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Neuronal Cytochrome P450IID1 (Debrisoquine/Sparteine-Type): Potent Inhibition of Activity by (−)-Cocaine and Nucleotide Sequence Identity to Human Hepatic P450 Gene *CYP2D6*[1]

R. F. TYNDALE, R. SUNAHARA, T. INABA, W. KALOW, F. J. GONZALEZ, and H. B. NIZNIK

Departments of Pharmacology (R.F.T., R.S., T.I., W.K.) and Psychiatry (H.B.N.), University of Toronto, Toronto, Ontario M5S 1A8, Canada; Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (F.J.G.); and the Molecular Neurobiology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, M5T 1R8, Canada (H.B.N.)

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SUMMARY

Catalytic, pharmacological, and molecular criteria have been used to identify cytochrome P450IID1 in mammalian brain (enzyme, P450IID; gene, *CYP2D*). Sparteine metabolism in canine striatal membranes was shown to be inhibited in a concentration-dependent and stereoselective manner by quinidine (K_i, ~51 nM), quinine (K_i, ~5.9 μM), and various other known substrates and inhibitors of hepatic P450IID1 activity. In addition, canine striatal P450IID1 was inhibited with high affinity by dopamine uptake blockers, such as (−)-cocaine (K_i, ~74 nM), *d*-amphetamine (K_i, ~4.5 μM), and methylphenidate (K_i, ~15 μM). Inhibitory constants (K_i) of numerous compounds for inhibition of sparteine metabolism in canine striatal membranes correlated well with (a) K_i values observed in human liver microsomes (r = 0.95), (b) [^{3}H]GBR-12935 binding to P450IID1 in canine striatal membranes (r = 0.85), and (c) the inhibition (IC_{50}) of sparteine metabolism in HepG2 cells expressing human CYP2D6 cDNA (r = 0.93). Moreover, antibodies raised against rat hepatic enzyme inhibited, in a concentration-dependent manner, sparteine metabolism in canine striatal membranes. Enzymatic activity was unevenly distributed throughout the canine brain and ranged from 0.5 to 21 pmol/mg of protein/hr in cerebellum and supraorbital cortex, respectively, with the striatum displaying moderate levels of activity (8 pmol/mg of protein/hr). The polymerase chain reaction was used to amplify cDNA from a human caudate λgt11 library encoding exons 6–9 of the human *CYP2D6* gene, which revealed, upon sequencing, 100% nucleic acid sequence identity. These data indicate that P450IID1 is expressed centrally and is similar, at the functional and molecular levels, to the human hepatic P450IID1 enzyme. Because the debrisoquine/sparteine monooxygenase is a polymorphic enzyme, in which 5–10% of caucasians are deficient in metabolism of various drugs, a genetic difference in human brain metabolism of P450IID1 substrates may possibly lead to differences in drug response and toxicity.

During the past decade, there has been a great deal of development in the field of CNS P450s, from which some conclusions can be drawn (2–4) (for review see Ref. 5). Many forms of P450 have been identified in the rat central nervous system using catalytic, spectrophotometric, and immunohistochemical techniques. The focus to date has been primarily on the P450s that are inducible and the catechol-forming/steroid-metabolizing P450. Some information is also available on the activity and localization of the NADPH-P450 reductase (6). However, little work has been done on the noninducible xenobiotic-metabolizing P450s beyond immunohistochemical studies, primarily because of a lack of assay techniques that are sufficiently sensitive to detect the very low levels of enzymatic activity. In order to comprehend neuronal metabolic and toxicological processes, an understanding of the P450 system in the CNS is required. The potential activation of substrates to reactive intermediates is extremely important in the brain, particularly because the magnitude and duration of a drug's effect depend on the concentration of the drug at a given receptor.

One of the main sources of variations in drug response is the variation in metabolism. Genetic polymorphisms such as the

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[1]The designation for the cytochrome debrisoquine/sparteine-type monooxygenase is P450IID1 and for the gene is *CYP2D1* in rat and is P450IID6 and *CYP2D6*, respectively, in human (1). For the sake of simplicity, we have used P450IID1 for the rat, human, and canine (currently unnamed) enzyme. This enzyme has previously been designated $P450_{db1}$, $P450_{bufI}$, and P450IID1. All of the cDNA work was from a human library and, therefore, we have referred to the gene as *CYP2D6* (1).

