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Cytochrome P450-dependent N-dealkylation of L-deprenyl in C57BL mouse liver microsomes: effects of in vivo pretreatment with ethanol, phenobarbital, β-naphthoflavone and L-deprenyl

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Abstract

The monoamine oxidase inhibitor L-deprenyl [(−)-deprenyl, selegiline] is an effective therapeutic agent for improving early symptoms of idiopathic Parkinson's disease. It appears to exert this action independently of its inhibition of monoamine oxidase B (MAO-B) and some of its metabolites are thought to contribute. Cytochrome P450 (CYP) activities are known to give rise to L-deprenyl metabolites that may affect the dopaminergic system. In order to clarify the interactions of L-deprenyl with these enzymes, C57BL mice were treated with L-deprenyl, ethanol, phenobarbital or β-naphthoflavone to induce different CYP isozymes. After preincubation of L-deprenyl with liver microsomes from control or treated mice, the metabolites were analysed by a GLC method. L-deprenyl (10 mg/kg i.p. for 3 days) caused a significant decrease in total CYP levels (0.315 ± 0.019, L-deprenyl; 0.786 ± 0.124, control, nmol/mg protein) and CYP2E1-associated *p*-nitrophenol hydroxylase activity (0.92 ± 0.04 vs. 1.17 ± 0.06 nmol/min/mg). Both phenobarbital and ethanol increased the N-depropynylation activity towards L-deprenyl that leads to the formation of methamphetamine (4.11 ± 0.64, phenobarbital; 4.77 ± 1.15, ethanol; 1.77 ± 0.34, control, nmol/min/mg). Ethanol alone increased the N-demethylation rate of L-deprenyl, that results in formation of nordeprenyl (3.99 ± 0.68, ethanol; 1.41 ± 0.31, control, nmol/min/mg). Moreover, the N-dealkylation pathways of deprenyl are inhibited by 4-methylpyrazole and disulfiram, two CYP2E1 inhibitors. None of the other treatments modified L-deprenyl metabolism. These findings indicate that mainly CYP2E1 and to a lesser extent CYP2B isozymes are involved in L-deprenyl metabolism. They also suggest that, by reducing CYP content, L-deprenyl treatment may impair the metabolic disposition of other drugs given in combination regimens.

Keywords: Deprenyl metabolism; Cytochrome P450; Parkinson's disease; N-dealkylation pathway; C57BL mouse liver microsomal preparation

1. Introduction

L-Deprenyl 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 (for a review see Gerlach et al., 1996). As an adjunct to levodopa therapy, L-deprenyl has been shown to improve function and reduce motor fluctuations in patients with advanced Parkinson's disease (see e.g.,Birkmayer et al.,1985). In patients with early untreated Parkinson's disease, controlled clinical trials have shown that L-Deprenyl delays the disability and slows the progression of signs and symptoms of the condition (Parkinson Study Group, 1989, 1993; Tetrud and Langston, 1989). Increased mortality has been reported when L-deprenyl was administered together with levodopa, as compared to levodopa alone, but the causes of this adverse effect are still obscure (Lees, 1995; Ben-Shlomo et al., 1998). However, a large meta-analysis of five long-term studies and four separate studies did not support these conclusions (for review see Heinonen and Myllyla, 1998).

The observation that the pretreatment with L-deprenyl protects against the neurotoxic effects of compounds like

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine and *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) has stimulated studies of the pharmacological and biochemical properties of this drug (see Tipton, 1994). Although it was initially assumed that the benefit of L-deprenyl in Parkinson's disease was a result of its MAO-B inhibitory property, more recent studies have suggested that it prevents neurodegeneration, in both in vivo and in vitro models, by mechanisms that are independent of the inhibition of MAO-B activity (see Tatton and Greenwood, 1991; Tatton and Chalmers-Redman, 1996; Olanow and Tatton, 1999). These studies also suggested that L-deprenyl metabolites contribute to its effect. In humans and experimental animals, L-deprenyl is rapidly metabolised by the liver cytochrome P450 (CYP) system, forming mainly L-nordeprenyl (L-desmethyldeprenyl) and L-methamphetamine (for a review see Baker et al., 1999). These two compounds are further metabolised to L-amphetamine (see Fig. 1).

