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Multiple Forms of Cytochrome P450 and Associated Monooxygenase Activities in Human Brain Mitochondria

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ABSTRACT. We have investigated cytochrome P450 (P450) and associated monooxygenase activities in human brain mitochondria isolated from eight regions of four human brain samples obtained at autopsy. P450-associated monooxygenase activities including aminopyrine *N*-demethylase (APD), 7-ethoxycoumarin *O*-deethylase (ECD), *p*-nitrophenol hydroxylase (PNPH), and *N*-nitrosodimethylamine *N*-demethylase (NDMAD) were detectable in the mitochondria from human brain regions. Immunoblot experiments using antisera to purified rat liver microsomal P450, namely P4502B1/2, P4501A1/2, and P4502E1, revealed immunoreactive bands in isolated mitochondria from different regions of the human brain. The antibody to P4502B1/2 and P4501A1/2 inhibited the human brain mitochondrial APD and ECD activities, respectively. The addition of antiserum to microsomal NADPH cytochrome P450 reductase did not affect the mitochondrial P450-associated monooxygenase activities, although it completely inhibited the corresponding activities in brain microsomes. Overall, the present study demonstrates, in human brain mitochondria, the presence of multiple forms of P450 belonging to the 1A, 2B, and 2E subfamilies that are involved in xenobiotic metabolism. BIOCHEM PHARMACOL **59**;5:573–582, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. human; brain; mitochondria; cytochrome P450; drug metabolism; monooxygenases

P450s§, a superfamily of heme-containing enzymes localized both in the endoplasmic reticulum and in mitochondria, are involved in the metabolism of a variety of xenobiotics and endogenous compounds such as fatty acids and steroid hormones. It is generally recognized that the microsomal P450 forms are involved predominantly in the metabolism of xenobiotics, including drugs. In contrast, the major functional role of mitochondrial P450s pertains to the metabolism of endogenous substrates such as steroid hormones [1]. Several isoforms of P450s have been characterized from the liver, the major organ involved in P450-mediated metabolism [2]. Recent interest has focused on the P450s in extrahepatic organs such as the lung, kidney, and brain in view of the consequences of *in situ* metabolism of both xenobiotics and endobiotics in specific cell types within specialized regions of these organs [3].

The multiple forms of P450 that are selectively inducible following exposure to a variety of drugs and other xenobiotics have been shown to be localized essentially in the endoplasmic reticulum; however, more current reports have demonstrated the constitutive presence of these forms of P450 in mitochondria from the liver [4, 5] and brain [6, 7] of rats. The mitochondrial P450 isoforms, such as P4501A1/2, 2B1, and 2E1, metabolize a variety of xenobiotics and are inducible by β-naphthoflavone, phenobarbital, and ethanol [5, 7, 8] in the rat. It has been demonstrated that a portion of the translated P4501A1 protein that escapes insertion into the endoplasmic reticulum membrane undergoes NH_2-terminal proteolytic cleavage, activating a mitochondrial targeting signal helping to localize the P450 to the inner mitochondrial membrane [9]. Thus, the same gene product with a chimeric signal sequence is targeted to both the endoplasmic reticulum and the mitochondria.

In humans, it is difficult to distinguish the constitutive and inducible forms of P450, since humans are exposed to a wide variety of xenobiotics, including phytochemicals from food and environmental contaminants. The microsomal forms of P450 in human liver have been well characterized [10], and their functional role in the metabolism of drugs and procarcinogens is relatively well understood [11]. However, the mitochondrial forms of human P450s are

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§ *Abbreviations*: P450, cytochrome P450; APD, aminopyrine *N*-demethylase; ECD, 7-ethoxycoumarin *O*-deethylase; PNPH, *p*-nitrophenol hydroxylase; NDMAD, *N*-nitrosodimethylamine *N*-demethylase; reductase, NADPH cytochrome P450 reductase; PB, phenobarbital; 3-MC, 3-methylcholanthrene; and ICDH, isocitrate dehydrogenase.

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TABLE 1. Case history of human subjects

Case number obtained	Sex	Age (years)	Cause of death	Time between death and autopsy (hr)	Drug treatments	Brain regions*
1	Male	55	Brain stem contusion/ infarction	4	Mannitol and glycerol	CT, CE, MB, MED, PN, ST, HP, and TH
2	Male	60	Brain edema with secondary brain stem lesions	8	Mannitol and glycerol	CT, CE, MB, MED, PN, ST, HP, and TH
3	Male	65	Spinal cord injury brain edema	4	Mannitol and glycerol	CT, HP, and MED
4	Male	22	Subarachnoid hemorrhage	5	Mannitol and epsolin	CT, CE, MB, MED, PN, ST, HP, and TH

* Abbreviations: CT, cortex; CE, cerebellum; MB, midbrain; MED, medulla; PN, pons; ST, striatum; HP, hippocampus; and TH, thalamus.

comparably less characterized. The mitochondrial P450s from human placenta [12] and adrenal [13] have been identified, and their role in the metabolism of steroids has been elucidated [14, 15]. However, the possible presence of mitochondrial P450 forms in human tissues that share similarity with the microsomal xenobiotic-metabolizing forms is yet to be explored.

It is generally well recognized that in the rat brain the specific content of mitochondrial P450 is higher than that in microsomes [16, 17]. In view of the above, if indeed mitochondria from the human brain had the capability to metabolize xenobiotics, it would have far-reaching implications. In the present study, we have examined the presence of 1A, 2B, and 2E forms of P450 in mitochondria prepared from human brain tissue obtained at autopsy of traffic accident victims. Their ability to metabolize a variety of xenobiotics through P450-associated monooxygenase and their possible immunological similarity with known microsomal forms have been studied. Due to tremendous regional heterogeneity exhibited by the brain, the distribution of the mitochondrial forms of P450 was studied in different regions of the human brain.

