/-Methamphetamine Pharmacokinetics and Pharmacodynamics for Assessment of In Vivo Deprenyl-Derived /-Methamphetamine

WILLIAM P. MELEGA, ARTHUR K. CHO, DEBRA SCHMITZ, RONALD KUCZENSKI and DAVID S. SEGAL
Department of Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, California (W.P.M., A.K.C., D.S.); and Department of Psychiatry, UCSD School of Medicine, San Diego, California (R.K., D.S.S.)

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ABSTRACT
This study evaluated whether the caudate-putamen dopamine response that has been observed after deprenyl administration could be attributed exclusively to metabolically generated /-methamphetamine (/MeAmp). Brain and plasma levels of deprenyl and /MeAmp were measured after deprenyl (10 mg/kg s.c.) from 10 to 60 min in conscious rats. Peak caudate-putamen levels were observed for deprenyl (15 nmol/g) at 10 min and for /MeAmp (3 nmol/g) at 30 min. In a parallel study, /MeAmp metabolism was evaluated. After /MeAmp (20 mg/kg s.c.), metabolite levels remained low relative to those of the parent compound: /amphetamine, ~5 to 12%; and para-hydroxy-/methamphetamine (OHMeAmp), ~0.25%. Accordingly, /MeAmp was considered to be the primary pharmacologically active deprenyl metabolite. A pharmacokinetic-pharmacodynamic analysis was then used to relate these pharmacokinetic data to the results of previous microdialysis studies in which increases in extracellular dopamine were measured in the caudate-putamen after /MeAmp (3–18 mg/kg) and after deprenyl (10 mg/kg). Dopamine response-area under curve versus dose plots were generated and used to show that an administered dose of 4 mg/kg /MeAmp would be necessary to effect a dopamine response-area under curve comparable to that observed after the deprenyl dose. However, the present pharmacokinetic results indicated that /MeAmp brain levels after deprenyl corresponded to those that would be obtained from 0.4 mg/kg /MeAmp (i.e., one tenth of the required dose). Collectively, these results suggest that the acute increases in extracellular dopamine observed after deprenyl are not due uniquely to metabolically generated /MeAmp but also to other actions of deprenyl at the dopamine terminal.

Deprenyl is used as an adjunct pharmacological therapy with /-dopa for the treatment of Parkinson’s disease (Golbe, 1988; Elsworth and Roth, 1993). Its long-term, putative therapeutic and neuroprotective actions are attributed to irreversible long-term monoamine oxidase (MAO) inhibition (Koller and Giron, 1990) and the consequent reduction in oxidative stress (Gerlach et al., 1992). In contrast, the acute pharmacodynamic (PD) effects of deprenyl may be more related to the actions of its metabolite, /methamphetamine (/MeAmp) (Heinonen et al., 1994).

The results of recent deprenyl studies in rodents have provided evidence that /MeAmp is pharmacologically active. For example, inhibitors of deprenyl metabolism reduced both the formation of /MeAmp and the acute behavioral effects normally observed after deprenyl (Engberg et al., 1991). In another report, concomitant microdialysis and behavioral assessment after deprenyl administration showed that an /amphetamine (Amp)-like response occurred, consisting of increases in both extracellular caudate-putamen dopamine (DA) levels and locomotor activity (Okuda et al., 1992). Although the behavioral actions observed after /MeAmp may not be exclusively related to changes in DA activity, the metabolically generated /MeAmp levels after deprenyl should be of sufficient magnitude to account for the observed DA PD response.

Accordingly, in the present study, we evaluated that hypothesis by determining the in vivo pharmacokinetics (PK) of deprenyl and its metabolite /MeAmp. Those data were then used in a PK/PD analysis in conjunction with the results of previous deprenyl and /MeAmp studies to evaluate the contribution of the metabolically generated /MeAmp to the DA response that has been observed after deprenyl.

