J. Pharm. Pharmacol. 1994, 46: 360-365
Received June 22, 1993
Accepted November 10, 1993

© 1994 J. Pharm. Pharmacol.

Interactions of Monoamine Oxidase Inhibitors, *N*-Acetylenic Analogues of Tryptamine, with Rat Liver Microsomal Cytochrome P450

MASSIMO VALOTI, MARISA COSTANZO, GIAN PIETRO SGARAGLI, VIRGILI PEREZ*, ELDIBERTO FERNANDEZ-ALVAREZ† AND MERCEDES UNZETA*

*Centro di Ricerca sul Metabolismo dei Farmaci Psicotropi, Istituto di Scienze Farmacologiche, Università di Siena, via Piccolomini 170, Siena, Italy, *Department de Bioquimica i Biologia Molecular, Unitat de Medicina, Universitat Autonoma de Barcelona (Bellaterra), and †Instituto de Quimica Organica C.S.I.C., Madrid, Spain*

Abstract—Interactions between some novel and potent monoamine oxidase inhibitors (MAOIs), acetylenic analogues of tryptamine, and rat liver microsomal cytochrome P450 (P450) as evidenced by visible spectra analysis were analysed. Compounds with a secondary aliphatic amine moiety throughout induced type II difference spectra and exhibited the highest affinity for P450, whereas tertiary amines induced type I spectral changes and showed diminished affinity. P450 dependent aniline hydroxylase activity was inhibited by all compounds in an irreversible time-dependent manner. Only tertiary aliphatic amines constituted the substrate for P450-dependent *N*-demethylase activity, with comparable kinetic parameters. The *N*-demethylated metabolites were identified by thin-layer chromatography and mass-spectrometric analyses. These findings describe the role of P450-dependent microsomal mono-oxygenase systems in the metabolism of some MAOI acetylenic tryptamine derivatives and the possible hepatic contribution to adverse interactions between MAOIs, endobiotics and sympathomimetic compounds.

Some recently synthesized *N*-acetylenic analogues of tryptamine have been shown to be potent monoamine oxidase-A inhibitors (MAOIs) and are therefore considered promising candidates for the pharmacological treatment of depression (Balsa et al 1990, 1991). Hitherto, clinical use of MAOIs has been restricted due to their adverse side-effects, i.e. provoking hypertensive crises after ingestion of fermented foodstuffs high in tyramine and other amines. It has been established that these drugs inhibit the pre-systemic MAO-dependent metabolism of tyramine, thus increasing tyramine concentration in the blood. After peripheral adrenergic nerve uptake, massive quantities of stored sympathomimetic amines are released, precipitating hypertensive episodes (Blackwell 1981).

It has been recently suggested (Callingham et al 1991) that oxidative enzymes other than MAO, yet responsive to MAOIs, mediate the metabolism of tyramine and may synergistically contribute to hypertension.

MAOIs have been linked to the inhibition of a wide spectrum of enzymes such as diphosphopyridine nucleotidase, succinic dehydrogenase, guanidine deaminase, spermine oxidase and histaminase (Bowman & Rand 1980). Only a few studies (Laroche & Brodie 1960; Kato et al 1964; Bélanger & Atitsé-Gbeassor 1981; Dupont et al 1987) have, however, addressed the effects of MAOIs on the microsomal mono-oxygenase systems. An assessment of MAOI oxidation by the microsomal cytochrome P450-dependent system (P450) would provide an indication of their persistence and bioactivity in the organism. The interaction of MAOIs with P450 enzymes is of more than theoretical concern, since their marked irreversible inhibition induced by phenelzine (Muakkassah & Yang 1981) and clorgyline may extend to the suppression of other P450 xenobiotic metabolites. The effect is cumulative and, upon repeated dosing, may lead to modification of the metabolism and disposition of other drugs given in combination regimens (Strolin Benedetti & Dostert 1992). An early study by Rand & Trinker (1968) suggested that phenelzine inhibition of P450-dependent amphetamine metabolism caused the enhanced pressor response of amphetamines.

