The Relative Contribution of Monoamine Oxidase and Cytochrome P450 Isozymes to the Metabolic Deamination of the Trace Amine Tryptamine

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ABSTRACT

Tryptamine is a trace amine in mammalian central nervous system that interacts with the trace amine TA2 receptor and is now thought to function as a neurotransmitter or neuromodulator. It had been reported that deamination of tryptamine to tryptophol was mediated by CYP2D6, a cytochrome P450 that is expressed in human brain, suggesting that tryptamine may be an endogenous substrate for this polymorphic enzyme. We were unable to confirm this report and have reinvestigated tryptamine metabolism in human liver microsomes (HLM) and in microsomes expressing recombinant human cytochrome P450 and monoamine oxidase (MAO) isozymes. Tryptamine was oxidized to indole-3-acetaldehyde by HLM and recombinant human MAO-A in the absence of NADPH, and indole-3-acetaldehyde was further reduced to tryptophol by aldehyde reductase in HLM in the presence of NADPH. Steady-state kinetic parameters were estimated for each reaction step by HLM and MAO-A. The CYP2D6 substrates bufuralol and debrisoquine showed strong inhibition of both tryptophol production from tryptamine in HLM and the formation of indole-3-acetaldehyde from tryptamine catalyzed by recombinant MAO-A. Anti-CYP2D6 monoclonal antibody did not inhibit these reactions. Pargyline, a nonselective MAO inhibitor, did not show cross inhibition to debrisoquine 4-hydroxylation and dextromethorphan O-demethylation by HLM and recombinant CYP2D6 enzyme. This is the first unequivocal report of the selective conversion of tryptamine to tryptophol by MAO-A. CYP2D6 does not contribute to this reaction.

CYP2D6 is a polymorphic human cytochrome P450 involved in the metabolism of approximately 20% of marketed drugs, such as antiarrhythmics, antihypertensives, β-blockers, antidepressants, and tricyclic antidepressants (Evans and Relling, 1999). In addition to this broad range of pharmaceuticals, CYP2D6 has also been shown to metabolize certain chemicals, including the neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Fonne-Pfister et al., 1987; Gilham et al., 1997) and 1,2,3,4-tetrahydroquinoline (Ohta et al., 1990). Over 70 variant alleles have been described for the CYP2D6 gene, at least 20 of which produce the poor metabolizer (PM) trait, occurring in 2 to 10% of different populations (Evans and Relling, 1999). Extensive metabolizers (EM) constitute the majority of the population. CYP2D6 protein is expressed primarily in the liver but also in respiratory and gastrointestinal epithelia (Madani et al., 1999; Ding and Kaminsky, 2002). As points of access for potentially toxic chemicals into the body, the function of CYP2D6 in these tissues is presumably one of detoxication of xenobiotics.

The presence of CYP2D6 in neurons of the human CNS has been demonstrated using a variety of techniques, including immunoblotting (Fonne-Pfister et al., 1987; Sieg et al., 2001), in situ hybridization (Gilham et al., 1997; Siegel et al., 2001), reverse transcription-polymerase chain reaction (McFayden et al., 1998), and metabolism of the CYP2D6 probe drug dextromethorphan (Voiri et al., 2000). One report lo-
calized the expression of CYP2D6 to the pigmented cells of the substantia nigra (Gilham et al., 1997), whereas another detected CYP2D6 mRNA in the neocortex, caudate nucleus, putamen, globus pallidus, hippocampus, thalamus, substantia nigra, and cerebellum (Sieglet al., 2001). CYP2D6 protein, however, was only detected in the large principal neurons in the cortex, hippocampus, and cerebellum (Sieglet al., 2001). If CYP2D6 was associated with the endothelial cells lining the 650 km of blood capillary found in the human brain, a case could be made that it functioned as part of the blood-brain barrier and its role was as a “last line of defense” preventing toxins from entering the brain. Many toxic alkaloids are CYP2D6 substrates. All studies, however, appear to show that CYP2D6 within the CNS is neuronal in origin (Gilham et al., 1997; McFayden et al., 1998; Sieglet al., 2001), and this brings in to question the function of this enzyme in the CNS. This is the first piece of evidence that CYP2D6 may have an endogenous substrate in the human brain.

A difference in personality between EM and PM individuals has also been reported (Bertilsson et al., 1989; Lilrennet al., 1993), suggesting that CYP2D6 may be involved in the metabolism of one or more endogenous neuroactive substances. In vitro studies have shown that CYP2D6 can metabolize 4-methoxyphenylethylamine to tyramine (Miller et al., 2001), followed by further CYP2D6-mediated hydroxylolation to yield dopamine (Hiroiet al., 1998; Miller et al., 2001).