MATERIALS AND METHODS

Animals

Male Wistar rats (3–4 months) were obtained from the stock colony of the Central Animal Research Facility of NIMHANS. Animals had free access to pelleted diet (Lipton India Ltd.) and water *ad lib*. To study the effects of post-mortem delay on mitochondrial P450, rats were killed by cervical dislocation and kept at room temperature for different time periods including 6, 12, and 24 hr before decapitation and removal of the brain. For control experiments, rats were killed by cervical dislocation, and their brains were sampled immediately. Brains from 2–4 rats were pooled for isolation of mitochondria.

Human Subjects

Four human brains, collected at autopsy from traffic accident victims having no known neurological disorders, were obtained from the Human Brain Tissue Repository, Department of Neuropathology, NIMHANS. The age, cause of death, and the interval between death and autopsy are given in Table 1. Information about the smoking and drinking habits of the patients was not available. Following

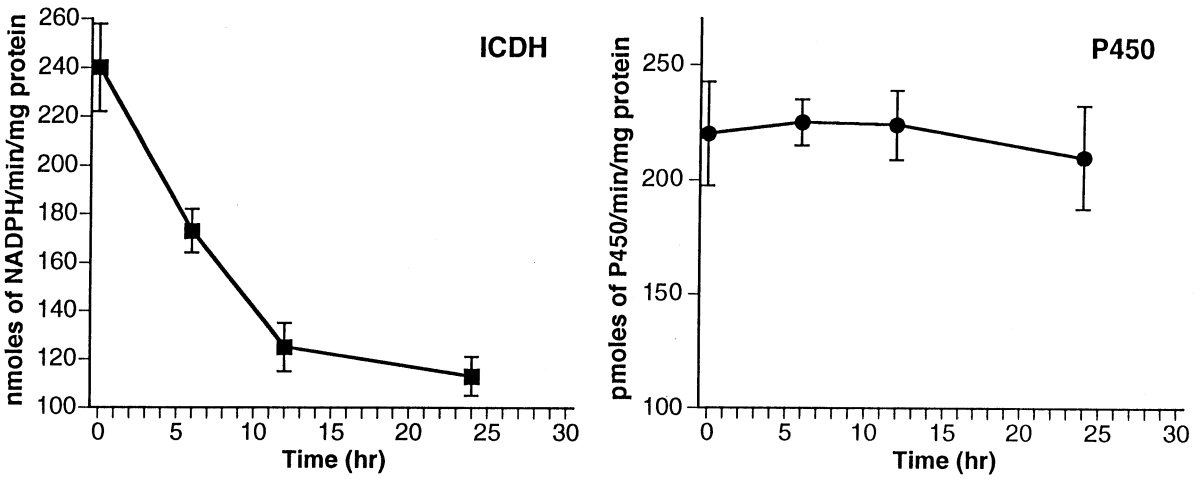

FIG. 1. Effect of post-mortem delay on mitochondrial ICDH activity and P450 levels in rat brain. ICDH activity and total P450 content were determined in brain mitochondria of rats subjected to various time intervals of post-mortem delay. Values are means ± SD (N = 3 preparations of mitochondria from pooled brains of 2–4 rats).

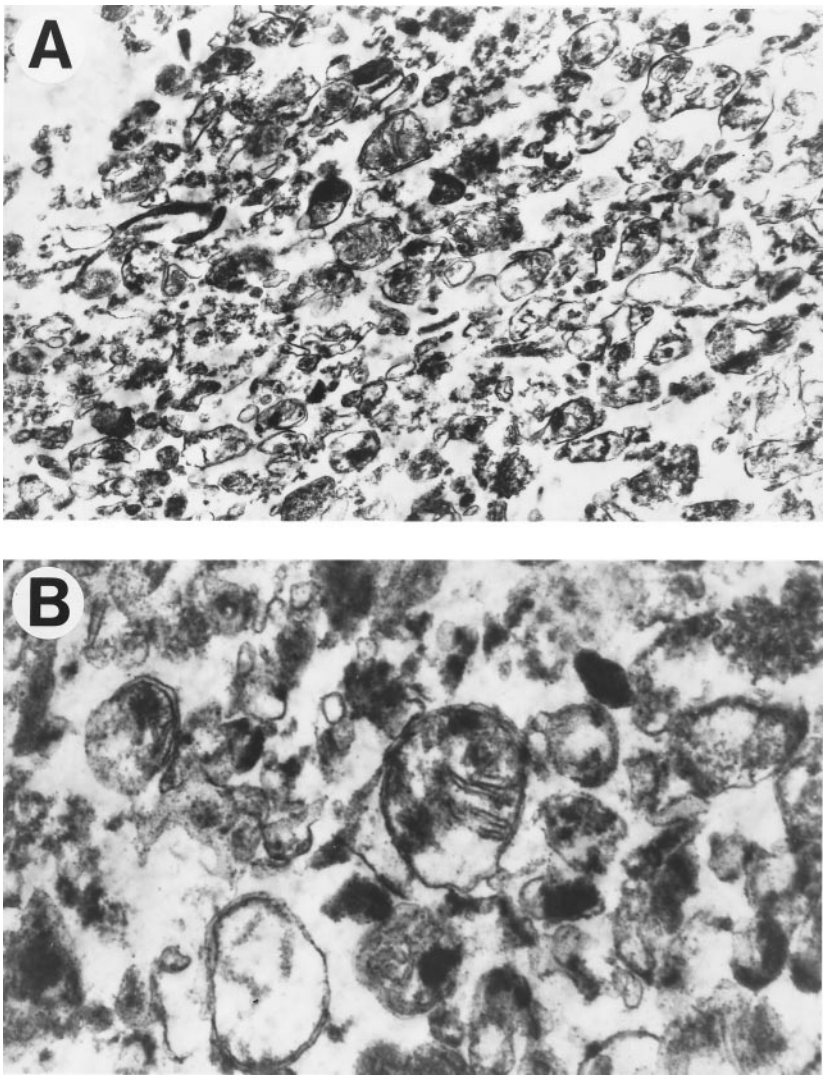

FIG. 2. Electron micrographs of brain mitochondria isolated from human brain cortex (case 1). (A) Lower magnification (18,000x). (B) Higher magnification (45,000x).