Ortiz de Montellano & Kunze (1980) reported that compounds having an acetylenic moiety throughout inactivate P450 mono-oxygenases in a time-dependent fashion and that during oxidative metabolism the haem group of the cytochrome undergoes attack by the allyl or ethynyl moiety throughout of unsaturated compounds. The products of haem alkylation have been seen to be powerful inhibitors of the enzyme ferrochelatase, thus causing porphyrin build-up and consequent hepatic protoporphyria (De Matteis 1987). These considerations regarding the common fate of P450 metabolites led us to define the interactions between nine newly synthesized potent MAO-AIs, *N*-acetylenic derivatives of tryptamine, with P450 hydroxylase and *N*-demethylase activity.

Correspondence: M. Valoti, Istituto Scienze Farmacologiche, Università di Siena, via Piccolomini 170, 53100 Siena, Italy.

Materials and Methods

Chemicals

Reagents of analytical grade were used without further purification. Chemicals were obtained from E. Merck (Darmstadt, Germany) except: NADPH, NADP, NADH and glucose-6-phosphate dehydrogenase (Boehringer & Soehne, Mannheim, Germany).

The MAOI, *N*-acetylenic analogues of tryptamine (compounds *26*, *27*, *30*, *42*, *43*, *45*, *102*, *69*, *70*; Fig. 1) were synthesized by Cruces et al (1990). Purity was of analytical grade, as confirmed by TLC and mass-spectrometric analyses.

Carbon monoxide, produced in a capped glass jar by

addition of sulphuric acid to formic acid at room temperature (21°C), was withdrawn by means of a plastic 50-mL syringe connected to the reaction chamber by plastic tubing.

Animal treatment and preparation of liver microsomes
Male Sprague-Dawley rats, 180–200 g, obtained from Charles River (Milan, Italy) were housed in stainless-steel cages in a temperature and light-controlled facility with free access to food and water (M.I.L. from Morini, S. Polo d'Enza, Italy). Rats were killed by diethyl ether anaesthesia. The abdominal cavity was opened and the liver perfused in-situ through the portal vein with an ice-cold NaCl physiological solution. The liver was excised, chopped, suspended in 3 vol 0·25 M sucrose and homogenized by a Potter-Elvejhem homogenizer fitted with a Teflon pestle.

The homogenate was centrifuged at 10 000 *g* for 20 min and the supernatant ultracentrifuged at 105 000 *g* for 1 h. The microsomal pellets were suspended in 0·01 M Tris-buffer, pH 7·6, containing 151 mM KCl, 1 mM EDTA and 20% glycerol, and stored in liquid nitrogen until use.

Spectrophotometric measurements
Difference spectra were recorded at 25°C with a Shimadzu dual wavelength UV-260 spectrophotometer. The quartz cuvettes, 10 mm wide, contained 2 mL microsomal suspension (2 mg mL^{-1}) in 100 mM phosphate buffer, pH 7·4 together with a series of MAOIs inhibitors at different concentrations.

P450 content was determined by CO-difference spectra of microsomes as described by Omura & Sato (1964).

Cytochrome C (P450) reductase activity was determined according to a procedure described by Phillips & Langdon (1962).

Enzyme assay
Levels of P450, cytochrome C reductase, aniline hydroxylase and ethylmorphine *N*-demethylase were determined in microsome preparations.

Incubations were performed at 37°C in a final reaction volume of 1 mL containing 50 mM Tris-HCl buffer, pH 7·6, substrates at varying concentrations, microsomes (1·5 mg protein) and a NADPH-generating system (1 mM NADPH, 4 mM glucose-6-phosphate and 1 unit glucose-6-phosphate dehydrogenase).

Aniline hydroxylase activity was recorded for 20 min and expressed as the measure of 4-aminophenol formation according to a procedure described by Lake (1987). After incubating the microsomes with different acetylenic tryptamine derivatives (concentration range 10^{-3}–10^{-8} M), inhibition was measured as the residual aniline hydroxylase activity. The reversibility of aniline hydroxylase inhibition was monitored by a dilution procedure as described by Gomez et al (1986).

N-Demethylase activity on ethylmorphine or MAOI substrates was determined by formaldehyde formation as described by Nash (1953).

Microsomal protein was estimated by the Bradford (1976) procedure using bovine serum albumin as standard.