Tryptamine is a trace amine found in very low concentrations in the mammalian CNS but localized in neurons with a very high turnover and short half-life (Jones, 1982). It has been speculated that tryptamine might be a neuromodulator, perhaps opposing the actions of serotonin (5-hydroxytryptamine; 5-HT) and, thus, playing a role in the regulation of mood, emotion, sleep, and appetite (Jones, 1981, 1982), the cardinal functions of 5-HT. Elevated tryptamine urinary excretion has been observed in schizophrenic patients, attributed to low MAO activity (Sullivan et al., 1980). Tryptamine may thus play a role in the pathophysiology of schizophrenia.

Only recently has a new family of 15 G protein-coupled receptors been described in humans, that have high affinity for the trace amines tryptamine, octopamine, β-phenylethylamine, and tryptamine (Borowsky et al., 2001). These receptors, called TA (trace amine) receptors, are distinct from the classical biogenic amine receptors, those for 5-HT, dopamine, and norepinephrine. The TA1 receptor is activated most potently by tyramine and β-phenylethylamine, whereas the TA2 receptor is activated by β-phenylethylamine and, to some extent, by tryptamine (Borowsky et al., 2001). Therefore, tryptamine may now be considered a true candidate neurotransmitter or neuromodulator, although its physiological function is still the subject of speculation. Clearly, the pathways and mechanisms of metabolism of tryptamine in the CNS are now of even greater interest.

It has been reported that CYP2D6 mediated the deamination of tryptamine (Martinez et al., 1997), which, prior to that, was understood to be an MAO-dependent pathway (Sullivan et al., 1986). This role of CYP2D6 in tryptamine metabolism has never been confirmed. Therefore, we have reinvigated tryptamine deamination in HLM and in microsomes expressing recombinant human MAO and P450 isoforms. Furthermore, the reason why CYP2D6 is expressed in human brain is not understood. Clarification of the role of CYP2D6 in the metabolism of tryptamine would add greatly to our understanding of the physiological potential of CYP2D6, beyond the detoxification of drugs and other exogenous chemicals.

Materials and Methods

Chemicals and Enzymes. Tryptamine, tryptophol, indole-3-acetaldehyde, sparteine, quinidine, pargyline, reduced NADPH, and 60% perchloric acid were purchased from Sigma-Aldrich (St. Louis, MO). Debrisoquine, 4-hydroxydebrisoquine, dextromethorphan, and dextropropoxyphene were purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). HPLC solvents and other chemicals were of the highest grade commercially available and were used as received. (±)-Buphuralol, recombinant human P450 Superoxymes, P450 insect control microsomes, pooled human liver microsomes (coded H161), recombinant human MAO-A, MAO-B, and MAO insect control were bought from BD Gentest (Woburn, MA). CYP2D6 Baculose reagents were purchased from Panvera Corp. (Madison, WI). Highly purified CYP2D6 isofrom reconstituted with P450 reductase was described elsewhere (Yu et al., 2001). The monoclonal antibody raised against human CYP2D6 (MAB-50-1-3) enzymes was characterized previously (Gelboin et al., 1997).

Incubation Reactions with Recombinant P450 Isoforms. Each incubation reaction was carried out in 100 mM potassium phosphate, pH 7.4, containing 20 pmol of cDNA-expressed P450 enzyme and 5 μM tryptamine in a final volume of 200 μl. The reaction was initiated by the addition of 20 μl of 10 mM NADPH after 5 min, preincubation at 37°C. Incubation was terminated by the addition of 10 μl of 60% perchloric acid after a further 5-min incubation. These reaction conditions were within the linear range with respect to enzyme content and incubation time. The mixture was vortexed for 20 s and then centrifuged at 14,000g for 10 min. The supernatant was transferred to a new vial and directly injected for HPLC analysis. Perchloric acid was also used to stop the reaction of dextromethorphan with CYP2D6 enzyme, and the supernatant obtained after centrifugation was directly injected for HPLC analysis. Reactions with debrisoquine were quenched with 50 μl of cold 400 mM sodium hydroxide solution and extracted with 3 ml of methyl tert-butyl ether. The extracts were evaporated and then reconstituted with 50% methanol solution. The final reconstitutes were transferred to new vials and injected for LC-MS/MS analysis. The final dextromethorphan concentration was 5 μM in the reactions for the inhibition study with pargyline, which ranged from 0 to 1,000 μM. The debrisoquine concentration was 5 μM in the incubation mixtures for the inhibition analysis with pargyline, ranging from 0 to 1,000 μM. All the reactions were performed in duplicate.