ABBREVIATIONS: CNS, central nervous system; P450, cytochrome P-450; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; GBR-12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenyl-2-propenyl)piperazine; GBR-12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl-propyl)piperazine; SKF 525A, 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride; bp, base pairs; SSC, standard sodium citrate.

debrisoquine/sparteine polymorphism leave 5–10% of the caucasian population deficient in their ability to metabolize >20 drugs (7, 8). Recently, we identified the debrisoquine/sparteine monooxygenase (P450IID1) in canine striatum, using radioligand binding and immunological techniques (9). This enzyme has also been detected in rat CNS, using total brain homogenates (10, 11). Among the drugs that are established P450IID1 substrates are many centrally acting drugs, such as neuroleptics and tricyclic antidepressants, as well as amphetamine, 4-methoxyamphetamine, and codeine. Interestingly enough, compounds that interact with the neuronal dopamine transporter appear to display significant overlap in substrate specificity with P450IID1 (9).

In this report, we document the existence of a neuronal P450IID1 enzyme that displays both pharmacological and molecular identity to hepatic P450IID1 and displays significant activity towards compounds that are substrates or inhibitors of the dopamine transporter, such as (−)-cocaine.

Experimental Procedures

Materials. Crystalline sparteine was purchased from Sigma Chemical Co. (St. Louis, MO). 17-*n*-Pentylsparteine (used as an internal standard), 5-dehydrosparteine, and cocaine HCl were gifts of Kali-Chemie Aktiengesellschaft (Germany), Dr. Michael Eichelbaum (M. Fischer-Bosch Institute, Stuttgart, FRG), and Dr. David Johnson (Sick Childrens Hospital, Toronto, Canada), respectively. Haloperidol and reduced haloperidol were purchased from Janssen Pharmaceutica (Olen, Belgium). All other drugs or reagents were obtained from sources previously described (9, 12). The antibody generated against rat P450db1 was characterized previously (13, 14) and was generously donated by J. P. Hardwick of the Argonne National Laboratories (Argonne, IL). This antibody cross-reacts specifically with the human P450IID1, as determined by immunoinhibition studies and SDS-PAGE immunoblots (14, 15).

Tissue preparation. Microsomes were prepared as previously described for hepatic tissue (12). Striata (total, $n = 20$; pooled preparations, $n = 2$–4) were dissected from canine brains (Pel-Freeze Biologicals, Rogers, AR) and homogenized in 6 volumes of ice-cold isotonic KCl for 15 sec, using a Brinkman Polytron. Sparteine-metabolizing capacity was found in both the 9000 × *g* pellet and the microsomal (100,000 × *g*) pellet from striata, either due to the enzyme being present in membranes other than microsomal membranes (perhaps mitochondrial or plasma membranes) (16) or due to incomplete separation of microsomes from the 9000 × *g* pellet in brain tissue by this method. Therefore, the 9000 × *g* pellet and 100,000 × *g* pellet were combined, and no further attempt was made to identify the enzyme sources (if any) in addition to the microsomal membrane.

Membranes from HepG2 cells expressing the CYP2D6 from a human cDNA clone (F. J. Gonzalez, National Institutes of Health) were prepared in a similar manner as CNS tissue, with the following modifications. Five milligrams of cells were added to 10 ml of phosphate buffer (pH 7.4, 0.2 M), sonicated for 10 sec, and then homogenized using a Brinkman Polytron for three cycles of 5 sec each, on ice. After centrifugation, the 9000 × *g* and 100,000 × *g* pellets were combined, resuspended in KCl, and rehomogenized. Protein was determined using a bicinchoninic acid protein assay kit (Pierce Chemical Co.).