injury, the patients were maintained on a respirator. The average interval between death and autopsy was 5 ± 2 hr (mean ± SD). The average age of the deceased subjects was 50 ± 19 yr (mean ± SD). At autopsy, none of the brains showed any features of infection, atrophy, or other anomalies, and the cerebral vessels were normal and intact. Immediately after removing the brain, blood was washed off thoroughly with ice-cold saline, and the brain was dissected free of meninges and blood vessels. Eight different regions, namely, cortex, cerebellum, midbrain, medulla, pons, striatum, hippocampus, and thalamus were dissected out using standard anatomical landmarks, and tissues were frozen at −70° until used. Before homogenization, tissue was thawed on ice, weighed, cut into small pieces, and used for preparation of mitochondria and microsomes.

Isolation of Mitochondria and Microsomes

The mitochondrial fraction was isolated using discontinuous Percoll density gradient centrifugation as described by Sims [18], and the microsomal fraction was isolated by the calcium aggregation method [19], with certain modifications as described earlier [7]. Briefly, the human brain tissue was homogenized using a Dounce homogenizer in 9 vol. of ice-cold Tris buffer (0.1 M, pH 7.4) containing dithiothreitol (0.1 mM), EDTA (0.1 mM), KCl (1.15%, w/v), phenylmethylsulfonyl fluoride (0.1 mM), butylated hydroxytoluene (22 μM), and 10% glycerol (buffer A, previously bubbled with nitrogen). The homogenate was centrifuged at 17,000 *g* for 30 min. The crude mitochondrial preparation obtained from the 17,000 *g* pellet was layered on a Percoll density gradient, and pure mitochondrial fraction was prepared as described [7]. In some experiments the supernatant from the 17,000 *g* centrifugation was collected, and an amount of solid calcium chloride sufficient to give a final concentration of 8 mM was added. The resulting suspension was centrifuged at 30,000 *g* for 1 hr, and the microsomal pellet thus obtained was washed twice in homogenization buffer to give the microsomal preparation. Microsomal and mitochondrial pellets were suspended

in a small volume of buffer A containing 20% glycerol (v/v), aliquoted, and stored at −70° until used.

The purity of the mitochondrial preparation was ascertained using electron microscopy. The isolated mitochondria from the cortex of a human brain (case 1) were processed for electron microscopy by fixing the mitochondrial pellet in 0.1 M sodium cacodylate buffer (pH 7.4) containing 3% glutaraldehyde. Both microsomal and mitochondrial marker enzyme activities were assayed in the subcellular fractions. Glucose-6-phosphatase was assayed as described by Swanson [20]. The assay was carried out in a final volume of 1 mL containing sodium acetate buffer (1 M, pH 5.75), glucose-6-phosphate (0.08 M, final concentration), and brain homogenate, microsomes, or mitochondria (0.5 to 3.0 mg protein). The reaction mixture was incubated for 5 min at 37°, stopped by adding 10% trichloroacetic acid (0.5 mL), and centrifuged. Ammonium molybdate solution (1.6%, w/v; 5 mL) and ferrous sulphate solution (10%, 0.8 mL) were added to the supernatant, and the absorbance was read at 660 nm. The specific activity was calculated from a standard curve using known amounts of sodium phosphate. ICDH [21] was assayed by measuring the substrate (isocitric acid)-stimulated rate of change in absorbance at 340 nm due to reduction of NADP to NADPH. Monamine oxidase activity was assayed using kynuramine as substrate [22]. NADPH cytochrome *c* reductase activity was measured in microsomal and mitochondrial preparations using cytochrome *c* as electron acceptor [23].

Assay of P450 Content and Associated Monooxygenase Activities

The total P450 content was measured from the carbon monoxide reduced minus oxidized difference spectrum [24]. Assays of APD [25], ECD [26], NDMAD [27], and PNPH [28] activity were measured as described. The monooxygenase activities were measured in the absence and presence of detergents (0.5% sodium cholate, w/v, and 0.4% Triton N-101, v/v). Protein was assayed by a dye-binding method using BSA as the standard [29].

Immunoblotting Experiments

Mitochondrial proteins (100–200 μg) from eight different regions of a human brain (case 1) were subjected to SDS–PAGE [30], and the separated proteins were electroblotted onto nitrocellulose membranes [31]. The membranes were immunostained with the antisera to purified rat liver P4502B1/2, P4501A1/2, or P4502E1. The immunoblots of both microsomes and mitochondria isolated from a human brain cortex (case 1) also were immunostained with antiserum to rat liver microsomal NADPH cytochrome P450 reductase (reductase).

Immunoinhibition Studies

The freeze–thawed mitochondrial or microsomal fractions from human brain cortex were preincubated with various concentrations of nonimmune or immune serum for 30 min at 4° prior to the assay of P450-associated monooxygenase activities as described above.

RESULTS

To determine the suitability of human post-mortem brain tissue for use in experiments involving the measurement of P450, we examined the effect of post-mortem delay on rat brain P450 content and compared it with changes in activity of the mitochondrial enzyme ICDH. ICDH activity in control mitochondria (with no post-mortem delay) was 238 nmol of NADPH formed/min/mg protein. However, ICDH activity decreased by 28 and 48% in brain mitochondria after 6 and 12 hr of post-mortem delay. Interestingly, P450 content in rat brain mitochondria was unaffected by post-mortem delay up to 24 hr. Rat brain mitochondrial P450 content was 0.22 nmol P450/mg protein in controls, and it remained at similar levels even after 24 hr of post-mortem delay (Fig. 1).