Incubation Reactions with Recombinant Human MAO. Incubation reactions were performed in 100 mM potassium phosphate, pH 7.4, containing 2.5 μg of protein and the inhibitors when necessary in a final volume of 200 μl. Reactions were preincubated at 37°C for 5 min and then initiated by the addition of the substrate. Incubations were terminated by the addition of 10 μl of 60% perchloric acid. The mixture was vortexed for 20 s and centrifuged at 14,000g for 10 min. A 5 min-assay with a final tryptamine concentration of 5 μM was applied to compare MAO activities with P450 isoforms. For the kinetic analysis, tryptamine concentrations ranged from 0 to 30 μM, and the reactions were incubated at 37°C for 5 min with recombinant MAO-A. The final tryptamine concentration was 5 μM for the inhibition study with dextromethorphan, debrisoquine, bufuralol, and pargyline, whose concentrations ranging from 0 to 100 μM. All reactions were performed in duplicate, and the supernatants were directly injected for HPLC analyses after spinning down the precipitated protein and salt. For LC-MS/MS analysis, incubations were stopped by the addition of 200 μl of cold 20 mM ammonium hydroxide, extracted with methyl tert-butyl ether, and reconstituted with 50% methanol.
Incubation Reactions with Human Liver Microsomes. Incubation reactions were carried out in 100 mM potassium phosphate, pH 7.4, containing 20 µg of protein, NADPH with a final concentration of 1 mM, and the inhibitors when necessary in a final volume of 200 µl. Reactions were preincubation at 37°C for 5 min and then initiated by the addition of the tryptamine or indole-3-acetaldehyde. For the incubations with debrisoquine and dextromethorphan, substrates were added before preincubation, and then NADPH was added to initiate the reactions. Reactions were terminated by the addition of 10 µl of 60% perchloric acid or 50 µl of 400 mM sodium hydrosulphide after a 15-min incubation. The concentrations of tryptamine and indole-3-acetaldehyde ranged from 0 to 150 µM, and a 15-min incubation was performed for the kinetic analysis. The tryptamine concentration was fixed at 5 µM for the inhibition study with the chemical inhibitors in which the concentration ranged from 0 to 1000 µM. Fifty microliters of monoclonal antibody against CYP2D6 was used for the immunoinhibition study. Antilysozyme (HyHel) was used as a control for nonspecific binding. Both debrisoquine and dextromethorphan concentrations were fixed at 5 µM for the analysis of pargyline inhibition of CYP2D6 activity. All reactions were performed in duplicate.

Quantitation of Metabolites by HPLC and LC-MS/MS. HPLC analysis was carried out on an Agilent 1100 series HPLC system (Palo Alto, CA) consisting of the online vacuum degasser, quaternary pump, autosampler, thermostated column compartment, fluorescence detector, and diode-array detector. The Agilent 1100 series HPLC System was controlled with an Agilent ChemStation and handheld control module. A Regis 250 × 4.6-mm i.d., rexchrom phenyl, 5-µm column (Morton Grove, IL) was used to separate the metabolites. The flow rate through the column at ambient temperature was 1 ml/min. Separation of tryptamine and its metabolites was achieved with a mobile phase containing 70% buffer A (0.1% trifluoroacetic acid in water) and 30% buffer B (40% v/v aqueous acetonitrile). The excitation and emission wavelengths of the fluorescence detector were set at 280 and 340 nm, respectively. Tryptamine, tryptophol, and indole-3-acetaldehyde eluted at 6.60, 9.56, and 10.54 min, respectively. The detection limit for tryptophol and indole-3-acetaldehyde was 5 pmol under the experimental conditions. For these HPLC analyses, an external standard was used for quantitation purposes. The calibration curves for tryptophol and indole-3-acetaldehyde were linear from 1 to 2000 pmol injected.

Analysis of dextromethorphan and dextropropoxyphene was performed with the HPLC method described previously (Yu et al., 2001). LC-MS/MS analysis of debrisoquine and its 4-hydroxy metabolite, using phenacetin (100 pmol) as internal standard, was performed on a PE SCIEX API 2000 ESI triple-quadrupole mass spectrometer (PerkinElmer/ABI, Foster City, CA) controlled by Analyst software, as described previously (Gravnil et al., 2002). The formation of tryptophol was confirmed by LC-MS. The chromatographic conditions were the same as those for 4-hydroxydebrisoquine. The detection and quantification of tryptophol were accomplished by selected ion monitoring with the protonated molecular ion of m/z 162.