Assay conditions. Sparteine monooxygenase activity was assessed using methods previously described (12), with the following modifications. Briefly, tissue homogenates were incubated with varying concentrations of sparteine and inhibitor, an NADPH-generating system, and 0.2 M phosphate buffer, pH 7.4, to 0.5 ml. HepG2 cell samples contained 0.6 mg of protein, brain incubates contained 3 mg of protein, and human liver incubates contained 0.2 mg of protein. The samples were incubated at 37° for 60 min, except for the human liver samples, which were incubated for 30 min. Background controls contained all of the assay components except the sparteine, which was incubated separately and added at the end of the incubation. In the standards, unincubated sparteine and dehydrosparteine were added at the end of the incubation. The reaction was terminated by immediate freezing after addition of 17-*n*-pentylsparteine (internal standard). After the samples were frozen at −20°, they were centrifuged in an Eppendorf microcentrifuge (10,000 × *g*, 10 min), and the supernatant was removed to conical tubes containing 100 μl of cold dichloromethane. The pellet was resuspended in 1.0 ml of phosphate buffer and recentrifuged. The resulting supernatant was then combined with the first supernatant. This procedure resulted in only minor losses (<5%) of the metabolites and produced clean gas chromatography tracings. Sparteine, 5- and 2-dehydrosparteine metabolites, and 17-*n*-pentylsparteine were extracted and analyzed by gas chromatography using a nitrogen-sensitive detector, as previously described (17). Sparteine, 5-dehydrosparteine, 2-dehydrosparteine, and 17-*n*-pentylsparteine had the following retention times, respectively: 3.52, 5.06, 5.69, and 7.46 min. A signal to noise ratio of greater than 3 was considered the minimal acceptable ratio. Protein was denatured by heating at 60° for 60 min.

Immunoinhibition studies. Immunoinhibition studies were carried out according to the method of Tyndale *et al.* (15). Essentially, the anti-rat P450IID1 IgG and preimmune IgG were preincubated with the tissue for 30 min at 4° before sparteine metabolism was assayed.

Regional distribution studies. For each brain region, a blank control and standard curve were assayed as described above. Each region was assayed with 3 mg of protein/sample.

[^{3}H]GBR-12935 binding studies. Inhibition of [^{3}H]GBR-12935 binding was assayed with haloperidol and reduced haloperidol on human liver microsomes, as described by Niznik *et al.* (9).

Data analysis. K_m and V_{max} values were estimated from Eadie-Hofstee graphs, which are linear rearrangements of the Michaelis-Menten rate equation. Curves defining the law of mass action were fitted to canine striatal inhibition studies in Fig. 1c. K_i values for the canine striata inhibition experiments were converted from IC_{50} values using the following rearrangement: $K_i = IC_{50}/\{1 + (\text{substrate}/K_m)\}$.

Oligonucleotides. The oligonucleotides used in the PCR were synthesized at the University of Toronto facility at the Banting Institute. One oligonucleotide coded for a sequence in exon 6 of the *CYP2D6* gene (18), corresponding to nucleotides 2908–2931 (5′-CCTGAGAGCAGCTTCAATGATGA-3′), and the second was complementry to nucleotides 4162–4186 (5′-AGGTGAAGAAGAGGAAGAGCTCCA-3′) in exon 9.

DNA amplification by PCR and subcloning. Five microliters of a λgt11 human caudate cDNA library (1×10^{10} plaque-forming units/ml; Clontech) were lysed by heating for 5 min at 95° in 100 μl of PCR reaction buffer (Cetus) containing 1 μg of each primer. After addition of the *Thermos aquaticus* polymerase (2.5 units; AmpliTaq), the cDNA was subjected to 25 cycles of PCR (Cetus-Perkin Elmer). The timing sequence used was 1.5 min at 94°, 2 min at 55°, and 4 min at 72° for denaturation, primer annealing, and extention, respectively, as described by Sakai *et al.* (19), followed by a 7-min extention at 72°. An aliquot of this reaction (10 μl) was removed and reamplified for an additional 25 cycles under the same conditions. DNA from this reaction was electrophoresed in soft agarose (0.8%); the DNA contained in four contiguous slices (300–700 bp) was extracted, blunt ended with Klenow, and subcloned into the *Sma*I multiple cloning site of the plasmid sp73 (Promega).

Southern blot hybridization and sequencing. Colonies were lifted onto hybridization transfer membranes (NEN) and prepared for screening according to the manufacture's instructions. The filters were hybridized at 42° with ^{32}P-labeled nick-translated (Amersham) hepatic *CYP2D6* probe (2.6×10^6 cpm/ml) (probe described in Ref. 14) for 17 hr in hybridization buffer (3× SSC, 20 mM $NaPO_4$, pH 6.8, 50% formamide, 1× Denhardt's (0.1 g Ficoll, 0.1 g polyvinylpyrrolidone, 0.1 g bovine serum albumin, 500 ml in H_2O), 1% glycine, 150 μg/ml salmon sperm DNA, 0.5% SDS). The membranes were washed at 20° for 20

min with 0.2× SSC, 0.1% SDS, followed by a 60° wash for 30 min with 0.2× SSC, 0.1% SDS.