Thin layer chromatography analyses
After 60 min microsomal incubation of the MAOIs, the reaction was stopped by adding 300 μL 1 M NaOH. Metabolites and parent compounds were extracted with 7 mL cyclohexane/isoamyl alcohol (98:2, v/v). After evaporation of the organic phase, the residues were suspended in 50 μL methyl alcohol and aliquots (4 μL) were subjected to TLC analysis on Silica Gel 60 F254 plates; 5 × 20 cm, 0·25 mm thick (E. Merck, Darmstadt, Germany). The chromatograms were developed for 15 min at room temperature with chloroform/ethyl ether/glacial acetic acid (75:20:5) and visualized under long-wavelength UV light as described by Stahl (1965) for TLC indole analysis.

Organic phase aliquots of the three microsomal reactants (compounds *27*, *43*, *69*), were analysed by gas chromatography-mass spectrometry (GC-MS).

GC-MS analysis
Mass spectra of the tryptamine analogues *26*, *42* and *102*, and the *N*-monodemethylated metabolites of *27*, *43* and *69* were determined by a quadruple QMD 1000 (Fison, Manchester, UK) mass spectrometer after injection into a gas-chromatograph equipped with an SE 54 fused-silica capillary column. Mass spectra were recorded in the electron ionization mode at 70 eV, 100 μA trap current, using an Intel SuperSinc 386 computer at a scan rate of 2 s $decade^{-1}$, at 1000 M/ΔM mass resolution and at 10% valley definition.

Compound *102* and the metabolite of *69* were trifluoroacetylated on the aliphatic amino group with *N*-methylbis-(trifluoroacetamide) before injection.

Results

P450 spectral changes induced by N-*acetylenic MAOI derivatives of tryptamine*
All the acetylenic analogues of tryptamine assayed in this study (Fig. 1) interacted with P450 giving rise to characteristic optical differential spectra. Compounds having a secondary amine moeity in the aliphatic chain (*26*, *42*, *102*) showed type II difference-binding spectra similar to that observed for phenylhydrazine (Jonen et al 1982). Compounds with a tertiary amine group caused type I spectral changes similar to clorgyline. Both type I and type II spectral binding changes were reversible in typical Michaelis–Menten fashion. Apparent saturation at high concentrations was achieved allowing half-maximal spectral changes or apparent dissociation constants to be determined for each compound. K_s values reported in Table 1 varied broadly. The compounds that induced type II spectral changes of P450 showed the highest affinity (K_s values ranging between 14·2 and 38·1 μM), whereas the other compounds with a tertiary amine group in the aliphatic chain showed reduced affinity (K_s values ranging between 21 and 358 μM) with characteristic type I spectral changes. In addition, clorgyline, a typical acetylenic MAO-A inhibitor, showed a K_s value of 65 μM and caused type I spectral changes.

The interaction of these compounds with dithionite-reduced P450 was also assayed, with no appreciable binding observed.

Inhibition of aniline hydroxylase activity
All nine MAOI acetylenic tryptamine derivatives inhibited

