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Effect of status epilepticus and antiepileptic drugs on CYP2E1 brain expression

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Abstract

P450 metabolic enzymes are expressed in the human and rodent brain. Recent data support their involvement in the pathophysiology of epilepsy. However, the determinants of metabolic enzyme expression in the epileptic brain are unclear. We tested the hypothesis that status epilepticus or exposure to phenytoin or phenobarbital affects brain expression of the metabolic enzyme CYP2E1.

Status epilepticus (SE) was induced in C57BL/6J mice by systemic kainic acid. Brain CYP2E1 expression was evaluated 18–24 hours after SE by immunohistochemistry. Co-localization with NEUN, GFAP and CD31 was determined by confocal microscopy. The effect of phenytoin, carbamazepine and phenobarbital on CYP2E1 expression was evaluated *in vivo* or by using organotypic hippocampal cultures *in vitro*. CYP2E1 expression was investigated in brain resections from a cohort of drug resistant epileptic brain resections and human endothelial cultures (EPI-EC).

Immunohistochemistry showed an increase of CYP2E1 expression limited to hippocampal CA2/3 and hilar neurons after severe SE in mice. CYP2E1 expression was also observed at the astrocyte-vascular interface. Analysis of human brain specimens revealed CYP2E1 expression in neurons and vascular endothelial cells (EC). CYP2E1 was expressed in cultured human EC and over-expressed by EPI-EC. When analyzing the effect of drug exposure on CYP2E1 expression we found that, *in vivo* or *in vitro*, ethanol increased CYP2E1 levels in the brain and liver. Treatment

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Conflict of Interest

All authors have no conflict of interest to declare. The authors have no relationships with organizations that could inappropriately influence, or be perceived to influence, the presented work.

with phenytoin induced localized CYP2E1 expression in the brain whereas no significant effects were exerted by carbamazepine or phenobarbital.

Our data indicate that the effect of acute SE on brain CYP2E1 expression is localized and cell specific. Exposure to selected anti-epileptic drugs could play a role in determining CYP2E1 brain expression. Additional investigation is required to fully reproduce the culprits of P450 enzyme expression as observed in the human epileptic brain.

Keywords

CYP2E1; status epilepticus; drug exposure; biotransformation

Introduction

Understanding the mechanisms regulating drug brain bioavailability is relevant to the development of new drugs and to possibly clarify cases of drug brain toxicity (Abbott et al., 2002, Brodie et al., 2013). This concept is more critical in brain pathological conditions, such as epilepsy, where therapeutic failure is significant (Loscher, 2007, Kwan et al., 2009). Recent evidence has suggested that cytochrome P450 (CYP) metabolic enzymes, expressed in the epileptic brain, could affect brain drug distribution and biotransformation (Dauchy et al., 2008, Ghosh et al., 2011b, Brodie et al., 2013). CYP are a superfamily of intracellular enzymes responsible for the metabolism of endogenous compounds and xenobiotics. CYP exert their primary functions in the liver (Brodie et al., 2013). In addition to hepatic drug metabolism and detoxification, a local brain drug biotransformation may also influence the pharmacokinetic and pharmacodynamic fate of CNS drugs (Gherzi-Egea et al., 1993, Gherzi-Egea et al., 2001, Bauer et al., 2006, Dauchy et al., 2008, Ghosh et al., 2010, Ghosh et al., 2011b).

Recent data have indicated the expression of selected P450 metabolic enzymes in human brain tissues, primary brain endothelial cell cultures and isolated cerebral micro-capillaries obtained from subjects affected by drug resistant seizures (Dauchy et al., 2008, Dauchy et al., 2009, Ghosh et al., 2010, Ghosh et al., 2011a). P450 metabolic enzymes expression was also found in neurons in human epileptic brain specimens (Ghosh et al., 2011a). Among the P450 enzymes recently studied, transcript CYP2E1 levels have been demonstrated in human brain specimens (Upadhyaya et al., 2000, Haorah et al., 2005, Dauchy et al., 2008). CYP2E1 is responsible for the metabolism of numerous xenobiotics, including some anti-epileptic drugs (AEDs) such as phenobarbital (Brodie et al., 2013). Hepatic CYP2E1 metabolizes ethanol and its expression is induced by ethanol (Upadhyaya et al., 2000). Increasing evidence suggests CYP2E1 expression in rodent brain (Hansson et al., 1990). CYP2E1 was found in astrocytes, cerebral vessels and neurons in control animals and experimental models of ischemia or traumatic brain injury (Hansson et al., 1990, Tindberg et al., 1996, Birnie et al., 2013).

Despite the evidence that CYP are expressed in the human epileptic brain, the exact determinants of enzyme expression remain to be elucidated. In particular, the possibility exists that seizure activity or repetitive exposure to drugs could modify CYP brain

expression. We have therefore used an *in vivo* model and *in vitro* organotypic hippocampal slices to investigate whether severe status epilepticus (SE) or exposure to phenytoin, carbamazepine or phenobarbital induces CYP2E1 brain expression. This investigation was paralleled by the use of a cohort of drug resistant human epileptic brains and primary brain endothelial cells, defining CYP2E1 expression in the human brain.

Experimental Procedures

Human tissue

Brain specimens were obtained conforming to the principles outlined in the Declaration of Helsinki and approved IACUC Protocol. Available patients' information is summarized in Table 1. We used endothelial cells obtained from the following sources: (1) primary endothelial cells previously established from brain specimens resected from drug-resistant epileptic patients, EPI-EC (isolation and characterization was previously detailed (Marchi et al., 2004, Ghosh et al., 2011a); see Table 1); (2) commercially available control human brain microvascular cerebral endothelial cells (Cell Systems, cat. number ACBRI 376, HBMEC).