Data Analysis. Enzyme Michaelis-Menten parameters, Kₘ and V₅₀, were estimated by nonlinear regression (GraphPad Prism 3.02; GraphPad Software, Inc., San Diego, CA). Initial estimates for nonlinear regression were generated graphically using Eadie-Hofstee plots (V₀ versus V₅₀/IS). Linear regression analyses were conducted using Microsoft Excel 2000 (Microsoft, Redmond, WA).

Results

Tryptamine Metabolism by HLM. Tryptophol was the major metabolite formed from tryptamine in pooled HLM with NADPH, whereas indole-3-acetaldehyde was detected as the major product in the absence of NADPH. Linear Eadie-Hofstee plots were obtained (not shown), and one-enzyme kinetic parameters were estimated for indole-3-acetaldehyde and tryptophol formation by pooled HLM, which are listed in Table 1. The Kₘ value for ttryptophol formation from indole-3-acetaldehyde was 10.2 µM, which was similar to the Kₘ value (4.9 µM) for tryptophol production from tryptamine in pooled HLM.

Tryptamine Oxidation by cDNA-Expressed Human P450 and MAO Isozymes. To investigate the relative involvement of major human P450 and MAO enzymes in tryptamine oxidative deamination, screening experiments were carried out using recombinant human P450 and MAO enzymes. Neither indole-3-acetaldehyde nor tryptophol was detected in the incubations of 5 µM tryptamine with any of the 13 individual recombinant human P450 isoforms tested. A CYP2D6 enzyme from another commercial source (Panvera Corp.) and a highly purified CYP2D6 reconstituted with P450 reductase were also used, with even higher substrate concentrations (up to 500 µM); yet, indole-3-acetaldehyde and tryptophol were not detected. Indole-3-acetaldehyde was only detected in the reactions of tryptamine with MAO-A and MAO-B (Fig. 1). These results demonstrate that none of the P450 isoforms tested, including CYP2D6, mediates tryptamine deamination. This metabolic inactivation of tryptamine is performed principally by MAO-A. The Kₘ value estimated for indole-3-acetaldehyde formation from tryptamine by MAO-A was 10.0 µM, which was very similar to the Kₘ value (11.5 µM) calculated using pooled HLM in the absence of NADPH.

Inhibition of Tryptamine Metabolism by CYP2D6 Substrates, Monoclonal Antibody, Quinidine, and Pargyline. The nonspecific MAO inhibitor pargyline showed strong inhibition (IC₅₀ < 0.1 µM) of tryptophol formation from tryptamine in pooled HLM (Figs. 2 and 3A). At a concentration higher than 50 µM, pargyline completely blocked tryptamine deamination in pooled HLM. However, quinidine, a potent CYP2D6 inhibitor (Granvil et al., 2002), inhibited less than 20% of the activity at a concentration up to 500 µM, while the CYP2D6 inhibitory potency of quinidine is reported in the nanomolar range (Granvil et al., 2002). Moreover, the anti-CYP2D6 monoclonal antibody inhibited only about 20% of tryptamine deamination in pooled HLM (Fig. 2). This

<table>
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<th>Substrate</th>
<th>Product</th>
<th>Material</th>
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<th>V₅₀</th>
<th>Kₘ</th>
<th>V₅₀/Kₘ</th>
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<td>H161</td>
<td></td>
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<td>4.9 ± 0.6</td>
<td>0.868</td>
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<td>4.74 ± 0.205</td>
<td>11.5 ± 2.0</td>
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<tr>
<td>Indole-3-acetaldehyde</td>
<td>Tryptophol</td>
<td>H161</td>
<td></td>
<td>8.86 ± 0.234</td>
<td>10.2 ± 1.1</td>
<td>0.869</td>
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<tr>
<td>Tryptamine</td>
<td>Indole-3-acetaldehyde</td>
<td>MAO-A</td>
<td></td>
<td>118 ± 12.2</td>
<td>10.0 ± 2.6</td>
<td>11.8</td>
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</tbody>
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monoclonal antibody did not show any inhibition to recombinant MAO-catalyzed tryptamine oxidation (data not shown).