It has been suggested that the L-metabolites, which do not have the toxicity of the corresponding D-isomers, contribute to the neuroprotective effects of L-deprenyl (see Yasar et al., 1996). Nordeprenyl, which is a less potent inhibitor of MAO-B assays than the parent drug in vitro and in vivo, is more efficacious in protecting dopamine neurones against toxic damage (Mytilineou et al., 1997; Olanow and Tatton, 1999). The metabolite L-methamphetamine is a more potent inhibitor of presynaptic noradrenaline and dopamine uptake than L-deprenyl, and it has been suggested that this effect contributes to its neuroprotective effects (Sziráki et al., 1994).

Although the CYP-dependent metabolism of L-deprenyl is of clinical importance, few studies have been done on this process. The aim of the present study is to clarify the N-dealkylation pathway of L-deprenyl metabolism and to investigate the possibility of interactions with other drugs at the level of the CYP system. C57BL mice were used in this study since this strain has been widely used as a rodent model for the induction of parkinsonism by MPTP. Moreover, mice present a CYPs pattern, which render them a suitable animal model for drug metabolism studies (Funae and Imaoka, 1993). The effects of pretreatment with the CYP isozyme inducers, phenobarbital and β-naphthoflavone (Whitlock and Denison, 1995) and L-deprenyl as well as an alcoholic diet, were studied. Furthermore, the effects of selective CYPs inhibitors, such as 4-methylpyrazole, disulfiram and ketoconazole, were investigated with the aim to identify the CYP isozymes involved in L-deprenyl metabolism.

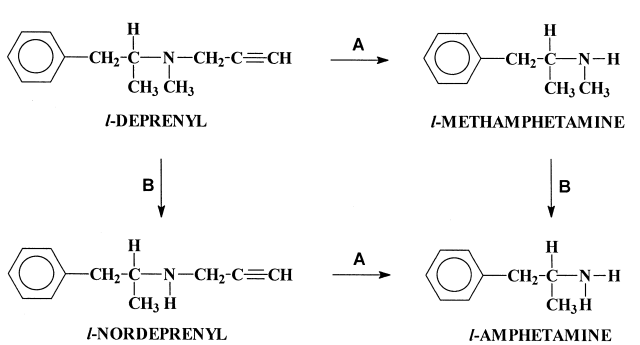

Fig. 1. Scheme of deprenyl sequential N-dealkylation pathways: (A) N-depropynylation reaction; (B) N-demethylation reaction.

2. Materials and methods

2.1. Chemicals

L-Deprenyl-HCl and L-nordeprenyl were gifts from Chinoin Chemical Works (Budapest, Hungary) and Prof. K. Magyar (Semmelweis University, Budapest, Hungary). NADPH, NADP, and glucose-6-phosphate dehydrogenase were from Boehringer (Mannheim, Germany). All other reagents were obtained from Sigma (Milan, Italy). Carbon monoxide was produced in a closed glass jar by adding sulphuric acid dropwise to formic acid at room temperature; it was withdrawn by means of a plastic 50-ml syringe connected to the reaction chamber by plastic tubing.

2.2. Drug treatments

The animal protocols used were reviewed and approved by the Animal Care and Ethics Committee of the Università degli Studi di Siena, Italy.

Male C57BL/6NCrlBR mice (20–25 g), obtained from Charles River (Milan, Italy), were housed in standard cages in a temperature and light-controlled facility, with free access to food (MIL from Morini, S. Polo d'Enza, Italy) and water. After an acclimatisation period of 10 days, a group of 20 control mice was maintained on the same diet without any further treatment; the other animals were divided into groups of 10 and subjected to one of the following six treatments, with the aim to induce different CYP family avoiding some toxic effects:

(i) phenobarbital: a single injection of 80 mg/kg i.p. and subsequently 1 mg/ml in the drinking water for 1 week as described by Lake (1987);
(ii) β-naphthoflavone: 40 mg/kg dissolved in olive oil, for 3 days i.p (Lake, 1987);
(iii) L-deprenyl: 10 mg/kg i.p in normal saline for 3 days;
(iv) ethanol: liquid diet containing ethanol (5%,w/v), representing 36% of the total calorie intake, for a period of 4 weeks, after an induction period of a week during which the ethanol concentration was gradually increased to this level (Della Corte et al., 1994);

(v) pair-fed isocaloric controls for the ethanol study on the same diet as (iv) except that dextrin-maltose (1:1) replaced the alcohol; dietary intake was monitored each day and the quantity available to this control group was adjusted to ensure that they received the same calorie intake as alcohol fed group;
(vi) ad libitum controls for the ethanol study: this group had unrestricted access to the same liquid diet as group (v).