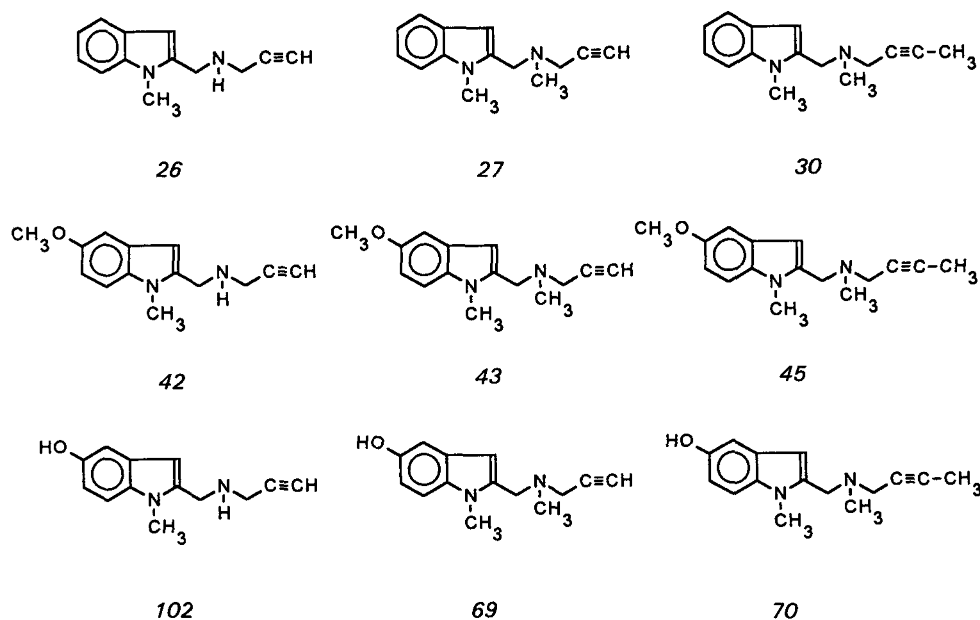

FIG. 1. Chemical structures of MAOI N-acetylenic tryptamine analogues, interacting with rat liver cytochrome P450-dependent oxidase systems.

Table 1. Spectral dissociation constants (K_s) and types of spectral changes for the N-acetylenic analogues of tryptamine.

Compound	K_s (μM)	Type of spectral change
26	18·7	II
27	137·4	I
30	21·4	I
42	14·2	II
43	357·9	I
45	109·7	I
102	38·1	II
69	73·0	I
70	37·7	I
Clorgyline	65·0	I

K_s values were determined by nonlinear regression analysis of the values of the absorbance changes with respect to substrate concentrations.

Table 2. Inhibition of aniline hydroxylase activity (IC50), NADPH-GS-dependence and apparent pseudo-first-order rate constant, K_{app}, of time-dependent irreversible inhibition of microsomal aniline hydroxylase activity by N-acetylenic analogues of tryptamine.

		% Inhibition		
Compound	IC50 (μM)	+GS	−GS	K_{app} (s^{-1})
26	68	53·7	62	$5{\cdot}5\times10^{-3}$
27	248	40·1	53·2	$2{\cdot}2\times10^{-2}$
30	191	30	50	$6{\cdot}2\times10^{-3}$
42	280	37·8	52·6	$7{\cdot}0\times10^{-3}$
43	154	17·0	55	$1{\cdot}4\times10^{-2}$
45	300	19·1	57	$9{\cdot}1\times10^{-3}$
102	1000	23·7	27	$1{\cdot}0\times10^{-2}$
69	347	30·8	36	$8{\cdot}8\times10^{-3}$
70	1240	23·7	35	$2{\cdot}9\times10^{-3}$

Each compound was preincubated with microsomes for 30 min. NADPH-dependence of the inhibition was tested only at 10^{-4} M concentration of each compound.

aniline hydroxylase activity of microsomal P450 in a dose-dependent manner with IC50 values reported in Table 2. The most potent inhibitors of this enzymatic activity were MAOIs with an −H or an $-OCH_3$ group in the 5 position of the indole ring (*26*, *27*, *30*, *42*, *43* and *45*), while weaker inhibition was sustained by MAOIs with a 5−OH group in the indole ring (*102*, *69* and *70*).

All inhibition was time-dependent (data not shown) and irreversible by dilution indicating a possible mechanism-based inhibition.

Influence of NADPH-generating system on aniline hydroxylase inhibition

Microsomal preincubation of MAOIs for 30 min in the presence of NADPH-GS partially reversed the inhibition by $5-OCH_3$ derivatives of aniline hydroxylase (*42*, *43* and *45*). Preincubation of other compounds (*26*, *27*, *30*, *102*, *69* and *70*) with NADPH-GS afforded diminished or zero protection of enzymatic inhibition. The inhibition curves of aniline hydroxylase activity plotted against preincubation time at varying concentrations (10^{-3}–10^{-5} M) were consistent with apparent first-order kinetics.

Table 3. Kinetic parameters of cytochrome P450-dependent *N*-demethylase activity towards the *N*-acetylenic analogues of tryptamine as substrates.

Compound	K_m (μM)	V_{max}[a]	V_{max}/K_m
27	47	5·14	45·4
30	164	11·90	30·5
43	193	12·85	27·9
45	139	11·52	34·7
69	45	2·85	26·8
70	110	6·42	24·4

[a] V_{max} is expressed in nmol HCOH formed (nmol cytochrome P450)$^{-1}$ min^{-1}.

All compounds exhibited a similar apparent pseudo-first-order rate constant (K_{app}), as shown in Table 2.

These effects on P450 were unrelated to NADPH-cytochrome P450 reductase inhibition; the microsomal incubation of MAOIs for up to 30 min with or without NADPH did not affect the reduction rate of cytochrome C.