It is interesting that some CYP2D6 substrates showed good inhibition of tryptamine metabolism. Debrisoquine (10 μM), bufuralol (100 μM), dextromethorphan (100 μM), and sparteine (1000 μM) inhibited more than 50% of the MAO activity (Fig. 2 and 3A). CYP2D6 substrates (bufuralol, debrisoquine, and dextromethorphan) and pargyline displayed a similar inhibition of indole-3-acetaldehyde formation from tryptamine by cDNA-expressed human MAO-A (Fig. 3B). The IC₅₀ for debrisoquine inhibition of tryptamine deamination was calculated as 4.8 μM, a value less than that for the Kₘ of debrisoquine 4-hydroxylation by CYP2D6 (12.1 μM) (Granvil et al., 2002), which in the absence of other data might cause one to believe that tryptamine was deaminated by CYP2D6. It thus occurred to us that debrisoquine itself might be metabolized by MAO, having an apparent affinity for MAO at least as great as for CYP2D6. We investigated this possibility using both HPLC and LC-MS, monitoring for the appearance of the deaminated urea analog of debrisoquine [(M + 1)⁺ = 177 m/z]. This putative debrisoquine metabolite (Idle et al., 1979) (kindly provided by Stefanie Lerch, University of Bern, Bern, Switzerland) was not detected in incubations of debrisoquine with recombinant MAO-A or MAO-B (data not shown), and thus, debrisoquine appears not to be metabolized by MAO.

**Effects of Pargyline on CYP2D6 Activity.** To further confirm the role of MAO in the catabolism of tryptamine, we eliminated the possibility of cross inhibition to CYP2D6 activity by pargyline. Debrisoquine 4-hydroxylation and dextromethorphan O-demethylation were used as index reactions for CYP2D6 activity. As shown in Fig. 4, 10 μM pargyline inhibited less than 20%, and 100 μM pargyline inhibited less than 40%, of debrisoquine 4-hydroxylation and dextromethorphan O-demethylation, respectively, by pooled HLM and recombinant CYP2D6. At these concentrations, pargyline inhibited more than 90% of tryptamine conversion to tryptophol (Fig. 3). These results indicate that pargyline is not a strong inhibitor of CYP2D6 and further exclude the involvement of CYP2D6 in tryptamine metabolism.

**Discussion**

It is now 25 years since the discovery of the debrisoquine 4-hydroxylation (CYP2D6) polymorphism (Mahgoub et al., 1977), and countless investigations have identified a plethora of pharmaceutical substrates for this enzyme, drugs that display widely variable pharmacokinetics and clinical responses in the population (Evans and Relling, 1999). The question still remains, however, why this cytochrome P450 gene, above all other P450 genes, harbors such a large number of diverse inactive alleles (Granvil et al., 2002), ranging from point mutations to deletions of the complete gene locus. Is evolution giving us a sign that CYP2D6 may have one or more endogenous substrates, the metabolism of which may sometimes be disadvantageous to the host? Answers to these
questions fall beyond the scope of this article, but all insights we can obtain on the physiological roles of CYP2D6 contribute useful steps toward the answers.

The earliest clues that CYP2D6 might have an endogenous substrate in the CNS came from the observation of personality differences between EMs and PMs (Bertilsson et al., 1989; Llerena et al., 1993). PMs were more anxiety prone and less successfully socialized than EMs, and the authors proposed the involvement of a neurotransmitter amine (Llerena et al., 1993). In vitro studies have reported that CYP2D6 catalyzes progesterone hydroxylation (Hiroi et al., 2001), tyramine hydroxylation (Hiroi et al., 1998; Miller et al., 2001), and tryptamine deamination (Martinez et al., 1997). Tryptamine catabolism to tryptophol, however, is known to be a two-step biotransformation mediated by MAO and aldehyde reductase (Neff and Yang, 1974). In an attempt to clarify this discrepancy and to better elucidate the data derived from experiments with chemical inhibitors, we carefully reinvestigated tryptamine metabolism by HLM and cell microsomes expressing human P450 and MAO enzymes. The results showed that neither CYP2D6 nor other P450 isoforms catalyze tryptophol formation and confirmed the role of MAO in tryptamine metabolism, in particular MAO-A.

