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SUBCELLULAR LOCALIZATION OF CYTOCHROME P450, AND ACTIVITIES OF SEVERAL ENZYMES RESPONSIBLE FOR DRUG METABOLISM IN THE HUMAN BRAIN

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Abstract—We studied the subcellular distribution of cytochrome P450 and related monooxygenase activities in six regions of human brains removed at autopsy. The content of total cytochrome P450 was found to be at least nine times higher in the mitochondrial fraction than in the microsomes in all the regions studied. However, cytochrome P450-dependent enzymatic activities which are representative of different isoforms metabolizing exogenous molecules exhibited a microsomal prevalence, a situation previously observed in rat brain. The other drug-metabolizing enzymes catalysing functionalization and conjugation reactions, presented the following characteristics in human brain: (i) a low activity of NADPH-cytochrome P450 reductase, which also catalyses the reduction of some xenobiotics; (ii) a high specific activity of the membrane-bound epoxide hydrolase; (iii) among the enzymes catalysing conjugation reactions, 1-naphthol-UDP-glucuronosyltransferase activity was barely or not detectable, whereas the mean glutathione-*S*-transferase activity was 15 times higher than the activity measured in rat brain. The presence of several drug-metabolizing enzyme activities in human brain microvessels, and particularly the high activity of epoxide hydrolase, suggests a participation of these enzymes in the metabolic blood–brain barrier.

The process of metabolism of drugs and other xenobiotics converts lipophilic compounds to more polar molecules either by modification or conjugation reactions. This metabolism is catalysed by different enzymatic systems, and usually transforms the parent compound to a more hydrosoluble and pharmacologically or toxicologically inactive metabolite, which is eliminated from the body [1]. Drug metabolism takes place primarily in the liver, and the multiple hepatic metabolizing enzymatic systems have been studied extensively. Several groups have demonstrated that it also occurs in extrahepatic organs, such as the kidney and organs which are in close contact with the external environment, i.e. the lung, gut and skin [2–5].

The brain is partially protected from circulating neuroactive or toxic molecules by the blood–brain barrier resulting from the specific properties of the cerebral capillary endothelial cells. This protective function results from both morphological (tight junctions, lack of pinocytic vesicles) and metabolic (presence of enzymes inactivating endogenous neuroactive substances) peculiarities of these cells [6]. In addition, the presence of several drug-metabolizing enzymatic systems has also been recently demonstrated both in brain tissue (for reviews, see Refs 7 and 8) and in cerebral microvessels [9] of laboratory animals. This suggests a possible protective function for these enzymes both in brain tissue and at the blood–brain interface (i.e. in the endothelial cells themselves), especially against foreign chemicals whose lipophilic properties and molecular mass allow their diffusion through the brain capillary walls. On the other hand, several endogenous molecules like neurosteroids are formed and metabolized by specific isoforms of the same enzymatic systems. These enzymes can also be involved in metabolic activation processes leading to neurotoxic events [10]. The potential physiological and toxicological consequences of these activities on brain functions should be taken into account in human health care, particularly in the case of neurodegenerative pathologies, originating possibly from environmental causes and metabolic activation processes [11]. To date, however, few studies have been devoted to these enzymes in the human brain, thus giving only a partial knowledge of cerebral human drug metabolism capacities. This paper deals with a regional study in the human brain of the main enzymes catalysing functionalization reactions such as cytochrome P450-dependent monooxygenases (EC 1.14.14.1), NADPH cytochrome P450 reductase (EC 1.6.2.4) and epoxide hydrolases (EC 4.2.1.63), as well as conjugation reactions like UDP-glucuronosyltransferases (UDPGTs§, EC 2.4.1.17) and glutathione-*S*-transferases (EC 2.5.1.18). Described here are the subcellular distribution of total cytochrome P450, and three activities of cytochrome P450-dependent monooxygenases

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§ Abbreviations: GGT, γ-glutamyltransferase; MAO, monoamine oxidase; UDPGT, UDP-glucuronosyltransferase; BROD, PROD, EROD, 7-benzoxy-, 7-pentoxy- and 7-ethoxyresorufin-*O*-dealkylase activities.

Table 1. Case and drug history of human subjects

Patient number	Symbol	Sex	Age (years)	Post-mortem time (hr)	Deceased from
1	◆	F	68	6	Heart infraction
2	▲	M	64	14	Lung oedema
3	○	F	64	23	Rectum carcinoma
4	□	F	64	20	Acute pancreatitis
5	▽	M	85	19	Pneumonia
6	■	M	64	8	Non-Hodgkins' lymphoma

involved in the metabolism of xenobiotics. The activities of the other main drug-metabolizing enzymes have also been investigated. Finally, in order to assess the role of these enzymes in the human metabolic blood–brain barrier, their activities in microvessels isolated from human brain have also been measured.

MATERIALS AND METHODS

Human tissue preparation. Investigations were performed on human brains removed at autopsy, 6–22 hr post-mortem, from six subjects without neurological or psychiatric disease, who died of non-neurological pathology (Table 1). None of these subjects received neurotropic drugs.

Six regions were investigated from each brain: frontal, temporal and parietal cortices, hippocampus, putamen–pallidum complex and cerebellum. The pial membranes, superficial blood vessels and most of the white matter were removed by dissection from tissues sampled from each region. These samples were separately homogenized in 3 vol. of the ice-cold isolation medium (sucrose 0.32 M, Tris 10 mM, EDTA 1 mM, pH 7.4) by the use of a manual Dounce glass homogenizer, which prevents the contamination of the microsomal fraction by membranes of mitochondrial origin [12].

A part (20 mL) of the homogenate obtained from each cortical region was centrifuged at 1500 *g* for 15 min. The pellets were mixed together in Krebs–Ringer medium and used for the preparation of the microvessel fraction by the method of Mrsulja *et al.* [13], modified as previously described [9]. Briefly, the resulting homogenate was filtered on a 500 μm nylon screen and centrifuged (58,000 *g*, 30 min) on a discontinuous 0.25 : 1 : 1.5 M sucrose gradient. The microvessels obtained were resuspended in Krebs–Ringer medium without albumin then washed and pelleted by a 1000 *g* centrifugation for 10 min.