The last two control groups were believed to be necessary since rats on ethanol and pair-fed diets often gain weight more slowly than the ad libitum group (see Della Corte et al., 1994) and it was thus important to distinguish the effects of semi-starvation from those of ethanol consumption.

2.3. Preparation of liver microsomes

All mice were fasted overnight prior to the sacrifice. After CO_2 asphyxia, the abdominal cavity was opened and the liver was perfused in situ through the vena cava with ice-cold normal saline solution. The livers were excised, and weighed. They were then combined in pairs, chopped, suspended in four volumes of 0.25 M sucrose and homogenised in a Potter-Elvejhem homogeniser fitted with a Teflon pestle. The homogenates were centrifuged at 10,000 × g for 20 min and the resulting supernatants then centrifuged at 105,000 × g for 1 h. The resulting microsomal pellets were suspended in 0.01 M Tris–HCl buffer, pH 7.6, containing 151 mM KCl, 1 mM EDTA and 20% glycerol, and stored in liquid nitrogen until use.

2.4. Assay procedures

CYP content was determined from the CO-difference spectra of the microsomal preparations, as described by Omura and Sato (1964).

NADPH cytochrome P450 reductase activity was determined as described by Phillips and Langdon (1962).

p-Nitrophenol hydroxylase activity was measured by determining *p*-nitrocatechol formation according to the procedure described by Reinke and Moyer (1985).

Alkoxyresorufin O-dealkylase activity towards four alkoxy-resorufins (ethoxyresorufin, pentoxyresorufin, methoxyresorufin, benzyloxyresorufin) by control and induced microsomes was measured as described by Nerurkar et al. (1993). Incubation was performed at 30°C in 50 mM Tris–HCl buffer, pH 7.5, containing 25 mM $MgCl_2$. The final concentration of protein was 100 μg/ml and the substrate concentrations were 2 mM for ethoxyresorufin, 10 mM for pentoxyresorufin and 5 mM for benzyloxyresorufin and methoxyresorufin. The reactions were started by addition of 150 mM NADPH. The resulting fluorescence was monitored using a Shimadzu RF-5000 spectrophotofluorimeter (Shimadzu Europa, Duisburg, Germany), at excitation and emission wavelengths of 522 and 586 nm, respectively.

L-Deprenyl N-dealkylase activities were measured as follows. L-Deprenyl, 150 μM, a concentration comparable to the K_m value obtained by Grace et al. (1994) for the formation of the methamphetamine promoted by CYP2D6, was incubated at 37°C for 30 min in 100 mM phosphate buffer, pH 7.4, containing 0.5 mg/ml of microsomal protein, in the presence of a NADPH-generating system (comprising 1 mM NADPH, 4 mM glucose-6-phosphate and 1 unit glucose-6-phosphate dehydrogenase); 1 ml total volume. The reaction was stopped by cooling in ice and adding 0.1 ml 5 M NaOH followed by 100 nmol clorgyline as internal standard. The reaction mixtures were then extracted once with ethylacetate and the parent drug and metabolites were subsequently determined by a modification of the GLC method described by Grace et al. (1994). After derivatisation for 30 min at 70°C with penthafluoropropionic anhydride, samples were dried under nitrogen, resuspended in ethylacetate and injected in a Perkin-Elmer 3B series gas chromatograph equipped with a DB-17 column (30 m × 0.32 mm ID) and a nitrogen–phosphorous detector. A temperature–programmed separation procedure was used, starting from an initial temperature of

Table 1
Effect of different treatments on cytochrome P450 levels and NADPH cytochrome P450 reductase activity in C57BL mouse liver microsomes
Values are means ± S.E.M.; *n* = number of individual microsome preparations.