After minipreparation, plasmid cDNA-containing inserts was restriction digested. The cDNA was subjected to Southern blot analysis using the human hepatic probe (3.0×10^6 cpm/ml). The membrane was washed under high stringency in 0.2× SSC, 1% SDS, at 20° for 15 min, followed by two 15-min washes at 60°, two 20-min washes at 65°, and a 75-min wash at 75° in 0.1× SSC, 0.5% SDS. The DNA fragment was then sequenced (Sequenase; United States Biochemical) using the dideoxy chain termination reaction (20).

Results

Assay characteristics in canine striata. Due to the lower velocity of 5-dehydrosparteine than of 2-dehydrosparteine formation, reliable kinetic parameters were obtainable from 2-dehydrosparteine only. Reaction rates were linear with respect to time for 120 min and with respect to protein concentration up to at least 6 mg. Metabolism of sparteine to 2-dehydrosparteine was drastically reduced by exclusion of the NADPH-generating system or the enzyme source (70%), by denaturation of the enzyme (>95%), by incubation of the sample at 4°C (>95%), or by inclusion of the P450 inhibitor SKF525A (10 μM, 49%; 100 μM, 73%, 1000 μM, 82%).

The K_m and V_{max} for canine striata 2-dehydrosparteine formation were 59 μM and 34 pmol of 2-dehydrosparteine/mg of protein/hr, respectively (Fig. 1a). At concentrations above 500 μM sparteine, a break in the points on the Eadie-Hofstee can be observed, but these points are not reliable (although they were reproducible), due to a signal to noise ratio that was below 1.5 and, therefore, they have not been included in the analysis.

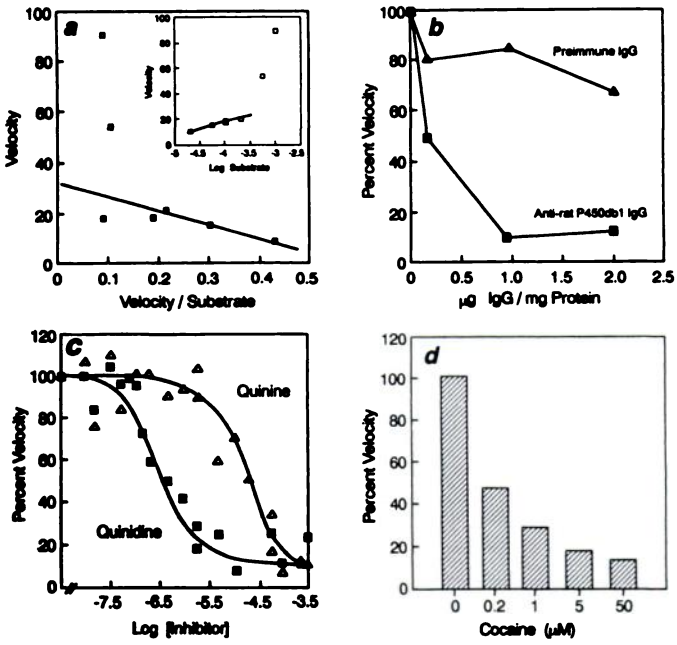

Fig. 1. Canine striatal sparteine metabolism catalyzed by P450IID1. a, Eadie-Hofstee plot of sparteine metabolism to 2-dehydrosparteine in the canine striatum. Velocity is given in pmol of 2-dehydrosparteine/mg of protein/hr. *Inset*, relationship between velocity and the logarithm of substrate. The duplicates are from experiments on two different days. b, Percentage of activity of sparteine metabolism remaining after inhibition by anti-rat P450IID1. c, Quinidine and quinine inhibition of striatal sparteine metabolism. The IC_{50} values were 140 nM and 16 μM for quinidine and quinine, respectively. d, (−)-Cocaine inhibition of sparteine metabolism in the canine striata. Sparteine concentration varied from 20 to 1000 μM in a and was 100 μM for b, c, and d. The 100% value was 12, 12, and 8 pmol of 2-dehydrosparteine/mg of protein/hr for b, c, and d, respectively.