N-Demethylation of some acetylenic tryptamine derivatives catalysed by rat liver microsomes

The nine compounds were assayed as substrates for microsomal P450-dependent oxidation. Endowment with a methylamino group in the aliphatic chain conferred substrate status for the P450-dependent *N*-demethylase activity, and gave rise to the formation of HCOH in a time- and enzyme-concentration-dependent fashion. The kinetic constants of this reaction were determined by initial rates of HCOH formation with respect to substrate concentrations (1×10^{-5}–2×10^{-4} M) and were established by nonlinear regression analysis (Enzfitter, Elsevier-Biosoft). K_m and V_{max} values of *N*-demethylase activity are shown in Table 3. Kinetic parameters and catalytic efficiency expressed as V_{max}/K_m ratio were similar for all compounds, suggesting similar *N*-demethylase P450-dependent affinity. In order to identify the oxidated methyl group, post-incubation TLC values for compounds *27*, *43* and *69* were compared against TLC values for their presumed metabolites *26*, *42* and *102*. Different R_f values were found for the test compounds. When the *N*-methylated compounds were incubated with microsomes up to 60 min, a second spot appeared with identical R_f values to the secondary amines *26*, *42* and *102*. GC-MS spectrometric analyses confirmed that *N*-desmethyl metabolites were formed from the corresponding *N*-methylated compounds. The mass spectra of the metabolites did not differ from those of authentic secondary amine derivatives (Fig. 2).

Discussion

Results show interaction between all acetylenic tryptamine derivatives tested and P450 rat liver microsomes. Secondary and tertiary aliphatic amines caused type II and type I spectral changes, respectively. Schenkman et al (1967) proposed that spectral changes as a function of the haemoprotein molar extinction reflect the influence on the haem-iron-protein interaction when a nucleophilic substrate binds near the haem. The spectral changes observed in our study were reversible and dependent on the concentration of substrates tested. Binding of these compounds to P450 which generates an enzyme-substrate complex according to Michaelis–Menten kinetics, probably precedes enzymatic oxidation. Calculation of spectral dissociation constants (K_s) shows that secondary amine derivatives have the highest bond affinity for P450, similar to that of phenylhydrazine ($K_s=25$ μM) (Jonen et al 1982). This is one order of magnitude greater than bond affinity reported by other authors for MAOIs such as pargyline, iproniazide, nialamide and tranylcypromine which induce type II spectral changes (Bélanger & Atitsé-Gbeassor 1981). We also found stronger MAOI bonding to P450 than to aniline ($K_s=360$ μM) (Schenkman et al 1967). Tertiary tryptamine derivatives, however, showed reduced affinity for P450, with a K_s value of the same order as clorgyline and oscillating over a wide range of concentrations.

All *N*-acetylenic indolalkylamine derivatives inhibited P450-dependent aniline hydroxylase activity. This inhibition, however, showed no correlation with observed K_s values. The main structural feature determining inhibition was the nature of the substituting group at the 5-position of the indole ring. Whereas compounds with an $-H$ and $-OCH_3$ group in the same position (*26*, *27*, *30*, *42*, *43* and *45*) had the lowest IC50 values comparable with clorgyline, the presence of an $-OH$ at 5-position of the indole ring (*102*, *69* and *70*), diminished inhibitory activity.

Complete inhibition of aniline hydroxylase activity was not observed with any of the compounds even at the highest (10^{-3} M) concentrations, thus indicating a low specificity towards aniline oxidation. A similar MAOI profile of antipyrine-4-hydroxylase activity was reported by Dupont et al (1987). Further study is required to ascertain whether the acetylenic derivatives of tryptamine would be more effective inhibitors of other P450-dependent oxidizing functions.

The addition of an NADPH-GS did not seem to affect aniline hydroxylase inhibition by $5-OH$ or $5-H$ indolalkylamine derivatives. On the contrary, NADPH-GS imparted heightened protection to the $5-OCH_3$ inhibition (*42*, *43*, *45*). The protection of aniline hydroxylase inhibition afforded by NADPH-GS may reside in promoting the formation of metabolites of diminished activity; consequently, NADPH-GS was omitted during kinetic trials. Other authors report a similar reduction in aniline hydroxylase inhibition when tranylcypromine was preincubated with NADPH-fortified rat liver microsomes (Bélanger & Atitsé-Gbeassor 1982). Bufuralol 1-hydroxylase inhibition (Dupont et al 1987) was also reportedly unaffected by the addition of NADPH-GS to phenelzine or tranylcypromine.