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ABBREVIATIONS: d-Amp, /-amphetamine; d-MeAmp, /-methamphetamine; /MeAmp, /-methamphetamine; MAO, monoamine oxidase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; OHMeAmp, para-hydroxymethamphetamine; HPLC, high-pressure liquid chromatography; GC/MS, gas chromatography-mass spectrometry; AUC, area under the curve.
Materials and Methods

Drugs and Chemicals
d-MeAmp HCl (fw 185.6; National Institute for Drug Abuse, Rockville, MD), deprenyl (RBI, Natick, MA), and l-MeAmp HCl (synthesized as described below) were dissolved in 0.9% saline. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) except for octanesulfonic acid (Eastman Chemicals, Rochester, NY) and perchloric acid (EM Science).

(\(R\))-\(\sim\)Metamphetamine (l-MeAmp) was prepared from (\(\sim\))-ephedrine (Kishi et al., 1983). Briefly, the ephedrine was converted to its chloro derivative with thionyl chloride in benzene. The crude chloro compound was hydrogenated over 10% palladium on charcoal for 48 h, the mixture was filtered, and the residue was evaporated. The residue was recrystallized from methanol ether, and the purity was determined by mass spectroscopy and NMR. The enantiomeric purity was determined to be >98% when analyzed after derivatization with \(N\)-trifluoroacetyl-l-proline (Regis Chemical Co., Morton Grove, IL) and gas chromatographic (GC) analysis of the resulting diastereomers.

Surgery

Male Sprague-Dawley rats (Harlan; 300–325 g) (\(n=3–7\) per group) were anesthetized with chloral hydrate (400 mg/kg i.p.) and then placed in a stereotactic apparatus for insertion of a microdialysis cannula above the caudate-putamen (from bregma, 0.6 mm anterior, 2.6 mm lateral, 3.0 mm ventral, with the incisor bar at −2.5 mm). Body temperature was monitored with a rectal thermometer and maintained at 37°C with a heating pad. After 7 to 10 days, the animals were used for microdialysis studies.

PK

For the PK experiments, conscious animals were divided into four groups and injected with either d-MeAmp HCl (2.5 mg/kg s.c.), l-MeAmp HCl (2.5, 20 mg/kg s.c.), or deprenyl (10 mg/kg s.c.). (All milligram per kilogram doses for the Amps refer to milligrams of salt per kilogram body weight.) The animals were then sacrificed by decapitation at 10, 20, 30, 50, and 60 min after drug administration. Caudate-putamen, cortex, and cerebellum were rapidly dissected on ice and frozen at −70°C until analyzed. Heparinized blood samples were obtained from trunk blood and centrifuged (5000 \(\times\) g for 10 min) at 70°C until analyzed.

Microdialysis

On the morning of each experiment, a 3-mm dialysis probe (CMA-12; BAS/Carnegie Medicin, West Lafayette, IN) was inserted into caudate-putamen via the previously implanted cannula and was perfused with a solution containing 145 mM NaCl, 1.2 mM CaCl\(_2\), 2.7 mM KCl, 1.0 mM MgCl\(_2\), 0.2 mM ascorbate, and 2.0 mM NaH\(_2\)PO\(_4\), pH 7.0 (Moghaddam et al., 1990) at a flow rate of 2.0 \(\mu\)l/min. After stable baseline levels were obtained for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) (\(~\sim 5–6\) h of perfusion), 20 min samples were collected at zero time point values. l-MeAmp (20 mg/kg s.c.) or d-MeAmp (1.0 or 2.5 mg/kg s.c.) was then administered; dialysate samples were collected at 10-min intervals for the succeeding 90-min postdrug injection. Samples were collected subsequently into polypropylene vials containing 45 \(\mu\)l of a solution containing 0.2 N perchloric acid, 0.1% sodium metabisulfite, and 0.05% EDTA; 50 \(\mu\)l of the 55-\(\mu\)l total sample/10 min was injected onto the high-pressure liquid chromatogram (HPLC). Quantification of DA, DOPAC, HVA, and 5-HIAA was obtained after correction for each dialysis probe (recoveries 14–20%) estimated from sampling; in vitro, a 0.9% saline solution at 22°C containing all analytes at 1 \(\mu\)g/ml. Probe placement was verified by histological examination of the caudate-putamen after each experiment.