Treatment	Cytochrome P450 (nmol/mg protein)	*n*	Cytochrome P450 reductase (nmol/min/mg protein)	*n*
Control	0.786 ± 0.124	9	103.68 ± 9.17	9
Ad libitum liquid diet	0.695 ± 0.183	5	67.62 ± 11.77*	5
Isocaloric liquid diet	0.684 ± 0.098	6	94.36 ± 8.45	6
Alcoholic liquid diet	0.660 ± 0.124	5	144.42 ± 15.78*	5
L-Deprenyl	0.315 ± 0.019*	4	86.04 ± 2.86	4
Phenobarbital	0.842 ± 0.157	6	128.15 ± 8.16	7
β-Naphthoflavone	0.765 ± 0.171	4	114.24 ± 14.83	4

*Significant difference from control: $P < 0.05$.

Table 2
Effects of different in vivo treatments on *p*-nitrophenol hydroxylase (PNPH), and ethoxy- (EROD), pentoxy- (PROD), benzyloxy- (BROD), and methoxy-resorufin (MR00OD) O-dealkylase activities
Values are means ± S.E.M.; *n* = number of individual microsome preparations.

Treatment	PNPH (nmol/min/mg)	*n*	EROD (pmol/min/mg)	*n*	PROD (pmol/min/mg)	*n*	BROD (pmol/min/mg)	*n*	MROD (pmol/min/mg)	*n*
Control	1.17 ± 0.06	9	8.31 ± 1.51	8	13.79 ± 2.75	7	24.20 ± 3.91	9	26.45 ± 1.61	4
Ad libitum liquid diet	1.29 ± 0.07	5	7.20 ± 1.57	5	4.03 ± 1.18*	5	7.77 ± 1.18*	5		
Isocaloric liquid diet	1.29 ± 0.09	6	16.62 ± 7.28	6	4.66 ± 0.75*	6	19.37 ± 7.82	6		
Alcoholic liquid diet	1.52 ± 0.12*	5	26.95 ± 5.72**	5	5.81 ± 0.55*	5	36.22 ± 9.73	5		
L-deprenyl	0.92 ± 0.04*	4	6.90 ± 1.46	4	12.10 ± 1.91	4	29.75 ± 4.08	4	31.00 ± 2.30	4
Phenobarbital	1.69 ± 0.05***	5	86.03 ± 10.19***	6	147.26 ± 23.89***	6	727.52 ± 95.35***	6	244.26 ± 33.72***	6
β-Naphthoflavone	1.42 ± 0.10	4	880.12 ± 135.02***	4	31.59 ± 4.49**	4	70.49 ± 6.66***	4	443.62 ± 46.91***	4

*Significant difference from control: $P < 0.05$.
**Significant difference from control: $P < 0.01$.
***Significant difference from control: $P < 0.001$.

100°C and increasing it 7°C/min, up to a final temperature of 230°C. Injector and detector temperatures were set at 230°C and 270°C, respectively, and the carrier-gas flow rate was 25 ml/min. Standard curves were obtained by adding varying amounts of the analytes to microsomal preparations from control rats.

The inhibition studies were performed by preincubating microsomes obtained from untreated mice with 50 μM 4-methylpyrazole, or disulfiram, or ketoconazole and NADPH-generating system at 37°C. After 10 min, 150 μM L-deprenyl was added and the reaction followed for 30 min. The samples were then processed as reported above.

2.5. Statistical analysis

All values are presented as means ± S.E.M. The significance of the differences between means from two treatment groups was established by Student's *t*-test.

3. Results

3.1. Effects of different treatments on CYP levels and NADPH cytochrome P450 reductase activity

None of the treatments significantly affected either the body or liver weight gain (results not shown). However, the alcoholic diet resulted in a slight, but not significant, reduction in body weight gain which was 21.8 ± 0.7 g (92% of the control value, 23.6 ± 0.5 g; $n = 10$) at the end of treatment. The CYP content and related enzyme activities were markedly affected by the different treatments. As shown in Table 1 the drug treatments did not significantly increase the CYP content in the mouse liver microsomal fractions whereas L-deprenyl treatment resulted in a decrease in CYP to less than 50% of its level in control microsomes. NADPH cytochrome P450 reductase activity was significantly increased by alcoholic diet (+40%) whereas the liquid diet given ad libitum resulted in a significant decrease (−35%). The other treatments had no significant effects on this activity (see Table 1).