The supernatants of the first centrifugation were mixed with the corresponding tissue sample homogenates. The resulting preparations were centrifuged at 2000 *g* for 3 min, the pellets were discarded and the resulting supernatant centrifuged at 12,500 *g* for 10 min. The pellet was homogenized and washed twice in the isolation medium by centrifugation at 11,000 and 10,000 *g* for 10 min each. The resulting resuspended pellet was used as the mitochondrial fraction. The supernatant of the 12,500 *g* centrifugation was centrifuged at 15,000 *g* for 20 min, the pellet discarded and the resulting supernatant centrifuged at 105,000 *g* for 90 min. The final pellet was homogenized in 100 mM potassium phosphate, pH 7.4 buffer containing 0.5 mM dithiothreitol, 0.2 mM EDTA, 20% glycerol, and used as the microsomal fraction. The supernatant was used as the soluble fraction.

Microsomes and mitochondria were also obtained from cortical surgical waste surrounding a brain tumor, by following a similar protocol, with respect to the weight/volume ratio.

Rat brain sampling, homogenization and fractionation. Male Sprague–Dawley rats (220 g) were killed by cervical dislocation, and kept in similar temperature conditions as used for dead humans prior to autopsy. The cerebellum and the two cortices were removed post-mortem after 0, 6, 12 or 24 hr. Both homogenization and fractionation procedures were done following the same protocol as for human tissues, with respect to tissue weight/buffer volume ratio.

Biochemical assays. Cytochrome P450 measurements were carried out on a Uvikon-810 (Kontron) spectrophotometer, using a second derivative spectrophotometric method which allows an accurate determination of cytochrome P450 content in turbid haem-containing suspensions [12].

NADPH-cytochrome P450-reductase activity was measured at 25° using cytochrome *c* as substrate, according to the method of Strobel and Dignam [14], slightly modified as previously described [15]. All other enzymatic measurements were carried out at 37°. 7-Benzoxy-, 7-ethoxy- and 7-pentoxyresorufin *O*-dealkylase activities (respectively BROD, EROD and PROD activities) were determined spectrofluorimetrically by the technique of Burke *et al.* [16] modified to allow accurate determinations of the low activities found in brain fractions, NADPH and NADH being used as cofactors for microsomal and mitochondrial fractions respectively, as previously described [17]. The detection threshold corresponded to a specific activity of 0.05 pmol/min · mg protein for all three substrates.

γ-Glutamyltransferase (GGT, EC 2.3.2.2) activity was determined by the method of Szasz [18] with L-γ-glutamyl-3-carboxy-4-nitroanilide as substrate, and in the presence of Triton X-100 (Triton/protein ratio 5:1, w/w).

The activity of UDPGT was measured in brain preparations using 1-naphthol as substrate. The formed glucuronide was quantified after separation

by HPLC as previously described [19]. The detection threshold corresponded to a specific activity of 0.1 nmol/hr · mg protein.

Membrane-bound epoxide hydrolase activity was measured by spectrofluorometry using benzopyrene-4,5-oxide as a substrate, as described by Dansette *et al.* [20].

Cytosolic epoxide hydrolase and glutathione-*S*-transferase were measured using the radiolabelled substrate *trans*-stilbene oxide, in the presence of 1-chloro-2,4-dinitrobenzene (which prevents the formation of glutathione conjugates) or glutathione, respectively, according to Wixtrom and Hammock [21]. Experimental conditions were adjusted to obtain linear reaction rates with respect to time and protein concentration. Protein concentration was measured by the method of Lowry *et al.* [22] using bovine serum albumin as a standard.

Western immunoblotting experiments. Antisera raised against rat hepatic microsomal epoxide hydrolase was provided by Dr T. Friedberg (Institut für Toxikologie der Universität Mainz, Germany). Anti-rat UDP-glucuronosyltransferase antibody was provided by Prof. B. Burchell (Dept of Biochemical Medicine, University of Dundee Medical School, Ninewells Hospital, Dundee, U.K.). It was directed against the phenol-UDPGT isoenzyme and recognized several forms of UDPGT including testosterone-UDPGT in rat and human liver [23].

Discontinuous SDS–PAGE was performed according to Laemmli [24]. The stacking and running gels were 5% and 10% (w/v) acrylamide, respectively. Protein electrotransfers to nitrocellulose sheets (Schleicher and Schull, Germany) were realized according to Burnette [25].

RESULTS

Due to their specific morphological properties, the microvessels obtained from both rat and human brains were not disrupted under our standard homogenization conditions and were sedimented during the first centrifugation. Therefore, the enzymatic activities we measured in the mitochondrial, microsomal and cytosolic fractions were not of cerebrovascular endothelial cell origin.

Effect of post-mortem delay before sampling on cerebral drug-metabolizing enzyme activities in rat brain. In order to check the effects of post-mortem delay on drug-metabolizing enzyme activities, we measured them in preparations of rat brain sampled 0–24 hr after the death of the animals. In microsomal and mitochondrial fractions, the activities of the studied enzymes were unchanged or slightly lowered following a post-mortem delay prior to sampling with the exception of EROD showing a 70 and 50% decrease, respectively, after a 12 hr post-mortem delay (Fig. 1A and B). The total cytochrome P450 content was moderately altered with a 30% decrease in both fractions. However, for most of the enzymes investigated, an increase of the interindividual variations was observed when the tissue sampling was done after a post-mortem delay. Cytosolic GST and EH activity measurements showed no apparent degradation over the various post-mortem delays before sampling (Fig. 1C). In the microvessel fraction, a decrease of the activities with post-mortem delay was seen which was not statistically significant, but with an increase of the interindividual variation, in particular concerning UDPGT (Fig. 1D). EROD was not detected in microvessels prepared from both brain sampled after a post-mortem delay and control brain, as previously reported [17].