Electron microscopic examination of mitochondria prepared from human brain cortex (case 1) revealed minimal

TABLE 2. Marker enzyme activities in isolated mitochondria and microsomes from (A) human cerebellum (case 1) and (B) human cortex (case 4)

	Homogenate		Mitochondria		Microsomes	
Enzymes	**A**	**B**	**A**	**B**	**A**	**B**
Monoamine oxidase (nmol 4-hydroxyquinoline formed/min/mg protein)	1.47	1.4	14.5	14.0	0.48	0.23
ICDH (nmol NADPH formed/min/mg protein)	79.8	74.1	261.8	294.0	5.8	5.8
NADPH cytochrome *c* reductase (nmol cytochrome *c* reduced/min/mg protein)	11.8	12.4	ND	ND	24.3	21.0
Glucose 6-phosphatase (nmol P_i formed/min/mg protein)	101	125	5.2	ND	448	482

Enzyme activities were measured in homogenate, mitochondria, and microsomes from (A) human cerebellum (case 1) and (B) human cortex (case 4). Values are means of quadruplicate analyses and did not vary by more than 10% between each determination. ND indicates that no enzyme activity was detectable.

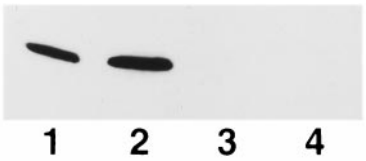

FIG. 3. Immunoblot analyses of microsomes and mitochondria from human brain cortex (case 4) using antiserum to rat liver NADPH cytochrome P450 reductase. Microsomal protein was loaded in lanes 1 and 2 (50 and 100 μg, respectively), and mitochondrial protein was loaded in lanes 3 and 4 (50 and 100 μg, respectively). The blot was immunostained with antiserum to rat liver NADPH cytochrome P450 reductase.

contamination with myelin or microsomes (Fig. 2, A and B). The specific activities of subcellular marker enzymes assayed in mitochondria and microsomes prepared from human brain cerebellum (case 1) and cortex (case 4) are given in Table 2. There was an enrichment of the activity of the mitochondria-specific marker enzyme monoamine oxidase in mitochondria from cerebellum as compared with homogenate. ICDH activity also was enriched by 3.3-fold in mitochondria as compared with homogenate. The activity of monoamine oxidase and ICDH in microsomes was only 3 and 2% of the mitochondrial activity, respectively. On the other hand, in mitochondrial preparations the activity of the microsome-specific marker enzyme glucose-6-phosphatase was not detectable or was less than 2% of the corresponding activity in microsomes. NADPH cytochrome P450 reductase activity was not detected in the mitochondrial preparations. Further, immunoblot analysis was carried out using antiserum to rat liver NADPH cytochrome P450 reductase to determine probable contamination of microsomes in the mitochondrial preparation. A single immunoreactive protein band was seen in microsomes but not in the mitochondrial preparation (Fig. 3). The carbon monoxide reduced minus oxidized spectrum of human brain mitochondria exhibited a characteristic absorption spectrum with two peaks at 450 and 430 nm (Fig. 4) in a manner similar to that seen with microsomal P450 from human brain [32]. The shoulder at 450 nm represents P450, and the specific content of P450 was calculated by measuring the absorbance at 450 nm. The peak at 430 nm was not taken into account for calculating the specific content of P450 in brain mitochondria. P450 measurements varied from 0.1 to 0.3 nmol of P450/mg protein; however, no statistically significant regional variation was seen in the mean P450 content (data not shown). The APD activity measured in mitochondria isolated from brain regions also did not show any significant variation among different regions (Fig. 5A). Considerable inter-individual variations were observed. APD activity determined in the

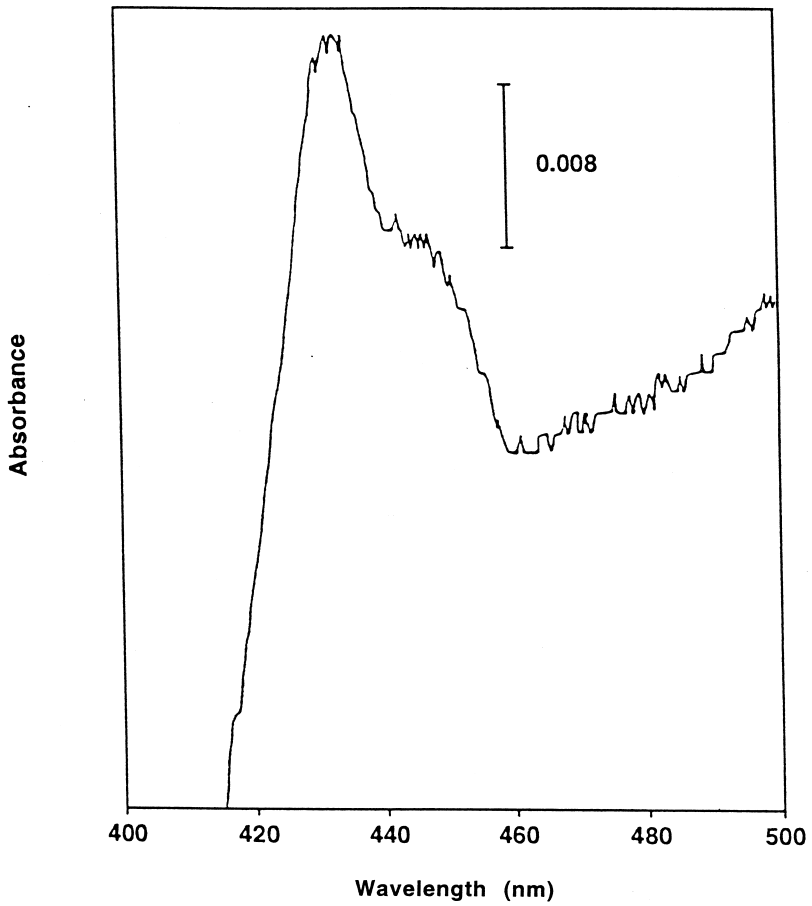

FIG. 4. Dithionite-reduced carbon monoxide binding spectrum of mitochondrial P450 from human brain cortex. Protein concentration was 0.2 mg/mL. The specific content of P450 in the mitochondrial preparation was estimated to be 0.23 nmol of P450/mg protein.