Animals

All animal procedures were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Ministère de la Recherche Française (protocol 00846.01). Animals (8–10 weeks old, male C57BL/6J mice; Janvier, France; 8–10 weeks) are housed in a controlled environment (21±1°C; humidity 60%; lights on 08:00 AM – 8:00 PM; food and water available *ad libitum*). Kainic acid (KA, intra-peritoneal injection, 10 mg/ml in PBS; 25 mg/kg, Sigma) was used to induce status epilepticus (SE). All animals were scored according to the Racine Scale (Racine et al., 1973). Animals were considered in severe SE only when repetitive generalized tonic-clonic events (lasting 90–120 minutes from SE onset) associated with a loss of balance was observed. Overall mortality was approximately 30% and benzodiazepine was not used. Stage IV/V indicates animals who did not experience SE or had one brief convulsive episode. After 18–24 hours from KA treatment, animals were sacrificed and brains removed after intracardiac perfusion with PBS (20 ml). Appropriate anesthesia and evaluation of signs of distress were performed. Brains were processed for western blot (snap frozen) or immunohistochemistry (immersion fixed in 4% PFA for 72 hours). We have used fixed brain slices (cryopreserved) and snap frozen brain tissues (hippocampi and cortex) from mice that experienced severe SE, Stage IV/V (Racine) changes and control animals. We also used 40 pups deriving from 5 pregnant rats for organotypic hippocampal cultures (OHC). Finally, a total of 20 mice was used for *in vivo* CYP2E1 drug induction study.

Organotypic slices and in vitro drug treatment

Methods have been previously described (Morin-Brureau et al., 2011). All pups were anesthetized prior to brain extraction. Briefly, hippocampi from 6 – 7 day old Sprague Dawley rats (Janvier, France) were dissected under aseptic conditions and transverse sections were obtained using a tissue chopper. Slices were placed on a 30 mm porous membrane (Millicell-CM, Millipore) and kept in 100 mm diameter dish. Petri dishes were filled with 5 ml of culture medium composed of 25% heat inactivated horse serum, 25%

HBSS, 50% MEM, 25 U/ml penicillin, 25 µg/ml streptomycin (Invitrogen). Cultures were maintained in a humidified incubator at 36°C and 5% CO₂. Two days later, media was changed and the temperature set to 33°C. After 2 weeks, the membranes were transferred to 6 well plates and each well was filled with 1 ml of medium. We have used 5 sets of OHC (1 × ethanol, 2 × phenytoin or 2 × phenobarbital). Each culture was prepared using 8 pups (derived from one pregnant rat) and consisting of approximately 160 hippocampal slices. Slices were then randomly distributed in 16 wells (10 slices / well). We have used 4 wells for each experimental condition (drugs and dosages). This approach was chosen to guarantee an adequate yield of protein extraction and representation of hippocampi obtained from different animals. OHC were exposed to phenytoin, phenobarbital or ethanol. Drugs were added at time 0, 24, 48 and 72 hours. Last treatment was performed 4–6 hours prior to slice processing. We evaluated the following dose dependency: ethanol 50 nM and 100 nM (Theile et al., 2013); phenobarbital 1 mM and 2 mM (Runge et al., 2000); phenytoin 20 µM, 100 µM and 200 µM (Berdichevsky et al., 2012). Dosages were chosen according to available protocols where the effect of these drugs on P450 enzymes was evaluated in hepatocyte cultures (Runge et al., 2000, Theile et al., 2013) or desired therapeutic range in human (Rambeck et al., 2006). Note that each Western Blot band and data point in Figure 6A–D represents CYP2E1 protein content from 30–40 slices (8 pups) pulled together from 4 culture wells. Western blot data obtained from each experiment were normalized to the respective non-treated control (set at 100%). Finally, all data obtained (relative to n = 5 experiments) were plotted together.

In vivo drug treatment

A group of 5 control mice was injected (i.p.) with sterile saline. Other animals were divided into 3 groups (n = 5 each) and subjected to one of the following treatments: i) phenobarbital 60 mg/kg (daily) in saline (4 injections) in 72 hours i.p.; ii) phenytoin 30 mg/kg (daily) in saline (4 injections) for 72 hours days i.p. (see (Seegers et al., 2002, Wen et al., 2008, Bankstahl et al., 2013)); iii) ethanol: mice were administered by gavage (daily; 3 g/Kg; 1 ml/kg of 25 % ethanol diluted in water; see (Howard et al., 2003). Phenobarbital and phenytoin dosages were chosen based on the reported anti-epileptic effects in rodents (Bankstahl et al., 2013) and studies where the effects on multi-drug transporters was evaluated (Seegers et al., 2002, Wen et al., 2008, Bankstahl et al., 2013). Another group of n=15 animals was used to evaluate the effect of carbamazepine on CYP2E1 levels. Mice were injected (72 hours, 4 injections) with 60 mg/Kg CBZ in PEG-400 (3ml/Kg), as previously described (Loscher and Honack, 1990). CYP2E1 brain and hepatic expression was evaluated in control, vehicle (PEG-400) and CBZ treated mice. Signs of animal distress and discomfort (e.g. body weight) were assessed daily. At the end of the treatments, mice were anesthetized using pentobarbital and perfused intracardially with PBS (15–20 ml) and hemispheres used for western blot or immunohistochemistry. Three animals (1 control, 1 ethanol and 1 phenobarbital) were not used due to inadequate perfusion. Western blot data in Figure 5 are indicated as percentage to the mean control value (set as 100%). Each individual values (control and treated) were then normalized accordingly.