HPLC Analysis of Doper, d-MeAmp, l-MeAmp, and Its Metabolites

The HPLC system consisted of a Beckman 210 solvent delivery system with the following mobile phase: 80% 0.1 M NaH\(_2\)PO\(_4\), 2.6 mM octanesulfonic acid, 0.1 mM EDTA, pH 3.1, and 20% MeOH, at a flow rate of 1 ml/min. A Beckman \(C_18\) reversed-phase column (250 \(\times\) 4.6 mm, 5 \(\mu\)m) was connected to an electrochemical detector (ESA: Coulochem II) set at an applied potential of +0.02 V at detector 1 and +0.40 V at detector 2. Detector response was linear for 0.05 to 10 ng (\(r=0.99\) for linear regression calculations of all compounds assayed; within-assay variance was less than 5%). Peak areas were quantified with the Rainin MAcIntegrator system.

GC/MS Analysis of Deprenyl, d-MeAmp, l-MeAmp, and Its Metabolites

Analyses were performed on a Hewlett Packard HP 5971A GC/MS system, consisting of a fused silica capillary column (0.2 mm i.d., 12.5 m length; Hewlett Packard) coated with crosslinked methylsilicone (thickness, 0.33 \(\mu\)m). The GC was operated with a thermal gradient from 65–190°C at a rate of 20°C/min with a flow rate of 1 ml/min.

The internal standards used in the selected ion mode were 1,1,2\(^{3}\)H\(_3\)-3-phenyl-2-amino-3-propanoic acid (amphetamine-d\(_3\)), 3-(4-hydroxy-phenyl)-2-(2-N,\(^3\)H\(_2\)-methylamino)-propane (4-hydroxy-metamphetamine-d\(_2\)), and 3-phenyl-2-(2-N,\(^3\)H\(_2\)-methylamino)-propane (methamphetamine-d\(_2\)). The compounds were synthesized in our laboratories: amphetamine-d\(_3\) by lithium aluminum deuteride reduction of phenylalanine (Gal, 1976) and methamphetamine-d\(_2\) and its 4-hydroxy derivative by lithium aluminum deuteride reduction of the N-formyl compounds. The mz values used for derivatized aniline and internal standard, respectively, were Amp, 139.9 and 142.9; OH-MeAmp, 153.9 and 156; and MeAmp, 153.9 and 156. These fragments were the base peaks in the electron impact mass spectra, obtained at an ionizing potential of 70 eV. The dynamic range of each analysis covered the range of concentrations so that no extrapolation of the standard curve was required. The lowest points on the standard curves were 25 pmol/sample for Amp, 10 pmol/sample for 4-hydroxy-MeAmp, and 250 pmol/sample for MeAmp. Standard curves were run with each analysis, and the slopes had a standard deviation of less than 5%.

For the deprenyl analysis, methamphetamine-d\(_2\) was used as an internal standard after extraction and derivatization by the same protocol. The mass fragment used for deprenyl was mz 96, the p-cleavage product of the tertiary amine. The standard curve range was 100 to 1000 pmol per sample, and all values were interpolated from the standard curve.

Sample Preparation

Plasma. Volumes of 0.2 ± 0.05 ml of plasma were used. To the plasma sample of known volume, we added 1 ml of 10% Na\(_2\)CO\(_3\), pH 11, 6 ml of isopropanol/CH\(_2\)Cl\(_2\) (1:4), 0.75 g of solid NaCl, and internal standards. The mixture was shaken and centrifuged, and the organic phase was transferred to another tube for evaporation at room temperature under a stream of nitrogen. The residue was dissolved in 50 \(\mu\)l of acetonitrile and derivatized by the addition of 100 \(\mu\)l of trifluoroacetic anhydride. The mixture was capped and heated at 60°C for 15 min and evaporated to dryness, and the residue taken up in 120 \(\mu\)l of acetonitrile for injection into the GC/MS.