These results, together with the enzymatic data obtained from human surgical waste, which are close to those obtained from human post-mortem tissue, show that these tissues can be useful to obtain information concerning the capacity of the human brain to metabolize xenobiotics.

Subcellular localization of cytochrome P450 in human brain tissue. The purity of the mitochondrial preparations was evaluated by electron microscopy, and by measuring the activity of monoamine oxidase as a mitochondrial marker enzyme. The photomicrographs obtained by electron microscopy showed a large number of organelles identified as being both condensed and non-condensed mitochondria, with well characterized double-membranes (Fig. 2). There was no apparent difference between the purity of the fractions obtained from post-mortem samples and from the peritumoral sample (not shown).

Monoamine oxidase (MAO) activity showed a 2.5–5-fold enrichment in the mitochondrial fractions when compared with the homogenates. This is in accordance with the enrichment measured in such mitochondrial fractions obtained by a differential centrifugation procedure in rat brain [26]. Differential spectra of CO-complexed, dithionite-reduced cerebral mitochondrial and microsomal fractions versus CO-complexed fractions, and their respective second derivatives spectra were recorded for each region. The spectra obtained with the mitochondrial fractions (Fig. 3) provided another index of their purity, as the profile obtained was similar to the one described in rat brain preparations [12] with the respiratory chain cytochromes evidenced at 418, 419 and 443 nm. The presence of cytochrome P450 was observed in the mitochondrial fractions obtained from all brain regions and was quantified from their second derivative spectrum.

The purity of the microsomal fraction was assessed by the measurement of NADPH-cytochrome P450 reductase activity as an enzyme marker. Its activity showed a 2–10-fold enrichment when compared with the crude homogenate, depending on the region and the case under investigation. However, no correlation was observed between the enrichment of activity and either the post-mortem delay or the region studied (not shown). Whatever the heterogeneity between the different microsomal fractions, a spectrum corresponding to low quantities of cytochrome P450 was observed with all microsomal fractions. The amount of cytochrome P450 measured in both microsomal and mitochondrial fractions is shown in Fig. 4. In all regions studied, the results clearly showed the predominant mitochondrial localization of the human cerebral cytochrome P450. A case-per-case analysis of the results in terms of regional variation also shows a decrease of 34 ± 14%

