, 21), effects of inflammatory factors on P450s may be on the transcriptional level in both hepatocytes and astrocytes. Interestingly, Chen *et al.* (36) recently showed sphingomyelin hydrolysis to be an obligatory event in IL-1β mediated down-regulation of *CYP2C11* transcription in hepatocytes. In our cortical glial cultures, however, we could not mimic the effects of LPS/IL-1β on CYP2E1 by addition of either sphingomyelinase or ceramide.[1]

IL-1β is released from both neurons and microglia after ischemic injury (37) and levels stay elevated for at least 10 days (17). Because injection of IL-1β into mammalian brain causes proliferation of astrocytes (38), it may be speculated that IL-1β is the stimulus for CYP2E1 expression after ischemia *in vivo*. Although IL-1β and LPS caused a substantial induction of 6-hydroxylation of CZN after 24 hr in cortical glial cultures, ischemic injury of the gerbil caused increasing numbers of CYP2E1 stained astrocytes up to 3 weeks, the longest period investigated.

Clearly, factors other than the simple presence of stimuli such as IL-1β must be important *in vivo*. This is exemplified by the induction of iNOS in response to ischemia/inflammation *in vitro* and *in vivo*. iNOS is induced by LPS in glial cultures within 4–8 hr, with a maximum at 24 hr (14), but

[1] Tindberg, N., and M. Ingelman-Sundberg, unpublished observation.

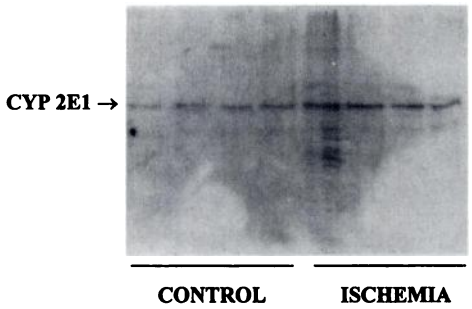

Fig. 6. Western blot analysis of the extent of CYP2E1 expression in rat cortex homogenates and the effect of focal cortical ischemia. Rat cortical homogenates from control or ischemic cortices were partly purified with *p*-chloroamphetamine chromatography as described in Experimental Procedures. Concentrated column eluates containing 40 μg of protein was loaded in each lane. After transfer, the polyvinylidene difluoride membrane was developed with a polyclonal antisera to CYP2E1.

does not show maximal induction until 2–4 days after cerebral ischemia (39).

iNOS and phospholipase A_2-II (15) are thus induced in LPS-exposed astrocytes; nitric oxide formation and arachidonic acid release are apparently important events during the activation of the astrocyte. Concerning the role of CYP2E1, hepatic CYP2E1 has been shown to be capable of hydroxylation and epoxidation of arachidonic acid (9). Specifically, 19(*R*)-, 19(*S*)-, and 18(*R*)- monohydroxyeicosatetraenoic acids and 14,15-, 11,12-, and 8,9- epoxyeicosatrienoic acids were formed in a reconstituted system with purified hepatic CYP2E1. In another study, Amruthesh *et al.* (40) detected, among other arachidonic acid metabolites, the formation of 14,15- and 8,9- epoxyeicosatrienoic acids in cultured rat astrocyte homogenates, which could be inhibited by the cytochrome P450 inhibitor SKF525A. Thus, there might be a role for CYP2E1 in astrocyte arachidonic acid metabolism.

In conclusion, our results suggest that catalytically active CYP2E1 is induced in a subset of astrocytes in response to LPS or IL-1β *in vitro* and during inflammation after ischemic injury *in vivo*. This is in contrast to the down-regulation of CYP2E1 seen during inflammation in hepatocytes. Because several related P450s were down-regulated or unaltered during inflammation in the brain, CYP2E1 is anticipated to have a distinct role in the astrocyte.

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