Brain. Tissue samples of 0.1 ± 0.05 g were used. Each tissue sample was weighed, internal standards were added, and the mixture was then homogenized in 0.5 ml of a 0.4 N perchloric acid solution containing 0.1% EDTA and 0.15% sodium metabisulfite. After centrifugation (10,000g for 5 min), the supernatant was alkalized with a sodium carbonate solution, extracted with 6 ml of the isopropanol-dichloromethane mixture, and processed for GC/MS analysis as described above.
Deprenyl concentrations in the caudate-putamen and plasma rose rapidly after s.c. doses of 10 mg/kg; peak concentrations occurred within 10 min after dosing. Deprenyl levels in these compartments decayed with apparent first order kinetics (Fig. 1). Metabolically generated \( l \)-MeAmp (Fig. 2) peaked at about 30 min with a caudate-putamen-to-plasma ratio of about 8:1, consistent with previous studies of MeAmp kinetics (Melega et al., 1995). The area under the curve (AUC; Fig. 2) for \( l \)-MeAmp after deprenyl was found to be 122 nmol/g*min (Table 1).

A separate study was conducted with \( l \)-MeAmp administration (20 mg/kg s.c.) to measure levels of MeAmp metabolites. In cortex, caudate-putamen, and cerebellum, levels of \( l \)-MeAmp, \( l \)-Amp, and OH-MeAmp were determined from 5 to 50 min (Fig. 3). Relative to striatal \( l \)-MeAmp levels, \( l \)-Amp caudate-putamen levels ranged from 5 to 10%, whereas OH-MeAmp levels were less than 0.25%.

To determine caudate-putamen drug levels after the administration of \( d \)- and \( l \)-MeAmp doses, total drug exposure was determined from the AUC (Fig. 4). The AUCs for 2.5 mg/kg \( d \)-MeAmp (795 nmol/g*min) and \( l \)-MeAmp (760 nmol/g*min) were similar, indicating that distribution and metabolism was not enantiomer sensitive (Table 1). Based on those data, PD, and not PK, factors were assumed to be related to differences in the magnitude of the \( d \)- and \( l \)-MeAmp responses at the DA terminal.

After 10 mg/kg deprenyl, the metabolically generated \( l \)-MeAmp AUC was also determined (122 nmol/g*min) (Table 1) and was approximately 15% (122/760*100) of that observed after the 2.5-mg/kg \( l \)-MeAmp dose. Based on our previous studies that showed a linear relationship between low doses (1–5 mg/kg) of \( d \)-Amp and \( d \)-MeAmp with brain levels (Melega et al., 1995), we then estimated that an administered \( l \)-MeAmp dose of 0.4 mg/kg (0.15*2.5 mg/kg \( l \)-MeAmp) would result in a caudate-putamen \( l \)-MeAmp AUC comparable to that observed after 10 mg/kg deprenyl.

PD. After \( d \)-MeAmp (1.0, 2.5 mg) and \( l \)-MeAmp (20 mg), peak DA levels were observed in the 20- to 30-min sample; the 20-mg \( l \)-MeAmp profile declined more slowly relative to the lower \( d \)-MeAmp doses (Fig. 5). The total DA responses between 10 and 90 min for \( l \)-MeAmp was ~8 fold less than that of \( d \)-MeAmp as estimated by trapezoidal analysis (Table 2).

DA metabolites were not qualitatively or quantitatively different after \( d \)-MeAmp and \( l \)-MeAmp; decreases in DOPAC levels were greater than those for HVA throughout the 90-min time period. Changes in 5-HIAA levels were not observed after either compound.

PK/PD Analysis. In a previous microdialysis study, the extracellular caudate-putamen DA response was examined over a 6-fold \( l \)-MeAmp dose range (Kuczenski et al., 1995) and, in a separate study, after 10 mg/kg deprenyl (Okuda et al., 1992). For the present study, their respective DA-AUC values were calculated by trapezoidal analysis. A linear response curve for DA-AUC versus dose was obtained from the \( l \)-MeAmp study (Fig. 6). That data were then used to estimate the \( l \)-MeAmp dose that was associated with the DA-AUC observed after deprenyl. Interpolation of the deprenyl DA-AUC of 12,486 nM*min onto the AUC-\( l \)-MeAmp curve (Fig. 6) corresponded to an administered dose of 4.2 mg/kg \( l \)-MeAmp.
Discussion