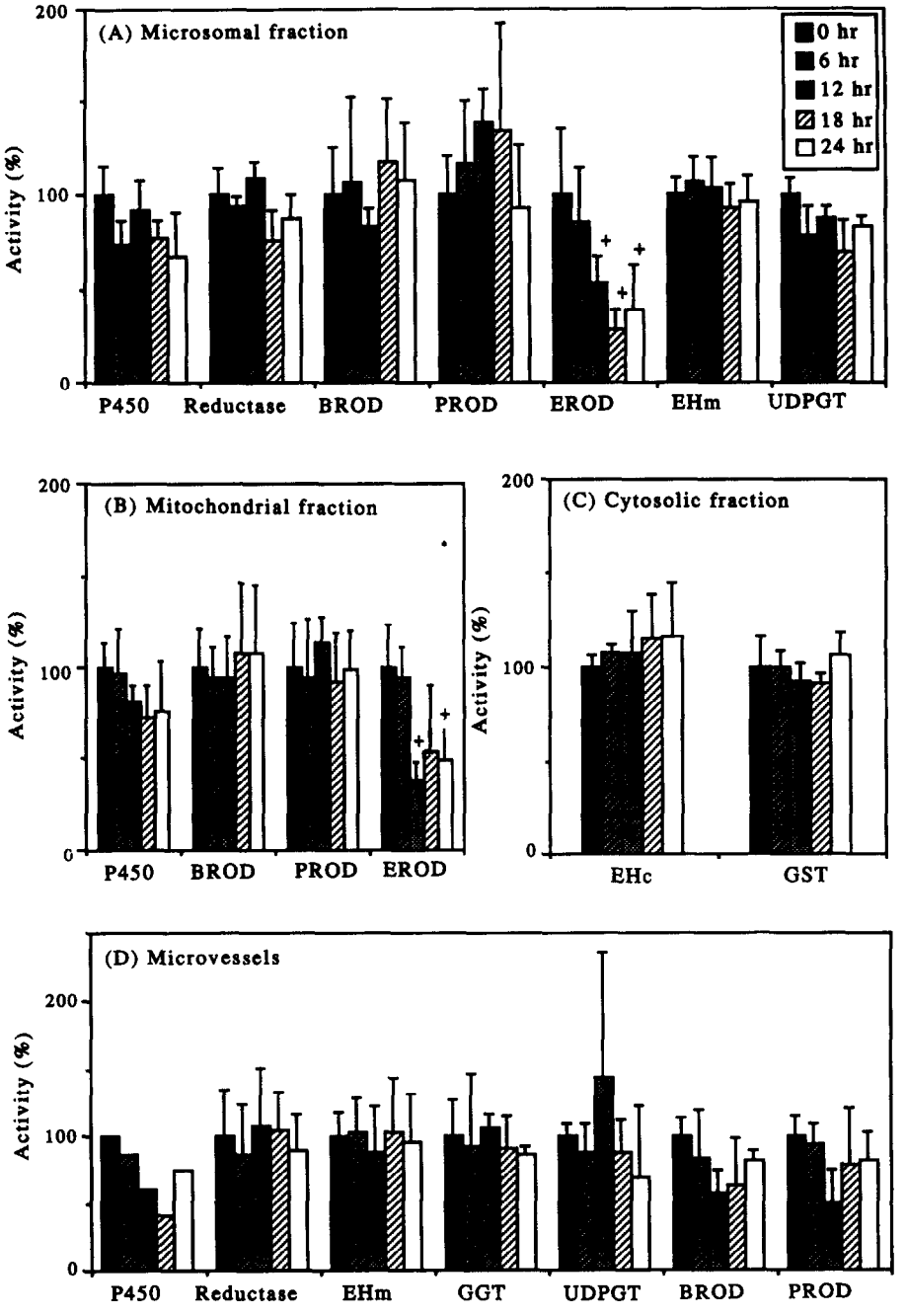

Fig. 1. Effect of post-mortem delay before brain sampling on drug-metabolizing enzyme activities in rat cerebral microsomal, mitochondrial, cytosolic and microvessel preparations. Values from three different pools of animals are expressed as a percentage of the values obtained from animals whose brains were sampled immediately after killing (mean ± SD). Cytochrome P450 content from microvessels: mean of two values. P450, cytochrome P450 content; Reductase, NADPH-cytochrome P450 reductase; EHm, membrane-bound epoxide hydrolase; EHc, cytosolic epoxide hydrolase; GST, glutathione-*S*-transferase activity. + Statistically different from value at 0 hr, Dunnett's *t*-test, $P < 0.05$.

of the mitochondrial cytochrome P450 in the hippocampus when compared with cortical regions.

Cytochrome P450-dependent activities in human brain tissue. 7-Benzoxyresorufin is a common substrate for several isoenzymes involved in xenobiotic metabolism. 7-Ethoxyresorufin is recognized as a specific substrate for cytochrome P450 IA1/2 (induced by arylhydrocarbons) in the rat liver, and the cytochrome homologous to rat cytochrome P450 IA2 in human liver [27]. 7-Pentoxyresorufin is

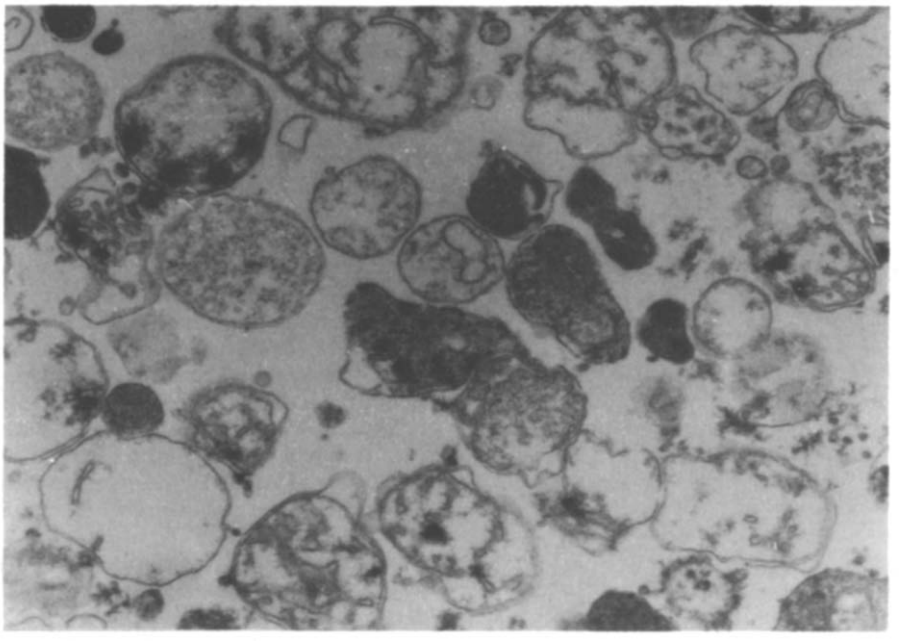

Fig. 2. Electron micrograph of the isolated human brain mitochondrial fraction obtained from post-mortem sample. Electron microscopy was realized on frozen samples of mitochondrial suspensions. (Shown: parietal cortex, case 6. Magnification 15,000.)

a specific substrate for the cytochromes P450 IIB1 and IIB2 in the rat (induced by phenobarbital), and cytochrome P450 IIIA4 in human liver [28]. Using these substrates, we measured the cytochrome P450-dependent specific activities in mitochondrial and microsomal fractions obtained from the six brain regions. The results are shown in Fig. 5.

In microsomal fractions, BROD was higher than both PROD and EROD activities. No significant variations in the mean activity of BROD were observed between the different brain regions. When the mean values of EROD and PROD were compared between regions, we did not observe any prevalence of an activity in a particular area of the brain. However, from case to case, we observed different isoenzymatic profiles, as the ratio PROD/EROD was higher, lower or equal to 1, depending on the case studied.

In mitochondrial fractions, BROD was also detected in all regions studied for most of the cases, with a higher activity in putamen–pallidum complex as compared with cortices. The data obtained with PROD and EROD were heterogeneous, as these activities were frequently not detected, depending on the cases and the regions considered.

Comparison of the mitochondrial with the microsomal data for the three activities, established in the different regions for each individual case, shows that microsomal BROD is higher in cortices, cerebellum and hippocampus when compared with the mitochondrial. Moreover, when the activities were expressed per cytochrome P450 content (data not shown), the data obtained were generally higher for all three substrates in microsomal fraction when compared with the mitochondrial, with a mean ratio ranging from 34 for EROD to 39 for BROD and to 90 for PROD, all regions analysed together. This indicates the prevalence of the microsomal localization of cytochrome P450 isoforms implicated in drug metabolism in the human brain. No evidence of sex-related differences were observed for either cytochrome P450 content or related activities.

Other drug-metabolizing enzymes in human brain tissue. The activities of three other membrane-bound enzymes involved in the metabolism of xenobiotics have also been measured in human brain microsomes (Table 2). NADPH-cytochrome P450 reductase was found to be active in all fractions, with the highest mean value in the putamen–pallidum complex. However, this activity was low in human brain microsomes, representing only 8% of the value reported in rat brain microsomes isolated from cortices or cerebellum. The variations in the purity of the microsomal fractions isolated from human brain cannot explain this finding as the mean value of activity measured in whole homogenates of human brain regions also represents 15% of the rat brain homogenate activity (not shown). Microsomes obtained from the peritumoral sample exhibited a higher activity.

