CYTOCHROME P450-DEPENDENT METABOLISM OF *L*-DEPRENYL IN THE BRAIN OF DIFFERENT EXPERIMENTAL MAMMALS

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Running title: *l*-deprenyl metabolism by brain of different mammals

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ABSTRACT

Although the CYP content of brain is too low to significantly influence the overall pharmacokinetics of drugs and hormones in the body, brain CYP could play a role in tissue- and/or cell-specific sensitivity to certain drugs or xenobiotics. We have characterized the CYP-dependent metabolism of *l*-deprenyl by brain microsomal preparations obtained from different experimental animals: monkey, C57BL/6 mouse and rabbit.

In monkey the kinetic analysis revealed a similar methamphetamine and nordeprenyl formation from *l*-deprenyl in striatum and cortex. Both metabolic pathways appeared to be more efficient in the cortex than in the striatum, since the V_{max} for microsomal preparations was lower in the striatum for the formation of both metabolites. Inhibition analysis suggested that *l*-methamphetamine formation is catalysed by CYP2A and CYP3A, whereas only CYP3A appears to be involved in nordeprenyl formation.

The kinetic study performed with microsomal preparations from whole brain of C57BL/6 mice highlighted that the only detectable *l*-deprenyl metabolite was methamphetamine with K_m and V_{max} values similar to those determined in monkey cortex. However, the inhibition studies suggested the involvement of CYP2E1.

In rabbit the apparent K_m value of both metabolic pathways obtained using whole brain microsomal preparations resulted about five fold higher to the values observed in previous mammalian preparations. Inhibition studies suggested the involvement of CYP2A family in deprenyl metabolism.

In conclusion, the present study indicates that *l*-deprenyl is effectively metabolised by CYP-dependent oxidases in the brain, giving rise mainly to the formation of methamphetamine and that there are differences between species in CYP-dependent metabolism of *l*-deprenyl.

INTRODUCTION

l-deprenyl, selegiline, is a selective inhibitor of monoamine oxidase-B (MAO-B). Because of its capacity to increase striatal dopamine levels, it has been used for treatment of Parkinson's patients (1). Furthermore, many studies have provided evidence that *l*-deprenyl protects neurones independently of its MAO-B inhibitory properties. Recently, a large body of evidence has accumulated indicating that the neuroprotection by *l*-deprenyl might be related to its inhibition of apoptotic pathway mechanisms due to the presence of N'-propargyl moiety (2).

l-deprenyl is primarily metabolized by cytochrome P450 (CYP) to the active metabolites *l*-methamphetamine, *l*-nordeprenyl (desmethyldeprenyl), *l*-amphetamine, and several other hydroxylated compounds. The metabolite *l*-methamphetamine is a more effective inhibitor of presynaptic noradrenaline and dopamine uptake than the parent drug and this improves the cognitive functions (3).

l-Nordeprenyl has been shown to promote antiapoptotic effect both in animal models as well as tissue culture (4,5).

The overall level of CYP in the brain is approximately 0.5-2% of that in liver microsomes (6). Although the CYP content of brain is too low to significantly influence the overall pharmaco-kinetics of drugs and hormones in the body, brain CYP could play a role in tissue- and/or cell-specific sensitivity to certain drugs or xenobiotics.

We have characterized in our laboratory the CYP-dependent metabolism of *l*-deprenyl by brain microsomal preparations obtained from different experimental animal models namely monkey (*Cercopithecus aethiops*), C57BL/6 mouse, extensively used in Parkinson's disease studies, and the rabbit that is another frequently used experimental animal models.

MATERIALS AND METHODS

Chemicals

l-deprenyl-HCl and *l*-nordeprenyl were kind gifts from Chinoin Chemical Works (Budapest, Hungary); ketoconazole was from Jannsen (Milan, Italy); NADPH, NADP, and glucose-6-phosphate dehydrogenase were from Boehringer (Mannheim, Germany). All other chemicals and solvents were of the highest grade available and obtained from common commercial sources.

In vitro assay of CYP-dependent *l*-deprenyl metabolism in microsomal preparations

Monkey, mouse and rabbit brain microsomal preapration were prepared as described in Dragoni *et al.* (7), and stored frozen at –80°C until use.

Protein content was measured by the method of (8) Lowry *et al.* using bovine serum albumin as a standard.

l-Deprenyl at various concentration were incubated in presence of microsomal preparations and the metabolic pathway studied as previously reported (9).

In order to clarify the CYP isozymes involved in *l*-deprenyl brain metabolism, microsomes were incubated in presence of selective inhibitors (ketoconazole, 8-methoxypsoralen, and 4-methylpyrazole) of specific CYP isoenzymes activities.

Statistical Analysis

Kinetic parameters were calculated according to Michaelis-Menten equation for one or two enzymes by nonlinear regression analysis (Prism 3.03 Graphpad Software Inc, San Diego, Ca, USA).Values are presented as means ± S.E.M. and one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons was performed.