3.2. Effects of in vivo treatments on cytochrome P450-dependent oxidase activities

As shown in Table 2, phenobarbital treatment resulted in an increase in alkoxyresorufin O-dealkylase activities towards the four resorufin derivatives and *p*-nitrophenol hydroxylase activity. Pentoxyresorufin, and benzyloxyresorufin O-dealkylase activities increased by one order of magnitude with respect to control. β-Naphthoflavone treatment also increased O-dealkylase metabolism towards all substrates, with the greatest induction (about two orders of magnitude) of ethoxyresorufin and methoxyresorufin O-dealkylase activities. Ethanol consumption increased *p*-nitrophenol hydroxylase and ethoxyresorufin O-dealkylase

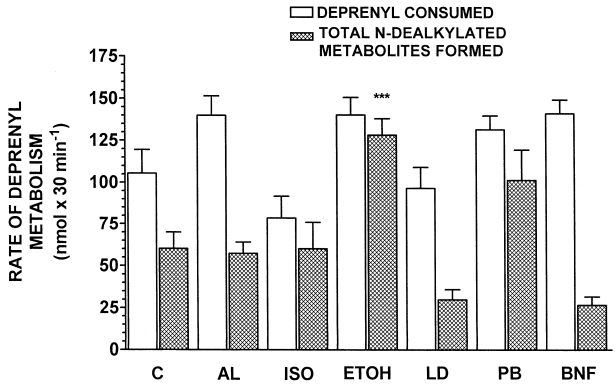

Fig. 2. Overall metabolism of L-deprenyl and formation of the respective NOR metabolites. L-Deprenyl (150 nmol) was incubated with different microsomal preparations. Columns represent the means and bars the S.E.M. of values determined in four or more individual microsomal preparations per group: C, control; AL, ad libitum liquid diet; ISO, pair-fed isocaloric liquid diet; ETOH, ethanol liquid diet; LD, L-deprenyl treatment; PB, phenobarbital treatment; BNF, β-naphthoflavone treatment. Significant difference from control: $^{***}P < 0.001$.

activities and decreased that of pentoxyresorufin O-dealkylase. The decrease, however, was similar to that observed with the other liquid diets. L-Deprenyl treatment significantly inhibited *p*-nitrophenol hydroxylase activity (by about 20% with respect to control), but did not affect the other enzyme activities.

3.3. Effects of in vivo treatments on L-deprenyl metabolism

Assays of N-dealkylase activity towards L-deprenyl showed that, under the conditions described in Materials and methods, the amount of product formed was a linear function with time up to 30–45 min, and that the initial rates were proportional to the amount of microsomal protein added.

Fig. 2 summarises the metabolic transformations of L-deprenyl. When 150 nmol L-deprenyl was added to the microsomal suspension, more than 50% was metabolised in a 30 min incubation period. The ad libitum liquid diet, alcohol and β-naphthoflavone treatments increased the rate of L-deprenyl metabolism, resulting in a loss of more than 90% of the added deprenyl in 30 min. In contrast, the rate of L-deprenyl metabolism was not enhanced in mice receiving the isocaloric liquid diet, with only 52% of the initial amount of drug being metabolised under the same conditions. Furthermore, the alcoholic diet channelled L-deprenyl metabolism towards the N-dealkylation pathway, to the extent that these metabolites accounted for almost 90% of the deprenyl utilised. β-Naphthoflavone treatment stimulated L-deprenyl metabolism to the greatest extent, but it did so in favour of non N-dealkylated metabolites. The formation of nordeprenyl, methamphetamine and amphetamine accounted for only about 20% of the deprenyl utilised, a value which is even lower than that observed with control microsomes. Pretreatment with L-deprenyl itself reduced the extent of N-dealkylation to about 27% of the deprenyl metabolised.

As shown in Table 3, the effect of alcohol treatment was to stimulate the N-demethylation and N-depropynylation metabolic pathways of L-deprenyl, which were 2.5-fold greater than those observed with control microsomes in both cases. Phenobarbital treatment stimulated only the methamphetamine formation rate (2.5-fold), without significantly affecting the rate of nordeprenyl formation. In contrast, treatment with L-deprenyl markedly depressed the rate of nordeprenyl formation, which was reduced to 30% of the control rate without affecting the rate of formation of methamphetamine. The rate of amphetamine formation was not increased in a statistically significant manner by any of the treatments. However, the β-naphthoflavone and L-deprenyl treatments resulted in a significant decrease in amphetamine formation, to about 25% of the control value.