By contrast, the microsomal (membrane bound) epoxide hydrolase was present in all regions (Table 2) with specific activities 6–11 times higher than those measured in rat brain microsomes. These activities showed no significant regional variations. The specificity of the recorded fluorimetric signal was assessed by the use of 1,2-epoxy-3,3,3-trichloropropane, a specific inhibitor of microsomal epoxide hydrolase [29]. The presence of 0.5 mM of inhibitor almost completely suppressed the signal in

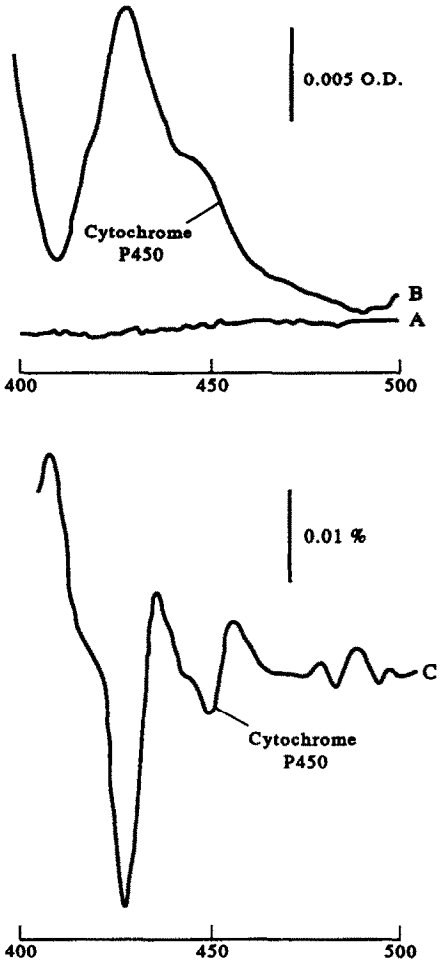

Fig. 3. Differential spectra of CO-complexed dithionite-reduced versus CO-complexed cytochrome P450 from cerebral fractions. Example of a mitochondrial fraction (parietal cortex, case 2). (A) Baseline; (B) zero order spectrum; (C) second derivative spectrum. Protein concentration in the cuvettes: 0.8 mg/mL.

each region studied (not shown). The immunoblot analysis of microsomal human and rat epoxide hydrolase is shown in Fig. 6. The good specificity of the antiserum raised against the rat hepatic epoxide hydrolase was assessed by the recognition of a single band in the rat hepatic preparation (Fig. 6, line A), even when 50 μg of protein was electrophoresed (not shown). The antiserum recognized the brain enzyme both in rat and human, with an apparent molecular mass, close to 50 kDa, similar in three preparations. The staining of the signal was much more intense in human as compared with rat preparations, and thus confirms the enzymatic data obtained. Among conjugation enzymes, UDPGTs are a family of membrane-bound isoenzymes responsible for the formation of glucuronides. The main isoform of UDPGT in rat brain metabolizes 1-naphthol [16]. This isoform was detected only in the temporal cortex from case 4, in frontal cortex from case 5, and in tissue obtained from surgical waste, where the microsomal activities, 0.32, 0.1 and 0.11 nmol/hr·mg protein, respectively, were low: by comparison, using similar analytical conditions, the activities we measured in the rat cortices and cerebellum were 6.2 ± 0.9 and 4.5 ± 1.0 nmol/hr·mg protein, respectively [30]. The immunoblotting experiments performed have revealed several bands in rat hepatic microsomes, but have not permitted visualization to a specific signal in either rat or human brain preparations (not shown).

Two main cytosolic enzymes metabolizing xenobiotics were characterized in human brain. As the activities measured differed from case to case, individual values are presented in Table 3. The activities of cytosolic epoxide hydrolase were similar between the regions, and for case 2, the activity was twice the mean activity of the other four cases. These activities were in the same range in human and rat brain.

The glutathione-*S*-transferase activities toward *trans*-stilbene oxide are heterogeneous between the different cases. However, except for case 2, they are 5–40 times higher in human than in rat brain cortices and cerebellum, with a mean ratio of 19:1, if the values from the five investigated cases were pooled and analysed together. No evidence of sex-related differences was observed for any enzyme activity in human brain tissue.

Drug-metabolizing enzymes in human brain microvessels. Human microvessel preparations were obtained from a pool of three cortical regions for each case. The purity of brain microvessel fractions was checked by both optical microscopy, and by determination of GGT activity. GGT was enriched 7–19-fold in these preparations compared with the corresponding homogenates, depending on the case under investigation, but without correlation with the post-mortem delay before sampling (not shown). The microscopic aspect of the preparation as well as the estimated enrichment of GGT activity was in accordance with the observations made on a rat brain preparation isolated by the same procedure.

Cytochrome P450 content, and the activity of related monooxygenases as well as some other drug-metabolizing enzymes were investigated in these preparations. The results are presented in Table 4.

Recording the spectra of the CO-complexed dithionite-reduced microvessel preparations has allowed the visualization and the quantification of the cytochrome P450 haemoprotein. Amounts of cytochrome P450 ranged from 5.5 to 13 pmol/mg protein. Microvessel preparations should be considered as whole homogenates. Therefore, and by comparison with the microsomal and mitochondrial contents reported in Fig. 3, the content of cytochrome P450 is at least as high in brain microvessels as in brain tissue itself.

The measurement of cytochrome P450-dependent activities showed that only BROD was detectable in the microvessels obtained in each case. PROD was only detected in one case and EROD was never detected. This is in close accordance with the situation observed in rat brain microvessels [17].