RESULTS AND DISCUSSION

The kinetic analysis in monkey microsomal preparations from cortex and striatum revealed a simple hyperbolic kinetic behaviour for the formation of both N-dealkylated metabolites, moreover CYP showed comparable affinity with respect to methamphetamine formation in both areas. The same was observed for nordeprenyl formation from *l*-deprenyl. Both metabolic pathways appeared to be more efficient in the cortex than in the striatum, since the V_{max} for microsomal preparations was lower in the striatum for the formation of both metabolites. Inhibition analysis of both pathways in monkey brain suggested that *l*-methamphetamine formation is catalysed by CYP2A and CYP3A, whereas only CYP3A appears to be involved in nordeprenyl formation. Our previous study, on *l*-deprenyl metabolism in monkey liver microsomal preparations, has shown that CYP-catalysed the formation of both *l*-methamphetamine and *l*-nordeprenyl by a high and a low affinity component. Both components however, showed the same V_{max}/K_m ratio, as index of intrinsic clearance, for both metabolic pathways, suggesting that CYP catalysed the l-deprenyl metabolite formation with a similar efficacy (9).

With microsomal preparations from C57BL/6 mice the only *l*-deprenyl metabolite detected was methamphetamine. The apparent K_m and V_{max} values were similar to those determined in monkey cortex (53.6±2.9 μM and 33.9±0.4 pmol/min/mg protein, respectively). Furthermore, 4-methylpyrazole selectively inhibited methamphetamine formation, suggesting the involvement of CYP2E1 as observed previously in mouse liver microsomal preparations (10).

In figure 1 has been reported the interaction of *l*-deprenyl with brain rabbit CYP. The formation rate of both methamphetamine and nordeprenyl followed a hyperbolic, Michealis-Menten, dependence. The apparent K_m value of both metabolic pathways resulted about five fold higher (292.4±68.3 and 232.6±10.3 μM for methamphetamine and nordeprenyl, respectively) to the values observed in previous mammalian preparations. Inhibitions studies highlighted that only 8-methoxipsoralen was able to decrease of about 40% formation of methamphetamine suggesting the involvement of CYP2A family in deprenyl metabolism.

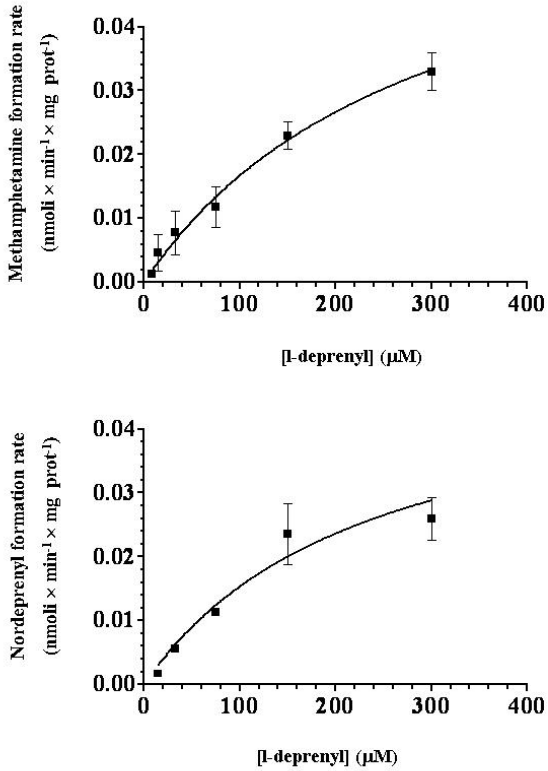

FIGURE 1. Kinetic analysis of *l*-methamphetamine (panel A) and *l*-nordeprenyl (panel B) formation by brain rabbit microsomal preparations. Data are expressed as the mean ± S.E.M. derived from 4 separate experiments.

In conclusion, the present study indicates that *l*-deprenyl is effectively metabolised by CYP-dependent oxidases in the brain, giving rise mainly to the formation of methamphetamine, which has been suggested to play a role in the pharmacological effects of the parent drug.

Although each mammalian species used in present work for the methamphetamine formation presented different K_m and V_{max} values, it was observed similar V_{max}/K_m values. This suggested that at *l*-deprenyl concentrations lower than the K_m value, as occurs *in vivo*, CYP enzymes present in brain have a similar efficacy in methamphetamine formation (table 1).

Animals	V_{max}/K_m (min^{-1})	
	Methamphetamine	Nordeprenyl
Monkey	0.422	0.305
Mouse	0.633	N.D.
Rabbit	0.225	0.232

Table 1. Intrinsic cleareance (V_{max}/K_m) for the formation of methamphetamine and nordeprenyl in different experimental animal models.

On the contrary nordeprenyl formation rate was different between monkey, rabbit and mice.

The results also demonstrate that there are differences between species in CYP-dependent metabolism of *l*-deprenyl and this makes danger of attempting to extrapolate results across species.

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