Brain Research, 496 (1989) 331–335
Elsevier

BRES 23683

Xenobiotic metabolism in human brain — presence of cytochrome *P*-450 and associated mono-oxygenases

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(Accepted 9 May 1989)

Key words: Human brain; Xenobiotic metabolism; Mono-oxygenase; Cytochrome *P*-450; Immunocytochemical localization

The cytochromes *P*-450, a family of heme proteins, play an important role in the oxidation of drugs and carcinogens, as well as endogenous substrates. We report the presence of cytochrome *P*-450 and associated mono-oxygenase activity in human brain regions and their selective enrichment in the brainstem. Immunocytochemical studies on human medulla with antibodies raised to phenobarbital-inducible rat liver cytochrome *P*-450 indicate that the enzyme is primarily localized in the neuronal cell bodies and to a lesser extent in the axons. These observations indicate that the human brain could be involved in metabolism of xenobiotics and endogenous compounds, mediated through cytochrome *P*-450.

The cytochrome *P*-450-containing mono-oxygenases (MOs) play an important role in the metabolism, with respect to both detoxification and activation, of a variety of endogenous compounds (steroids, fatty acids, eicosanoids, etc.) and xenobiotics including drugs[2]. The cytochrome *P*-450 (*P*-450), a family of heme proteins, have been well characterised in liver of both experimental animals and humans[3,5], as liver is the primary organ involved in the metabolism of xenobiotics. Recent interest has centered on the capability of the extra-hepatic tissues to metabolize foreign compounds and the consequences of this action. The presence of *P*-450 has been reported in several extra-hepatic tissues of experimental animals such as rat and mice[10]. However, *P*-450 and associated mono-oxygenases have not been reported in human brain. If established, it raises the possibility of local regulation of pharmacological action of drugs and other endogenous compounds. More recently, exposure to environmental toxins has been linked to the pathogenesis of several neurodegenerative disorders[1,9]. In the light of these findings and limited regenerative capability of the nervous tissue, the consequences of cerebral metabolism mediated by *P*-450 may be far reaching, in causing disruption of neuronal function and contributing to drug action[10]. The present study demonstrates the presence of *P*-450 and associated MOs in human brain and its selective enrichment in the brainstem.

Microsomes were prepared from discrete regions of 6 human brains obtained at autopsy, from victims of traffic accidents with no known neurological disorders. The average interval between death and autopsy was 8.16 ± 1.6 h; the average age of the deceased was 55 ± 19.8 years. Following injury, the patients were kept on artificial respiration for a period of 12–144 h. All patients received mannitol and glycerol but no corticosteroids as antiedema measures. One patient (case VI) received 1 g of phenobarbital over 24 h. None of the others received any anticonvulsant medication. Following autopsy the brain was dissected into discrete regions using standard anatomical landmarks. Liver was obtained from 5 of the above cases and kidney from two cases. The tissues were washed free of blood, blood vessels