The other drug-metabolizing enzyme activities measured in human brain microvessel preparations

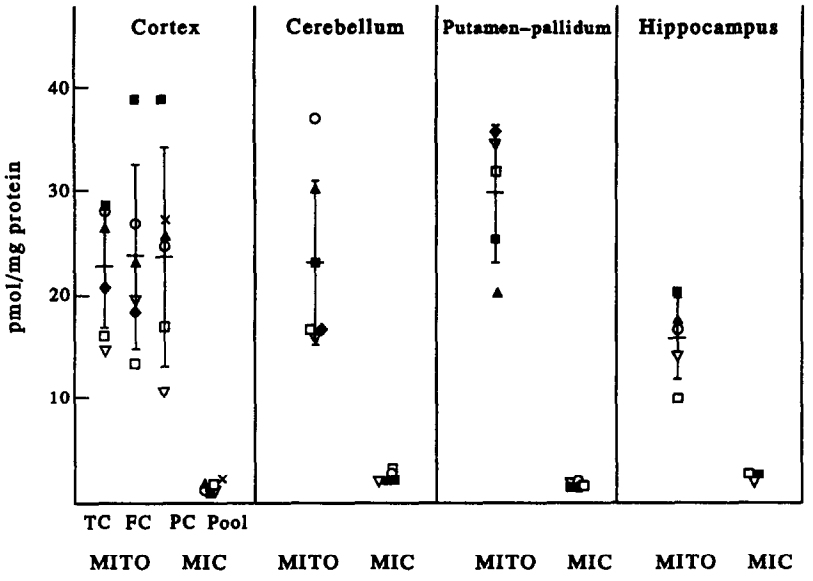

Fig. 4. Cytochrome P450 content in mitochondrial and microsomal fractions isolated from human brain. MITO, mitochondrial fraction; MIC, microsomal fraction. FC, frontal cortex; TC, temporal cortex; PC, parietal cortex. Symbols represent the cases investigated as described in Table 1. X represents the data obtained with parietal cortex sample originated from surgical waste. Results are expressed as pmol/mg protein. Statistical analysis: all cytochrome P450 contents from mitochondrial fractions differ from these contents of the corresponding microsomal fractions, Student's t-test, $P < 0.01$.

displayed large differences when compared with previous data obtained with rat preparations [9]. A 5–10-fold lower NADPH-cytochrome P450 reductase activity was measured in the preparations obtained from each case. The activity of UDPGT was not detected in human microvessels. By contrast, a high activity of microsomal epoxide hydrolase was observed in human brain microvessels, as in human brain microsomes isolated from cells which are not of cerebrovascular endothelial origin (Table 2). Epoxide hydrolase activity was lower in cases 3 and 5 than in other cases, this also being observed for GGT, a marker of the preparation.

DISCUSSION

The results reported here describe the activities and localization of typical drug-metabolizing enzymes in human brain. These enzymes catalyse oxidation, reduction or hydrolysis among modification reactions, and glucuronide or glutathione, conjugate formation among conjugation reactions.

Cytochrome P450 and associated xenobiotic monooxygenases have been detected in the brain of rodent species by several groups (for a review, see Ref. 8). We reported here for the first time the spectral evidence and the subcellular distribution of cytochrome P450 in human brain, thus leaving no doubt about the main mitochondrial localization of the human cerebral cytochrome P450 haemoprotein, a situation previously observed in the brain of rodent species [26]. On the other hand, we also showed the occurrence of cytochrome P450-dependent side-chain cleavage of cholesterol in rat brain mitochondria [31]. So, a possible function of mitochondrial cytochrome P450 is related to steroid metabolism, although the use of antibodies raised against bovine cytochrome P450-side-chain cleavage has permitted the visualization of an immunoresponsive protein only in the white matter of the cerebellum of human brain [32].

NADPH-cytochrome P450 reductase, an enzyme known to supply electrons not only to the microsomal cytochrome P450 but also to enzymes responsible for desaturation and elongation of fatty acids [33, 34], was evidenced in human brain throughout the regions studied, with a lower specific activity than in the rat brain. This result is corroborated by the work of Iscan *et al.* [35] on non-human primate showing that NADPH-cytochrome P450 reductase activity in monkey brain represents only 20% of the activity measured in rat brain. Together with NADPH-cytochrome P450 reductase, human brain microsomal xenobiotic metabolizing cytochrome P450 isoforms were evidenced in all regions investigated, by the measurement of BROD activity. This activity is considered as a useful probe for the detection and measurement of xenobiotic transforming cytochrome P450 abilities, especially in extrahepatic organs [36]. Cytochrome P450 IA2 is involved in the metabolism of arylhydrocarbons [27], and cytochrome P450 IIIA4 is able to catalyse the oxidation of numerous xenobiotics in addition to endogenous steroids [37]. So, the measured rates of 7-ethoxyresorufin and 7-pentoxyresorufin biotransformations have allowed us to differentiate at least two different isoenzymes of cytochrome P450 in the human brain. This finding was previously observed in the rat brain, where both

