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Microsomal cytochrome P450 in human brain regions

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Abstract—Cytochrome P450 (P450) levels were quantitated in microsomes from human brain regions obtained at autopsy. The reduced carbon monoxide binding spectra of cortical microsomes showed two absorption maxima at 449 and 425 nm. On solubilization of the microsomes, essentially a single peak was observed at 449 nm. The P450 levels in human brain cortical microsomes varied from 0.03 to 0.12 nmol/mg protein among the seven samples examined. The concentration of the hemeprotein present as nmol/g tissue was highest in the brain stem and cerebellum and lowest in the striatum and hippocampus.

Cytochromes P450 (P450, EC 1.14.14.1), a family of heme proteins, are the major xenobiotic-metabolizing enzymes in the body. They are also involved in steroid and fatty acid metabolism [1]. The liver contains the largest amount of P450 and the hepatic enzymes have been well characterized both in humans [2] and experimental animals [3]. The extrahepatic tissues have not been investigated extensively, in humans particularly [4].

During recent years a definitive role has been ascribed to environmental toxins in the aetiopathogenesis of neurodegenerative disorders [5]. In view of this, a cerebral P450-mediated metabolism leading to bioactivation or detoxification of xenobiotics "*in situ*" in the brain would be of great importance. Furthermore, a diverse functional role for brain P450 has been suggested. The heme protein is present in close association with the dopamine uptake site [6], and the sigma/dopamine 1 binding site has been shown to bear close homology with P450 [7].

The presence of cytochrome P450 and associated monooxygenase activity was first reported in rat brain [8], and several laboratories have subsequently confirmed this observation [9]. More recently, the presence of multiple forms of P450 and their selective inducibility have also been demonstrated [10]. Monooxygenase activities associated with P450 have been detected in different regions of the human brain collected at post-mortem. The human brain P450 cross-reacted immunologically with antisera to purified rat liver P450 (IIB1+IIB2). Using this antisera, P450 was found to be localized preferentially in the neuronal soma by immunohistochemistry [11].

The actual amount of the heme protein P450 in the human brain has not been reported hitherto [12]. While monooxygenase activities give an indication of the capability to metabolize specific substrates, determination of P450 levels is essential to evaluate the total xenobiotic-metabolizing capability of the human nervous tissue and to demonstrate the presence of the heme protein unequivocally. We hereby report the quantitative regional distribution of P450 in seven human brain tissues and present the reduced carbon monoxide binding spectra from solubilized and unsolubilized microsomes.

Materials and Methods

Human brain tissues were collected at autopsy from seven male traffic accident victims after informed consent of close kith and kin. None of the cases had any known neurological or metabolic disorders prior to death. Following injury, the patients were maintained on artificial respiration for a period of 0–144 hr. The average interval between death and autopsy was 5.2 ± 3.0 hr (mean ± SD). The average age of the deceased subjects was 39.6 ± 18.9 years (mean ± SD). Some of the patients received mannitol and glycerol as antiedema measure, but no anticonvulsant medication was administered. After death, the bodies were kept at 10–14°. At autopsy, the brain did not reveal any features of infection, atrophy or developmental anomalies. The cerebral vessels were normal and patent. Immediately after removing the brain, the blood was thoroughly washed off with cold buffered saline. The liver was also collected at autopsy from three of the cases. The tissues were dissected free of meninges, blood vessels and other fibrous elements. The brain was dissected into discrete regions using standard anatomical landmarks. The tissues were stored at −70° prior to analysis.

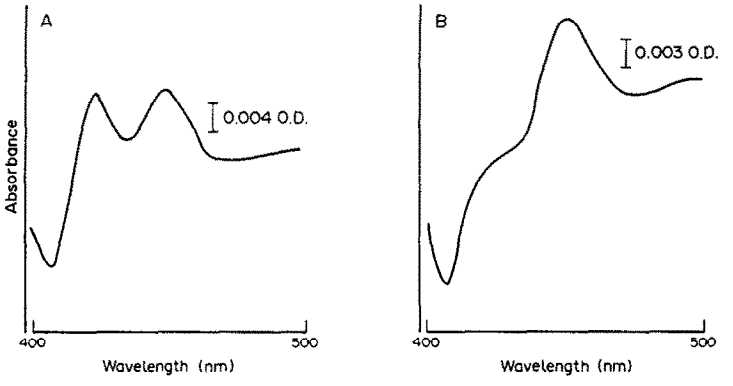

Fig. 1. Reduced carbon monoxide spectra of (A) human brain cortical microsomes and (B) human brain cortical microsomes solubilized with sodium cholate. The protein concentration was 850 μg/mL in (A) and 410 μg/mL in (B). The human brain cortical microsomes were prepared from the tissue obtained from case III.

Table 1. Cytochrome P450 levels in human brain regions

Case	Interval between death and autopsy (hr)	Age (years)	CT	CE	MD	Pons	MB	ST	HP	TH	Liver
I	4	35	0.08	—	0.09	—	—	—	—	—	0.32
II	4	18	0.04	0.04	0.05	—	—	—	—	—	—
III	4	58	0.12	—	—	—	—	—	—	—	—
IV	12	50	0.11	0.064	0.13	0.05	0.04	0.05	0.04	0.101	0.11
V	4	35	0.04	0.05	0.04	0.06	0.03	0.03	0.02	0.06	—
VI	4	65	0.03	0.04	0.06	0.07	0.07	0.03	0.05	0.03	—
VII	4	16	0.12	0.14	0.17	0.12	0.20	0.16	0.07	0.10	0.11

P450 levels in the microsomal preparation were determined as described and P450 content is expressed as nmol/mg protein.