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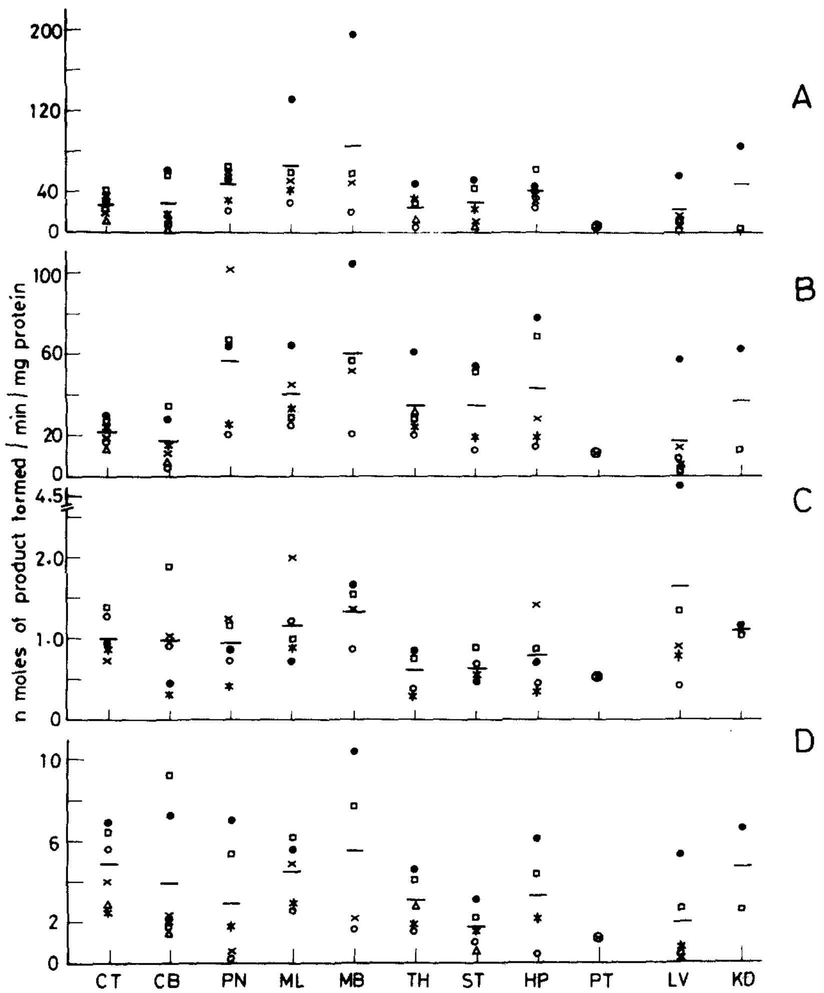

Fig. 1. Microsomal mono-oxygenase activity in human liver, kidney and brain regions. A: aminopyrine *N*-demethylase. B: morphine *N*-demethylase. C: aniline hydroxylase. D: ethoxycoumarin *O*-deethylase. The ages of the deceased were as follows: case I, 70 yrs (△); case II, 73 yrs (×); case III, 55 yrs (○), case IV, 35 yrs (□); case V, 27 yrs (●) and case VI, 70 yrs (*) and pituitaries were pooled from 10 autopsied cases (⊗). The horizontal bars (–) indicate the mean enzyme activity in the tissues. Abbreviations: CT, cortex; CB, cerebellum; PN, pons; ML, medulla; MB, midbrain; TH, thalamus; ST, striatum; HP, hippocampus; PT, pituitary; LV, liver; KD, kidney.

and meninges removed, and stored at −70 °C prior to analysis. Microsomes were prepared from tissues[12] and the mono-oxygenase activities were measured as described[6]. P-$450_{(b+e)}$ was purified from phenobarbital-treated rat liver[6] and the antisera to it was raised in rabbits. Immunoblot analysis of human brain microsomes was carried out using this antisera. Microsomes from human brain regions and liver were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)[8] and the proteins were transferred to nitrocellulose membrane[14]. The proteins were then immunostained with the antisera to rat liver P-$450_{(b+e)}$[7].

Frontal cortex, cerebellum and medulla oblongata were collected from an 11-year-old child, who had died of spinal cord injury. The tissues were fixed in 5% buffered formalin and processed for paraffin sectioning. Serial sections (6 μm thick) were collected on 0.1% gelatinized glass slides. The sections were immunostained by the peroxide–antiperoxide technique using preimmune rabbit serum, polyclonal rabbit antiserum to rat liver P-$450_{(b+e)}$ and antiserum preadsorbed with antigen. Sections were incubated for 24 h at 4 °C. The immune reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide.

TABLE I

Effect of various inhibitors on cytochrome P-450 associated mono-oxygenase activity in human cortical microsomes

Assays were carried out with cortical microsomes from case VI at 37 °C, unless otherwise specified (see Fig. 1 legend). Control incubations were carried out in an atmosphere of air. Blanks containing no NADPH and no substrate were run and their values substracted from experimental values. The control activities of (A) aminopyrine *N*-demethylase and (B) ethoxycoumarin *O*-deethylase were 45.04 and 2.06 nmol of product formed/min/mg protein respectively. Values are mean ± S.D. ($n = 3$).