Immunoinhibition studies. Fig. 1b illustrates the percentage of inhibition of the 2-dehydrosparteine formation in canine striata by the anti-rat P450IID1 and preimmune IgG. The anti-rat P450IID1 IgG inhibited maximally (90%) at 1.0 μg of IgG/mg of protein, inasmuch as no further inhibition was observed at concentrations as high as 10 μg of IgG/mg of protein.

Quinidine/quinine inhibition studies. The stereoselective nature of the quinidine/quinine inhibition of P450IID1 has been established for humans (21, 22), where the quinidine isomer is 100–250 times more potent than quinine. In previous studies using [^{3}H]GBR-12935 to label the P450IID1 in canine striatum, the K_i values for quinidine and quinine were 250 nM and 168 μM, respectively, indicating that the same stereoselective inhibition exists in dog (9). Similarly, as depicted in Fig. 1c, quinidine and quinine stereoselectively inhibited canine striatum P450IID1 activity, with quinidine being 115 times more potent than quinine at inhibiting the 2-dehydrosparteine formation. The IC_{50} value for quinidine was 140 nM and for quinine was 16 μM.

Inhibitor studies. Various compounds were tested as inhibitors in the canine striatal sparteine metabolism assay. Among the compounds of interest that were tested were a number of compounds that are either inhibitors or substrates for the dopamine transporter. (−)-Cocaine was a very potent inhibitor of the striatal sparteine metabolism, with a K_i value of 74 nM (Figs. 1 and 2). Methylphenidate and GBR-12909 had K_i values of 15 μM and 39 nM, respectively (Figs. 2 and 3). Amphetamine displayed stereoselective inhibition of canine striatal sparteine metabolism, whereby *d*-amphetamine had a K_i value of 4.5 μM and the *l*-isomer did not inhibit metabolism at 200 μM.

Inhibition of P450IID1 by these compounds was confirmed using human liver microsomes (Fig. 2a). Fig. 2a demonstrates the correlation between K_i values for the inhibition of sparteine metabolism in canine striatum and human liver microsomes ($r = 0.95$). Any aberrations in the rank order we would suggest are due to species differences, which are commonly observed for this enzyme. Fig. 2b illustrates that K_i values of various compounds for inhibiting [^{3}H]GBR-12935 binding to striatal wheat germ agglutinin pass-through fractions (9), corresponding to P450IID1, correlate well with the K_i values of these compounds for inhibiting canine striatal sparteine metabolism ($r = 0.85$). Further evidence that the enzyme activity being measured in the striatal membranes was due to debrisoquine hydroxylase was obtained through a correlation of the inhibition by five compounds of the striatal sparteine metabolism with the inhibition of pure enzyme activity from CYP2D6 cDNA expressed in HepG2 cells ($r = 0.93$) (Fig. 3b). Stereoselective inhibition of the enzyme activity by quinidine and quinine, with IC_{50} values of 32 nM and 4.0 μM, respectively, was observed (Fig. 3a).

Regional distribution of P450IID1 in canine CNS. Due to the highly specialized functions of different brain regions, it is potentially important to know in which regions P450IID1 is active. Eight canine brain regions were examined for their relative P450IID1 activities, and the results are illustrated in Fig. 4. There was a 40-fold difference in activity, ranging from 0.5 to 21 pmol/mg of protein/hr, in the cerebellum to supraorbital cortex.