Western blot

Protein isolation (rodent tissue)—Cortical, hippocampal tissues or *in vitro* OHC were homogenized in buffer (500 µl) containing: 0.1% SDS, protease inhibitor cocktail (Promega), 50 mM Tris-HCL (pH 7.4), 10 mM EDTA, 1 mM Na₃VO₄, 40 mM sodium pyrophosphate, 50 mM NaF, and 1 mM DTT. After centrifugation (12,000 rpm for 10 min), the samples were separated by electrophoresis and then transferred onto a nitrocellulose membrane (Life science). After 1 hour of blockage in skimmed milk, the membranes were probed overnight at 4 °C with a rabbit anti-mouse CYP2E1 (1:1000; ab151544, Abcam) or a mouse Actin (1:10000; ab6276, Abcam). Secondary goat anti-rabbit and goat anti-mouse HRP conjugated (1:4000) antibodies were used for CYP2E1 and actin respectively.

Protein isolation (human)—Total proteins were extracted from EPI-EC and HBMEC as previously described (Ghosh et al., 2010, Ghosh et al., 2011a). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoridate (PVDF) membrane (Millipore Corporation). The membranes were probed overnight at 4°C with the primary antibody (rabbit polyclonal CYP2E1, 1:1000, Abcam Inc, Cambridge, MA, U.S.A.). Secondary HRP rabbit immunoglobulin G antibodies was added (1: 2,000; Gibco Laboratories, Carlsbad, CA, U.S.A.) for 1 h. Specific protein bands were visualized by enhanced chemiluminescence reagent (Amersham Pharmacia, Little Chalfont, Buckinghamshire, United Kingdom). Mouse monoclonal anti-actin antibody (1:10,000; Oncogene, Cambridge, MA, U.S.A.) was also used. Secondary antibody HRP mouse IgG (1:2,000; Gibco Laboratories) were used.

Immunohistochemistry

Human Tissue—Staining was performed on paraffinized blocks of neocortical epileptic tissues obtained during surgery (Table 1). Human drug-resistant epileptic brains (n = 3) were evaluated for the study. Free floating sections were stained with CYP2E1 (1:200), GFAP (1:100), and NeuN (1:500). We used: rabbit polyclonal anti-human CYP2E1 (AB84598, Abcam, Cambridge, MA, USA); mouse monoclonal anti-GFAP (G 3893, 1:100; Sigma, St Louis, MO, U.S.A.); mouse monoclonal anti-NeuN (MAB377, 1:500; Chemicon, U.S.A.). Secondary antibodies: Texas red affininipure donkey anti-mouse IgG (1:100; Jackson Laboratories Inc., West Grove, PA, U.S.A.), and fluorescein isothiocyanate (FITC)–conjugated affininipure donkey anti-rabbit IgG (1:100; Jackson Laboratories Inc., West Grove, PA, U.S.A.). Auto-fluorescence was blocked with Sudan black B. Sections were imaged using fluorescent microscopy.

Mouse brains—Coronal sections were collected and washed three times with PBS. Antigen unmasking was performed for CD31 using a PBS solution with 1 µg/mL proteinase K (15 minutes at room temperature). Sections were blocked using PBS containing 0.25% triton, and 20% normal Horse Serum (used for CD31 and NeuN) or Goat Serum (used for GFAP), 90 min at room temperature. Slices were then incubated overnight at 4°C with primary antibody (PBS containing 0.1% triton) against CYP2E1 (polyclonal rabbit, 1:500, Abcam, Ab28146), NeuN (mouse, 1:300, Millipore, MAB377), GFAP (polyclonal chicken, 1:300, Abcam, Ab 4674) or CD31 (monoclonal rat, 1:100, Abcam, Ab 56299). After three washes, secondary antibodies were incubated 2 hours at room temperature according to the

primary host: Goat anti-rabbit Alexa-488 1:2000 (Lifetechnologies, A11008), Donkey anti-mouse AlexaFluor 568 1:2000 (Invitrogen, A10037), Goat anti-chicken conjugated with AMCA 1:100 (Jackson ImmunoResearch, 103-155-155) or Donkey anti-rat conjugated with Cy3 1:1000 (Jackson ImmunoResearch, 712-165-150). After washes with PBS, slices were mounted with Mowiol.

Fluorescence quantification and 3D reconstruction

CYP2E1 signal was imaged (10X, 20X and 40X) using a fluorescent microscope (Zeiss, AxioImager Z1). We have selected areas from cortices and dorsal/ventral hippocampal regions. Quantification of CYP2E1 fluorescence (pixel) was performed analyzing a total of 71 images (10X and 20X) from hippocampal and cortical areas (n = 20 mice). All images were acquired maintaining comparable exposure and contrast. Post-acquisition image analysis and quantification of CYP2E1 fluorescent pixels was performed using Image J. Briefly: i) region of interest (ROI) was defined (e.g., CA2/3 642 × 692 pixels; CA3/DG 976 × 570); ii) images were converted to RGB stack format; iii) signal threshold was adjusted to 100 units for each image; iv) area of signal (black) was calculated setting threshold sensitivity equal for each image. Data are expressed as a percentage of total pixel image area. Briefly, we calculated the mean value for the control group and we assigned it the nominal value of 100%. Each individual value (group/category) was then normalized accordingly.

Confocal microscope analysis was performed using a sequential laser scanning confocal microscope (Zeiss LSM780). Double- or triple-labeled images from each region of interest were obtained using: green 480 – 534 nm; red 575 – 620 nm, blues 415 – 470 nm. The objectives and the pinhole setting remained unchanged during the acquisition of all images (40X). Post-acquisition processing and 3D reconstruction were performed using IMARIS 7.2.

Statistical analysis

We used Origin Microcal (Northampton, MA, USA) for all statistical analyses. Data are indicated as mean ± sem. One-way ANOVA (Bonferroni post-hoc) was used on paired populations (e.g., CTR vs. SE). p<0.05 was considered statistically significant. Data are expressed using a Statistical Box Chart showing mean ± SE, 5% and 95% percentile and individual data distribution. X–Y linear regression (Pearson's r) was performed.