PK. Previous studies on deprenyl pharmacology have shown that Amp-like striatal DA responses and behavior occur acutely after drug administration. These effects were attributed to the actions of the major metabolite of deprenyl, $l$-MeAmp. In the present study, we used a PK/PD analysis to assess whether those $l$-MeAmp levels were of sufficient magnitude in brain to account for the associated DA response. The rationale for this type of analysis was based on our previous studies in which the DA response (Kuczenski et al., 1995) and brain levels of Amp and MeAmp (Melega et al., 1995) were each shown to be proportional to the administered drug dose. Accordingly, to obtain PK/PD data, the PK of

Fig. 3. Concentrations of $l$-MeAmp (top left) and two of its metabolites, $l$-Amp (bottom left) and OH-MeAmp (right), after $l$-MeAmp administration (20 mg/kg s.c.). Levels (nmol/g tissue wet weight) were determined in caudate-putamen, cortex, and cerebellum of rats ($n = 3$ for each time point). □, striatum; ▲, cerebellum; ▽, cortex.

Fig. 4. Concentrations of $l$-MeAmp and $d$-MeAmp in caudate-putamen after $l$-MeAmp (20 mg/kg s.c.) and $d$-MeAmp (1, 2.5 mg/kg s.c.), respectively. Levels (nmol/g tissue wet weight) were determined for each compound ($n = 3$ for each drug at each time point).
Deprenyl and its major metabolite, \(l\)-MeAmp, were first determined.

Deprenyl, a nonpolar, weak organic base, was expected to penetrate the brain rapidly and efficiently, and the plasma and brain data of Fig. 1 were consistent with that prediction. Peak caudate-putamen deprenyl levels were observed at the first time point for analysis, 10 min, and declined thereafter. In contrast, \(l\)-MeAmp levels increased throughout 10 to 30 min after deprenyl, indicative of its deprenyl metabolism-dependent appearance. Peak caudate-putamen MeAmp levels at 30 min (3 nmol/g) were approximately 20% of those of peak deprenyl (15 nmol/g). Both drugs were apparently ion-trapped (Zaczek et al., 1991) as demonstrated by maximal caudate-putamen: plasma ratios of 8 for \(l\)-MeAmp and 2.5 for deprenyl. The lower value of deprenyl was probably due to less protonation at intracellular \(pH\) \(\sim 7\) because of its lower \(pK_a\) (7.5) relative to that of \(l\)-MeAmp (\(pK_a\) 10). These results indicate that for deprenyl pharmacology, its metabolism will generate Amp-like metabolites that will be distributed uniformly throughout the brain, and these may contribute significantly to multiple PD responses.

Although PK differences were not observed between enantiomers at low doses, dose-dependent differences in PK were apparent between 2.5 and 20 mg/kg \(l\)-MeAmp. Peak caudate-putamen \(l\)-MeAmp levels appeared later (\(\sim 30\) min) than those observed after 2.5 mg/kg (\(\sim 10\) min). The relatively slow appearance of \(l\)-MeAmp in brain after high-dose \(l\)-MeAmp may have been related to norepinephrine release in blood vessels. In a previous microdialysis study, \(d\)- and \(l\)-MeAmp-induced norepinephrine responses appeared equipotent in the hippocampus in contrast to the \(d\)- to \(l\)- effect on DA and serotonin in the caudate-putamen (i.e., \(l\)-MeAmp appeared to be relatively more potent on norepinephrine systems) (Kuczenski et al., 1995). If this potency occurred peripherally as well, \(l\)-MeAmp-induced norepinephrine release would result in vasoconstriction and a subsequent reduction in blood flow to the brain. Because the delivery of Amps into the brain is flow limited, the rate of entry and the overall brain availability would then be reduced, particularly at the high dose that was administered in this study and in others (Yasar et al., 1996). However, after therapeutic or experimental deprenyl

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**Fig. 5.** Temporal pattern of changes in extracellular caudate-putamen levels of DA (left) and DOPAC, HVA, and 5-HIAA (right) in response to \(d\)-MeAmp (1, 2.5 mg/kg s.c.) and \(l\)-MeAmp (20 mg/kg s.c.). Each value represents the mean dialysate concentration (\(n = 3\)).