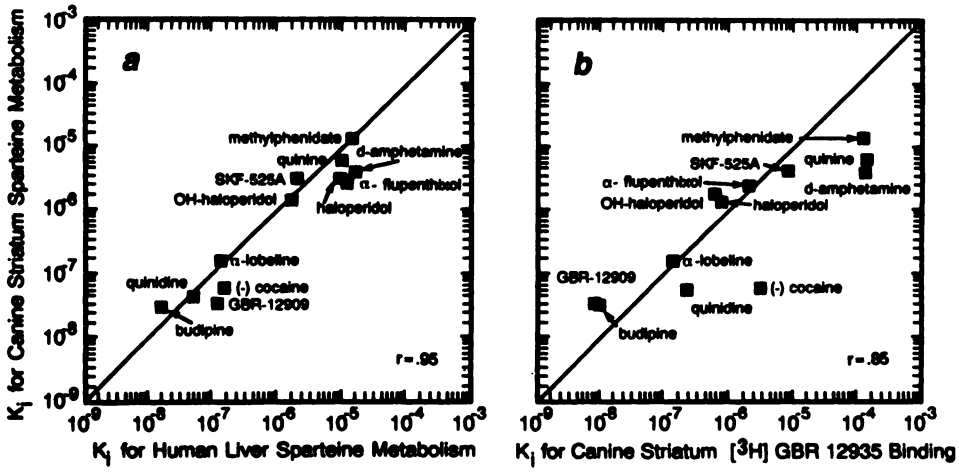

Fig. 2. Inhibition correlates of striatal sparteine metabolism. Correlation between K_i values for the inhibition of canine striatal sparteine metabolism by various compounds and K_i values for inhibition of human liver sparteine metabolism (a) or K_i values for inhibition of [^{3}H]GBR-12935 binding to wheat germ agglutinin pass-through fractions (b). Line of unity is drawn. Some of the K_i estimates for human liver P450IID1 activity are found in Refs. 9 and 37. K_i estimates for [^{3}H]GBR-12935 binding are from Niznik *et al.* (9), except for haloperidol and OH-haloperidol, which are from hepatic inhibition studies as described.

Sequence of human caudate cDNA clone. Multiple ethidium bromide-stained DNA bands from the PCR reamplification were visualized on a 0.8% soft agar gel (Fig. 5A). After subcloning of DNA from four contigious gel slices (300–700 bp) and colony screening, a single colony hybridized with the ^{32}P-labeled *CYP2D6* probe. Plasmid cDNA from this colony was isolated, restriction digested (Fig. 5B), and subjected to Southern blot analysis with nick-translated ^{32}P-labeled hepatic *CYP2D6* probe (Fig. 5C). A hybridizing band of approximately 600 bp (Fig. 5c) was sequenced and displayed 100% nucleic acid sequence identity (exons 6–9, nucleotides 2918–4186, coding region for 171 amino acids) (18) with the *CYP2D6* gene encoding the human polymorphic debrisoquine hydroxylase (data not shown).

Discussion

Although the role of P450IID1 in the brain is unknown, it may include such different functions as metabolism of xenobiotics and catabolism and processing of neurotransmitters. In this paper, we have provided evidence that P450IID1 is catalytically active and functionally expressed in the canine brain and is able to bind centrally stimulating drugs such as cocaine and amphetamine.

Evidence consistent with the contention that sparteine metabolism measured in the striata is due to P450IID1 was obtained as follows. (a) Sparteine metabolism in canine striata was inhibited by SKF525A, was temperature sensitive, and required an NADPH-generating system for optimal activity. (b) The anti-rat P450IID1 antibody used previously to inhibit human P450IID1 activity (14, 15) inhibited sparteine metabolism in the dog brain (Fig. 1b). (c) The characteristic stereoselective inhibition by quinidine and quinine of hepatic sparteine metabolism was observed in canine striatal membranes (Fig. 1c). (d) Excellent correlations were observed between K_i values for inhibition of sparteine metabolism in dog brain and those in human liver ($r = 0.95$) (Fig. 2a) and cells expressing human hepatic cDNA for *CYP2D6* ($r = 0.93$) (Fig. 3b). (e) Lastly, a cDNA fragment (exons 6–9) 100% homologous to the *CYP2D6* gene has been amplified from a human caudate cDNA library.

Our previous work indicated that there was an overlap of substrate/inhibitor specificities between the dopamine transporter and P450IID1 (9). Specifically, cocaine displayed a K_i of 74 nM for the canine striatal P450IID1, higher than that observed for the dopamine transporter (9, 23), where it is believed to cause its primary pharmacological effects. Because P450IID1 is a polymorphic protein in humans, there may be differences between phenotypes in the pharmacological actions of cocaine. Similarly, another dopamine transporter ligand, *d*-amphetamine, binds to striatal P450IID1 (Fig. 2). Amphetamine is polymorphically metabolized to *p*-hydroxyamphetamine by P450IID1 *in vivo* in humans (24, 25), and *p*-hydroxyamphetamine can be detected after incubations of amphetamine with brain slices (26). Central metabolism of amphetamine to *p*-hydroxyamphetamine is of interest because of the suggested association between the formation of *p*-hydroxynorephedrine, a "false neurotransmitter" that is derived from *p*-hydroxyamphetamine, and the development of tolerance to the drug (25, 26). The hallucinogen 4-methoxyamphetamine is also polymorphically *O*-demethylated by P450IID1 *in vivo* in humans (27), and it has been speculated that poor metabolizers may be unable to demethylate *O*-methylated psychotoxins, perhaps contributing to the symptoms/etiology of schizophrenia. It is certainly tempting to speculate about the role of P450IID1 in the central metabolism and/or neurotoxicity of psychotoxins, in particular cocaine and amphetamine-like compounds.