The discrepancies between our findings and those of others underscore the potential pitfalls inherent in the use of chemical inhibitors as a means of dissection of the role of individual isozymes in the metabolism of a particular substrate. Only through the use of cDNA-expressed P450s and highly specific inhibitory monoclonal antibodies were we able to ascertain that CYP1A1, in addition to CYP2D6, contributed to the 4-hydroxylation of debrisoquine in a manner also inhibited by quinidine (Granvil et al., 2002), the usual hallmark of CYP2D6 activity. Previous work (Martinez et al., 1997) had reported that the CYP2D6-dependent pathway, dextromethorphan O-demethylation, was inhibited by tryptamine, and conversely, tryptamine deamination to tryp-

![Graph](Fig. 2. Effects of pargyline, quinidine, anti-CYP2D6 monoclonal antibody, and CYP2D6 substrates on tryptophol formation from tryptamine in pooled human liver microsomes. Reaction was performed using pooled human liver microsomes containing 20 μg of protein, 10 μM tryptamine substrate, 100 μM chemical inhibitor, or 50 μl of anti-CYP2D6 monoclonal antibody (CYP2D6 MAB) and was incubated at 37°C for 10 min.)
tophol was inhibited by quinidine (IC$_{50}$, 16 µM) and by the CYP2D6 substrates debrisoquine (IC$_{50}$, 6 µM), bufuralol (IC$_{50}$ 45 µM), dextromethorphan (IC$_{50}$, 500 µM), and sparteine (IC$_{50}$ 3800 µM). These inhibitory potencies fall short of what might be expected for a CYP2D6 substrate and are more reminiscent of the inhibition of CYP1A1 debrisoquine 4-hydroxylase by quinidine (IC$_{50}$, 0.018 µM) (Granvil et al., 2002). Interestingly, the inhibition of tryptamine deamination by debrisoquine reported by Martinez et al. (1997) (IC$_{50}$, 6 µM) is almost exactly what we have reported here for the inhibition of MAO-mediated tryptamine deamination by debrisoquine (IC$_{50}$, 4.2 µM; Fig. 2, A and B). What these workers were observing with debrisoquine versus tryptamine in human liver microsomes was almost certainly MAO-mediated deamination. Although

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 3. Inhibition of tryptophol formation from tryptamine with CYP2D6 substrates, quinidine, and pargyline by human liver microsomes (A) and recombinant human monoamine oxidase A enzyme (B). The reaction was conducted with pooled human liver microsomes containing 20 µg of protein or MAO-A cDNA-transfected cell microsomes containing 2.5 µg of protein and 5 µM tryptamine in potassium phosphate buffer, pH 7.4, at 37°C for 5 min.
MAO is a mitochondrial enzyme, microsomal fractions may sometimes be contaminated with mitochondrial enzyme activities, and this may be the basis of the discrepancy between our findings and the earlier report (Martinez et al., 1997).

Interactions of debrisoquine with MAO have been observed on numerous occasions. Patients treated with debrisoquine were reported to have lower excretion of vanillylmandelic acid, the terminal metabolite of norepinephrine, due to inhibit...
bition of peripheral neuronal MAO (Silas et al., 1979). In other studies, debrisoquine administration was also found to inhibit the formation of homovanillic acid from dopamine by its action on MAO (Kendler et al., 1982). Using highly purified human MAO-A and MAO-B, debrisoquine was shown to inhibit kynuramine metabolism with a $K_i$ of 0.5 and 8.8 $\mu$M for placental MAO-A and hepatic MAO-B, respectively (Javors et al., 1989). Our finding of an $IC_{50}$ of 4.2 $\mu$M for the inhibition by debrisoquine of tryptamine deamination by recombinant MAO-A is of the same order. Finally, MPP$,^+$, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine, has been shown to be displaced from its binding sites in mouse brain by debrisoquine. These binding sites appear to be MAO-A (Del Zompo et al., 1990). Thus, debrisoquine appears to bind reversibly to two proteins found in neurons within the CNS, CYP2D6, and MAO-A.

The high affinity of debrisoquine for MAO-A, the enzyme responsible for the rapid turnover of tryptamine, has been the source of misinterpretation of the earlier metabolic findings, leading to the false conclusion that CYP2D6 was responsible for the deamination of tryptamine (Martinez et al., 1997). We have shown, using recombinant cDNA-expressed P450 and MAO isoforms together with a highly specific anti-CYP2D6 monoclonal antibody, that CYP2D6 and 12 other human P450s are not involved in the deamination of tryptamine. This reaction is essentially performed by MAO-A.

The role of CYP2D6 in central neurons remains no closer to a solution. This is nevertheless a subject of intense interest, if only because the polymorphic expression of this enzyme in the population may contribute to intersubject differences in, for example, mood, anxiety, sleep, and behavior, and may contribute to the pathophysiology of various disorders of the CNS that appear to be chemical in origin.

References


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