Results

CYP2E1 brain expression after severe status epilepticus in mice

In naive mice, CYP2E1 immunoreactivity was detected in hippocampal CA2/3, hilus (Figure 1A) and in the cortex (Figure 2A). These data are consistent with earlier findings (Upadhya et al., 2000, Hao et al., 2010) where CYP2E1 was found in GFAP⁺ cells and, occasionally, in vessels. After severe *status epilepticus* (SE), CYP2E1 immunoreactivity was regionally increased in CA2/3 and hilar but not in CA1 hippocampal neurons (Figure 1A). Examples of confocal images showing CYP2E1 – NEUN colocalization are provided in Figure 2C. Quantification of the immunofluorescent signals is provided in Figure 1B–C. Note that mice

experiencing behavioral Stage IV or one short-lasting SE (see Methods) did not display significant changes in CYP2E1 expression compared to control (Figure 1C – C1). Analysis of the cortical areas shows vascular CYP2E1 staining after severe SE (Figure 2A). Figure 2B–C shows examples of confocal images and neuronal expression of CYP2E1 (NEUN), glial cells (GFAP) and endothelial cells (CD31). Taken together, these results indicate that severe SE induces expression of CYP2E1 only in selected hippocampal areas.

CYP2E1 expression in human TLE

We used brain sections derived from temporal lobe epileptic brain specimens (see Table 1 and (Ghosh et al., 2011a). Immunofluorescent staining revealed neuronal and astrocytic CYP2E1 expression (Figure 3A). Perivascular astrocytes were immunopositive for CYP2E1 in regions of reactive gliosis. CYP2E1 expression was also evaluated by western blot in primary cultures of brain endothelial cells (n = 4 EPI-EC; see Table 1) and commercially available HBMEC (see Methods and Figure 3B). CYP2E1 expression levels were normalized by β -actin. CYP2E1 was overexpressed in EPI-EC (see Methods for details; Figure 3B).

In vitro and in vivo CYP2E1 induction by drug exposure

We evaluated whether brain CYP2E1 expression is modified following *in vivo* exposure to phenytoin, carbamazepine or phenobarbital. These drugs are used clinically and experimentally to control seizures (Bankstahl et al., 2013). Drug dosages were selected based on reports where the effect of these drugs on multidrug transporters was examined (Seegers et al., 2002, Wen et al., 2008, Bankstahl et al., 2013). Ethanol was used as a positive control for CYP2E1 induction. Ethanol increased vascular CYP2E1 immunoreactivity (Figure 4A cortex and Figure 4A1 hippocampus). Animals exposed to phenytoin displayed increased CYP2E1 brain levels. Immunohistochemistry analysis revealed expression of CYP2E1 in selected cortical vessels and hippocampal sub-regions (arrowheads in Figure 4A and A1). Western blot indicated that exposure to phenytoin resulted in an increase of CYP2E1 specifically in the hippocampus but not in the liver (Figure 5 A–C). Exposure to phenobarbital produced isolated changes in CYP2E1 brain expression detectable by immunohistochemistry (Figure 4A and immunohistochemistry quantification in B1–B2). However, the latter result was not accompanied by a significant increase in total tissue CYP2E1 expression as measured by western blot (Figure 5 A, B and C). Carbamazepine did not provoke a significant effect on CYP2E1 brain expression (Figure 5E). As previously reported, phenobarbital did not affect hepatic CYP2E1 levels (Runge et al., 2000). Examples of western blots used for the quantification are shown in Figure 5.

We then evaluated whether exposure to ethanol, phenytoin or phenobarbital induces CYP2E1 expression using chronic hippocampal cultures (Figure 6 A–D). We found increased CYP2E1 expression after exposure to ethanol and, to a lesser extent, phenytoin. Figure 6B and D shows the dose dependent effects of ethanol and phenytoin treatments. Note that phenobarbital did not alter CYP2E1 expression (Figure 6C). Each data point in Figure 6A–D refers to CYP2E1 expression (normalized by b-actin) in 16 hippocampal slices pooled together from n=4 animals (see Method for details). Data are normalized by the respective OHC non-treated control mean value (set at 100%).

Discussion

We attempted to elucidate whether severe status epilepticus (SE) or exposure to selected AED could influence CYP2E1 brain expression. This investigation is based on previous data showing CYP2E1 expression in the human epileptic brain. Our results suggest that the effect of experimental severe SE on CYP2E1 brain expression is limited to specific hippocampal regions. We found that cortical or hippocampal CYP2E1 levels are increased following *in vivo* and *in vitro* exposure to ethanol or the anti-epileptic drug phenytoin. Phenobarbital and carbamazepine provoked negligible effects. Brain and hepatic CYP2E1 levels may also be differently affected by drug exposure.

CYP2E1 in healthy and diseased rodent and human brain

Despite of evidences showing P450 expression in the human drug resistant epileptic brain, the pathophysiological and pharmacological causes of this expression remain unknown. Our results are in agreement with previous data showing CYP2E1 transcript levels in specific areas of the normal rodent brain, including the hippocampus (Hansson et al., 1990, Upadhyaya et al., 2000, Joshi and Tyndale, 2006). CYP2E1 staining was described in cerebral blood vessels and associated with astrocytic end-feet (Hansson et al., 1990, Upadhyaya et al., 2000, Joshi and Tyndale, 2006). Regional variability and cell specific over-expression was also reported in the primate brains (Joshi and Tyndale, 2006). It was also shown that changes in CYP2E1 brain expression occur in response to pathological conditions. For instance, augmented CYP2E1 levels were found in brains after experimentally induced cerebral ischemia (Tindberg et al., 1996). CYP2E1 expression was also augmented in astrocytes in response to pro-inflammatory factors (Tindberg et al., 1996). CYP2E1 transcripts are elevated in the rodent hippocampus following traumatic brain injury (Birnie et al., 2013). In addition, chronic consumption of drugs, including alcohol or nicotine, increased CYP2E1 brain levels and activity (Howard et al., 2003, Haorah et al., 2005). In our study, the effect of severe SE on brain CYP2E1 expression was region specific, mostly localized to the hippocampus. Kainic acid triggers CA neuronal activation possibly contributing to the regional nature of CYP2E1 induction. Repetitive *in vivo* intraperitoneal treatments with ethanol or phenytoin induced CYP2E1 expression in the cortex or hippocampus (Figures 4 and 5). Further studies are required to isolate specific brain cell types (microdissection, isolated vessels, etc.) to unequivocally quantify local CYP2E1 expression. Additional studies are also required to evaluate the combined effect of SE and drug treatment.