Codeine is *O*-demethylated to morphine by the polymorphic P450IID1, which results in differences in pain thresholds between extensive and poor metabolizers (28). Animal studies indicate that the morphine formed in the brain from codeine may be responsible for the analgesic effects of codeine (29). These two findings suggest that the central form of P450IID1 is also polymorphic and that the central form of P450IID1 may be very important in the pharmacological effects of codeine.

Some neuroleptics have been demonstrated to be metabolized by P450IID1 (30, 31). Perhaps the lack of a good correlation between serum concentrations of neuroleptics and the clinical effects can be attributed in part to central metabolism of these drugs, whereby the actual concentration of the drug at the CNS receptor is not related to the concentration of the drug in the serum.

Because of the highly specialized functions and cell types in different CNS regions, we examined the regional distribution of P450IID1, in order to better understand whether P450IID1 plays a ubiquitous or specialized role in the brain (Fig. 4). Although there is no relationship between the levels and distribution of the dopamine transporter (32) and P450IID1 in the brain, both proteins may be localized to the plasma mem-

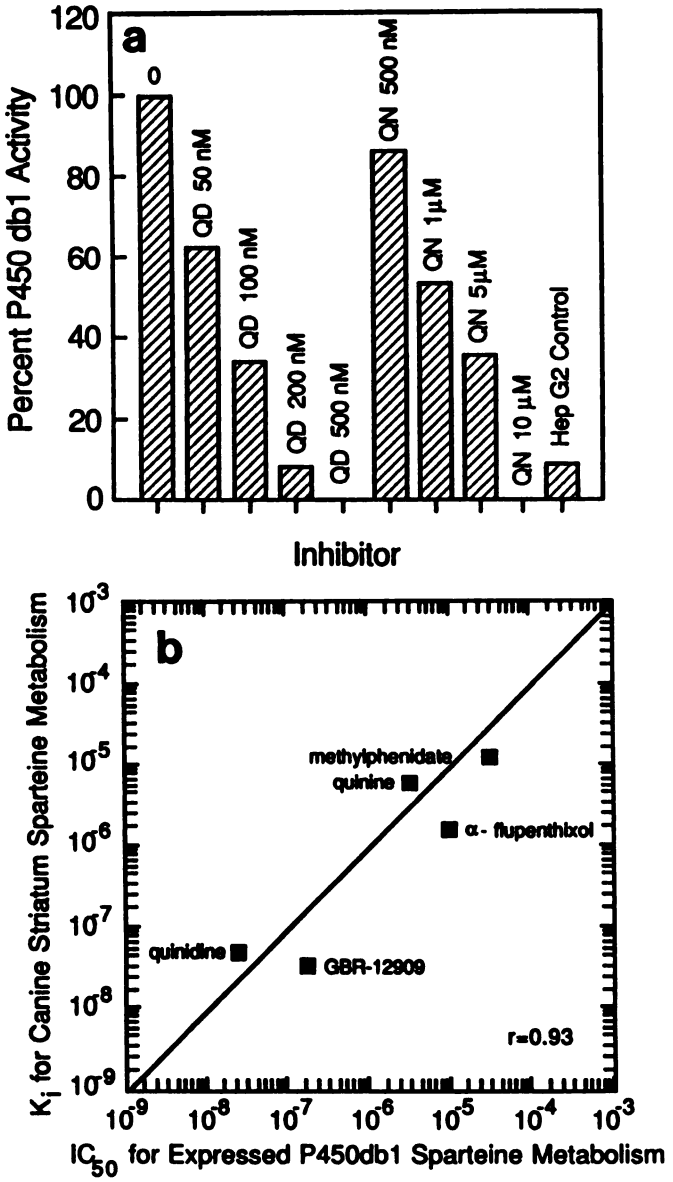

Fig. 3. Inhibition of sparteine metabolism in cDNA-expressed CYP2D6. a, Stereoselective inhibition of cDNA-expressed CYP2D6 by quinidine (*QD*) and quinine (*QN*). HepG2 cells containing plasmid without the *CYP2D6* act as a control. Sparteine concentration was 100 μM; the 100% value was 380 pmol/mg/hr. b, Correlation between K_i values for the inhibition of canine striatal sparteine metabolism by various compounds and IC_{50} values for inhibition of cDNA-expressed human CYP2D6. Line of unity is drawn.