Treatment	*Percent control*	
	A	*B*
1. Control	100	100
2. 25 °C	29.17 ± 1.26	13.14 ± 1.00
3. 100% nitrogen	20.47 ± 6.50	22.75 ± 8.32
4. 100% oxygen	125.27 ± 27.0	116.87 ± 11.0
5. 100% carbon monoxide	13.47 ± 6.35	13.14 ± 1.00
6. Carbon monoxide + air	18.72 ± 5.36	13.14 ± 1.00
7. Piperonyl butoxide	26.38 ± 5.70	6.35 ± 1.60
8. Antisera to $P\text{-}450_{(b+e)}$	67.98 ± 5.70	21.91 ± 9.79

Significant amounts of microsomal MOs were detectable in all regions of the human brain examined (Fig. 1). Maximal activity of aniline hydroxylase was observed in medulla oblongata and midbrain, followed by pons, cerebral cortex and cerebellum; corpus striatum and thalamus had lesser activity. Ethoxycoumarin *O*-deethylase was highest in mid-

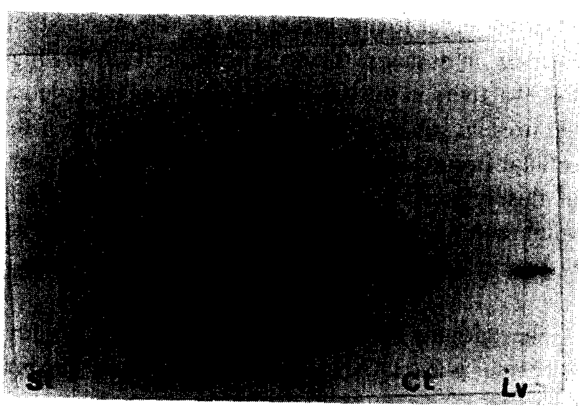

Fig. 2. Immunoblot of microsomal protein from human brain and liver (case VI, 70 yrs old) with antisera to $P\text{-}450_{(b+e)}$: striatum (St, 50 μg), medulla (Ml, 26 μg), thalamus (Th, 42 μg), cortex (Ct, 49 μg) and liver (Lv, 43 μg). The amount of microsomal protein loaded on the gel is given in parentheses. Similar immunoblots were obtained from microsomal proteins from case IV (35 yrs old).