Similar to rodent brain, evidence shows expression CYP2E1 in human brain (Howard et al., 2003, Haorah et al., 2005, Dauchy et al., 2009). For instance, CYP2E1 is over-expressed in autaptic brains derived from alcoholics and alcoholic smokers (Howard et al., 2003). We report overexpression of CYP2E1 protein in the drug resistant epileptic human brain. CYP2E1 transcript levels were previously found in isolated human brain microvessels while others have reported CYP2E1 in isolated human brain endothelial cells (Dauchy et al., 2008, Dauchy et al., 2009). Our data show over-expression of CYP2E1 in primary cultures of endothelial cells derived from TLE human brains compared to commercially available human brain endothelial cells (Figure 3). Taken together, these data support the expression of CYP2E1 in the human epileptic brain. Whether CYP2E1, or other CYP enzymes, directly

participate to the pathophysiology of the epileptic condition by interfering with local drug biotransformation remains to be fully elucidated. It is unclear whether and how CYP2E1 interferes with brain pharmacology; the changes in brain CYP2E1 activity in response to SE remain to be evaluated.

Multiple roles of CYP2E1 brain expression

Brain CYP metabolic enzymes were previously suggested to interfere with drug brain biodistribution (Gherzi-Egea et al., 1995, Gherzi-Egea et al., 2001, Dauchy et al., 2008, Dauchy et al., 2009, Ghosh et al., 2011b), to be involved in detoxification processes, or in the production of neurotoxins (Ghosh et al., 2012). Brain CYP could also play a role in brain drug resistance (Ghosh et al., 2011b). The possibility that, depending on the substrate, CYP takes a detoxification or a detrimental role is experimentally supported. For instance, CYP2E1 was proposed as a mechanism of free radical production by brain endothelial cells after exposure to ethanol (Haorah et al., 2005). A link between astrocytic aquaporin-4, decreased CYP2E1 and protection to reactive oxygen species was recently proposed (Hao et al., 2010). Reactive oxygen species induced by ethanol parallel CYP2E1 over-expression in the hippocampus and cell damage, suggesting that CYP2E1 mediates the toxic effects of ethanol on neurons (Zhong et al., 2012). On the other hand CYP2E1 brain expression was beneficial in an experimental model of Parkinson's' Disease (Pardini et al., 2008). This evidence supports the notion that the balance between beneficial and toxic effects of CYP enzymes expression could occur in function of the disease state.

Pattern of brain CYP induction

Numerous studies have described the pattern of drug induction of CYP enzymes in the liver. Several anti-epileptic drugs are known to induce hepatic levels and the activity of CYP enzymes (Levy, 1995, Brodie et al., 2013). Our data suggest that brain and hepatic CYP2E1 could be differentially regulated (Figure 5). Recent evidences support this hypothesis. For instance, primates treated with nicotine showed significant induction of CYP2D in the brain and no changes in the liver (Mann et al., 2008). Conversely, CYP2B was induced in the liver but not in the brain following nicotine exposure (Schoedel et al., 2001). It was previously reported that phenobarbital does not increase CYP2E1 in human hepatocytes *in vitro* (Runge et al., 2000). Commonly prescribed anti-epileptic drugs include carbamazepine and levetiracetam. Our results show no effect of carbamazepine on CYP2E1 brain levels. Others have demonstrated that levetiracetam blood levels are independent of P450 hepatic metabolism and that levetiracetam does not inhibit or induce hepatic CYP enzymes (Patsalos, 2000). However, the possibility remains that levetiracetam may have specific effects on brain CYP levels. A comprehensive study on the effect of various anti-epileptic drugs on CYP levels using isolated human brain cells is required.

Finally, nuclear transcription factors (Pregnane Xenobiotic Receptor and Constitutive Androstane Receptor) in the brain were proposed to control the expression of metabolic enzymes and drug transporters (Bauer et al., 2004, Bauer et al., 2006). The possibility exists that, in the epileptic brain, the expression of CYP enzymes could be influenced by *epileptic by-products* (e.g., glutamate) produced during seizure activity. The latter hypothesis needs to be investigated. Interestingly, the expression of the drug transporter p-glycoprotein was

found to be influenced by glutamate levels (Bankstahl et al., 2008). Ad hoc experiments are needed to fully elucidate the exact molecular determinants of CYP2E1 expression in the epileptic brain.

Conclusion

The presented data and available evidences (Tindberg et al., 1996, Upadhya et al., 2001, Pardini et al., 2008, Hao et al., 2010, Mann et al., 2012, Zhong et al., 2012, Birnie et al., 2013) support the hypothesis of drug biotransformation in healthy and diseased brain (*e.g.*, traumatic brain injury, ischemia, Alzheimer's' disease, epilepsy). Further studies are required to fully elucidate the functional relevance of selected P450 enzymes, such as CYP2E1, in the epileptic brain.