**TABLE 2**
Areas under the microdialysate concentration versus time curve between 0 and 60 min after methamphetamine.

<table>
<thead>
<tr>
<th>Enantiomer dose</th>
<th>AUC (nM*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d)-MeAmp 1.0 mg/kg</td>
<td>9,586 ± 3,101</td>
</tr>
<tr>
<td>(d)-MeAmp 2.5 mg/kg</td>
<td>25,939 ± 5,970</td>
</tr>
<tr>
<td>(l)-MeAmp 20.0 mg/kg</td>
<td>21,120 ± 3,484</td>
</tr>
</tbody>
</table>

The area under the microdialysate concentration-time curve for each of the four animals was determined by trapezoidal analysis. Values are mean ± S.E.

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**Fig. 6.** Integrated DA response versus \(l\)-MeAmp dose. For \(l\)-MeAmp doses (3, 6, 12, 18 mg/kg s.c.), the increases in caudate-putamen extracellular DA levels between 10 and 90 min as previously determined by microdialysis (Kuczenski et al., 1995) were converted to cumulative DA response area under the curve values by trapezoidal analysis and then plotted versus the administered dose. The line represents a linear regression analysis of the data. The resultant function was used to interpolate the DA AUC (12,486 nM*min) after 10 mg/kg deprenyl (Okuda et al., 1992) that corresponded to an \(l\)-MeAmp dose of 4.2 mg/kg.
doses, it is unlikely that resultant \( l \)-MeAmp levels would reach a magnitude sufficient to affect blood flow to the brain.

For our PK/PD analysis, we had assumed that \( l \)-MeAmp was the major deprenyl metabolite, based on previous metabolic studies in rodents (Phillips, 1981) and humans (Reynolds et al., 1978). Nevertheless, if \( l \)-MeAmp metabolites \( l \)-Amp and OH-\( l \)-Amp were generated in sufficient quantity, they too could contribute to the DA response (Kuczenski et al., 1995). We evaluated this possibility in a separate PK study in which a relatively high dose of \( l \)-MeAmp (20 mg/kg) was administered to allow for the detection of those metabolites, especially at early time points. However, throughout 50 min post-\( l \)-MeAmp, brain levels of \( l \)-Amp and \( p \)-OH-\( l \)-Amp did not exceed 12% and 0.25% of those of \( l \)-MeAmp, respectively. Accordingly, we concluded that \( l \)-MeAmp was the primary active metabolite during the initial 50 min after deprenyl administration. Although another deprenyl metabolite, desmethyldeprenyl, was not measured, other studies had shown that its levels remained less than 5% those of deprenyl (Yoshida et al., 1986; Lajtha et al., 1996). Thus, we restricted our PK/PD analysis to deprenyl and \( l \)-MeAmp after a single deprenyl dose.

**PD.** We first established that the PK design and experimental methods used in our laboratory (W.P.M., A.K.C.) yielded DA response results that were similar to the prior \( l \)-MeAmp microdialysis studies performed in another laboratory (R.K., D.S.). The concordance of results for the DA-AUCs that were observed provided evidence that the present PK data were relevant to the interpretation of those previous data sets. Specifically, for both sets of studies, an \( \sim 8:1 \) potency difference was observed between low \( d \)- and high \( l \)-MeAmp doses, whereas increases in \( d \)-MeAmp effect led to proportional increases in the corresponding DA-AUCs. Similar alterations in the respective DA and DA metabolite (DOPAC, HVA) profiles were also observed; for both drugs, extracellular DOPAC and HVA levels were decreased by \( \sim 50\% \) between 20 and 90 min after drug, whereas 5-HIAA levels remained unchanged. Overall, these results suggest that MeAmp isomers act through a common set of mechanisms to effect increases in extracellular DA levels. The actions of \( l \)-MeAmp apparently differ from those of \( d \)-MeAmp only in potency as this and the previous microdialysis studies have demonstrated.