That all nine compounds inhibited aniline hydroxylase in a time-dependent and irreversible manner suggests a mechanism-based action consistent with apparent first-order kinetics. Consequently, comparable pseudo-first-order rate constants for aniline hydroxylase inhibition were obtained for all *N*-acetylenic derivatives of tryptamine (K_{app}). Incomplete solubility of these compounds prevented the calculation of the kinetic constants involved in the two steps of mechanism-based inhibition (Dixon & Webb 1979).

It has been said that the potential for destructive interaction with P450 is intrinsic to the acetylenic group and that this may be attenuated by modifying the moiety throughout incorporating the triple bond (Ortiz de Montellano & Kunze

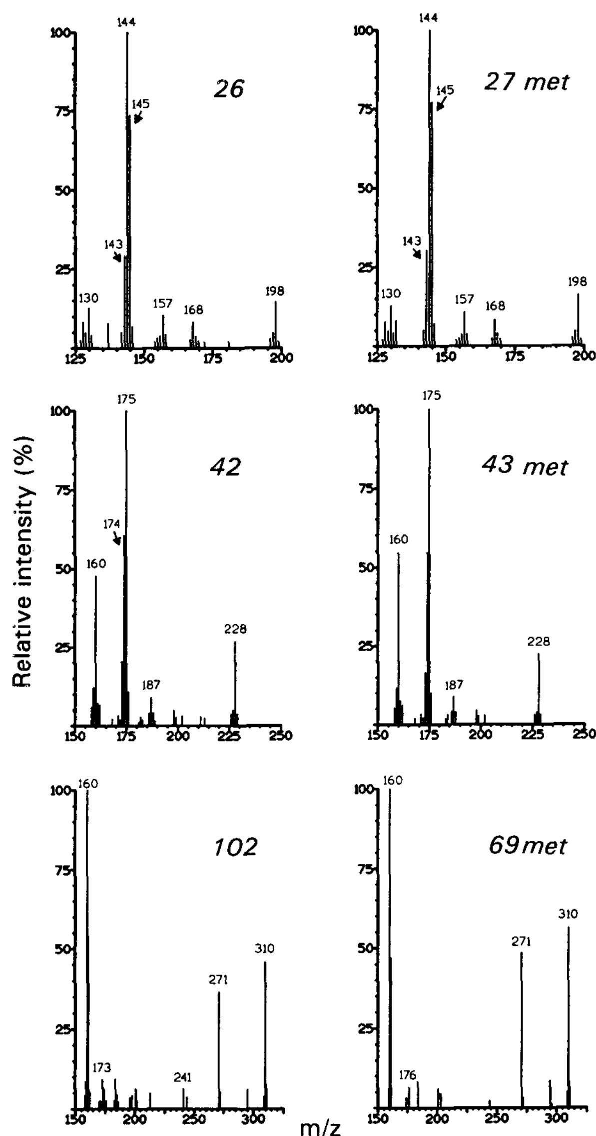

FIG. 2. Electron-impact mass-spectra of the *N*-demethylated metabolites of *27*, *43* and *69* obtained by incubating the *N*-methyl-derivatives *27*, *43* and *69* with microsomes, compared with the mass-spectra of the authentic compounds *26*, *42* and *102*. Experimental conditions are detailed in the text.

1980). However, the inhibitory capacity of the test compounds bore no relation to metabolic activation of their acetylenic moiety throughout and, as in the case of other compounds, might be ascribed to interference with the substrate-binding capacity or to disruption of electron transport and spin control of P450 (Murray & Reidy 1990).

Only the tertiary aliphatic amine derivatives performed as substrates for *N*-demethylase activity, all with similar K_m and V_{max} values. These parameters correlate well with V_{max} values of *N*-demethylase activity shown by ethylmorphine (13·7 nmol HCOH formed (nmol P450)$^{-1}$ min^{-1}), as reported elsewhere (Gibson & Skett 1986). TLC and MS spectrometric analyses demonstrated that demethylation involves only the methyl group attached to the *N* of the aliphatic chain and not that on the indole ring. Similarly, in the case of deprenyl, the *N*-dealkylation pathway was found to be very active, whether in-vivo or in-vitro, where this MAO-B inhibitor undergoes extensive metabolism to nordeprenyl and metamphetamine (Yoshida et al 1986).