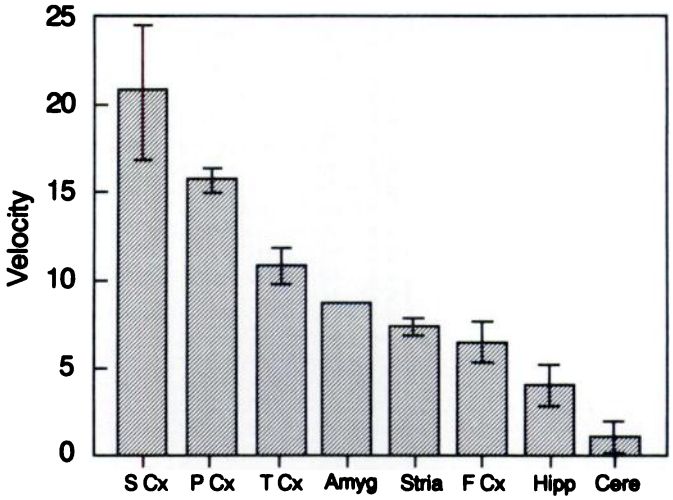

Fig. 4. Regional localization of P450IID1 in canine brain. Sparteine substrate was 100 μM; velocity is given in pmol of 2-dehydrosparteine/mg of protein/hr. *Bars*, range found in duplicate experiments (amygdala only done once). *S Cx*, supraorbital cortex; *P Cx*, parietal cortex; *T Cx*, temporal cortex; *Amyg*, amygdala; *Stria*, striatum; *F Cx*, frontal cortex; *Hipp*, hippocampus; *Cere*, cerebellum.

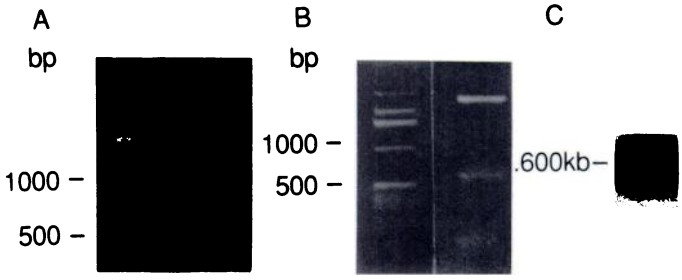

Fig. 5. A, Ethidium bromide staining of electrophoretically separated PCR products (*right lane*). B, Ethidium bromide staining of *BglII*/*BamHI*-digested plasmid cDNA containing insert (*right lane*). Shown in the *left lanes* of both A and B are molecular size markers, using a 1-kilobase DNA ladder (BRL). C, Southern blot of B, using ^{32}P-labeled human hepatic *CYP2D6* probe, as described in Experimental Procedures.

brane, because P450IID1 has been identified on these membranes in liver cells (16).

It is also possible that P450IID1 may be inducible to a much greater extent in brain than in liver, a phenomenon demonstrated by Guengerich and Mason (33) with 3-methylcholanthrene induction of 7-ethoxy-coumarin *O*-deethylase. Therefore, although P450IID1 is not thought to be an inducible enzyme (34), induction of the CNS rather than the hepatic P450IID1 has not been directly tested. There are also differences in induction between neuronal and glial cells in the CNS (35), and manganese increases *d*-amphetamine hydroxylation to a greater extent in striatal mitochondrial membranes than in microsomal membranes (36). Because manganese is known to selectively deplete dopamine in the striatum and *d*-amphetamine is a substrate of P450IID1, this latter observation is particularly interesting. Future studies are needed to investigate the relative induction of P450IID1 in the CNS versus the liver and between different cell types and different cell membranes within the CNS.

In conclusion, evidence is presented here suggesting that P450IID1 is present and functional in the central nervous system. It can be speculated that the genetic variation of this enzyme may have consequences for neurological diseases and toxicity as well as in therapeutic drug response.

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Send reprint requests to: R. F. Tyndale, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.