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REFERENCE

- Abbott NJ, Khan EU, Rollinson CM, Reichel A, Janigro D, Dombrowski SM, Dobbie MS, Begley DJ. Drug resistance in epilepsy: the role of the blood-brain barrier. *NovartisFoundSymp.* 2002; 243:38–47.
- Bankstahl JP, Hoffmann K, Bethmann K, Loscher W. Glutamate is critically involved in seizure-induced overexpression of P-glycoprotein in the brain. *Neuropharmacology.* 2008; 54:1006–1016. [PubMed: 18394657]
- Bankstahl M, Bankstahl JP, Loscher W. Pilocarpine-induced epilepsy in mice alters seizure thresholds and the efficacy of antiepileptic drugs in the 6-Hertz psychomotor seizure model. *Epilepsy research.* 2013; 107:205–216. [PubMed: 24161515]
- Bauer B, Hartz AM, Fricker G, Miller DS. Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. *MolPharmacol.* 2004; 66:413–419.
- Bauer B, Yang X, Hartz AM, Olson ER, Zhao R, Kalvass JC, Pollack GM, Miller DS. In vivo activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. *MolPharmacol.* 2006; 70:1212–1219.
- Berdichevsky Y, Dzhalal V, Mail M, Staley KJ. Interictal spikes, seizures and ictal cell death are not necessary for post-traumatic epileptogenesis in vitro. *Neurobiology of disease.* 2012; 45:774–785. [PubMed: 22115940]
- Birnie M, Morrison R, Camara R, Strauss KI. Temporal changes of cytochrome P450 (Cyp) and eicosanoid-related gene expression in the rat brain after traumatic brain injury. *BMC genomics.* 2013; 14:303. [PubMed: 23642095]
- Brodie MJ, Mintzer S, Pack AM, Gidal BE, Vecht CJ, Schmidt D. Enzyme induction with antiepileptic drugs: Cause for concern? *Epilepsia.* 2013; 54:11–27. [PubMed: 23016553]
- Dauchy S, Dutheil F, Weaver RJ, Chassoux F, Daumas-Duport C, Couraud PO, Scherrmann JM, de WI, Decleves X. ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood-brain barrier. *JNeurochem.* 2008; 107:1518–1528. [PubMed: 19094056]
- Dauchy S, Miller F, Couraud PO, Weaver RJ, Weksler B, Romero IA, Scherrmann JM, de WI, Decleves X. Expression and transcriptional regulation of ABC transporters and cytochromes P450 in hCMEC/D3 human cerebral microvascular endothelial cells. *BiochemPharmacol.* 2009; 77:897–909.
- Ghera-Egea JF, Leininger-Muller B, Cecchelli R, Fenstermacher JD. Blood-brain interfaces: relevance to cerebral drug metabolism. *ToxicolLett.* 1995; 82–83:645–653.

- Gherzi-Egea JF, Perrin R, Leininger-Muller B, Grassiot MC, Jeandel C, Floquet J, Cuny G, Siest G, Minn A. Subcellular localization of cytochrome P450, and activities of several enzymes responsible for drug metabolism in the human brain. *BiochemPharmacol.* 1993; 45:647–658.
- Gherzi-Egea JF, Strazielle N, Murat A, Edwards J, Belin MF. Are blood-brain interfaces efficient in protecting the brain from reactive molecules? *AdvExpMedBiol.* 2001; 500:359–364.
- Ghosh C, Gonzalez-Martinez J, Hossain M, Cucullo L, Fazio V, Janigro D, Marchi N. Pattern of P450 expression at the human blood-brain barrier: Roles of epileptic condition and laminar flow. *Epilepsia.* 2010
- Ghosh C, Marchi N, Desai NK, Puvenna V, Hossain M, Gonzalez-Martinez J, Alexopoulos AV, Janigro D. Cellular localization and functional significance of CYP3A4 in the human epileptic brain. *Epilepsia.* 2011a; 52:562–571. [PubMed: 21294720]
- Ghosh C, Marchi N, Hossain M, Rasmussen P, Alexopoulos AV, Gonzalez-Martinez J, Yang H, Janigro D. A pro-convulsive carbamazepine metabolite: quinolinic acid in drug resistant epileptic human brain. *NeurobiolDis.* 2012; 46:692–700.
- Ghosh C, Puvenna V, Gonzalez-Martinez J, Janigro D, Marchi N. Blood-Brain Barrier P450 Enzymes and Multidrug Transporters in Drug Resistance: A Synergistic Role in Neurological Diseases. *CurrDrug Metab.* 2011b
- Hansson T, Tindberg N, Ingelman-Sundberg M, Kohler C. Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. *Neuroscience.* 1990; 34:451–463. [PubMed: 2333153]
- Hao C, Liu W, Luan X, Li Y, Gui H, Peng Y, Shen J, Hu G, Yang J. Aquaporin-4 knockout enhances astrocyte toxicity induced by 1-methyl-4-phenylpyridinium ion and lipopolysaccharide via increasing the expression of cytochrome P4502E1. *Toxicology letters.* 2010; 198:225–231. [PubMed: 20615459]
- Haorah J, Knipe B, Leibhart J, Ghorpade A, Persidsky Y. Alcohol-induced oxidative stress in brain endothelial cells causes blood-brain barrier dysfunction. *Journal of leukocyte biology.* 2005; 78:1223–1232. [PubMed: 16204625]
- Howard LA, Miksys S, Hoffmann E, Mash D, Tyndale RF. Brain CYP2E1 is induced by nicotine and ethanol in rat and is higher in smokers and alcoholics. *British journal of pharmacology.* 2003; 138:1376–1386. [PubMed: 12711639]
- Joshi M, Tyndale RF. Regional and cellular distribution of CYP2E1 in monkey brain and its induction by chronic nicotine. *Neuropharmacology.* 2006; 50:568–575. [PubMed: 16368115]
- Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen HW, Mathern G, Moshe SL, Perucca E, Wiebe S, French J. Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia.* 2009
- Levy RH. Cytochrome P450 isozymes and antiepileptic drug interactions. *Epilepsia.* 1995; 36(Suppl 5):S8–S13. [PubMed: 8806399]
- Loscher W. Mechanisms of drug resistance in status epilepticus. *Epilepsia.* 2007; 48(Suppl 8):74–77. [PubMed: 18330007]
- Loscher W, Honack D. The effect of interstimulation interval on the assessment of anticonvulsant drug potency in fully kindled rats. *Epilepsy research.* 1990; 7:182–196. [PubMed: 2289478]
- Mann A, Miksys S, Lee A, Mash DC, Tyndale RF. Induction of the drug metabolizing enzyme CYP2D in monkey brain by chronic nicotine treatment. *Neuropharmacology.* 2008; 55:1147–1155. [PubMed: 18687346]
- Mann A, Miksys SL, Gaedigk A, Kish SJ, Mash DC, Tyndale RF. The neuroprotective enzyme CYP2D6 increases in the brain with age and is lower in Parkinson's disease patients. *Neurobiology of aging.* 2012; 33:2160–2171. [PubMed: 21958961]
- Marchi N, Hallene KL, Kight KM, Cucullo L, Moddel G, Bingaman W, Dini G, Vezzani A, Janigro D. Significance of MDR1 and multiple drug resistance in refractory human epileptic brain. *BMC Med.* 2004; 2:37. [PubMed: 15473912]
- Morin-Brureau M, Lebrun A, Rousset MC, Fagni L, Bockaert J, de Bock F, Lerner-Natoli M. Epileptiform activity induces vascular remodeling and zonula occludens 1 downregulation in organotypic hippocampal cultures: role of VEGF signaling pathways. *JNeurosci.* 2011; 31:10677–10688. [PubMed: 21775611]