Moreover, although we showed that the DA-AUCs and \( l \)-MeAmp brain levels were proportional to dose, the DA-AUC calculated from the previous deprenyl study (Okuda et al., 1992) could not be directly related only to metabolically generated \( l \)-MeAmp levels. It was possible that deprenyl also contributed to the DA response because our PK results indicated that deprenyl and \( l \)-MeAmp were both present in caudate-putamen between 10 and 60 min. Recently, evidence for PD actions of deprenyl at the DA terminal was demonstrated by its induced modulation of \( K^+ \)-ATP channels in caudate-putamen tissue slices that resulted in an enhanced DA outflow (Neusch et al., 1997). Accordingly, if the observed DA response in the microdialysis studies were temporally related to the kinetics of each drug in brain, the DA efflux between 0 to 10 min could be attributed to deprenyl insofar as \( l \)-MeAmp levels were relatively low during this time interval. As \( l \)-MeAmp levels subsequently increased, both drugs would then contribute to the DA response. At later times when deprenyl had essentially cleared from the brain, \( l \)-MeAmp effects would then predominate because of its longer half-life, after its peak levels in brain at \( \sim 30 \) min.

Conversely, if the DA response were attributed essentially to \( l \)-MeAmp, then our PK/PD analysis indicated that a 4.2 mg/kg \( l \)-MeAmp dosage would be necessary to effect a DA response AUC comparable to that calculated from the 10 mg/kg deprenyl study (Okuda et al., 1992). However, our results showed that the metabolically generated \( l \)-MeAmp AUC value after deprenyl corresponded to that which would be obtained after a 0.4 mg/kg \( l \)-MeAmp dose (i.e., only one tenth the required dose). We conclude, therefore, that neither the time course for the appearance nor the magnitude of the metabolically generated \( l \)-MeAmp can account uniquely for the observed DA response after deprenyl. Rather, the data suggest that deprenyl and \( l \)-MeAmp additively or synergistically effect increases in extracellular DA.

In addition, it may be expected that at relatively high deprenyl doses, a potentiated DA response to \( l \)-MeAmp may be expected due to MAO A inhibition. However, the inhibitory actions of deprenyl on MAO A do not appear very potent. For example, it has been shown that only after 8 mg/kg deprenyl was MAO A activity inhibited by \( \sim 20\% \) (Patterson et al., 1991). Also, in a microdialysis study that did measure Amp-induced DA-AUC in the presence and absence of 10 mg/kg deprenyl, similar DA levels were obtained in the dialysates (Butcher et al., 1990). Thus, it appeared that MAO inhibition by deprenyl did not affect Amp-induced DA release. Furthermore, in the microdialysis study in which clorgyline- and deprenyl-induced DA responses were compared (Okuda et al., 1992), 4 mg/kg clorgyline (MAO A inhibitor) resulted in DA levels that remained elevated for \( \sim 6 \) h. In contrast, after 10 mg/kg deprenyl, the elevation of the DA concentration appeared more rapidly and returned to baseline within 2 h. Thus, it was unlikely that the short-term DA dynamics observed after deprenyl were due to significant irreversible inhibition of MAO A. In the same report, DOPAC and HVA dialysate levels after clorgyline markedly decreased, but after deprenyl, the decreases were significantly less. Also, 3-MT dialysate levels increased after clorgyline but remained low and equivalent to control values after deprenyl. These data suggest that the effects of deprenyl on MAO A are minimal and that the deprenyl-induced DA response includes factors other than deprenyl metabolites and MAO B inhibition.

Although the 10 mg/kg deprenyl used in this PK/PD study far exceeded that usually administered to humans (10 mg), our results showed that even at this dose, relatively low levels of \( l \)-MeAmp were generated by metabolism. Consequently, it is unlikely that lower deprenyl doses would result in an \( l \)-MeAmp-induced PD response. Rather, our results suggest a direct deprenyl effect on the DA system, and these actions may have implications for therapeutic applications. For example, the uses of deprenyl have been extended to long-term treatment for aging-related diseases (Gerlach et al., 1996) and for the reduction in the subjective response to cocaine (Newton et al., 1996). In these instances, deprenyl, particularly in multiple exposures, may result in an even more pronounced DA response as well as other central nervous system actions due to bioavailability increases resulting from the inhibition of its cytochrome P-450-dependent metabolism (Grace et al., 1994; Sharma et al., 1996).
References


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Send reprint requests to: William P. Melega, Ph.D., Department of Molecular and Medical Pharmacology, 23-120 CHS, UCLA School of Medicine, Box 931735, Los Angeles, CA 90095-1735. E-mail: wmelega@mednet.ucla.edu