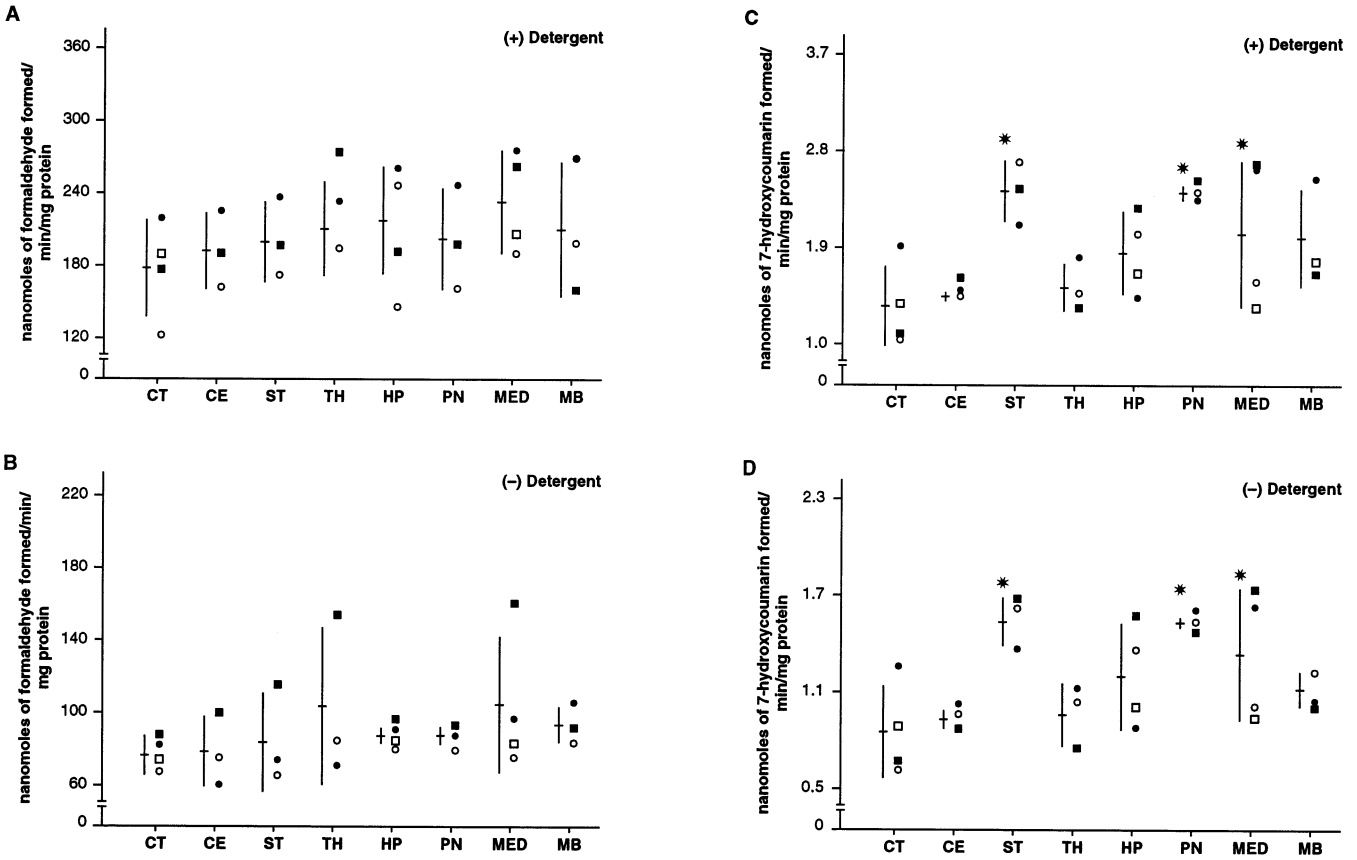

FIG. 5. APD (A, B) and ECD (C, D) activity in mitochondria from different regions of the human brain. The enzyme activities were determined in the presence of detergents [(+) detergent, A and C] and in the absence of detergents [(−) detergent, B and D]. The ages of the deceased were case 1, 55 years (○); case 2, 60 years (●); case 3, 65 years (□); and case 4, 22 years (■). Abbreviations: CT, cortex; CE, cerebellum; ST, striatum; TH, thalamus; HP, hippocampus; PN, pons; MED, medulla; and MB, midbrain. Asterisks represent values significantly different from the activity in the CT and CE ($P < 0.05$).

presence of detergents in all the brain regions was 1.5- to 2-fold higher than the activity in the absence of detergents. Mitochondrial P4501A1/2-mediated ECD activities in the human brain regions (Fig. 5C) varied from 1 to 3 nmol of 7-hydroxycoumarin formed/min/mg protein. Also, there was a significant difference in the mean activity of ECD between different brain regions. The striatum and pons showed the highest ECD activity as compared with the cortex and cerebellum. ECD activity determined in the presence of detergents increased by 1.5-fold in all brain regions, and regional variations persisted.