N-Demethylation of compounds *27* and *69* gave rise to compounds *26* and *102*, already shown to be only one quarter as potent as MAO-A inhibitors compared with their parent compounds (Tipton et al 1993). Compound *42*, however, which may be considered the *N*-demethylated metabolite of *43*, showed a K_i value for MAO-A comparable with its parent (Avila et al 1993), seemingly due to the -OCH_3 group at position 5 of the indole ring.

Further study is required regarding the effects of MAOIs

on other enzymes implicated in the presystemic metabolism of xenobiotics such as intestinal peroxidase, whose involvement in the metabolism of certain direct and indirect sympathomimetic amines, such as L-dopa and tyramine, was previously established by these authors (Valoti et al 1992).

Interactive relations between MAOIs and oxidative enzymes such as P450 and peroxidases still need to be elucidated, as do their implications for the metabolism and toxicity of tyramine and other substances known to produce adverse reactions when administered in conjunction with MAOIs.

Acknowledgements

M. Costanzo was supported by an ERASMUS fellowship (ICP-91-1-0047/12). The authors thank Dr A. Franchi, U.O. Chimica Ambientale USL, Siena, for assistance in MS analysis. Dr R. Raikes is acknowledged for her help in improving the manuscript. This work has been supported in part by the CICYT Grant FAR 88-0194-CO2-02 from the Minesterio de Industria y Energia of Spain and MURST, CNR, Italy.

References

Avila, M., Balsa, D., Fernandez-Alvarez, E., Tipton, K. F., Unzeta, M. (1993) The effect of side chain substitution at position 2 and 3 of the heterocyclic ring of *N*-acetylenic analogues of tryptamine as monoamine oxidase inhibitors. Biochem. Pharmacol. 45: 2231–2238

Balsa, D., Fernandez-Alvarez, E., Tipton, K. F., Unzeta, M. (1990) Inhibition of MAO by substituted tryptamine analogues. J. Neural Transm. 32 (Suppl.): 103–105

Balsa, D., Fernandez-Alvarez, E., Tipton, K. F., Unzeta, M. (1991) Monoamine oxidase inhibitory potencies and selectivities of 2-[*N*-(2-propynyl)-aminomethyl]-1-methyl indole derivatives. J. Biochem. Soc. Trans. 19: 215–218

Bélanger, P. M., Atitsé-Gbeassor, A. (1981) The inhibitory effect of monoamine oxidase inhibitors on hepatic microsomal enzyme system of rat liver. Pharmacol. Res. Commun. 13: 819–828

Bélanger, P. M., Atitsé-Gbeassor, A. (1982) Inhibitory effect of tranylcypromine on hepatic drug metabolism in the rat. Biochem. Pharmacol. 31: 2679–2683

Blackwell, B. (1981) Adverse effects of antidepressant drugs. Part I. Monoamine oxidase inhibitors and tricyclics. Drugs 21: 201–219

Bowman, W. C., Rand, M. J. (1980) Textbook of Pharmacology. 2nd edn, Blackwell Scientific Publishers, Oxford, chapter 11, pp 9–11

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–250

Callingham, B. A., Valoti, M., Sgaragli, G. P. (1991) Drug and enzyme interactions with meclobemide. In: Racagni, G., Brunello, N., Fukuda, T. (eds) International Congress Series 968, Biological Psychiatry. vol 2; Excerpta Medica, Amsterdam, pp 846–849

Cruces, M. A., Elorriaga, C., Fernandez-Alvarez, E., Nieto, O. (1990) Acethylenic and allenic derivatives of 2-(5-methoxyindolyl) methylamine: synthesis and evaluation as selective inhibitors of monoamine oxidase A and B. Eur. J. Med. Chem. 25: 257–265

De Matteis, F. (1987) Drugs as suicide substrates of cytochrome P-450. In: De Matteis, F., Lock, E. A. (eds) Selectivity and Molecular Mechanism of Toxicity. The Macmillan Press, London, pp 183–210