As shown in Fig. 3, the formation of both methamphetamine and nordeprenyl were significantly inhibited when control microsomes were incubated with 4-methylpyrazole or disulfiram, while ketoconazole promoted a less marked inhibition of deprenyl metabolism. The inhibition

Table 3
Effects of different in vivo treatments on the rates of formation of methamphetamine, nordeprenyl and amphetamine promoted by C57BL mouse liver microsomes

Treatment	*n*	Methamphetamine	%	Nordeprenyl	%	Amphetamine	%
Control	8	1.77 ± 0.34	100	1.41 ± 0.31	100	0.80 ± 0.16	100
Ad libitum liquid diet	3	2.17 ± 0.74	123	1.73 ± 0.51	122	0.86 ± 0.04	107
Isocaloric liquid diet	6	2.04 ± 0.52	115	1.49 ± 0.39	108	0.81 ± 0.15	101
Alcoholic liquid diet	5	4.77 ± 1.15**	269	3.99 ± 0.68**	282	1.12 ± 0.06	140
L-deprenyl	4	1.27 ± 0.18	72	0.53 ± 0.28*	37	0.20 ± 0.01**	25
Phenobarbital	4	4.11 ± 0.64**	232	1.92 ± 0.89	136	0.68 ± 0.15	85
β-Naphthoflavone	4	1.16 ± 0.29	65	0.61 ± 0.34	43	0.20 ± 0.06**	25

Activities in nmol/min/mg protein. Values are means ± S.E.M.; $n =$ number of individual microsome preparations.
*Significant difference from control: $P < 0.05$.
**Significant difference from control: $P < 0.01$.

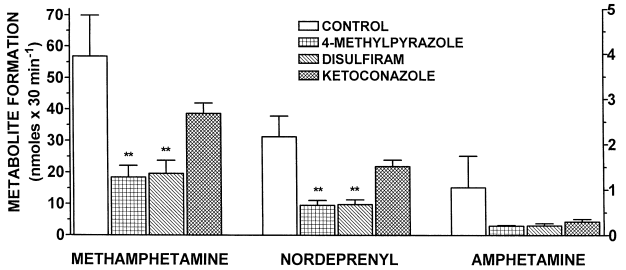

Fig. 3. Effects of 4-methylpyrazole, disulfiram and ketoconazole on methamphetamine, nordeprenyl and amphetamine formation rates. The inhibitors (50 mM) were preincubated with control microsomal preparations in the presence of a NADPH-generating system; after 10 min, 150 mM L-deprenyl was added to the assay mixture and the reaction was followed for 30 min. Columns represent the means and bars the S.E.M. of values determined in four microsomal preparations per group. Left axis, methamphetamine and nordeprenyl formation; right axis, amphetamine formation. Significant difference from control: $^{**}P < 0.01$.

exerted by 4-methylpyrazole or disulfiram was about 70% while that exerted by ketoconazole was about 30%.

4. Discussion

Acetylenic compounds, including those that are MAO inhibitors, have been associated with modulation of CYP activities. It has been suggested that they interact with other drugs by inhibiting CYP isoenzymes (Ortiz de Montellano and Correia, 1995). It was recently demonstrated that L-deprenyl and clorgyline inactivated the 7-ethoxy-4-trifluoromethylcoumarin O-deethylase activity of purified CYP2B1 without affecting the activities of CYP1A1 and CYP2E1 (Sharma et al., 1996). In the present study, in vivo treatment of C57BL mice with L-deprenyl caused a notable decrease in microsomal CYP content. This effect, however, did not greatly modify the CYP activities assayed and was not accompanied by inhibition of the pentoxyresorufin O-depentylation activity characteristic of CYP2B1, although *p*-nitrophenol hydroxylase activity, characteristic of CYP2E1, was inhibited. L-Deprenyl treatment elicited the lowest rates of formation of the three N-dealkylated metabolites and the highest inhibitory effect observed was that on the L-deprenyl N-demethylation reaction. The decrease in total CYP levels resulting from L-deprenyl treatment requires further investigation in terms of dose- and time-dependence, since it may have profound implications for the long-term use of this drug.