We determined P4502E1-associated monooxygenase activity including NDMAD and PNPH in mitochondria isolated from human brain regions. A robust amount of NDMAD activity was detected, which varied from 5 to 10 nmol of formaldehyde formed/min/mg protein among brain regions of different individuals (Fig. 6A); there was no significant variation in the mean activity of NDMAD among the different brain regions examined. In the presence of detergents in the assay buffer, NDMAD activity was increased by 1.3- to 1.4-fold in all brain samples examined. Unlike NDMAD activity, significant variations were observed in the activity of PNPH between different brain regions (Fig. 6C). PNPH activity varied from 75 to 200 pmol of *p*-nitrocatechol formed/min/mg protein in the mitochondria isolated from different regions. The thalamus and striatum showed the highest PNPH activity as compared with the cortex and cerebellum. Like all other mitochondrial monooxygenase activities examined by us, the PNPH activities increased by 1.5- to 2-fold in the presence of detergents such as sodium cholate and Triton N-101 in the assay mixture (Fig. 6C).

Immunoblot analysis of mitochondria from different regions of a human brain (case 1) using antisera to various microsomal forms of rat hepatic P450s revealed the presence of immunoreactive protein bands in all regions of the human brain that were examined (Fig. 7). When the blot was immunostained with antiserum to rat liver P4502B1/2, a single immunoreactive protein band was seen in mitochondria from all brain regions examined (Fig. 7A). When a similar blot was immunostained with antiserum to rat liver P4501A1/2, it immunostained two protein bands (corresponding to 1A1 and 1A2) in the brain regions studied (Fig. 7B). Further, in a similar experiment, immunostaining of the blot with antiserum to rat liver P4502E1 revealed the presence of a single immunoreactive protein band in mitochondria in all brain regions examined (Fig. 7C).

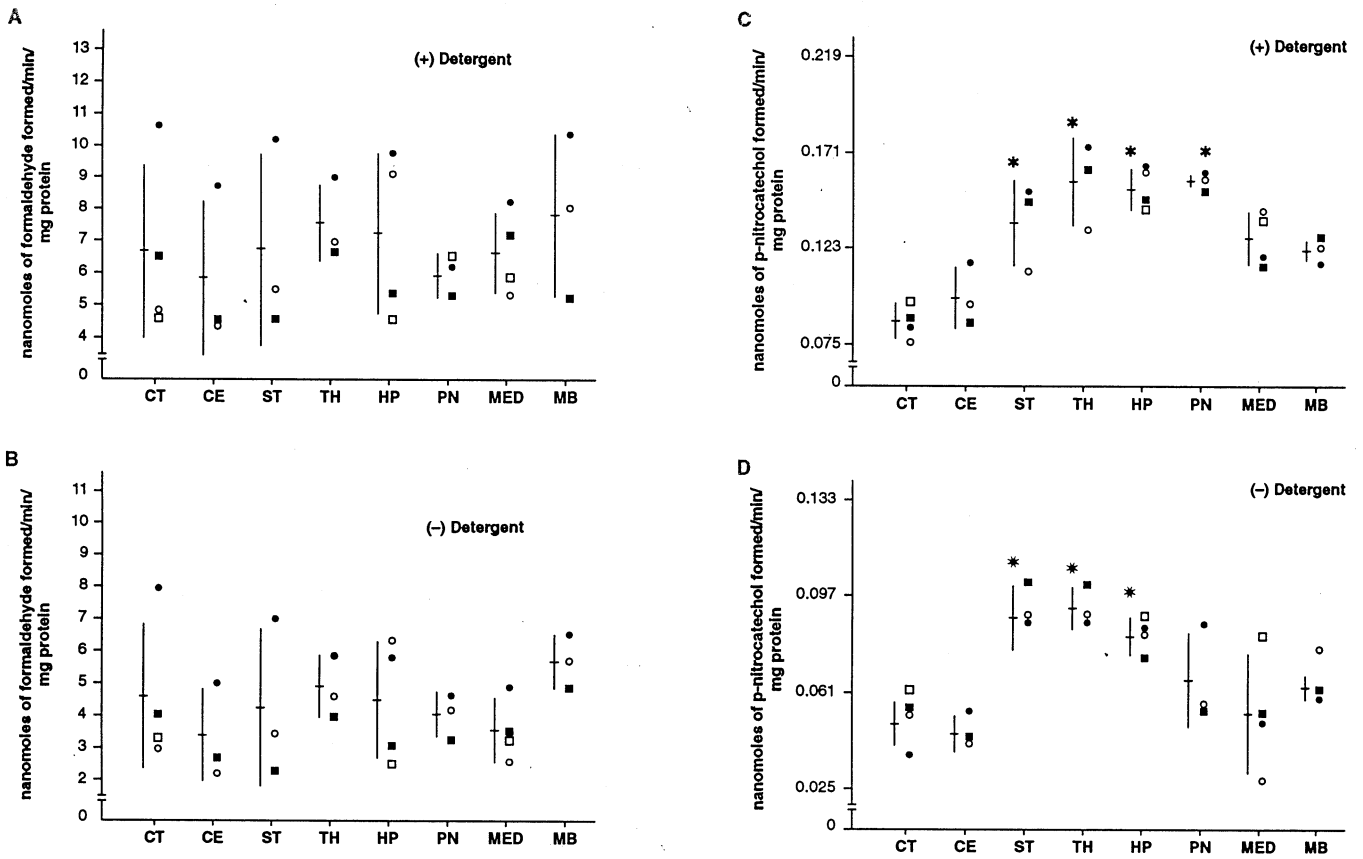

FIG. 6. NDMAD (A, B) and PNPH (C, D) activity in mitochondria from different regions of the human brain. The enzyme activities were determined in the presence of detergents [(+) detergent, A and C] and in the absence of detergents [(−) detergent, B and D]. The ages of the deceased were: case 1, 55 years (○); case 2, 60 years (●); case 3, 65 years (□); and case 4, 22 years (■). Abbreviations: CT, cortex; CE, cerebellum; ST, striatum; TH, thalamus; HP, hippocampus; PN, pons; MED, medulla; and MB, midbrain. Asterisks represent values significantly different from the activity in the CT and CE ($P < 0.05$).