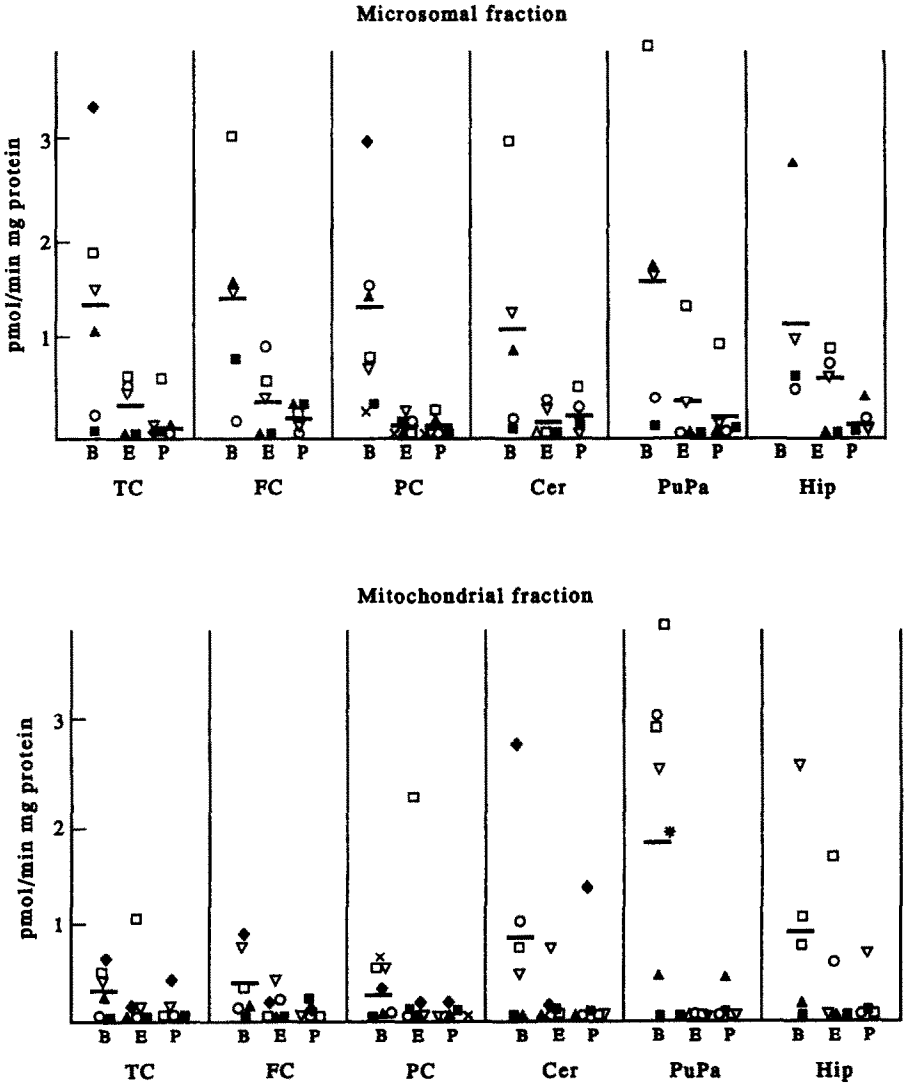

Fig. 5. BROD (B), EROD (E) and PROD (P) activities in mitochondrial and microsomal fractions isolated from six regions of human brain. Symbols represent the cases investigated as described in Table 1. X represents the data obtained with parietal cortex sample originated from surgical waste. Specific activities are expressed as pmol/min·mg protein. *Statistically different from the values obtained from each cortical region, $P < 0.05$, paired *t*-test. TC, temporal cortex; FC, frontal cortex; PC, parietal cortex; Cer, cerebellum, PuPa, putamen–pallidum; Hip, hippocampus.

cytochrome P450 IIB and IA families were detected either by immunological studies [38] or by measurement of their specific activities [17]. Finally, the evidence for a 7-benzoxyresorufin-O-dealkylation in mitochondrial fractions is consistent with previous reports showing that metabolism of arylhydrocarbons occurs in rat brain mitochondria [39]. Epoxide hydrolases are important enzymes in the metabolism of olefinic and aromatic substrates since they represent a major enzymatic pathway for the further bioactivation of intermediate epoxides and arene oxides. This specific hydrolytic capacity is probably of paramount importance in human brain, as both the microsomal enzymatic activity and the immunoreactive protein are much higher in human brain when compared to rat brain. On the other hand, cytosolic enzyme activities were similar in both species. In other human extrahepatic organs, Waziers *et al.* [28] reported low epoxide hydrolase activities as compared with those found in the liver, and suggested that this was related to a low production of epoxide due to the low cytochrome P450 content in these organs. The high microsomal epoxide hydrolase activities in human brain are therefore intriguing. Whether or not a role of this enzyme in specific endogenous cerebral biochemical pathways exists remains to be established.

The two systems of conjugation measured in human brain display a different pattern when compared with rat brain. UDPGT activity toward

Table 2. NADPH-cytochrome P450 reductase and epoxide hydrolase activities in human brain microsomes

Regions	Reductase (nmol/min·mg protein)					EHm (pmol/min·mg protein)				
	FC	TC	PC‡	Cer	PuPa	Hip	FC	TC	PC‡	Cer	PuPa	Hip
Human	1.5 ± 1.0*	1.3 ± 0.7*	1.4 ± 0.5*	1.4 ± 0.7†	2.1 ± 1.1	1.3 ± 0.5	333 ± 45*	291 ± 106*	284 ± 140*	381 ± 114†	357 ± 92	377 ± 58
Rat		18.3 ± 2.0		10.5 ± 1.1	—	—		48.8 ± 9.7		33.4 ± 10.8	—	—

Reductase, NADPH-cytochrome P450 reductase activity; EHm, membrane-bound epoxide hydrolase activity; FC, frontal cortex; TC, temporal cortex; PC, parietal cortex; Cer, cerebellum; PuPa, putamen–pallidum complex; Hip, hippocampus.
The data reported in human brains are the mean ± SD of values measured in the 6 cases. Individual data were the mean of 2 or 3 determinations.
Rat brain data were exposed as mean ± SD, N = 4.
* and †: statistically different from cortex and cerebellum rat values, respectively. Student's *t*-test, $P < 0.01$.
‡ The value obtained from microsomes issued from surgical waste tissue was 9.4 nmol/min·mg protein for reductase and 370 pmol/min·mg protein for EHm.