CT, cortex; CE, cerebellum; MD, medulla; MB, midbrain; ST, striatum; HP, hippocampus; TH, thalamus.

Microsomes were prepared from human brain regions and liver [13], and the P450 content determined from the reduced carbon monoxide spectra [14, 15] using dithionite as a reducing agent. The microsomal protein concentration used for P450 assay varied from 0.15 to 1 mg/mL. The detection limit was 9 pmol/mg protein. The microsomes were solubilized using sodium cholate [16] and the P450 content was determined from the reduced carbon monoxide spectra. All buffers were bubbled with nitrogen gas prior to use.

Results and Discussion

The reduced carbon monoxide spectrum of microsomes from the human cerebral cortex (case III) is depicted in Fig. 1A. The spectrum exhibited two absorption maxima at 449 nm, corresponding to P450, and 425 nm, possibly corresponding to its denatured form, cytochrome 420. The peak at 425 nm could also be contributed to by cytochrome b_5. The optical density of the peak at 425 nm varied from sample to sample. The reduced carbon monoxide spectra from solubilized microsomes exhibited a single peak at 449 nm. The P450 levels in solubilized microsomes (0.19 nmol/mg protein) was 150% of that present in unsolubilized microsomes (0.12 nmol/mg protein).

The P450 levels in different regions of the human brain and the liver are shown in Table 1. The P450 levels could not be estimated in all brain regions from all samples due to non-availability of tissues. Detectable P450 levels were observed in all available samples. The P450 level in the cerebral cortex of case I was 0.08 nmol/mg protein which was 25% of the corresponding hepatic level (0.32 nmol/mg protein). However, the hepatic P450 levels were comparable to that in the cerebral cortex in case VII.

In experimental animals, the P450 levels in the whole brain have been typically 4–10% of the corresponding hepatic levels [8–10]. In human tissues, the cerebral P450 levels are proportionally higher in spite of the possible autolytic changes that could have taken place between death and autopsy, but this observation needs to be substantiated with more samples. The P450 levels in mouse and rat brain are 40 and 100 pmol/mg microsomal protein, respectively [8, 10]. The P450 levels in human brain regions ranged from 30 to 170 pmol/mg protein. The P450 levels in the human cerebral cortex ranged from 0.03 to 0.12 nmol/mg protein indicating the differential levels of the hemeprotein in the samples examined. The P450-mediated monooxygenase activities in the human brain also show variation [11]. Genetic polymorphism of P450 in the human liver is well established [17]. It remains to be determined if similar genetic polymorphism exists in the human brain as well.

Brain P450 is present in both microsomes and mitochondria [18]. In the present study, only the microsomal P450 concentrations have been determined. Thus, the total concentration of P450 in the human brain (microsomal and mitochondrial) would be substantially higher.

P450 levels also exhibited regional variation, and ranged from 0.04 to 0.13 nmol/mg protein (case IV) among the different regions of the brain that were examined. The maximal levels of P450 in case IV and V were detected in the cortex, medulla and thalamus within the respective individual sample. Maximal P450 levels were detectable in the pons, medulla and midbrain in case VI and case VII. In addition, in case V, P450 levels were higher in the pons and, in case VII, P450 levels were higher in the cortex and striatum. While higher P450 levels were observable in the pons (cases V, VI and VII), the corresponding levels in case IV were substantially lower than those in the cortex; 0.05 and 0.11 nmol/mg protein in the pons and cortex, respectively. Since the yield of microsomes from different regions of the brain varied, the brain P450 levels of cases IV, V, VI and VII are expressed as nmoles per gram tissue in Fig. 2. The highest levels of P450 were detectable in the pons, cerebellum, medulla and midbrain. The lowest concentration of P450 was found in the striatum and hippocampus. The post-mortem changes could also have contributed to these differences to a certain extent. The microsomal yield did not vary significantly between samples, except for case VII wherein the microsomal yield was substantially lower (50% lower) as compared to the other cases (data not shown).

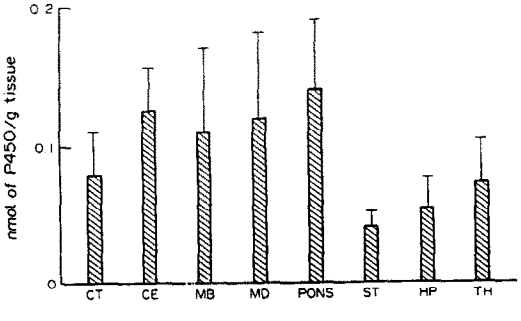

Fig. 2. Concentration of P450 in various regions of the human brain. The P450 levels in various regions of the brain from case IV, V, VI and VII are expressed as nmol/g tissue. The values are means ± SD (N = 4). Abbreviations are as for Table 1.

The concentration of P450 per gram tissue is highest in the brain stem region comprising of the midbrain, pons and medulla. This region is known to possess low levels of glutathione [19] and hence may be vulnerable to damage through P450-mediated bioactivation of xenobiotics.

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