- Pardini C, Vaglini F, Viaggi C, Caramelli A, Corsini GU. Role of CYP2E1 in the mouse model of MPTP toxicity. *Parkinsonism & related disorders*. 2008; 14(Suppl 2):S119–S123. [PubMed: 18583171]
- Patsalos PN. Pharmacokinetic profile of levetiracetam: toward ideal characteristics. *Pharmacology & therapeutics*. 2000; 85:77–85. [PubMed: 10722121]
- Racine RJ, Burnham WM, Gartner JG, Levitan D. Rates of motor seizure development in rats subjected to electrical brain stimulation: strain and inter-stimulation interval effects. *Electroencephalography and clinical neurophysiology*. 1973; 35:553–556. [PubMed: 4126463]
- Rambeck B, Jurgens UH, May TW, Pannek HW, Behne F, Ebner A, Gorji A, Straub H, Speckmann EJ, Pohlmann-Eden B, Loscher W. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. *Epilepsia*. 2006; 47:681–694. [PubMed: 16650134]
- Runge D, Kohler C, Kostrubsky VE, Jager D, Lehmann T, Runge DM, May U, Stolz DB, Strom SC, Fleig WE, Michalopoulos GK. Induction of cytochrome P450 (CYP)1A1, CYP1A2, and CYP3A4 but not of CYP2C9, CYP2C19, multidrug resistance (MDR-1) and multidrug resistance associated protein (MRP-1) by prototypical inducers in human hepatocytes. *Biochemical and biophysical research communications*. 2000; 273:333–341. [PubMed: 10873607]
- Schoedel KA, Sellers EM, Tyndale RF. Induction of CYP2B1/2 and nicotine metabolism by ethanol in rat liver but not rat brain. *Biochemical pharmacology*. 2001; 62:1025–1036. [PubMed: 11597571]
- Seegers U, Potschka H, Loscher W. Lack of effects of prolonged treatment with phenobarbital or phenytoin on the expression of P-glycoprotein in various rat brain regions. *European journal of pharmacology*. 2002; 451:149–155. [PubMed: 12231384]
- Theile D, Schmidt TT, Haefeli WE, Weiss J. In-vitro evaluation of chronic alcohol effects on expression of drug-metabolizing and drug-transporting proteins. *The Journal of pharmacy and pharmacology*. 2013; 65:1518–1525. [PubMed: 24028619]
- Tindberg N, Baldwin HA, Cross AJ, Ingelman-Sundberg M. Induction of cytochrome P450 2E1 expression in rat and gerbil astrocytes by inflammatory factors and ischemic injury. *Molecular pharmacology*. 1996; 50:1065–1072. [PubMed: 8913336]
- Upadhya SC, Boyd MR, Ravindranath V. Characterization and localization of cytochrome P450 mediated metabolism of MPTP to nor-MPTP in mouse brain: relevance to Parkinson's disease. *Neurotoxicity research*. 2001; 3:369–380. [PubMed: 14715467]
- Upadhya SC, Tirumalai PS, Boyd MR, Mori T, Ravindranath V. Cytochrome P4502E (CYP2E) in brain: constitutive expression, induction by ethanol and localization by fluorescence in situ hybridization. *Archives of biochemistry and biophysics*. 2000; 373:23–34. [PubMed: 10620320]
- Wen T, Liu YC, Yang HW, Liu HY, Liu XD, Wang GJ, Xie L. Effect of 21-day exposure of phenobarbital, carbamazepine and phenytoin on P-glycoprotein expression and activity in the rat brain. *Journal of the neurological sciences*. 2008; 270:99–106. [PubMed: 18440557]
- Zhong Y, Dong G, Luo H, Cao J, Wang C, Wu J, Feng YQ, Yue J. Induction of brain CYP2E1 by chronic ethanol treatment and related oxidative stress in hippocampus, cerebellum, and brainstem. *Toxicology*. 2012; 302:275–284. [PubMed: 22960445]

Highlights

- Brain CYP2E1 expression is locally induced after **severe status epilepticus**
- Brain CYP2E1 expression can be induced by ethanol or phenytoin
- CYP2E1 expression in the brain and liver may be differently regulated

Figure 1. Increased CYP2E1 expression is localized in the hippocampus after severe SE
A) Control mice display CYP2E1 staining in glial cells and sporadic vessels throughout the hippocampus (see also co-localization in Figure 2). CYP2E1 immunoreactivity was negligible in the CA1. A number of hilar and CA2/3 neurons were positive for CYP2E1 (see quantification in C). Mice experiencing severe SE display increased immunoreactivity specifically in the CA2/3 regions and in the hilar portion of the dentate gyrus (neurons and reactive astrocytes indicated by *arrowheads* and vascular staining indicated by *arrows*). **B–B1)** Example of RGB stacks images utilized for the quantification of CYP2E1 fluorescence.