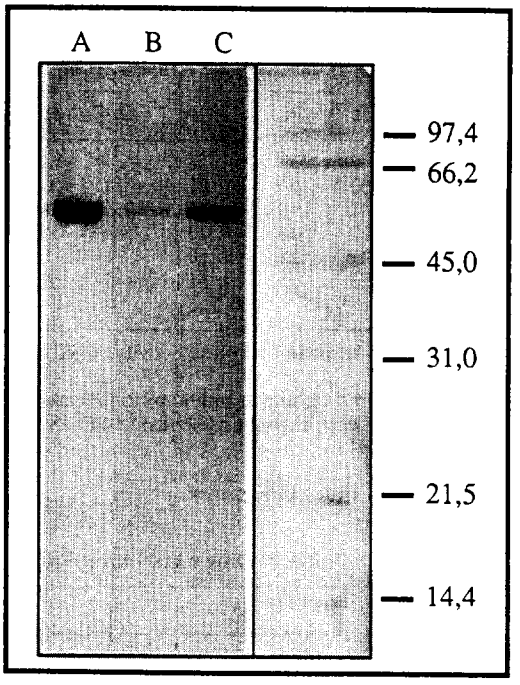

Fig. 6. Immunoblotting analysis of hepatic and cerebral microsomal fractions using polyclonal antibodies raised against rat liver microsomal epoxide hydrolase. Lane A: liver microsomes (10 μg protein loaded onto the gel); Lane B and C: rat and human brain microsomes, respectively (20 μg protein loaded onto the gel).

1-naphthol was barely or not detectable in human brain, whereas it was easily measurable in the different rat brain regions [30], even after a post-mortem delay before sampling (Fig. 1A). This result is not in accordance with those of Viani *et al.* [40] who reported in a recent study on human brain surgical waste UDPGT activity in different regions of each case investigated. However, the activities reported by these authors were largely lower than the one we measured in the rat [30]. By contrast, we observed a glutathione-*S*-transferase activity toward *trans*-stilbene oxide higher in human than in rat brain. At least three different forms, characterized by their isoelectric points and their immunological properties, exist in human brain [41], *trans*-stilbene oxide being more specific of the *mu* form. Although the whole conjugating capacity of the brain cannot be completely assessed by the use of a single substrate, this high glutathione-*S*-transferase activity is of importance as, together with hydrolysis of epoxides, glutathione conjugation is a major pathway for inactivating highly reactive metabolites. Also, the cellular localization and binding properties toward hormones and exogenous molecules point out possible specialized functions for glutathione-*S*-transferase in this organ, possibly in transport processes [42]. The large variation in glutathione-*S*-transferase activity toward *trans*-stilbene oxide observed between cases suggests a possible polymorphism of the human brain form(s) conjugating

Table 3. Glutathione-*S*-transferase and epoxide hydrolase activities in human and rat brain cytosolic fraction

Case number	EHc (pmol/min·mg protein)						GST nmol/min·mg protein					
	FC	TC	PC	Cer	PuPa	Hip	FC	TC	PC	Cer	PuPa	Hip
2	56	103	138	78	149	127	0.11	0.11	0.09	0.10	0.09	0.12
3	89	53.5	54	62	32	62	6.5	8.5	7.5	6.6	4.1	12
4	37	42	40	41	35	21	2.6	2.9	0.8	0.8	0.7	0.7
5	33	27	13	23	34	43	7.8	10.1	5.5	16	10.2	17
6	27	39	28	50	44	15*	9.6	11.5	6.3	5.5	3.2	1.5
Mean	48	53	55	51	59	53						
Rat		29 ± 9		30.5 ± 6	—	—		0.26 ± 0.07		0.36 ± 0.07	—	—

EHc, cytosolic epoxide hydrolase activity; GST, glutathione-*S*-transferase activity. Other abbreviations as in Table 2.

The data reported in human brain are the mean of two determinations except: * only one determination.

Rat brain data were expressed as mean ± SD, N = 4.

Table 4. Drug-metabolizing enzymes in human brain microvessel preparations

Case number	1	2	3	4	5	6	Mean
Cytochrome P450 content (pmol/min·mg protein)	6.7	5.5	7.5	12.2	9.7	13	9.1
Enzyme activities*							
BROD (pmol/min·mg protein)	0.62	0.39	0.70	0.75	0.47	0.58	0.58
PROD (pmol/min·mg protein)	—	ND	0.152	ND	ND	ND	—
Reductase (nmol/min·mg protein)	0.47	0.38	1.2	0.2	1.5	1.9	0.9
EHm (pmol/min·mg protein)	283	115	66	—	60	124	130
GGT (μmol/min·mg protein)	—	60	33	22	27	66	42

Reductase, NADPH-cytochrome P450 reductase; EHm, membrane-bound epoxide hydrolase

The data reported are the mean of two determinations—not determined; ND, not detected.

* Ethoxyresorufin-*O*-deethylase and 1-naphthol UDP-glucuronosyltransferase activities were not detected, whatever the case under investigation.

this substrate, probably related to a neutral (*mu*) form, as evidenced in a tissue non-specific manner for other human organs [28, 43].

The importance of drug-metabolizing enzymes in the microvessels of human brain stems from the large range of circulating exogenous molecules which can be in contact with cerebral endothelial cells. Thus, some biotransformation resulting in an inactivation and/or an increase of their polarity can take place before they cross the abluminal membranes. The cytochrome P450 content and related activities toward exogenous molecules show a similar pattern in human brain microvessels as compared with the rat [17]. However, the low NADPH-cytochrome P450 reductase activity, the absence of detectable glucuronidation and the high epoxide hydrolase activity observed in human cerebral microvessels contrasted with the data of the rat, where we found a particularly high UDPGT activity in cerebral microvessels, which was 15-fold higher than in brain tissue [9]. The role of GGT from brain microvessels, whose activity is slightly higher in human than in rat (unpublished observation), is not really understood. Its implication in detoxication processes is an interesting question, as this enzyme catalyses the first step of mercapturic acid formation from glutathione conjugates [44], and so could play a role in the efflux from the brain of these metabolites.

In conclusion, the human brain contains to some extent the drug-metabolizing enzyme equipment needed for complete detoxification pathways, with particularly high activities of microsomal epoxide hydrolase and cytosolic glutathione-*S*-transferase. Their cellular localization or co-localization in human brain is a critical factor in the understanding of the true toxicopharmacological impact of these enzymes, and remains to be determined.

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