Dixon, M., Webb, E. C. (1979) Enzyme inhibition and activation. In: Dixon, M., Webb, E. C. (eds) Enzymes. 3rd edn, Longman, London, pp 332–467

Dupont, H., Davies, D. S., Strolin-Benedetti, M. (1987) Inhibition of cytochrome P-450-dependent oxidation reactions by MAO inhibitors in rat liver microsomes. Biochem. Pharmacol. 36: 1651–1657

Gibson, G. G., Skett, P. (1986) In: Gibson, G. G., Skett, P. (eds) Introduction to Drug Metabolism. Chapman and Hall, London, pp 85–90

Gomez, N., Unzeta, M., Tipton, K. F., Anderson, M. C., O'Carroll, A. M. (1986) Determination of monoamine oxidase concentrations in rat liver by inhibitor binding. Biochem. Pharmacol. 35: 4467–4472

Jonen, H. G., Werringloer, J., Prough, R. A., Estabrook, R. W. (1982) The reaction of phenylhydrazine with microsomal cytochrome P-450. Catalysis of heme modification. J. Biol. Chem. 257: 4404–4411

Kato, R., Chiesara, E., Vassanelli, P. (1964) Further studies on the inhibition and stimulation of microsomal drug-metabolizing enzymes of rat liver by various compounds. Biochem. Pharmacol. 13: 69–83

Lake, B. (1987) Preparation and characterisation of microsomal fractions for studies on xenobiotic metabolism. In: Snell, K., Mullock, B. (eds) Biochemical Toxicology: a Practical Approach. IRL Press, Oxford, pp 183–215

Laroche, M. J., Brodie, B. B. (1960) Lack of relationship between inhibition of monoamine oxidase and potentiation of hexobarbital hypnosis. J. Pharmacol. Exp. Ther. 130: 134–137

Muakkassah, S. F., Yang, W. C. T. (1981) Mechanism of the inhibitory action of phenelzine on microsomal drug metabolism. J. Pharmacol. Exp. Ther. 219: 147–155

Murray, M., Reidy, G. F. (1990) Selectivity in the inhibition of mammalian cytochromes P-450 by chemical agents. Pharmacol. Rev. 42: 85–101

Nash, T. (1953) The collorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55: 416–421

Omura, T., Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2378

Ortiz de Montellano, P. R., Kunze, K. L. (1980) Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. J. Biol. Chem. 255: 5578–5585

Phillips, A. H., Langdon, R. G. (1962) Hepatic triphosphopyridine nucleotide-cytochrome C reductase: isolation, characterization, and kinetic studies. J. Biol. Chem. 237: 2652–2660

Rand, M. J., Trinker, F. R. (1968) The mechanism of augmentation of responses to indirectly acting sympathomimetic amines by monoamine oxidase inhibitors. Br. J. Pharmacol. Chemother. 33: 287–303

Schenkman, J. B., Remmer, H., Estabrook, R. W. (1967) Spectral studies of drug interaction with hepatic microsome cytochrome. Mol. Pharmacol. 3: 113–123

Stahl, E. (1965) In: Stahl, E. (ed.) Thin-layer Chromatography. A Laboratory Handbook. Springer-Verlag, Berlin, pp 292–301

Strolin Benedetti, M., Dostert, P. (1992) Monoamine oxidase: from physiology and pathophysiology to the design and clinical application of reversible inhibitors. Adv. Drug Res. 23: 65–125

Tipton, K. F., Balsa, D., Unzeta, M. (1993) Newer approach to the study of monoamine oxidase inhibition. In: Yasuhara, H., Parvez, S. H., Sandler, M., Oguchi, K., Nagatsu, T. (eds) Monoamine Oxidase: Basic and Clinical Aspects. VSP Press, Zeist, Holland, pp 1–13

Valoti, M., Tipton, K. F., Sgaragli, G. P. (1992) Oxidative ring-coupling of tyrosine and its derivatives by purified rat intestinal peroxidase. Biochem. Pharmacol. 43: 945–951

Yoshida, T., Yamada, Y., Yamamoto, T., Kuroiwa, Y. (1986) Metabolism of deprenyl, a selective monoamine oxidase (MAO) B inhibitor in rat: relationship of metabolism to MAO-B inhibitory potency. Xenobiotica 16: 129–136