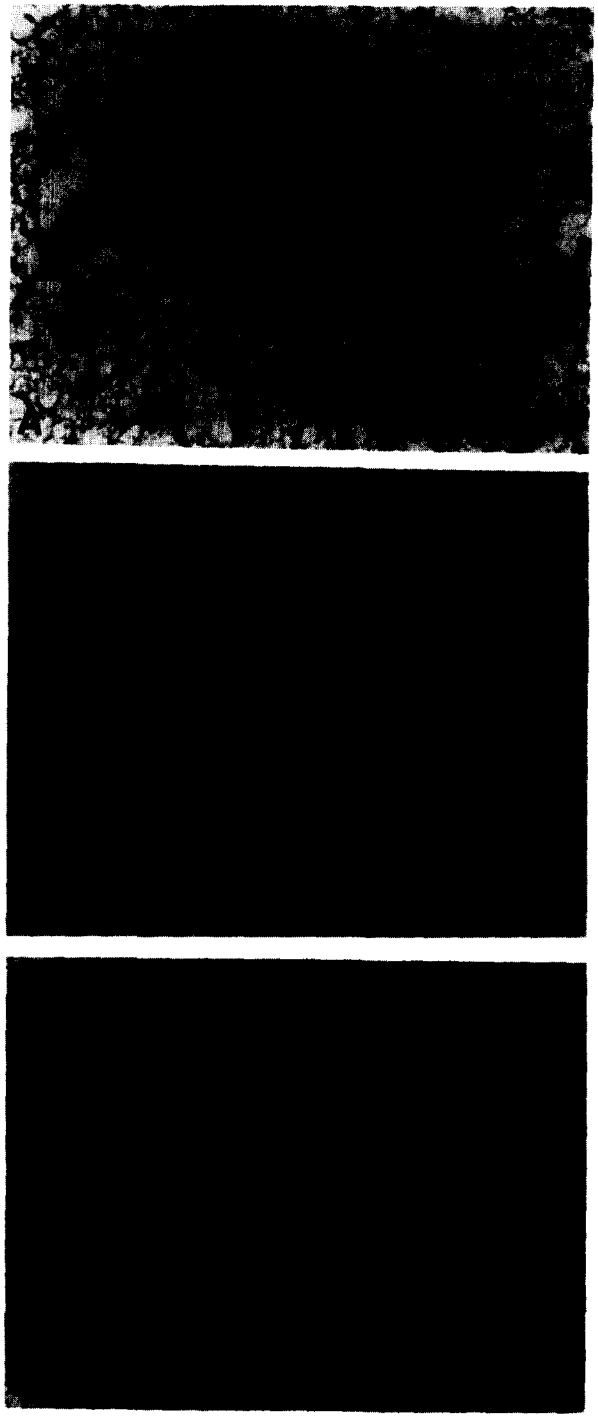

Fig. 3. Immunocytochemical localization of cytochrome *P*-450 in human brain medulla oblongata using antisera to rat liver $P\text{-}450_{(b+e)}$. A: neurons of the lower cranial nerve nuclei showing strong immunoreaction in neuronal somata (×160). B: absence of immunoreaction with antisera preadsorbed with antigen (×144). C: large neurons of the reticular formation and long fiber tracts in medulla oblongata showing positive immunoreactivity (×192).

brain and medulla, followed by cerebral cortex, cerebellum and pons. Morphine *N*-demethylase activity was highest in midbrain, pons and medulla, followed by hippocampus, corpus striatum and thalamus, while cerebral cortex and cerebellum had lesser activity. All the regions of the brain examined, except cortex and cerebellum had higher capability to *N*-demethylate morphine as compared to the liver from the same individual. In fact, the specific activity of all the MOs examined was higher in the brainstem region as compared to the liver, in spite of the possible autolytic changes that would have taken place in both the organs during the interval between death and collection of the tissue at autopsy. The pituitary gland (pooled from 10 autopsied cases) was found to have low levels of MO activity. Another extra-hepatic organ, the kidney, was collected from 2 of the 6 cases for evaluation of renal MOs. Renal MO activity was comparable to that of the cerebral cortex.

The MO activity was inhibited by carbon monoxide and piperonyl butoxide, both inhibitors of *P*-450. The enzyme activity was also dependent upon the presence of both oxygen and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), and a considerable increase in activity was observed when the enzyme assays were carried out in the presence of 100% oxygen (Table I). These observations indicate that the mono-oxygenase reaction are indeed mediated by *P*-450. Further, addition of the rabbit antisera, raised to phenobarbital-inducible rat liver *P*-450 (P-$450_{(b+e)}$), partially inhibited aminopyrine *N*-demethylase (32%) in cortical microsomes. Ethoxycoumarin *O*-deethylase was inhibited to a greater extent (78%) by the same antisera.

The immunoblot analysis of human brain and liver microsomes indicated the presence of a protein that cross-reacted with antisera to rat liver P-$450_{(b+e)}$ (Fig. 2). Maximal staining was observed in the microsomal protein from medulla oblongata, where 3 bands were observed on immunostaining, while only one band was observed in the other regions and liver. These results indicated the presence of an immunologically related form of P-$450_{(b+e)}$ in the human brain. Immunological cross-reactivity between human liver, human lymphocytes and P-$450_{(b+e)}$ from rat liver, has been detected earlier[4,13,15]. In the present study, similar immunological reaction is observed between human brain and rat liver *P*-450. Hence, the antisera raised against P-$450_{(b+e)}$ was used for the cellular localization of *P*-450 in human brain using immunocytochemistry.

Immunohistochemical studies on the 3 human brain regions with antisera raised to P-$450_{(b+e)}$ revealed maximal staining in the medulla oblongata, while the cerebral cortex and cerebellum showed minimal staining reaction. In the medulla oblongata, immunoreactivity was detected in the neuronal cell bodies, dendrites of the reticular formation and lower cranial nerve nuclei (Fig. 3A,C). The inferior olivary nucleus neurons were stained light. In addition, the axons of the long fibre tracts were lighted up variably. No staining was observed when antisera preadsorbed with antigen was used for staining (Fig. 3B). Antisera to rat liver NADPH cytochrome *P*-450 reductase stained in the same region within the medulla oblongata (data not shown), indicating the co-localization of the two enzymes.