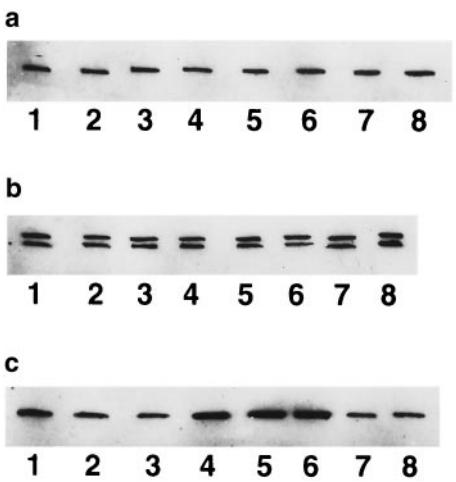

FIG. 7. Immunoblot analysis of mitochondrial protein from human brain regions (case 1) using: (A) antiserum to rat liver P4502B1/2, (B) antiserum to rat liver P4501A1/2, and (C) antiserum to rat liver P4502E1. Each lane was loaded with 50 μg of mitochondrial protein. Lane 1, cortex; lane 2, cerebellum; lane 3, striatum; lane 4, thalamus; lane 5, hippocampus; lane 6, pons; lane 7, medulla; and lane 8, midbrain.

Immunoinhibition studies were carried out using mitochondria and microsomes isolated from a human brain cortex (case 1) using antisera to (i) rat liver P4502B1/2, (ii) rat liver P4502E1, and (iii) rat liver reductase (Fig. 8). The mitochondrial APD activity was immunoinhibited by up to 70% by the antiserum to rat liver microsomal P4502B1/2, whereas the antiserum to rat liver reductase did not have any effect on the mitochondrial APD activity (Fig. 8A). On the other hand, the microsomal APD activity was immunoinhibited completely by the antiserum to rat liver reductase (Fig. 8B). NDMAD activity in human cortical mitochondria was immunoinhibited (70%) upon preincubation with antiserum to rat liver microsomal P4502E1, whereas it was unaffected by antiserum to rat liver reductase (Fig. 8C). However, NDMAD activity was inhibited completely upon preincubation of human cortical microsomes with the antiserum to rat liver reductase (Fig. 8D). Thus, all the microsomal monooxygenase activities examined were inhibited completely by the antiserum to rat liver reductase (Fig. 8, B and D), whereas the corresponding mitochondrial activities were unaffected by this antiserum (Fig. 8, A and C).