CYP2E1 is expressed as % pixels to control mean (see Methods). **C–C1)** Note the significant increase in the CA2/3 regions of SE animals, while in the hilar/DG portion a trend increase was measured. Expression of CYP2E1 in animals experiencing Stage IV/V Racine changes did not increase. Data are indicated by a box plot as mean $\pm$ SE (ANOVA).

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Figure 2. CYP2E1 colocalization with GFAP, NEUN and CD31

A) The pattern of CYP2E1 cortical expression was slightly modified after severe SE as compared to control. A number of CYP2E1⁺ vessels were visible after severe SE only (*arrows*). In mice experiencing behavioral stage IV–V CYP2E1 immunoreactivity was identical to control. **B)** CYP2E1 co-localized with activated astrocytes (GFAP⁺; *arrowheads*). An example of hippocampal staining is provided (control and SE). **C)** CYP2E1 expression was associated with CD31⁺ vessels in the cortex and hippocampus after SE. Examples of confocal 3D reconstructions (CD31 and NEUN) are provided.

Figure 3. Evaluation of CYP2E1 expression in the human TLE brain tissues and endothelial cells
A) CYP2E1 staining was observed at the vasculature, astrocytes (GFAP⁺) and neurons (NEUN⁺). Images depict examples obtained from gliotic regions. **B)** Example of CYP2E1 expression in a brain specimens obtained from a patient receiving phenytoin (see also Table 1). **C–C1)** CYP2E1 is expressed in brain endothelial cells (HBMEC and EPI-EC; see Methods for details) and it is over-expressed in EPI-EC (n = 4, see Table 1). While CYP2E1 levels were increased in EPI-EC as compared to HBMEC, variable CYP2E1 levels were

found within the pool of EPI-EC analyzed. Data are indicated by a box plot as mean $\pm$ SE (ANOVA).

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Figure 4. Immunohistochemistry of CYP2E1 brain expression after *in vivo* drug exposure
A–A1) Treatment with ethanol, a known CYP2E1 inducer, increased enzyme brain immunostaining. Note the appearance of distinct vascular signal in the cortical and hippocampal areas (*arrowheads*). Interestingly, *in vivo* treatment with phenytoin also increased CYP2E1 staining. Treatment with phenobarbital provoked minor effects on CYP expression, mostly confined to the hippocampus. **B)** Images depict examples of cortical RGB stacks used for CYP2E1 quantification (see also Methods). Note the increased on CYP2E1 immunoreactivity in animals after treatment with phenytoin. Data are indicated by a box plot as mean $\pm$ SE (ANOVA).

Figure 5. Western blot analysis of CYP2E1 brain expression after *in vivo* drug exposure
A) CYP2E1 tissue levels were increased (significant or trend) in the cortex after ethanol or phenytoin treatments. **B)** In the hippocampus phenytoin determined an increase of CYP2E1 expression. **C)** Ethanol, and not phenytoin or phenobarbital, determined a trend increase in CYP2E1 expression in the liver. **D)** Examples of western blots are shown. Data are indicated by a box plot (mean $\pm$ SE; ANOVA). **E)** In vivo, carbamazepine treatment did not affect brain or hepatic CYP2E1 levels. Note that treatment with vehicle (PEG 400) alone was associated with increased enzyme expression.

Figure 6. Western blot analysis of CYP2E1 brain expression after *in vitro* drug exposure
A) Data are relative to $n = 5$ *in vitro* OHC (1 ETOH; 2 Phenytoin; 2 Phenobarbital; see Methods). Phenytoin and ethanol induced CYP2E1 expression in organotypic hippocampal cultures. Box plot depicts the CYP2E1 differences between treatments while x-y plots in **B**, **C** and **D** depict CYP2E1 levels in function of drug dosage. Each value refers to CYP2E1 quantification from 16 slices pulled together from 4 wells ($n = 8$ pups). Data are normalized by respective OHC control (100%; see Methods for details). Linear regression was used to analyze data in B, C and D (Pearson's coefficient).

Table 1

Patients' data

LD.	Pathology	Age (yrs)	Gender	AEDs	Use
1	TLE	46	F	LCM; PGB	WB
2	TLE	49	F	LMT; LEV; PRM; OXC	WB
3	TLE	27	M	CBZ, LEV	WB,IHC
4	TLE	52	F	LEV; OXC; LMT	WB,IHC
5	TLE	16	M	NA	IHC
6	TLE	1	M	NA	IHC
7	TLE	53	F	PGB; LEV	IHC
8	TLE	28	F	CBZ; LEV; PHT	IHC
9	TLE	3	F	PHT; PB; LEV	IHC
10	TLE	31	F	LEV, PHT	IHC

Abbreviations: AEDs, antiepileptic drugs; PGB, pregabalin; **PHT, phenytoin**; PB, phenobarbital; GBP, gabapentin; DZ, diazepam; CBZ, carbamazepine; LEV, levetiracetam; LCM, Lacosamide; LMT, lamotrigine; OXC, oxcarbamazepine; TLE, temporal lobe epilepsy; IHC, immunohistochemistry; WB, western blotting; NA, not available.