The marked decrease of NADPH-cytochrome P450-reductase activity caused by liquid diet suggests that this diet could interfere with hormonal homeostasis which regulates the expression of this enzyme (Ram and Waxman, 1992; Chen et al., 1999).

The highest rate of formation of deprenyl metabolites was observed after ethanol treatment. The alcoholic diet increased methamphetamine and nordeprenyl formation rates, consistent with the involvement of CYP2E1 in these metabolic pathways. In contrast, phenobarbital treatment increased only the rate of methamphetamine formation, indicating that the CYP2B family is also involved in the N-depropynylation of deprenyl. The induction of methamphetamine formation in rat microsomes by phenobarbital treatment has been reported by Yoshida et al. (1986) who also found that phenobarbital, but not 3-methylcholanthrene treatment, increased the in vitro formation rate of methamphetamine and amphetamine but not that of nordeprenyl. Moreover, they demonstrated that L-deprenyl metabolism was gender-dependent in three rat strains (Yoshida et al., 1987).

In the present study, we have shown that the rate of N-dealkylation is strongly inhibited by 4-methylpyrazole and disulfiram, two inhibitors of the CYP2E1 subfamily in rats and humans (Feierman and Cederbaum, 1986; Brady et al., 1991), whereas ketoconazole, a potent but less specific inhibitor related to phenobarbital- and 3-methylcholantrene-inducible CYP isozymes (Rodriguez et al., 1987), caused weaker inhibition. This provides additional support for the hypothesis that a member of the CYP2E1 subfamily plays a role in L-deprenyl N-dealkylation metabolism in mice.

Treatment of C57BL mice with β-naphthoflavone, an inducer of CYP1A, resulted in an overall increase in L-deprenyl metabolism without affecting N-dealkylation pathways, as observed with 3-methylcholanthrene in the rat (Yoshida et al., 1987). This suggests that β-naphthoflavone may affect L-deprenyl metabolism towards other metabolites, such as hydroxylated derivatives. The presence of hydroxylated metabolites has been demonstrated in human and rat urine after deprenyl administration Yoshida et al., 1986; Shin, 1997). This suggests that three distinct pathways are involved in L-deprenyl metabolism: N-dealkylation, β-carbon hydroxylation and ring hydroxylation. The involvement of other CYP isozymes in N-dealkylation of L-deprenyl metabolism cannot be excluded. Grace et al. (1994) demonstrated that human recombinant CYP2D6 catalyses the formation of nordeprenyl and methamphetamine in a molar ratio of 13:1. However, neither quinidine nor quinine, two competitive inhibitors of the CYP2D family in man and rat, reduced the formation rate of these two metabolites in control or phenobarbital-induced rat microsomes (Grace et al., 1994). Furthermore, Scheinin et al. (1998) have demonstrated that CYP2D6 is not important in the primary elimination of deprenyl, and that the biological effect of selegiline seems to be similar in poor and efficient metabolisers of debrisoquin. The inhibitory effect of selegiline and its main metabolites on CYP2D6 activity seems to be negligible.

The observation that the rate of amphetamine formation was not increased by any treatments used in the present study, suggests that different CYP isozymes, rather than those induced by these drugs, are involved in this process. In contrast, the decrease in amphetamine formation following L-deprenyl and β-naphthoflavone treatments might re-

sult from a decrease in the substrate (methamphetamine and nordeprenyl) levels available to the enzymes or from a direct inhibition of the enzyme reactions by β-naphthoflavone and L-deprenyl.

Since nordeprenyl has been reported to contribute to the therapeutic profile of L-deprenyl (Mytilineou et al., 1997; Olanow and Tatton, 1999), the decreased formation of the former during chronic administration of the parent drug, as observed in the present study, may have major implications for long-term L-deprenyl treatment. These experiments also suggest that by reducing CYP content L-deprenyl treatment may impair the metabolic disposition of other drugs given in combination regimens. The changes in L-deprenyl metabolism, as a result of ethanol consumption, may also have implications for the treatment of subjects with histories of long-term alcohol consumption or abuse.

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