The presence of significant amount of *P*-450 and associated MOs in the human brain regions indicates that this organ also possesses considerable xenobiotic metabolizing capability. Thus, nervous tissue is likely to play an important role, through *P*-450-mediated metabolism, in the local pharmacological action of drugs and in the biotransformation of environmental toxins acting on the nervous system. Further, in humans, the presence of a relatively high *P*-450 activity in the brainstem region, as observed in the present study, coupled with the low levels of the cellular protectant, glutathione known to be present in this region[11], may render this area particularly vulnerable to damage by neurotoxins which act through bioactivation in situ in the target cell.

The authors thank Prof. G.N. Narayana Reddy, Director, NIMHANS, for his support and encouragement.

1 Calne, D.B., McGeer, E., Eisen, A. and Spencer, P., Alzheimer's disease, Parkinson's disease and motor neuron disease: abiotropic interaction between aging and environment?, *Lancet*, 11 (1986) 1067–1070.
2 Conney, A.H., Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons, *Cancer Res.*, 42 (1982) 4875–4917.
3 Guengerich, F.P., Enzymology of rat liver cytochromes *P*-450. In F.P. Guengerich (Ed.), *Mammalian Cytochromes P-450, Vol. I*, CRC Press, Boca Raton, 1987, pp. 1–54.

4 Guengerich, F.P., Wang, P., Mason, P.S. and Mitchell, M.B., Immunological comparison of rat, rabbit and human microsomal cytochromes *P*-450, *Biochemistry,* 20 (1981) 2370–2378.
5 Guengerich, F.P., Umbenhauer, D.R., Churchill, P.F., Beaune, P.H., Bocker, R., Knodell, R.G., Martin, M.V. and Lloyd, R.S., Polymorphism of human liver cytochrome *P*-450, *Xenobiotica,* 17 (1987) 311-316.
6 Guengerich, F.P., Microsomal enzymes involved in toxicology — analysis and separation. In A.W. Hayes (Ed.), *Principles and Methods of Toxicology,* Raven Press, New York, 1984, pp. 609–634.
7 Guengerich, F.P., Wang, P. and Davidson, N.K., Estimation of isozymes of microsomal cytochrome *P*-450 in rats, rabbits and humans using immunochemical staining coupled with sodium dodecyl sulphate–polyacrylamide gel electrophoresis, *Biochemistry,* 21 (1982) 1698–1706.
8 Laemmli, U.K. and Favre, M., Maturation of the head of bacteriophage T_4, I DNA packaging events, *J. Mol. Biol.,* 86 (1973) 575–599.
9 Langston, J.W. and Irwin, I., MPTP: current concepts and controversies, *Clin. Neuropharmacol.,* 9 (1986) 485–507.
10 Mesnil, M., Testa, B. and Jenner, P., Xenobiotic metabolism by brain mono-oxygenases and other cerebral enzymes .In B. Testa (Ed.), *Advances in Drug Research, Vol. 13,* Academic Press, London, 1984, pp. 95–207.
11 Perry, T.L., Godin, D.V. and Hansen, S., Parkinson's disease: a disorder due to nigral glutathione deficiency?, *Neurosci. Lett.,* 33 (1982) 305–310.
12 Ravindranath, V. and Anandatheerthavarada, H.K., High activity of cytochrome *P*-450 linked aminopyrine *N*-demethylase in mouse brain microsomes and associated sex-related difference, *Biochem. J.,* in press.
13 Robie-Suh, K., Robinson, R., Gelboin, H.V. and Guengerich, F.P., Arylcarbon hydroxylase is inhibited by antibody to rat liver cytochrome *P*-450, *Science,* 208 (1980) 1031–1033.
14 Towbin, H., Staehelin, T. and Gordon, J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, *Proc. Natl. Acad. Sci. U.S.A.,* 76 (1979) 4350–4354.
15 Wang, P., Mason, P.S. and Guengerich, F.P., Purification of human liver cytochrome *P*-450 and comparison to the enzyme isolated from rat liver, *Arch. Biochem. Biophys.,* 199 (1980) 206–219.