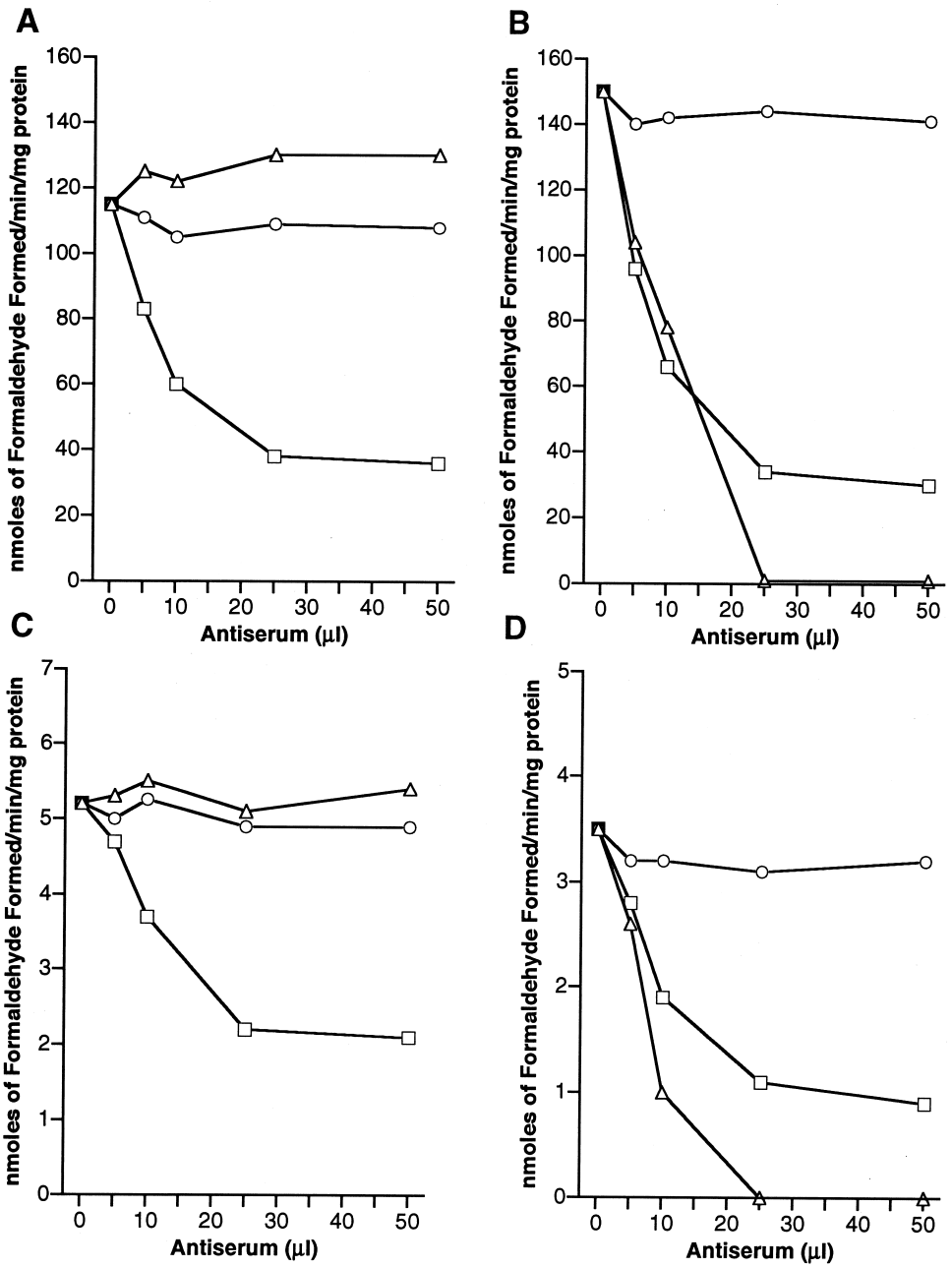

FIG. 8. Immunoinhibition of human brain cortical mitochondrial and microsomal APD (A, B) and NDMAD (C, D) activities. Mitochondria (A) and microsomes (B) from human brain cortex (case 1) were preincubated with various amounts of nonimmune serum (○—○) or antiserum to rat liver P4502B1/2 (□—□) and reductase (△—△) for 30 min at 37° prior to initiation of enzymatic reaction for measurement of APD activity. The values are means of duplicate analyses. Mitochondria (C) and microsomes (D) from human brain cortex (case 1) were preincubated with various amounts of nonimmune serum (○—○) or antiserum to rat liver P4502E1 (□—□) and reductase (△—△) for 30 min at 37° prior to initiation of enzymatic reaction for measurement of NDMAD activity. The values are means of duplicate analyses.

DISCUSSION

Significant amounts of P450-associated monooxygenase activities were detectable in mitochondria prepared from human brain tissue obtained at autopsy, as calculated using the absorption at 450 nm in the carbon monoxide reduced minus oxidized spectrum. Our studies on effects of post-mortem delay using rats demonstrated that the mitochondrial P450s were relatively intact during post-mortem delay, in contrast to other mitochondrial enzymes such as ICDH (Fig. 1), indicating the suitability of using post-mortem tissue for P450 studies. The purity of the mitochondria used in the present study has been assessed by marker enzyme studies, electron microscopy, and immunoblot analysis, ensuring that the mitochondrial preparation used was essentially free of microsomal contamination.

Several monooxygenase activities known to be mediated

by well-characterized hepatic P450 isoforms such as P4501A1/2, 2B1, and 2E1 were detectable in human brain mitochondrial preparations (Figs. 5 and 6), and the measurable activities were increased when the assays were carried out in the presence of detergents. Brain mitochondrial P450 has been shown to be localized in the inner mitochondrial membrane [16]. Thus, accessibility of the substrate to the protein increases upon addition of detergents that help solubilization of membrane proteins. However, the location of P450 in the inner mitochondrial membrane makes it difficult for it to interact with the immunoglobulins added for immunoinhibition experiments. Therefore, it becomes necessary to freeze–thaw the mitochondria prior to immunoinhibition experiments to facilitate antigen–antibody interaction, as performed in the present study. Similar observations have been made with liver mitochondria [32]. The immunological similarity between the mitochondrial and microsomal forms of P450 was apparent from the immunoblotting and immunoinhibition experiments. Further, the immunoinhibition studies on human brain mitochondria and microsomes carried out using the antiserum to rat liver NADPH cytochrome P450 reductase clearly demonstrated the difference in the electron transport systems used by the two subcellular compartments. Whereas microsomal monooxygenase activities were inhibited completely by the antiserum to reductase (Fig. 8), the mitochondrial activities were unaffected; this indicated that mitochondrial P450s did not receive the required electrons from NADPH cytochrome P450 reductase, which is an essential component of the microsomal P450 systems. In the well-characterized liver and adrenal mitochondrial P450 systems, ferredoxin/ferredoxin reductase and adrenodoxin/adrenodoxin reductase carry out the electron transfer during P450-mediated monooxygenase reactions [4, 15]. However, characterization of similar functional electron transport systems in human liver mitochondria is nearly absent. The mitochondrial P450scc present in the human placenta is known to be limited by electron transport from adrenodoxin reductase to adrenodoxin [33].

The presence of mitochondrial P450 forms in the human brain that share immunological similarity with well-characterized microsomal P450 forms such as 1A1/1A2, 2B1, and 2E1 may have far-reaching implications, since these forms are involved in metabolism of a variety of carcinogens, and, furthermore, may be inducible by many environmental contaminants (e.g. aryl hydrocarbons), drugs (e.g. phenobarbital), and ethanol. Rat brain mitochondrial P450s are known to be induced by phenobarbital and ethanol [7], and it seems probable that they are also inducible in the human brain. These isoforms, in functionally active forms, are known to exist in human brain microsomes [34].

Significant amounts of P450-associated monooxygenase activity were detected in human brain mitochondria in the present study; in fact, the mitochondrial activities were comparable to those seen in human brain microsomes [34, 35], indicating the existence of a significant amount of P450 in human brain mitochondria, in a manner similar to that seen in rat brain [7]. Although the mitochondrial P450 content of the human liver is not established with certainty, in the livers of D2 mice mitochondrial P450 levels are typically 2.5% of the corresponding microsomal levels [36].

The identification of multiple forms of P450s belonging to subfamilies 1A, 2B, and 2E in human brain mitochondria by measurement of catalytic activity and immunoblot analysis, to our knowledge, has not been reported earlier. If the total P450 content in the human brain (both mitochondrial and microsomal) is taken into consideration, the human brain seems to have significant capability to metabolize a variety of xenobiotic substrates. Since these isoforms of P450 are involved in drug metabolism, carcinogenesis, and generation of oxygen free radicals, the presence of a functionally active P450 system might have far-reaching physiological, pharmacological, and toxicological implications in the human brain. More importantly, the present findings on the differential distribution of cytochrome P450-dependent functions in brain regions and subcellular organelles in humans may be useful in understanding localized tissue damage caused by exposure to chemicals, which probably has a role in idiopathic neurodegenerative diseases in humans.

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